Mycorrhizal colonisation of dry season cotton grown on virgin soil in northern Australia

Brian L Duggan[1] and Megan H. Ryan[2]

[1]CSIRO Plant Industry, Locked Bag 59, Narrabri, NSW 2390, Australia Email brian.duggan@csiro.au
[2]School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia Email mryan@agric.uwa.edu.au

Abstract

Colonisation by arbuscular mycorrhizal fungi (AMF) was assessed on cotton grown during the dry season on soil that had no previous history of fertiliser application or cropping in the Ord River Irrigation Area (ORIA) of northern Western Australia. Colonisation by AMF was low (<11% of root length) 17 days after sowing in the first year cotton was grown while in the second year colonisation improved dramatically (>62% in phosphorus-fertilised plots). However, second year crops that had received no phosphorus fertiliser in either year displayed lower colonisation levels (25%). As the response of cotton dry matter and node development to application of phosphorus fertiliser was the same in both years, irrespective of AMF colonisation being negligible or high, it appeared the fungi were not playing a major role in crop nutrition or growth at time of sampling. Colonisation levels also did not differ between Bt transgenic and conventional plants.

Media summary

Cotton plants, both transgenic and conventional, were more readily infected with AMF when grown the year after a cotton crop than on virgin soil.

Key words

tropical, superphosphate, clay, Kimberley, fallow

Introduction

The Ord River Irrigation Area (ORIA) is located in the Kimberley region of northern Western Australia and despite the tropical semi-arid environment grows an array of crops during the winter/'dry season' (April-November) due to the abundant supply of water from Lake Argyle. The current area under cultivation is 15,000 ha although a further 45,000 ha of land, known as Ord Stage II, is being investigated for further development. Cotton is a candidate crop to be grown in this area. In its virgin state the soil is known to be low in phosphorus and zinc and if cotton is to be grown in Ord Stage II strategies for fertiliser management will be required. In addition, the dynamics and effect on cotton of arbuscular mycorrhizal fungi (AMF), which have been reported elsewhere to be important for phosphorus nutrition of crops in the tropics (Ahiabor and Hirata 1994) may require elucidation.

Materials and Methods

Experiments to investigate phosphorus requirements and AMF dynamics for cotton grown on soils similar to those in Ord Stage II were conducted at the Frank Wise Institute of Tropical Agriculture, Kununurra, WA, Australia (15?39'S, 128?43'E) in the 2002 and 2003 dry season (winter). The soil is predominantly a uniform dark brown medium to heavy clay with swelling and shrinking characteristics (Gunn 1969), known locally as a Cununurra clay (Ug 5.34; Northcote 1971) (Montomorillinitic Typic Happlustert).

The land was cleared of trees (*Lysiphyllum cunninghammi*) in 1988, cleared of native grasses (*Chrysopogon fallax, Aristida sp*. and *Astrebla squarrosa*) and shrubs (*Abelmoschus ficulneus* and *Hibiscus panduriformus*) and laser leveled in 1996 and again in 2001 (G Plunkett and P McCosker, personal communication). Soil analysis was conducted on the field during the 2001 dry season. In the top 30 cm of soil there was 3 mg/kg of available P (Colwell bicarbonate extraction (Colwell 1963)), 31 mg/kg

of total P, 0.58 mg/kg of DTPA Zn and the soil pH (1:5 $CaCl_2$) was 6.91 with an increasing trend with depth. Prior to sowing in 2002 and 2003, five rates of phosphorus (0, 40, 80, 120 and 160 kg/ha) were applied as double superphosphate 20 cm deep and 2 cm outside the proposed plant line. Sulphur (51 kg/ha), zinc (40 kg/ha) and nitrogen (200 kg/ha) were balanced across the trial using $ZnSO_4$ and urea.

The experiment was designed as a randomised block with four replicates. In 2003 each plot in the area on which the 2002 trial had been conducted was further divided into five subplots and each randomly allocated one of the five rates of phosphorus fertiliser. However, only plots that received no additional phosphorus fertiliser in 2003 were sampled. This area will from here-on be referred to as the 'old area', while the area which was sown to cotton for the first time in 2003 will be known as the 'new area'. The 'new area' had been left fallow during the 2002 dry season. The crops were sown into dry soil on the 28th of April 2002 and on the 28th of March 2003. There were two rows, spaced 90 cm apart, grown on 100 cm wide beds. Beds were watered up two days after sowing and nine plants per metre of row were established in each year.

The cultivar chosen in 2002 was Sicot 289i which is transgenic for Bt toxins *Cry 1Ac*, while in 2003 the cultivar was Sicot 289B which is transgenic for two Bt toxins *Cry 1Ac* and *Cry 2Ab*. Sampling for AMF consisted of removing five plants with roots intact from each plot 17 days after watering up. Roots were removed , washed and stored in 70% ethanol, and later stained with aniline blue (Grace and Stribley 1991) and the percentage of root length colonised by AMF calculated using the line-intersect method (Giovannetti and Mosse 1980). The number of main-stem nodes for each plant sampled for AMF was determined and the above ground dry weight of the plants measured after drying in a fan-forced oven at 70?C for 48 hours. Refuge areas of conventional cotton cultivar Siokra 1-4 were also grown and in 2003 samples were taken from these areas which had ('new area') and had not ('old area') had crops and fertiliser applied in 2002.

Results

AMF colonisation in 2002 was low and variable (Table 1). The higher than expected value for the 40P treatment was possibly due to weedy shrubs such as *Abelmoschus ficulneus* or *Hibiscus panduriformus* which may have been growing in the sampling area during the 2001 dry season.

Table 1. AMF colonisation of transgenic cotton grown on virgin soil in 2002 ('old area')

Fertiliser treatment	Root length colonised by AMF (%)
0P	0.4
40P	11.2
80P	1.1
120P	1.9
160P	1.9
LSD (P=0.05)	NS

In 2003, AMF colonisation was as low or lower in the 'new area' as in 2002 (Table 2). Above ground dry matter and node production were lower for the 0P treatments compared to those treatments that had received any amount of phosphorus fertiliser.

Table 2. AMF colonisation and plant development of transgenic cotton on virgin soil in 2003 ('new area')

Fertiliser treatment	Root length colonised by AMF (%)	Above ground dry matter (g/plant)	Nodes
0P	2.0	0.37	2.8
40P	1.5	0.57	3.3
80P	0.8	0.58	3.3
120P	0.5	0.68	3.5
160P	1.2	0.67	3.5
LSD (0.05)	NS	0.08	0.3

Sampling from the 'old area' in 2003 demonstrated that AMF readily colonised the roots of cotton plants in the second year in which they were grown at this site (Table 3). However, plants that had not been exposed to any phosphorus fertiliser in the two years had less colonisation than those that had received some phosphorus fertiliser and, in addition, produced less above ground biomass and fewer nodes at sampling.

Table 3. AMF colonisation and plant development of transgenic cotton in 2003 on soil previously sown to cotton in 2002 ('old area'). Variable fertiliser rates were applied in 2002, no fertiliser was applied in 2003.

Fertiliser treatment	Root length colonised by AMF (%)	Above ground dry matter (g/plant)	Nodes
0P	25.0	0.37	2.9
40P	63.7	0.64	3.7
80P	70.5	0.55	3.4
120P	67.7	0.62	3.9
160P	62.2	0.71	3.8

LSD (0.05)	26.8	0.18	0.4

Conventional cotton also displayed significantly greater AMF colonisation when grown in the 'old area' than the 'new area' (Table 4). Both of these areas were well fertilised with phosphorus and displayed no difference in above ground dry matter production or node production.

Table 4. AMF colonisation and plant development in conventional cotton grown in 'old' and 'new' areas in 2003. Both areas had 160P applied in the first year cotton was grown. Numbers in brackets are transformed data (√X+1).

Fertiliser treatment	Root length colonised by AMF (%)	Above ground dry matter (g/plant)	Nodes
'New area'	0.0 (1.00)	0.76	3.9
'Old area'	50.0 (7.05)	0.86	4.0
LSD (0.05)	(2.09)	NS	NS

Discussion

Results from 2002 and 'new area' experiment in 2003 demonstrate that AMF colonisation in the first year a cotton crop is grown on virgin soil in the ORIA is extremely poor. However, AMF colonisation was substantially better on cotton grown in the second year, irrespective of the amount of phosphorus fertiliser applied in the first year. Colonisation following no application of phosphorus fertiliser in the first year was lower than following the application of any rate of phosphorus. This may reflect the lower amount of cotton root biomass produced in the previous year, and thus production of less AMF inoculum. Inoculum levels following the laser levelling and fallowing in 2001 were likely to be low (Thompson 1987; Pattinson and McGee 1997). It may also reflect AMF themselves being limited by phosphorus on these low phosphorus soils (Pairunan *et al* 1980) or the absence of AMF species which readily colonise cotton. Above ground biomass and node production was restricted for plants that had not been provided with any phosphorus fertiliser, irrespective of AMF colonisation level. In the 'old area' the similar responses to addition of phosphorus fertiliser in the poorly colonised 2002 and highly colonised 2003 crops suggests AMF were playing little role in crop phosphorus nutrition at this stage of their development. While poor AMF colonisation has been found to restrict the growth of cotton in temperate areas (Rich and Bird 1974) and of some crops in tropical regions (Ahiabor and Hirata 1994), the fungi have been shown to play no role in crop nutrition in some crop species in other regions (Ryan and Angus 2003). Further research is required to define the role of AMF in crop growth in the ORIA. The transgenic and conventional cotton did not differ in colonisation level indicating that the capacity to host AMF was not compromised in the transgenic cotton in spite of the insertion of genes involved in production of Bt toxins to aid with insect control. In an attempt to promote the presence of AMF in virgin soil it may be beneficial to grow a wet season cover crop that promotes the production of AMF inoculum capable of colonising cotton prior to the dry season in the ORIA. However, the affects of AMF on cotton production in the ORIA require further research.

Conclusion

Cotton grown on virgin soil had low colonisation rates with AMF although this was dramatically increased in the second year that crops were grown in that area. The application of phosphorus fertiliser increased the colonisation by AMF in the second year of cropping although at the early stages of growth plants did not appear to be relying on AMF for their uptake of phosphorus. AMF colonisation occurred to the same degree in both transgenic and conventional cotton plants in the second year that cotton was grown.

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