FULL REPORT

Project Title: **QUANTIFICATION OF VAM FUNGI IN SOIL FOR SUSTAINABLE PRODUCTION OF COTTON**

INTRODUCTION

Our previous research indicated that in normal soils, fungi that formed VAM were present in the upper 15cm of soil, and that VAM were established rapidly in root systems. Thus in seasons where crop followed crop, more than adequate quantities of VAM fungi were always present. However, Long Fallow Disorder and similar syndromes are a serious concern to farmers, and we lacked any understanding of what levels of VAM fungi are necessary in soil to establish adequate levels of VAM in the roots of cotton plants. We also needed to know where in the soil profile the VAM fungi survived following long fallows.

OBJECTIVES

The research had three parts:

- Quantify the VAM fungi at depth in the soil
- Determine the rate of colonisation of roots of cotton by VAM fungi, and
- Develop understanding of the relationship between rate of colonisation and quantity of VAM fungi.

RESULTS AND DISCUSSION

VAM fungi at Depth.

VAM fungi were quantified using bioassays in cores from pits opened up at ACRI. We found that potential to form VAM was similar in soil from the surface to that we have examined previously. However, the soil from 50 and 100cm below the surface had very little potential to colonise soil, and the VAM fungi were distributed unevenly through the soil where quantities of fungi were low. David Nehl examined the colonisation of roots of cotton and other crops from these profiles and found that the roots contained moderate levels of VAM, an extend we would consider normal. The level of colonisation in the roots did not correlate with the amount of VAM fungus present in the soil.

In a related project, we found that VAM fungi were entirely absent in soil which had been fallowed for two years.

Quantification

By using pot cultures of a single VAM fungus from Auscott, we determined how much was needed to give a single colony of VAM by dilution. We then inoculated seedlings in long tubes and determined the quantity of VAM fungus to provide a rate of colonisation similar to or less than what we have observed in the field. Inoculation of 100 propagules in 100g soil gave approximately normal rates of colonisation. Inoculation with 10 propagules resulted in delayed colonisation. By 6 weeks, the level of colonisation had caught up with that of the larger inoculum.

Inoculation with one propagule in one hundred gram of soil resulted in severely delayed colonisation, and a severely reduced rate of spread. By 6 weeks, the roots of these experimental plants remained largely uncolonised, indicative of the situation in long fallow disorder or arising from colonisation at 1 m below the soil surface.

We were unable to clarify which parts of the fungus initiated VAM in field crops. This is important because spores have been used by other researchers to indicate the amount of fungus in the soil. In our experimental system, VAM arose from fragments of hyphae in soil. VAM in old roots and spores of the fungi did not contribute in any way. I believe that a similar situation exists in the field. Spores are unlikely to contribute to VAM in field grown plants because less than 1% are viable at the end of the cropping season. Fewer would be viable when a new crop was sown. Further, spores are only located in the upper soil profile thus limiting their contact with roots. In our opinion, VAM mostly arise fro fragments of hyphae in soil in the field.

Rate of Colonisation

Spread of VAM through root systems of cotton is extremely rapid. Inoculation at 25cm below the surface resulted in complete colonisation of the root system within 4 weeks. Even where colonisation was initiated from 45cm below the surface, the root system was rapidly colonised, both up and down the root system from the point of inoculation. Recovery from LFD is probably rapid in cotton due to the fast spread of VAM.

This experiment also resulted in the observation that initiation of VAM results in a proliferation of roots at the point of colonisation. In other words, the VAM fungus causes the root system to branch more following initiation of VAM. We repeated this experiment and are certain that VAM cause root proliferation of cotton.

CONCLUSIONS, RECOMMENDATIONS AND APPLICATION

The research contributes to our understanding of the pattern of survival and growth of VAM in cotton. We now know that under normal conditions, the soil contains many thousands of survival units of VAM fungi per gram of soil, when around 1 per gram is all that is needed to colonise roots at the maximum rate. The soil normally contains a huge excess of survival units. Thus **conditions such as Long Fallow Disorder are a consequence of a HUGE reduction in the population of VAM fungi.** As the fungi are concentrated in the upper soil profile, the reduction mostly takes place at the surface. What happens below the surface is more important for recovery of the fungal population.

Recovery from Long Fallow will be relatively fast in cotton, and probably be complete within a single growing season provided a few units of the VAM fungi survive. The VAM fungi appear to survive in pockets down the soil profile. The VAM reestablish quickly and spread rapidly through the root system. They also cause the roots to multiply where VAM have been initiated. This means fresh roots are constantly forming in the contact zone with the VAM, where the VAM are most concentrated, thus maximising the benefit from VAM. The effect on crop production is at this stage still unclear, even when plant growth is retarded due to a lack of VAM early in the season. Field research by Allen and Nehl will clarify this issue.

Finally, VAM fungi are concentrated in the surface of soils used to grow cotton. The size of the fungal population declines extremely rapidly down the soil profile. VAM in roots found at depth are present because the fungi have grown down the root system as the roots elongate, rather than form from VAM fungi in the soil. Further, few survival units are found at depth, suggesting that the VAM fungi are not growing out from the roots and thus not taking up nutrients at depth. **Thus the functional part of a root system is concentrated in the upper part of the soil profile.**

The difficulties of quantifying VAM fungi are clear from our research. In the past researchers have used spore counts to quantify the fungi. We have found that spore numbers give a very poor indication of the quantities of VAM fungi. Others have used the amount of VAM in root systems. Again, this measure does not indicate whether the fungi have grown beyond the surface of the root. At depth, the fungi are clearly only associated with the root. The use of bioassays is slow, tedious and requires controlled growth conditions. However, the data are reliable and consistent, and we have used them extensively for experimental purposes. Experimentally, it is possible to measure the proportion of VAM after 3 weeks to provide an indication of high densities, but at low densities, the measure is unreliable because of the clumped distribution of propagules. Bioassays are inappropriate for field estimates. The development of a linseed indicator crop is a much more practical approach to determining if few units of VAM fungi are present. Comparison of the size of seedlings growing in the test soil is compared with others in inoculated soil. If the seedlings in the test soil are small, then few VAM fungi are likely to be present. Nehl and Allen developed the linseed bioassay.

We can only speculate on the causes of Long Fallow Syndrome and similar problems. The most obvious cause is that rainfall events cause germination of VAM fungi while a crop is absent. The fungi will die in the absence of a host, be it a weed or crop plant. Cultivation is also important, because it reduces the hyphal network. However, some soils appear susceptible and others are not, and the critical issues relate to these differences. The causes are probably complex and may include physical, chemical and biological components which interact. Our research indicates that anoxia, or absence of oxygen, is likely to be a major contributor. Anoxia is common in soils at depth, it is associated with increased availability of Mn, and is particularly important in flooded (eg irrigated) soils. Anoxia is also likely to inhibit or encourage differing groups of bacteria, some of which effect either the plant or VAM fungi. We can now measure the quantities of VAM fungi in soil. We know how much fungus is normally present in soil, how much is needed for maximum rates of plant growth and how much is present in disordered soils. Thus we can design experiments in which details of the effect of each factor on plant and VAM fungi can be determined, and the consequence on plant and fungus predicted in the field.

COMMUNICATION OF RESULTS

A. 1998 Australian Cotton Conference,
"VAM Fungi in Cotton Soils of eastern Australia".
This paper summarised the research we have completed at University of Sydney over the last 5 years.

We have also prepared the following research publications:
B. Torrisi V, Pattinson GS, & McGee PA. "Proliferation of roots of cotton follows establishment of arbuscular mycorrhiza" accepted for publication in New Phytologist.
C. McGee PA, Torrisi V, & Pattinson GS. "Initiation and spread of arbuscular mycorrhizas in roots of cotton from known quantities of propagules." To be submitted to New Phytologist
D. Nehl DB, McGee PA, Torrisi V, Pattinson GS, & Allen SJ. "Rapid decline of arbuscular mycorrhizal fungi down the soil profile, but not mycorrhizas in roots of crop plants." To be submitted to new Phytologist

We also propose to complete an article for submission to Cotton Grower on the latest work.

ABSTRACT

The research quantified a severe decline in the population of VAM fungi down the soil profile beneath cotton crops. To maximise formation of VAM in cotton, soil at the surface should contain at least 1 propagules per gram soil. Most soils used to grow cotton contain thousands of propagules. However, the main population of fungi are in the surface 30cm. By 50cm, the density is below 0.1 propagules and at 1m approximately 0.01 propagule per gram of soil, and the populations are unevenly dispersed. Long Fallow Disorder and other VAM problems are presumably the result of severe loss of fungal propagules in the surface soils. Recovery of the population of VAM fungi is likely to be rapid under cotton. The fungi rapidly spread through the roots, and they also cause the roots to proliferate.

SUMMARISED REPORT

VAM fungi were quantified down the profile to determine what were normal densities, and how few could establish normal VAM in cotton. More than 100 survival units of VAM fungi (propagules) per g soil are present in the surface 20cm of normal soils. However, the density declines rapidly. Less than 0.1 propagules at 50cm and less than 0.01 propagules per gram soil at 100cm below the surface were found. While huge densities of VAM fungi are present, they are mostly located in the top 20cm.

Approximately 1 propagule of the fungus per gram of soil initiated maximum rates of VAM formation. Thus Long Fallow is a result of the loss of the huge population mostly found at the surface. We suggest that recovery from Long Fallow results from establishment of VAM from the few propagules located lower in the soil profile.

Spread of VAM in roots was found to be rapid, such that a cotton crop after Long Fallow would probably establish normal levels of VAM fungi in the soil within the growing season. Further, VAM cause the roots of cotton to multiply in the zone where VAM are established. This means that VAM will function at their maximum effectiveness when in low densities because they are causing root increases. In normal soils, VAM may be causing root proliferation at the soil surface, suggesting that application of fertiliser and watering should be concentrated in this zone to maximise uptake.

VAM at the surface are associated with a mass of fungi growing in the soil around the roots. However, in the field the fungi do not grow into the soil from VAM below about 50cm from the surface. We do not know why. The absence of hyphae explains why land planing results in Long Fallow-like signs in plants growing in the areas where soil has been removed. The roots growing lower in the soil profile are unlikely to be as functional as the those in the upper profile, because the VAM lack a supply of minerals from the soil.

In this and previous research funded by CRDC, we have been able to develop a model of the population of fungi in soil that form VAM. Essentially, high densities of fungus are normally present in surface soils, and they decline dramatically down the soil profile. The fungi grow rapidly up and down the root system, causing roots to proliferate, especially in the surface soil. Where the population of VAM fungi declines dramatically in the surface soils, Long Fallow Syndrome results. Recovery from Long Fallow is likely to be rapid with cotton, VAM probably being initiated from the few fungi located down the soil profile.