Mycorrhizal inoculants for cotton: Doing less with more

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Abstract
This study investigates the value of mycorrhizal inoculants for cotton production in southern NSW. Cotton seeds were inoculated with one of seven commercially available mycorrhizal products at three times the recommended rate (RR) and at twenty times RR for comparison with non-inoculated controls. Seedlings were grown under two nutrient regimes. Inoculated cotton plants were inspected for mycorrhizal colonisation after 6 weeks growth. However, although there was some colonisation of roots in some products at the higher rate, the extent of that colonisation was very low and inadequate to make a production difference in the seedlings. Additionally, soils were collected from adjacent field plots growing wheat, barley, canola, field peas and along the fence line to provide further assessment of the background mycorrhizal levels. An ex rice soil from Coleambally, NSW, was included as a known positive control. The colonisation of seedling cotton was significant in all cases and well above any colonisation achieved with commercial AMF inoculants. Of the winter crop species backgrounds, wheat was clearly superior and field peas least effective. Our assessment of the commercial inoculant products showed them to be ineffectual at both three times RR and twenty times RR outlined on the label. Management of crop rotations provides an alternative and more reliable means for boosting background mycorrhizal levels in the soil.

Keywords
Cotton mycorrhiza, arbuscular mycorrhizal fungi, commercial inoculants.

Introduction
Cotton grown in southern New South Wales needs to emerge quickly and strongly to ensure that yields in the short summer season are maximised. Mycorrhizal associations assist emerging cotton seedlings to access phosphorus and zinc more efficiently and are essential for normal cotton growth (Allen and Nehl 1999). If phosphorus and zinc are limiting, the developing cotton seedlings become more susceptible to diseases, competition from weeds and temperature fluctuations. What is unclear is the extent to which AMF inoculation of cotton enhances colonisation of mycorrhizal fungi to compensate for the slower establishment conditions in southern NSW. Commercial AMF products are not regulated under the Australian Pesticides and Veterinary Medicines Authority (APVMA) and so evidence of their effectiveness is not readily available. The science is clear that advantages may be gained by boosting mycorrhizal presence in the soil, but there is no consensus as to the effectiveness of commercial inoculants for improving crop production, and their effects are often difficult to measure. Resilient propagules of AMF, such as spores, mycorrhizal root pieces and organic matter containing hyphae, can be produced. Dry inoculant can remain viable for many years at room temperature (Sylvia and Jarstfer 1994), whereas refrigerated moist inoculants have a shorter life-span of approximately two years. Further, the widespread occurrence of these fungi in nearly all Australian agricultural soils may negate their usage completely. The feasibility of using commercially available inoculants for broadacre cotton to enhance seedling nutrient acquisition and thus improve speed of establishment is the primary aim of this research.

Methods
Evaluation of commercial AMF products was undertaken in the laboratory and glasshouse. A glasshouse trial of 7 commercial products considered the colonisation outcomes on cotton seedling roots. For comparison and as validation of techniques in the glasshouse, the AMF background levels in a comparable soil were measured based on a range of current crop types.

Inoculation experiment
The impact of the AMF inoculants on cotton germination and growth were evaluated in glasshouse experiments. All products were sealed, kept at 4 °C and utilised within 2 weeks of acquisition. In this experiment three cotton seeds (variety Sicot 71BRF) were sown into each pot and seven inoculants were applied at two rates, at three times the recommended rate (3RR) and at 20RR. The inoculants were applied

directly to each seed and subsequently planted in the soil mix at a depth of 20 mm. Cotton seedlings were later thinned to the strongest seedling per pot after germination in order to reduce variation. Cotton seedlings were grown until six leaves were present and roots were sampled and processed as previously described. The soil mix comprised sand:loam at 7:3 to facilitate steam penetration during the pasteurisation process with a soil steamer with temperature maintained at 70 °C for 1 hour. Each inoculant treatment was exposed to two nutrient regimes relating to phosphorus and trace elements. Phosphorus was applied as superphosphate at the rates of 0.1g/pot and 0.4g/pot together with a micronutrient supplement. Soluble nitrogen in the form of urea (urea, 46 % w/w nitrogen) was applied at a rate of 0.15 g per pot every 2 weeks from the start of the experiment for its duration. Soluble sulphate of potash (Potassium as sulphate 41.5 % w/w and sulphur as sulphate 17 % w/w) was applied at the start of the experiment to each pot at the rate of 0.11 g per pot. Germination was recorded each day with growth and root colonisation by AMF evaluated at time of harvest, 6 weeks after sowing. Root and shoot lengths, dried whole plant biomass and number of nodes were also taken. Experimental design comprised a randomised complete block design with three replicates in a temperature controlled glasshouse at 30/20 °C for 12 h/12 h, day/night cycle. Pots were maintained at field capacity for the duration of the experiment. The experimental control contained pasteurised sandy loam soil without the application of inoculants. The staining method was adapted from Koske and Gemma (1989). Colonisation of cotton roots (as percent of root length infected) were then assessed using the line intersect method described by Giovannetti and Mosse (1980).

Background AMF

Background soil level of AMF under different crop regimes Soils were collected from adjacent field sites containing wheat, barley, canola, field peas and along the fence-line. In addition, an ex-rice soil of known mycorrhizal infectivity was added as a positive control. The collected soils were dried at 40 °C for 48 hours and then ground to fit through a 1mm sieve. Sand was added to provide a sand:soil ratio at 7:3 to facilitate steam sterilisation of control treatments. Experimental pots were filled with the appropriate sand/soil mix (1.2 kg). Three cotton seeds (variety Sicot 71BRF) were sown into each pot and one of two inoculants was applied at either 3RR or 20RR directly to each seed. Seeds were subsequently planted in the soil mix at a depth of 20 mm. Plants were later thinned to one plant per pot and were maintained and grown for six weeks under controlled glasshouse conditions (20/30 °C 12hr/12hr night day cycle). The experiment utilised a randomised complete block design (four blocks, seven treatments, two AMF inoculants and two rates of inoculants). Shoot length, root length, shoot dry weights and mycorrhizal colonisation of roots were measured at time of harvest.

Results

Glasshouse inoculation

Germination, plant growth and root colonisation by AMF at time of harvest were measured in all treatments but none of these parameters produced a significant response to any of the seven mycorrhizal products used (Table 1). Minor AMF root colonisation occurred in M6 and M7 (< 1%) treatments but these levels were miniscule and unlikely to be contributing to plant performance. This outcome suggests that the inoculants tested were either grossly below the concentration of propagules needed or were non-viable. A nutrient growth response achieved for the positive control rice soil (CR) ($P < 0.001$; LSD = 0.0685; data not shown) indicated that there was scope for mycorrhizal influence in growth response should colonisation have been affected. In other studies, properly stored and applied commercial inoculants have also failed to show responses under varying conditions (Corkidi et al. 2004).

Table 1. List of parameters and the P value attained for each parameter (3 way ANOVA; Fertiliser rate x Inoculant rate x Inoculant type; Dependant variable AMF infection %).

Parameter	P value
Fertiliser rate	0.703
Inoculant	0.154
Inoculant rate	0.580
Fertiliser x Inoculant rate	0.068
Fertiliser x Inoculant	0.520
Inoculant rate x Inoculant	0.184
Fertiliser rate x Inoculant rate x Inoculant	0.385

Plant growth parameters showed a highly significant response to the phosphorus fertiliser regime ($P < 0.001$; data not shown). Fertiliser effects on shoot height and dry matter weight were highly significant for the

experimental soil. As expected the CR (positive control rice soil) treatment did not produce a fertiliser response as it was a different soil, that was already high in phosphorus, and only included as a positive AMF control. The general fertiliser response was consistent across all seven inoculant treatments.

Background soil level of AMF under different crop regimes
This experiment was undertaken as a yardstick to AMF levels to increase the confidence in the validity of the previous experiments. In all measurements there were no differences in the + and – inoculant treatments for any soil background and so data have been combined. Wheat background gave highest shoot length (data not shown) and dry matter of cotton seedlings (data not shown) and was superior to all other species than rice in root colonisation by AMF. The peas background appeared to inhibit AMF colonisation (Figure 1) although this was not reflected in shoot length or dry matter production. While variation in AMF colonisation occurred relative to species background there were no measurable effects from the inoculants treatments.

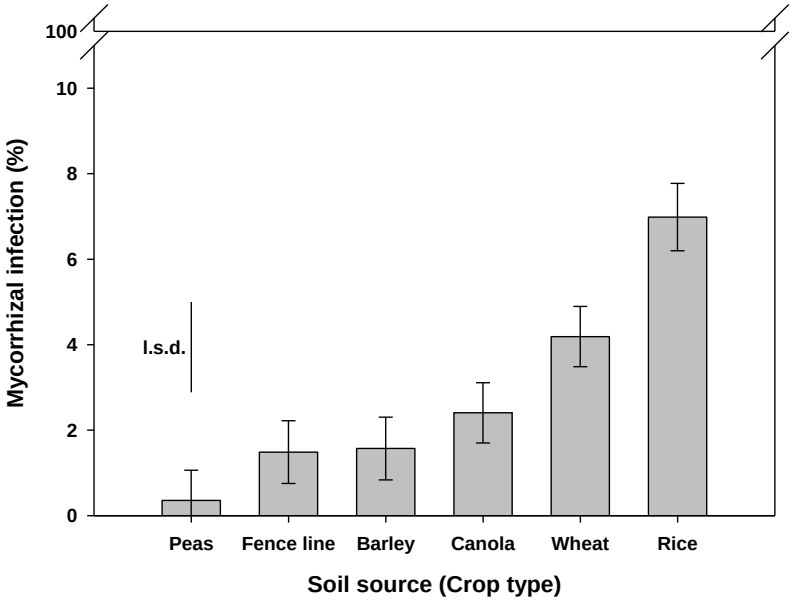

Figure 1. Effect of soil sample location on mycorrhizal inoculation (P <0.001; LSD = 2.112) bars on each column denote standard error of measurement. Soils collected from adjacent field plots growing wheat, barley, canola, field peas and along the fence line. An ex rice soil from Coleambally, NSW, was included as a known positive control.

Conclusion

Early vigour in cotton is an imperative in the shorter season southern climes of Australia. AMF associated with establishing cotton play an important role in fossicking, particularly for phosphorus and zinc, to contribute to that vigour. Poor growth of AMF-dependent crops occurs where the number of propagules are depleted through long fallows and land levelling that eliminates propagule rich topsoil (Thompson 1994) or through management practices such as wetting/drying cycles and soil disturbance (McGee et al. 1997; Pattison and McGee, 1997). It follows therefore that where a soil is depleted of AMF, or where there are lowly effective fungal species, then introducing the appropriate AMF through plant inoculation may provide the impetus needed for early initial colonisation (Abbott and Robson 1982). Ortas (2012) found that 1000 spores per plant in the form of chopped roots and spores (10 g of inoculant 50 mm under the seed) was only effective in increasing plant growth in the field in one out of three years. Thompson et al. (2012) showed that field soil was just as effective in AMF colonisation of cotton plants as inoculation at the rate of 6000 spores per pot.

In the inoculation experiment three of the seven products showed some colonisation at 20x the recommended rate. However the colonisation was poor and did not result in improved plant performance. Some products contained hyphae and fruiting bodies but the quantity was very small, highly variable and insufficient to make an impact in a field situation. These results therefore raise questions about the validity of product claims. The relatively high cost of these products and their poor efficacy suggest that they represent poor value for money. Being biological products means that they do not require the approval of AVPMA as do

chemical products and so there is no check on the authenticity of the claims made. The background soil experiment showed that AMF populations can be generated by choosing rotation crops wisely and this would be a much better way of ensuring such populations were at critical levels. Wheat, was shown to be effective and would be a desirable crop to grow preceding a cotton crop. Management of crop rotations provides a simple and effective way to ensure AMF populations and negates the need for application of expensive products that have inadequate performance.

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