Cotton Research and Development Corporation

FINAL REPORT

Influence of vesicular arbuscular mycorrhizas on growth, development and yield of cotton

UNE7C & UNE17C
1991-1995

David B. Nehl

UNE
The University of
NEW ENGLAND

Cotton Research and Development Corporation

Influence of vesicular arbuscular mycorrhizas on growth, development and yield of cotton

Projects UNE7C & UNE17C

UNE
The University of
NEW ENGLAND

Principal researcher: **David Nehl BSc (Hons)**, Postgraduate Student, Department of Botany, University of New England, Armidale NSW 2351.

Current address: Australian Cotton Research Institute, Myall Vale, via Narrabri, NSW, 2390. Ph. 067 991500.

Project supervisor: **Dr John F. Brown**, Associate Professor, Department of Botany, University of New England, Armidale NSW 2351. Ph. 067 732343

A final report prepared for the Cotton Research and Development Corporation

Summary

Influence of vesicular arbuscular mycorrhizas on growth, nutrition and yield of cotton

A growth disorder of cotton has been observed in irrigated cracking clay soils in fields at Galathera Creek, north of the Australian Cotton Research Institute (ACRI) for several years. The condition has since been found at other sites. Affected plants were stunted and grew slowly during the first half of the season and yielded poorly. Growth of stunted plants improved in mid-season but too late for substantial recovery of yield. Yield showed little or no improvement when P and Zn fertilisers were applied. Preliminary observations suggested that a lack of vesicular arbuscular mycorrhizal (VAM) development may have been adversely affecting cotton nutrition The aim of projects UNE7C and UNE17C was to determine the cause of the early season growth disorder (Galathera syndrome) and the role of VAM with regard to growth, development and yield of cotton.

Interactions among cotton growth, VAM fungi, soilborne pathogens and chemical and physical characteristics of the soil were investigated. The causes of the early season growth disorder were thus identified by a process of elimination.

Three soil groups (A, B, and C) which corresponded to patterns of yield and early season growth were identified in fields near Galathera Creek. Stunting of cotton was greatest in group A and B soils. Cotton growth and yield was relatively good in group C soils. Group B sites showed a recovery of yield later in the season while group A sites did not. Group A and B soils had lower pH, finer texture and higher P, Zn, Mn and exchangeable Mg, K and Na than group C soils. Thus, paradoxically, the greatest stunting occurred in the more fertile soils with the more favourable pH for cotton growth. The stunting of seedlings at an early stage was not consistent with some potential physical and chemical causes of stunting, including sodicity, compaction, manganese toxicity and waterlogging.

At some sites cotton had a high relative dependency (up to 92%) on VAM fungi for successful growth. In other words, 92% of the shoot growth of cotton was enabled by the presence of the VAM fungi. This growth benefit was primarily due to transport of P and Zn from the soil to the plant by the VAM fungi.

Cotton growth in the field was closely related to levels of VAM colonisation in roots: the more stunted the plants, the lower the level of colonisation. However, the lack of VAM colonisation in stunted cotton was not due to low numbers of VAM fungi in the soil. Rotation with dolichos lablab (a green manure crop used in fields with stunted cotton) did not affect the VAM development, growth and yield of cotton in comparison to bare fallow. Since soil P was higher in soil with stunted cotton, those plants were less dependent on VAM fungi for P supply and this accounted partly for the lower levels of VAM development. However, soilborne microorganisms were also inhibiting VAM development.

Sterilisation of soils in which cotton growth was stunted consistently increased cotton growth, which indicated that pathogenic soilborne microorganisms were causing the stunting. Viruses and nematodes were discounted as possible pathogens. Few fungi other than VAM fungi were observed colonising cotton roots from poor growth sites. Black root rot and verticillium wilt were least frequent in the stunted cotton. Therefore pathogenic fungi were not a cause of the disorder.

Several observations indicated that soilborne bacteria were responsible. First, the application of bacterial antibiotics (penicillin and streptomycin) to soil increased cotton

growth. Secondly, under the microscope bacteria were observed inside browned root cells and bacteria streamed out from the cut surfaces of browned roots. Rapid development of root browning was a symptom of stunted cotton. Thirdly, in laboratory studies bacteria isolated from browned cotton roots were shown to be pathogenic to cotton seedlings, causing root browning and stunted root growth. All the isolates of bacteria from cotton roots that were highly pathogenic belonged to a species of *Pseudomonas*. These bacteria were shown to inhibit VAM development in cotton. These pathogenic bacteria occur widely through cotton growing soils. The pathogenic species of *Pseudomonas* has been isolated form soil at the Australian Cotton Research Institute where early season stunting of cotton does not occur.

All these observations have contributed to a picture of the effects of soil ecosystems on cotton growth. The VAM fungi had the dominant effect on cotton growth in soils which did not show early season stunting (group C soils) and were less important in the more fertile soils that support stunted cotton growth (group A soils). Conversely soilborne pathogens had the dominant effect on cotton growth in group A soils, but also cause a slight reduction of growth in apparently healthy cotton.

In conclusion, the early season growth disorder is caused primarily by certain soilborne bacteria which inhibit growth and VAM development of cotton in nutrient rich, heavy clay soils. There may be further species of pathogenic soilborne bacteria than those isolated and tested so far. The severity of the early season growth disorder is undoubtedly linked to soil properties but the specific properties which tip the balance of the soil ecosystem to favour the pathogenic soilborne bacteria are yet to be identified. Current knowledge of the distribution of the disorder is inadequate. Potential directions for research of control procedures have been identified.

Abstract

Influence of vesicular arbuscular mycorrhizas on growth, nutrition and yield of cotton

Nehl, D.B. and Brown, J.F., Department of Botany, University of New England,

Armidale, 2351.

Allen, S.J., NSW Agriculture, Australian Cotton Research Institute, Narrabri, 2390.

A growth disorder of cotton (*Gossypium hirsutum*) occurs in irrigated cracking clay soils in cotton growing regions of northern New South Wales. Observations of early season cotton growth were made at 100 m intervals along transects in fields that showed gradients in severity. Ordination analysis of physical and chemical properties of the soil distinguished three groups of sites (A, B, and C) which corresponded to patterns of yield and early season growth. Early season growth and arbuscular mycorrhizal colonisation of cotton at group A and B sites was much slower than at group C sites. Group B sites showed a recovery of yield later in the season while group A sites did not.

Group A and B soils had lower pH, finer texture and higher P, Zn, Mn and exchangeable Mg, K and Na than group C soils. Thus, paradoxically, the greatest stunting occurred in the more fertile soils with putatively more favourable pH for cotton growth. Shoot growth also decreased with increasing manganese availability, soil clay content and soil water holding capacity. The stunting of seedlings at an early stage was not consistent with some potential physical and chemical causes of stunting, including sodicity, compaction, manganese toxicity and waterlogging.

Cotton had a high relative field mycorrhizal dependency in group C soil (up to 92% at six weeks after sowing). A series of bioassays in pots showed that slow mycorrhizal development in group A soils was not due to a reduction in the number of propagules of mycorrhizal fungi. Cotton was less dependent on mycorrhizal fungi in group A soils (15 to 20 %), which partly accounted for the lower levels of colonisation, but the soil microflora also inhibited mycorrhizal development.

Sterilisation of group A soils consistently eliminated root browning, a symptom of the disorder, and increased cotton growth in pots and in the field. This was despite the negative effects of sterilisation on P and Zn nutrition due to the elimination of mycorrhizal fungi. Fungi, viruses and nematodes were discounted as possible pathogens. Soil borne bacteria were shown to be causal 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 species of fluorescent *Pseudomonas* isolated from cotton roots was pathogenic to cotton. Variation in cotton growth within fields could be accounted for by the additive effects of non-biological and biological properties of the soil, including both beneficial and pathogenic microorganisms.

Full Report

Influence of vesicular arbuscular mycorrhizas on growth, nutrition and yield of cotton

INTRODUCTION

A growth disorder of cotton (*Gossypium hirsutum* L.) has been observed by farmers and agronomists in irrigated cracking grey clay soils in cotton growing regions of northern New South Wales since at least the early 1980s. The disorder was first observed in fields at Galathera Creek, an ephemeral stream, north of the Australian Cotton Research Institute (ACRI). Cotton has been grown in these fields for approximately 30 years. The condition has since been found in other areas of NSW.

Investigation of the growth disorder near Galathera Creek (Figure 1.1) was initiated by researchers at the ACRI. Affected plants were stunted and grew slowly during the first half of the season and yielded poorly. Gradients in severity of the stunting were present within individual fields. Cotton farmers observed that growth of stunted plants improved in mid-season but too late for substantial recovery of yield. Severely affected plants had lower phosphorus content, inadequate zinc content (as low as 9 mg kg^{-1} in young leaves) and showed visual symptoms of zinc deficiency (G. A. Constable, personal communication). Trial applications of N, P and Zn to areas with stunted cotton resulted in marginal or no increases in yield (G. A. Constable, personal communication).

Early in the 1990/91 season, vesicular arbuscular mycorrhizal colonisation of stunted cotton near Galathera Creek was 80% lower than in larger plants in the same field. This suggested that the stunting may have been caused by a lack of mycorrhizal colonisation and associated negative effects on plant nutrition. There was no obvious explanation for the reduction mycorrhizal development. Hence the causes of this early season growth disorder were not clear. The stunting of cotton may have been caused by biological or non-biological agents, or a combination of both.

OBJECTIVES

The aim of projects UNE7C and UNE17C was to determine the cause of the early season growth disorder and the role of mycorrhizas in the syndrome with regard to growth, development and yield of cotton.

RESULTS AND DISCUSSION

Projects UNE7C and UNE17C investigated many of the potential relationships among cotton growth, mycorrhizal fungi, soilborne pathogens and chemical and physical characteristics of the soil (Figure 1). The cause of the early season growth disorder was identified by a process of elimination.

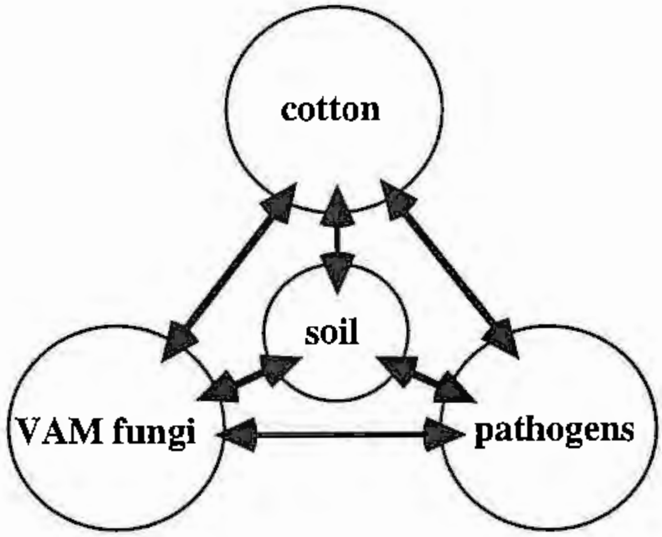

Figure 1. Potential interactions among cotton, some soilborne microorganisms and chemical and physical properties of the soil. (VAM fungi = vesicular arbuscular mycorrhizal fungi).

Cotton-soil Observations of early season cotton growth were made at 100 m intervals along transects in fields 18 and 20 on the Auscott Narrabri farm. Ordination analysis of physical and chemical properties of the soil distinguished three groups of sites (A, B, and C) which corresponded to patterns of yield and early season growth. Early season growth and mycorrhizal colonisation of cotton at group A and B sites was much slower than at group C sites. Group B sites showed a recovery of yield later in the season while group A sites did not.

Group A and B soils had lower pH, finer texture and higher P, Zn, Mn and exchangeable Mg, K and Na than group C soils. Thus, paradoxically, the greatest stunting occurred in the more fertile soils with the more favourable pH for cotton growth. Shoot growth also decreased with increasing manganese availability, soil clay content and soil water holding capacity. This suggested that reduced cotton growth might be related to either manganese toxicity or anaerobic conditions in the soil.

While it was certain that the early season growth disorder was related to soil characteristics, none of the cotton-soil associations described above prove causality. Hence experiments were designed to determine whether biological or non-biological characteristics of soil, at poor growth sites, were causing the disorder. These experiments were performed in the glasshouse and in the field. Sterilisation of these soils stimulated increased cotton growth. This was despite the fact that mycorrhizal colonisation was eliminated by sterilisation. In pots, the concentration of phosphorus and zinc in non-mycorrhizal plants was half that of the mycorrhizal plants and yet the mycorrhizal plants were stunted. This suggested that something in unsterilised soil was holding plant growth back, namely soilborne microorganisms that were pathogenic to cotton. Sterilisation of soil in the field increased cotton seedling growth and these seedlings contained twice as much Mn as stunted seedlings in unsterilised soil. Therefore Mn toxicity was not a cause of stunted seedling growth.

Mycorrhizal fungi-cotton On the transects in fields 18 and 20 shoot growth was closely related to mycorrhizal fungal colonisation: the more stunted the plants, the lower the level of colonisation of their roots. It was feasible that low levels of mycorrhizal colonisation at poor growth sites were due to an absence of viable propagules of the mycorrhizal fungi at the start of the season. A series of bioassays in pots showed that at the start of the season the colonisation of cotton roots in soil from poor growth sites was no lower than in soil from anywhere else. Hence, the early season growth disorder was not caused by a reduction in the number of propagules of mycorrhizal fungi in the soil.

Cotton was grown followin either a bare fallow or rotation with dolichos lablab (a green manure crop used in fields with stunted cotton) at sites severely affected by the early season growth disorder. At the start of the following season mycorrhizal colonisation, growth and yield of cotton was no different between these two treatments.

Mycorrhizal fungi-soil At some sites cotton had a high relative field mycorrhizal dependency (up to 92% at six weeks after sowing). In other words, 92% of the shoot growth of cotton was enabled by the presence of the mycorrhizal fungi. This dependency decreased as the level of available phosphorus in the soil increased. On the transects in fields 18 and 20 mycorrhizal colonisation in cotton roots declined as phosphorus availability increased. Hence the lack of mycorrhizal colonisation in stunted plants reflected a lower level of dependency on mycorrhizal fungi (15 to 20 % in group A soils). In a pot experiment mycorrhizal plants in pasteurised soil had double the phosphorus content of non-mycorrhizal plants in pasteurised soil. Therefore, although the dependency of cotton is lower in the high phosphate soils the mycorrhizal fungi still contribute to uptake of nutrients. However, in the same pot experiment, colonisation of roots inoculated with mycorrhizal fungi in pasteurised soil was 58% higher than colonisation of roots in unpasteurised soil. Therefore the high phosphorus availability was not the sole cause of reduced mycorrhizal development: microorganisms in the unpasteurised soil were inhibiting cotton growth.

Pathogens-cotton A symptom of the early season growth disorder is root browning. In pot and field experiments root browning was eliminated by soil pasteurisation which indicated that browning was caused by microorganisms. In fields 18 and 20 root browning was closely related to shoot growth: the stunted plants had the brownest roots. Therefore it seemed likely that root browning resulted directly from the activity of pathogenic microorganisms. Viruses and nematodes were discounted as possible pathogens. Few fungi other than mycorrhizal fungi were observed colonising cotton roots from poor growth sites. Colonisation of roots by *Thielaviopsis basicola* and chytrid fungi increased as shoot growth increased. Furthermore, the incidence of verticillium wilt symptoms in mature plants increased as boll yield increased. Therefore fungi were not causal in the disorder.

Several observations indicated that soilborne bacteria play a causal role in the early season growth disorder. First, the application of bacterial antibiotics (penicillin and streptomycin) to live soil gave increased growth of cotton in pots. Secondly, under the microscope bacteria were observed inside browned root cells and bacteria streamed out from the cut surfaces of browned roots. Thirdly, bacteria isolated from browned cotton roots were shown to be pathogenic to cotton seedlings, causing root browning and stunted root growth. All the isolates of bacteria from cotton roots that were highly pathogenic belonged to a species of *Pseudomonas*. These pathogenic bacteria occur widely through cotton growing soils. The pathogenic species of *Pseudomonas* has been isolated form soil at the Australian Cotton Research Institute where early season stunting of cotton does not occur.

Pathogens-mycorrhizal fungi An interaction between the pathogenic bacteria and the mycorrhizal fungi was found. In a pot experiment the colonisation of cotton roots by mycorrhizal fungi was reduced 10% by the application the pathogenic species of *Pseudomonas* to the soil. The lack of mycorrhizal colonisation observed in the field is caused partially by a negative interaction with the pathogenic bacteria and partially by the lower mycorrhizal dependency of cotton in high phosphate soils (as mentioned above).

In conclusion, the observations above have demonstrated that cotton growth is determined by the additive effects of non-biological and biological properties of the soil, including both beneficial and pathogenic microorganisms (Figure 2). The mycorrhizal fungi had the dominant effect on cotton growth in soils which did not show early season stunting (group C soils) and were less important in the more fertile soils that in which cotton growth was stunted (group A soils). Conversely soilborne pathogens had the dominant effect on cotton growth in group A soils, and only cause a slight reduction of growth in group C soils (Figure 2).

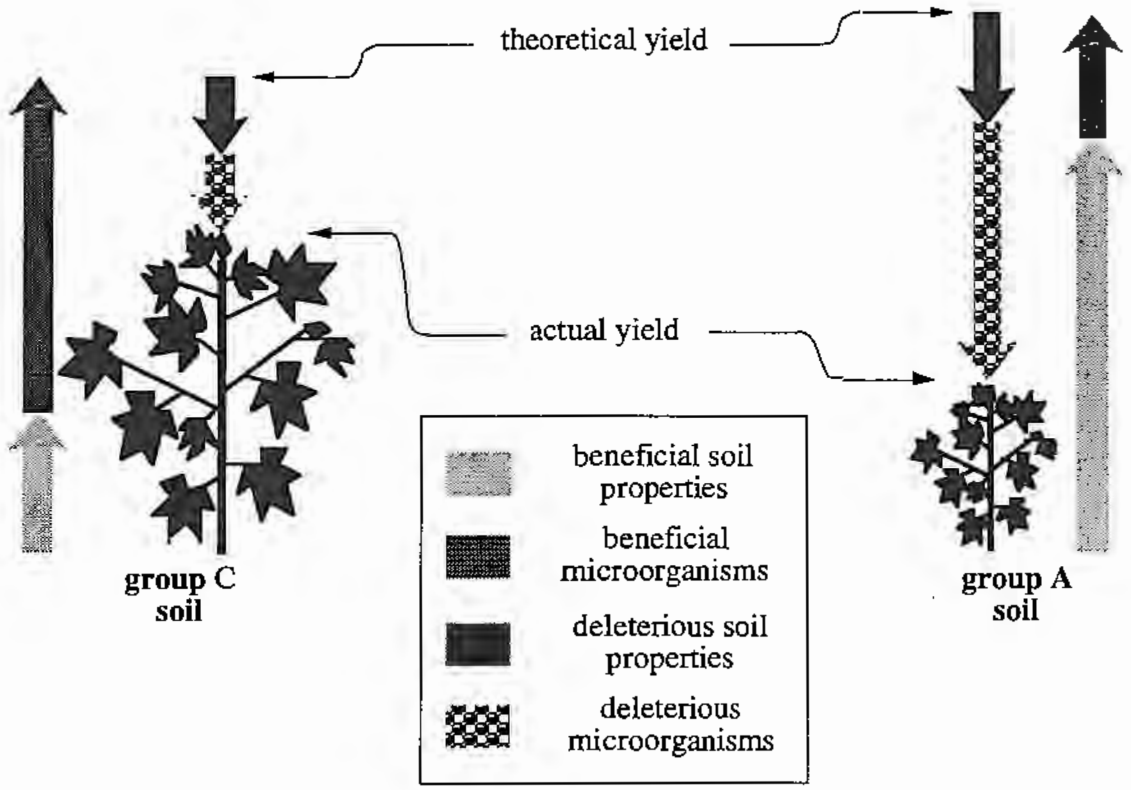

Figure 2 **A qualitative depiction of the relative contribution of biological and non-biological soil properties to cotton growth in soils that support slow early season growth (group A) and faster early season growth (group C). Arrows represent changes in growth and/or yield.**

This model of the factors affecting cotton growth (Figure 2) depicts an ecosystem which is dynamic. Field experiments showed that pathogens in group A soils were active throughout the growing season and therefore contribute a constant impediment to cotton growth.

In conclusion, the early season growth disorder is caused by bacteria which inhibit growth and mycorrhizal development of cotton in nutrient rich, heavy clay soils. Cotton is less dependent on mycorrhizal fungi in these nutrient rich soils than elsewhere. However, mycorrhizal colonisation is inhibited by bacteria in these soils and this contributes, in part, to poor cotton nutrition.

DISCUSSION RELATING TO OBJECTIVES AND OTHER WORK

The objective of UNE7C and UNE17C has been achieved. The early season growth disorder was shown to be a disease in which certain rhizosphere bacteria inhibit growth and mycorrhizal development of cotton in nutrient rich, heavy clay soils.

Other research on the early season growth disorder has included CRDC projects DAN 47L (*High yield packages for cotton*, researched by Dr Greg Constable) and DAN61C (*Involvement of phytotoxins, probably herbicides, in the Galathera syndrome*, researched by Dr Paul Milham). Research in DAN 47L showed that while 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, whilst apparently present in some soils, were not a cause of the disorder. It is possible that the phytotoxic effects found in DAN61C were due to toxins released by bacteria in the cotton rhizosphere.

Attendance at international conferences was enabled by travel under projects UNE15C and UNE24C. These conferences provided interaction with plant pathologists and mycorrhizal researchers which was highly beneficial to the research in projects UNE7C and UNE17C.

CONCLUSIONS, RECOMMENDATIONS AND APPLICATION TO INDUSTRY

This research has shown that cotton growth and nutrition is not determined by physical and chemical properties of the soil in isolation: the balance between beneficial and pathogenic soilborne microorganisms has a major influence cotton growth. There are two areas where our knowledge of the ecology of the early season growth disorder is lacking. First, there may be pathogenic species of bacteria other than those isolated and tested so far. Secondly, the unique properties of the soil which predispose development of a pathogenic population of soilborne bacteria have not been identified.

It has been estimated that the area affected by the growth disorder near Galathera Creek is approximately 10000 ha (G. A. Constable, personal communication). Current knowledge of the distribution of the early season growth disorder is inadequate. The fact that the disorder was first identified at Galathera Creek is a reflection of the severity of the disorder in that area. At other sites the disorder is less prominent or the severely affected areas are small. Therefore identification at other sites has been less prompt. Knowledge of the distribution of the disorder is likely to increase in future for two reasons. First, confirmation of its presence by researchers is now more certain because symptoms of the disorder are more fully understood. Procedures suitable for rapid, cost effective detection and monitoring of populations of pathogenic bacteria in cotton roots and soil are needed in further research of the early season growth disorder. Secondly, increased grower awareness of the problem is likely to lead to more frequent reporting of it. This trend has been observed since projects UNE7C and UNE17C commenced. User friendly protocols for detection the early season growth disorder by growers and/or consultants should be developed.

There is potential for development of control procedures in three areas: (i) selection of tolerant or resistant cotton cultivars; (ii) investigation of chemical controls; (iii) manipulation, by cultural practices, of those soil properties which specifically favour a population of pathogenic bacteria in and on cotton roots. Control procedures should be integrated with current disease management programs.

COMMUNICATION OF RESULTS

The principal researcher and Dr Steve Allen discussed aspects of mycorrhizas in cotton with growers at a Narrabri Agricultural Research Station field day in March, 1992. Results of research in UNE7C and UNE17C have been presented by the principal researcher at ACGRA cotton conferences in 1992 and 1994 and in four seminars at the University of New England. The principal researcher has participated in all CRDC Cotton Soil Coordination Meetings (formerly Soils and Tillage Review) since 1991. Presentations of results were made at three of these meetings.Dr John Brown presented research results in a seminar at the University of Florida in the USA in October, 1994.

Published papers

Allen, S. J., Constable, G., Nehl, D. B. and McGee, P. (1992). Mycorrhizas, early season cotton growth and the Galathera syndrome. *The Australian Cotton Grower*, 13 (1), 16-18.

Nehl, D. B. and Brown, J. F. (1992). A growth disorder of cotton associated with poor mycorrhizal development. In *Third Graduate Seminar in Plant Pathology and Mycology*, Eds. Brown, J. F. and Ponter, C. J., University of New England: Armidale, Australia.

Nehl, D. B. and Brown, J. F. (1992). Mycorrhizas in Cotton. In *Proceedings of the Sixth Australian Cotton Conference*, pp. 169-178, Australian Cotton Growers Research Association: Broadbeach, Australia.

Nehl, D. B. and Brown, J. F. (1993). Arbuscular mycorrhizal fungi: their potential as pathogens. pp. 21-24. In *Fourth Graduate Seminar in Plant Pathology and Mycology*, Eds. Ogle, H.J. and Brown, J. F., University of New England: Armidale, Australia.

Nehl, D. B. and Brown, J. F. (1993). Does phosphorus application benefit arbuscular mycorrhizal fungi?. pp. 25-30. In *Fourth Graduate Seminar in Plant Pathology and Mycology*, Eds. Ogle, H.J. and Brown, J. F., University of New England: Armidale, Australia.

Nehl, D.B. (1994). Culture of arbuscular mycorrhizal fungi using a constant water table technique. pp. 112-114. In *Fifth Graduate Seminar in Plant Pathology and Mycology*, Ed. Ogle, H.J., University of New England: Armidale, Australia.

Nehl, D.B. (1994). A simple bioassay for seedling pathogens using gnotobiotic chambers. pp. 109-111. In *Fifth Graduate Seminar in Plant Pathology and Mycology*, Ed. Ogle, H.J., University of New England: Armidale, Australia.

Nehl, D. B., Brown, J. F. and S.J. Allen (1994). Mycorrhizas and early season growth disorder: the lazy cotton plant gets into trouble. In *Proceedings of the Seventh Australian Cotton Conference*, pp. 337-347, Australian Cotton Growers Research Association: Broadbeach, Australia.

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

Abstracts

Nehl, D. B. and Brown, J. F. (1993). Biological and non-biological factors associated with a growth disorder in cotton. *9th Biennial Conference Australasian Plant Pathology Society*: Hobart. p. 55.

Nehl, D.B., Allen, S.J. and J.F. Brown (1994) Microbial suppression of plant growth and mycorrhizal development in cotton grown in heavy clay soils. *World Cotton Research Conference-1*: Brisbane.

Posters

Nehl, D. B., Allen, S. J, and Brown, J. F. (1992). Reduced arbuscular mycorrhizal development associated with a growth disorder of cotton. *The International Symposium on Management of Mycorrhizas in Agriculture, Horticulture and Forestry, Abstracts*, p.79, University of Western Australia: Perth, Australia.

Nehl, D.B., Allen, S.J. & Brown, J.F. (1993). *"Plants ain't plants" when they're mycorrhizal*. Moree Agricultural Trade Exhibition: Moree, NSW, Australia.

Publications in preparation

The doctoral dissertation of the principal researcher is undergoing final review and corrections before submission (expected late October 1995). A copy of this thesis will be provided to the CRDC and further copies will be lodged in libraries at the University of New England and the Australian Cotton Research Institute.

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

> Temporal variation in the arbuscular mycorrhizal colonisation potential of cotton growing soils. *Mycological Research*
>
> The role of biological and non-biological properties of the soil in a growth disorder of cotton. *Phytopathology.*
>
> Increased growth and arbuscular mycorrhizal colonisation of cotton caused by application of antibiotics to soil. *Soil Biology and Biochemistry.*
>
> Bacterial stunt of cotton caused by a fluorescent species of *Pseudomonas*. *Phytopathology.*

APPENDIX - BUDGET

Total funds contributed to UNE7C and UNE17C by the CRDC.

	UNE7C	UNE17C
1990-91	29,281	-
1991-92	46,924	-
1992-93	47,148	-
1993-94	26,358	22,785
1994-95	14,077	23,104
Total	$163,788	$45,889