Mycorrhizal status in the rotation: the importance to subsequent cotton establishment

J R Moore [1], J E Pratley[1], R Malone[3] K O'Keeffe[2] and K A Kirkby[4]

[1]Graham Centre for Agricultural Innovation (An alliance between NSW Department of Primary Industries and Charles Sturt University). Wagga Wagga Agricultural Institute, NSW DPI, Private Mail Bag, Pine Gully Road, Wagga Wagga, NSW, 2650, http://www.csu.edu.au/research/grahamcentre. jmoore@csu.edu
[2]Elders Rural Services, 11 Banna Avenue, Griffith, NSW, 2680.
[3]CottonInfo, Regional Development Officer, Southern NSW
[4]Australian Cotton Research Institute, Department of Primary Industries, Narrabri, 2390

Abstract
Early vigor in cotton is related to its ability to access nutrients present in the soil. Mycorrhizal associations are known to benefit cotton establishment by enhancing nutrient and moisture acquisition, particularly phosphorus and zinc. If the colonisation of arbuscular mycorrhizal fungi (AMF) is incomplete, the cotton crop may be restricted in its establishment and growth. The need for vigorous germination, strong emergence and establishment at appropriate densities becomes paramount to subsequent crop development and ultimate yield. Commercial mycorrhizal testing of 'pre-plant' soil samples showed a wide range of spore counts (4-100 spores/g), dependant on the crop rotational circumstances. Additionally, a field site previously used for rice that displayed growth differences between old bank lines and the adjacent rice bay area was utilised for soil sampling. Paired soil core samples from these areas were taken in PVC tubes (100mm diameter, 250mm depth). Plant height and establishment rates were measured in the field at time of sampling. Plant heights at time of soil core sampling between bank and bay areas were significantly different (p <0.001). Subsequently, PVC tubes were then sown with cotton and utilised in a pot experiment. Cotton plants were grown for six weeks in a temperature controlled glasshouse (day/night cycle 30°C/20°C). Roots were then washed, stained and mycorrhizal colonisation was determined in each sample. Mycorrhizal colonisation was greater in soil cores sampled from old bank areas compared with the adjacent bay ($p < 0.05$).

This paper discusses the relationship between crop rotations and their effect on colonisation of mycorrhiza in cotton and the implications of converting old rice bays into raised beds for cotton production.

Key words
Crop rotations, cotton emergence, soil biology

Introduction

Cotton is one of the leading plant fibre crops worldwide and is grown commercially in the temperate and tropical regions of more than 50 countries (Smith 1999). In Australia, the bulk of the cotton industry is concentrated in northern New South Wales and southern Queensland. However the industry has made steady inroads to expanding the southern NSW area planted. Cotton is now grown commercially from the Victorian border to Emerald in central Queensland, and as far west as Bourke and Lake Tandou in New South Wales. Cotton is grown either as a dryland crop, relying on rainfall, or as an irrigated crop where a reliable water supply is available. The total area planted to cotton in Australia was about 583,000 hectares in 2011/12 season. Cotton in southern regions is grown in rotation with rice and winter crops, thus experiences a different set of agronomic challenges specific to the south. The shorter season associated with southern cotton production necessitates strong germination, emergence and establishment as there is little time for compensatory growth resulting in lowered yields. The switch in crop from rice to cotton also presents unique growth and development problems to cotton in southern Australia, which are not yet elucidated.

As mycorrhizal associations are known to benefit cotton establishment, determining their colonisation subsequent to rice in the rotation was investigated. Mycorrhiza are ubiquitous plant symbionts which colonise the root systems of most terrestrial plants (Nehl *et al*. 1996). The fungus relies on the plant to provide carbohydrates and in exchange provides the plant with an underground hyphal network that extends the root system and allows increased uptake of nutrients and moisture (Ho and Trappe 1973). The predominant type of mycorrhiza found associated with 60-70% of plant species are arbuscular mycorrhizal fungi (AMF) and these are associated with the roots of cotton.

The two objectives of this study were-

1. To measure the AMF content in soil to determine the level and variability of colonisation following different crop rotations in the southern NSW region.
2. Investigate mycorrhizal colonisation in soil cores taken from an area which exhibited poor cotton growth after rice bay conversion to raised beds.

Method

Pre-plant field collection

Field soil and cotton roots for pre-plant testing and in crop testing were collected from two farms in the Coleambally and Darlington Point areas. Pre-plant soil was sampled according to Forecasta pre-plant® requirements and commercially tested by Microbiology Laboratories Australia. Briefly, supplied containers were filled with field collected soil and sampled at a rate of one sub sample per hectare using a diagonal transect through each cotton field. Each sample was bulked into the final sample and subsequently sealed and immediately sent for commercial testing.

Glasshouse experiment

Paired soil cores utilised in the pot experiment were taken from a cotton field in the Coleambally irrigation area where the old bank lines were and adjacent rice bay areas were apparent. Core samples were taken in PVC tubes (100mm diameter, 250mm depth). Subsequently, the PVC tubes were sown with cotton as a pot experiment. Cotton plants were grown for four, five and six weeks in a temperature controlled glasshouse (day/night cycle 30°C/20°C) before being sampled. Soil cores were held in individual plastic bags and placed in a large watertight container and each bag filled with a 2g L^{-1} sodium hexametaphosphate solution and allowed to soak for one hour to facilitate the separation of roots and soil. Soil was then transferred to a bucket and agitated vigorously with a jet of water. After allowing the sample to settle for several seconds, the supernatant was poured onto a fine sieve (250µm). Roots were then recovered and stored in 70% ethanol until the staining process was performed. The staining method is adapted from Koske and Gemma (1989). Briefly, 0.3-0.5g fresh roots were transferred to custom staining tubes and submerged in 10% KOH and placed in a 90° C water bath for one hour. Roots were subsequently acidified with 2% HCL for 5 minutes before the staining solution (acidified glycerol, water and 0.05% trypan blue) was added and placed back in a 90° C water bath for 20 minutes. Stained roots were then de-stained for 30 minutes in a 90° C water bath with acidic glycerol to remove excess stain. Colonisation of cotton roots (as percent of root length infected) were then assessed using the line intersect method described by Giovannetti and Mosse (1980).

Experimental design and statistical analysis

Paired soil cores were arranged in a randomised block design with three sampling times.
Effects of sampling time and location in the field (bank or bay) were tested using a two way analysis of variance. For the field measurements, the effect of location in the field was tested using a one way analysis of variance. Correlation between shoot dry matter and mycorrhizal colonisation was tested using a Pearson product moment correlation. All statistical analyses were performed in R statistical package (R Development Core Team 2008*)*.

Results and discussion

Pre-plant field collection

Canola crops had the lowest mycorrhizal spore and colonisation levels followed by rice (Table 1). Canola does not associate with mycorrhizal fungi. Canola crops are known to produce a biofumigation effect on the soil with toxic chemicals such as glucosinolates and their breakdown products isothiocyanates likely to be responsible for decreased mycorrhiza in the soil (Glenn *et al*. 1988; Vierheilig *et al*. 2000). Variation in spore counts are likely to have been affected by different management practices between between sampling locations.

Table 1 Results from commercial (VAMwise) 'Forecasta pre-plant' tests of soil collected from cotton fields in 2014/15 season for mycorrhizal spore content (spores/g) and predicted colonisation percentage.

2013/14 crop	Spore count (spores g^{-1})	Colonisation (%)
Canola	4	12.8
Canola	5	16
Canola	6	19.2
Canola	7	22.4
Canola	9	28.8
Canola	13	41.6
Rice	20	64
Fallow	35	100
Wheat	40	100
Cotton	51	100
Cotton	70	100

Glasshouse experiment

At the time of soil core sampling plant heights were significantly different (p <0.001; Table 2), Bank areas sampled exhibited higher growth as compared with old rice bay areas. There was a clear effect seen in the field at the time of sampling and the old bank line was easily distinguished from the adjacent bay. However there was no effect on establishment rate per metre of row (Table 2) or the mean number of nodes found per plant per metre row when the soil cores were taken.

There was a significant main effect between combined sampling time means of bank and bay shoot matter (p <0.05). There were also differences in mycorrhizal colonisation (p <0.05) between combined means of bank and bay soil cores. Mycorrhiza have been reported to always be associated with the roots of cotton and found to increase the growth and development of cotton plants, as well as cause earlier flowering and boll formation (Rich and Bird, 1974; Price *et al*. 1989; Nehl *et al*. 1996). There was a strong positive correlation between combined sampling time means of shoot dry matter and AMF colonisation (Pearson correlation coefficient =0.781; $p \leq 0.01$).

Table 2 Mean observations comparing old bank lines and the adjacent rice bay area

Observations	Bank	Bay	
In-crop heights (mm)	733	499	$p < 0.001$
Establishment (plants m^{-1})	12.2	13.1	NS
Nodes (nodes $plant^{-1}$ m^{-1})	13	11	NS
Colonisation (%)	43.88	21.80	$p < 0.05$
Shoot dry matter (g $plant^{-1}$)	0.460	0.374	$p < 0.05$

Where rice has been the prior crop, rice bay area's had a lower mycorrhizal colonisation rate compared with bank areas. The inundation of rice crops changes the soil biology and chemistry, previous research having shown that phosphorus and zinc are immobilised in the soil post-rice (Willet *et al*. 1978) leading to slower early growth.

Conclusions

AMF spores were lower in soil of cotton fields that had previously canola. This may be due to the biofumigation effect canola produces. The lower spore counts found in cotton fields previously sown with rice are likely due to the inundation of the soil whereby the anaerobic conditions may have inhibited mycorrhizal spore production. AMF are essential for normal cotton growth and yield. If the number of AMF propugales in the soil is low, colonisation of the roots is delayed and plant growth may be depressed, with subsequent delays in maturity and reductions in yield. The importance of mycorrhizal associations in southern NSW, and their potential for enhancing cotton establishment, require further investigation.

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