Title: Management of VA mycorrhizal fungi for sustainable production of Cotton.

Project Number: US 21C

Research Organisation: University of Sydney

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A final report prepared for the Cotton Research and Development Corporation.

FULL REPORT

Project Title: **MANAGEMENT OF VA MYCORRHIZAL FUNGI FOR SUSTAINABLE PRODUCTION OF COTTON**

INTRODUCTION

INDUSTRY SIGNIFICANCE:
Late or slow initiation of VAM in seedlings reduces production of cotton. A system of managing the fungi will allow more predictable production across fields, and higher production in problem soils, and in virgin country planted for the first time.

RESEARCH PROPOSAL - SUMMARY:
The project aimed to increase our understanding of VAM fungi so that their management may be improved. Good management is particularly important where poor infection of roots by VAM fungi is associated with reduced production of cotton. In this application, information was obtained on how VAM fungi survive common managerial practices.

OBJECTIVES

1993/4 Determine the common species of VAM fungi in the cotton areas of eastern Australia. Place the fungi in pot culture. From areas where 3 - 5 of the common fungi are found, collect further soil samples and determine the infectivity of the soils and individual fungal species. This gives information on the importance of the common species in individual sites and their usefulness as predictors of the population.
1994/5 Determine the sources of fungal infection (survival structures), their location in the soil profile and their density. This experiment would need to include representative soil samples from several climates. From individual fungi in pot culture, determine the survival structures, subject the fungi to factors like cultivation, fungicide application, fallow etc. Follow the fungi through to recovery. This work would continue into 95/6.
1995/6 Experimentally, determine the fungal structures that survive long fallow or disturbance, the rates of recovery of fungal populations, and the effects on mycorrhizal infection and plant growth. We will also determine the location of propagules of VAM fungi in the soil profile.

RESULTS AND DISCUSSION

In each field we examined, at least three and up to six different species of VAM fungi were found in each collection of soil. Several species of VAM fungi were commonly found across the cotton growing areas of eastern Australia. They were present in soils currently used for cropping and grazing. The fungi were all small spored species of *Glomus* and *Acaulospora*. The most common fungi were *Glomus intraradices*, *G. mosseae* and an unidentified species of *Acaulospora*. With selected isolates of these fungi, we examined the response of seedlings of cotton to the different isolates of VAM fungi.

Some general conclusions can be made from the initial research. Presence of VAM fungi in roots increased the uptake of phosphate by cotton seedlings, and increased the growth of seedlings in our experimental conditions. All fungi reduced the ratio of roots to shoots (Pattinson et al, submitted to Mycorrhiza). These results are consistent with published research on most plant species. We also compared the response of several varieties of cotton to VAM. The responses of each variety to infection by one isolate of *G. mosseae* and one of *G. intraradices* were similar, though the variety CS50 responded with least variation and was used subsequently for experimental research.

Each of the three common fungal species was found in soils collected from Warren to Emerald, leading us to believe that we could develop models from understanding host response to each fungus. However, when we compared isolates of a single species of fungus from adjacent locations, we found that they caused different plant responses (McGee et al, submitted to Mycorrhiza). We therefore abandoned use of single species for prediction of responses in the field. The results indicated that a different system is required to predict field responses of cotton to mycorrhizal infection. We examined the relationship between quantity of VAM fungus and host response to different quantities of inoculum.

Several approaches are used to quantify VAM fungi. The methods include counting fungal spores, measuring hyphal lengths and using an infectivity bioassay which measures the rate mycorhizas develop in a trap plant. Each procedure has disadvantages and advantages. For instance, counting spores indicates the number of spores but not other propagules, it does not indicate which spores are viable nor whether viable spores can initiate infection. We used a combination of approaches to quantify VAM fungi in field soils from Warren, Narrabri and Dalby. The soil samples were collected from approximately 5 to 15 cm down the soil profile in cores. A mean of approximately 200 spores per g soil were extracted from each soil, though less than 5% of spores were able to establish infection when adjacent to a growing root tip. Approximately 0.5m hyphae per g soil was also present in freshly collected soil. The viability of spores and hyphae declined over time as determined by bioassays and stains, respectively, though we could not determine the significance of these reductions using these methods (McGee et al, 1997, New Phytologist). We found that the infectivity bioassay we developed provided information on the density of fungi in soil as well as the rate at which infection spreads in the root system.

To quantify VAM fungi in soil, we used the infectivity assay in undisturbed soil cores collected from Warren, Narrabri and Dalby. We found that in soils collected from the surface, similar quantities and rates of mycorrhizal infection developed in cotton seedlings, in soils freshly collected or stored dry for up to 18 months. If the soils were disturbed after collection, to simulate cultivation, and then stored, the quantity of VAM fungi and the rate of infection declined slightly. If the soils were wetted and then dried, to simulate rainfalls, then quantities and the rate of initiation of infection declined significantly especially if the soil had also been disturbed (Pattinson et al, submitted to New Phytologist). These data indicate that the development of Long Fallow Disorder is possibly associated with infrequent rainfall events. Experimentally, the amount of VAM in 3 week old seedlings is also a good indicator of the amount of VAM after 8 weeks growth.

We initiated attempts to determine the effects of different quantities of VAM fungi in soil on the growth to maturity and production of lint by cotton variety CS50. In the time available, we were unable to develop a method which provided satisfactory experimental conditions. Preliminary results indicated that lint production was delayed by low initial quantities of VAM fungi, but that final lint production was similar in plants grown from low and high initial quantities of the fungus. Similar trends are thought to occur in the field (Allen, unpublished). The field work is progressing. Steve Allen is using an experimental field approach at ACRI.

We have also initiated examination of the quantity of VAM fungi in soil cores collected from different depths. Preliminary data indicate that the quantity of the fungi declines rapidly down the soil profile. This result was unexpected, as the decline in quantities of VAM fungi in sandy and loamy soils is gradual. Most research relies on the establishment of VAM from fungal propagules distributed through the profile in which roots grow. While considerably more work is required to support this conclusion, it has implications for establishment of new fields and use of various pesticides which may reduce infection in roots of cotton as they grow through the surface soil. If infection is delayed in the roots as they grow through the upper soil profile, then the densities of VAM fungi at depth may be inadequate to ensure optimum rates of plant growth, and thereby delay the formation of lint. I hope to continue the work in my laboratory. In 1997, I may have an Honours student examining the rates of growth of VAM in cotton under a variety of soil conditions.

We examined the effect of three pesticides on the rates of initiation of VAM in variety CS50. We chose CS50 because of its reported general tolerance to fungal pathogens and insect pests. We examined the seed dressing Terraclor, the nitrification inhibitor Terrazole and the nematicide Fenamiphos. Each of these pesticides has been reported elsewhere to cause significant delays in the initiation of VAM in other plants. We found that though initiation of infection might be delayed, the effect of all pesticides on seedling growth and amount of infection at 6 weeks was insignificant, provided that the pesticides were applied at recommended rates (Pattinson et al, submitted to Mycorrhiza). Previous research reported significant reductions in development of mycorrhizas and reductions in the reproduction of VAM fungi. However, in all these cases, excessive amounts of pesticides were used in the experiments.

We also found that complex interactions complicated interpretation of data. For instance, we found that infestations of mites were reduced in seedlings treated with Fenamiphos, and in the absence of mites seedlings grew faster (Pattinson et al, submitted to Mycorrhiza). Field experiments would not detect this type of interaction and careful interpretation of field data is indicated.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

In most soils used to grow cotton, under normal conditions the quantity of VAM fungi in the surface soil was more than adequate to ensure maximum rates of initiation of VAM in roots. We hypothesise that the fungi grow down the roots as the roots grow into the soil, leading to a cycle in which fungi and roots invade the soil profile from the surface each growing season. If correct, potential causes of reduced plant growth associated with a lack of VAM would include the following factors:

1. removal of topsoil, such as during landplaning
2. suppression of germination and growth of VAM fungi, such as might occur where pesticides were used excessively
3. reduction of rates of elongation of hyphae of VAM fungi during root growth such as apparently takes place in soils subject to "Galathera Syndrome"
4. suppression of growth of hyphae of VAM fungi such as may happen when soils become anoxic, especially in clay soils during flooding.

Of these factors, we know that the effects of landplaning can be overcome by cropping for one season. We would suggest that a crop such as wheat be used as the first crop, because it is less sensitive than cotton to low densities of VAM. We were able to eliminate three pesticides as the cause of suppression of VAM fungi. However, the effects of each new pesticide will need to be assessed for its effects on VAM fungi. The association between bacteria and VAM fungi is subject of another research project. The effects of anoxia on hyphae, especially in clay soils, on VAM fungi and related plant growth have not been assessed.

Long Fallow Decline (LFD) is still a potential issue but the research completed so far does not indicate clearly how the decline in production is related to VAM fungi. Clearly, incidental rainfall germinates fungal propagules, reducing their density in the surface soil. However, we could not induce sufficient reduction in the density of fungal propagules to say that rainfall is the sole cause of LFD. Anecdotal evidence from southern Queensland suggests that LFD is only evident in heavy clay soils. If this is correct, then it may be associated with several factors including soil texture, soil aeration or microbial interactions with VAM fungi.

The precise relation between densities of VAM fungi at the beginning of the season with production of lint is also unclear. The results from our research indicate that in typical soils, adequate densities of VAM fungi are found in the surface layers to establish maximum levels of VAM in cotton, that the fungi are hardy, and able to tolerate the current practices associated with cotton cropping. Low densities of VAM fungi are likely to be associated with reduced production where the growing season is short, provided other factors are normal. The link between fungal densities and lint production needs to be clarified in both experimental conditions where other factors can be controlled and under field conditions.

The research reported above has been carried out in the laboratory. Field experiments are required to support the general conclusions. The following questions also require clarification:

Related to depth:

- what is the relationship between quantity of propagules of VAM fungi at depth, the development of mycorrhizas, uptake of nutrients and production of lint
- what factors influence the spread of VAM in root systems of cotton

Related to anoxia:

- what effect does anoxia associated with flooding or heavy clay soils have on the development of VAM in roots, and uptake of nutrients by the cotton plant
- what factors determine how development of infection is reduced in anoxic soils.

COMMUNICATION OF RESULTS

To the grower:

An article from S. Allen, D. Nehl and P. McGee has been submitted to Australian Cotton Grower.

I have also presented a poster on this work to the World Cotton Research Conference, and talks to the 1994 Cotton Soil Coordination Meeting.

To the scientific audience:

1. McGee PA, Pattinson GS, Heath RA, Newman CA & Allen SJ. (1997) Survival of propagules of arbuscular mycorrhizal fungi in soils in eastern Australia used to grow cotton. New Phytologist (in press)
2. McGee PA, Morrow JL, Lyon BR & Masuhara G. Variation between isolates of *Glomus mosseae* (Nicol & Gerd) Gerd & Trappe. Submitted to Mycorrhiza
3. Pattinson GS & McGee PA. High densities of arbuscular mycorrhizal fungi maintained during long fallows in soils used to grow cotton except when soil is periodically wetted Submitted to New Phytologist
4. Pattinson GS, Warton DI, Misman R & McGee PA. The fungicides Terrazole and Terraclor and the nematicide Fenamiphos had little effect on the growth of cotton seedlings and their infection by *Glomus mosseae*. Submitted to Mycorrhiza

I have also presented aspects of this research to the first International Conference on Mycorrhiza in 1996 and the 9th North American Conference on Mycorrhiza in 1993.

Personnel who have worked on this project:

GS Pattinson was employed as a research assistant on most of this project. A Ford and D Warton were also employed casually to assist with the work. Assistance was also provided by Dr G. Masuhara, Prof R. Misman, C. Newman and R. Heath using support from the University of Sydney. J. Morrow was employed on another grant funded by CR&DC and was supervised by Dr B. Lyon. Dr S Allen collaborated with us in this project. I express my appreciation for the contribution made by each of the people associated with the project and the support provided by CR&DC.

SUMMARISED REPORT

Project Title: **MANAGEMENT OF VA MYCORRHIZAL FUNGI FOR SUSTAINABLE PRODUCTION OF COTTON**

AIM: The project aimed to increase our understanding of VA mycorrhizal fungi so that their management may be improved. Good management is particularly important where poor infection of roots by VAM fungi is associated with reduced production of cotton. In this application, information was obtained on how VAM fungi survive common managerial practices.

RESULTS AND CONCLUSIONS: Several species of VAM fungi were common in the cotton growing areas of eastern Australia. The fungi were all small spored species, the most common fungi being *Glomus intraradices*, *G. mosseae* and an unidentified species of *Acaulospora*. Presence of VAM fungi in roots increased the uptake of phosphate by cotton seedlings, and increased the growth of seedlings in our experimental conditions. All fungi reduced the ratio of roots to shoots. All varieties of cotton gave similar responses. These results are consistent with published research on most plant species.

We compared isolates of a single species of fungus from different locations and found that they caused different plant responses. This necessitated the use of mixed fungal populations for most experimental work.

In soil samples collected from approximately 5 to 15 cm down the soil profile we found approximately 200 spores per g soil. Less than 5% of spores were able to establish infection when adjacent to a growing root tip. Approximately 0.5 m hyphae per g soil was also present in freshly collected soil. The viability of spores and hyphae declined over time. When undisturbed soil was examined in a bioassay, we found similar quantities of VAM fungi in soils collected from the surface, and that similar rates of mycorrhizal infection developed in cotton seedlings, in soils freshly collected or stored dry for up to 18 months. If the soils were disturbed after collection, to simulate cultivation, and then stored, the rate of infection declined slightly. If the soils were wetted and then dried, to simulate rainfalls, then the rate of initiation of infection declined significantly especially if the soil had also been disturbed.

We initiated attempts to determine the effects of different quantities of VAM fungi in soil on the growth to maturity and production of lint. Preliminary results indicated that lint production was delayed by low initial quantities of VAM fungi, but that final lint production was similar in plants grown from low and high initial quantities of the fungus. Similar trends are thought to occur in the field. The methods used in this experiment need to be improved.

We also initiated examination of the quantity of VAM fungi in soil cores collected from different depths. Preliminary data indicate that the quantity of the fungi declines rapidly down the soil profile. This result was unexpected, as the decline in quantities of VAM fungi in sandy and loamy soils is gradual. This conclusion has implications for establishment of new fields and use of various pesticides which may reduce infection in roots of cotton as they grow through the surface soil. If infection is delayed in the roots as they grow through the upper soil profile, then the densities of VAM fungi at depth may be inadequate to ensure optimum rates of plant growth, and thereby delay the formation of lint.

We examined the effect of three pesticides on the rates of initiation of VAM. We examined the seed dressing Terraclor, the nitrification inhibitor Terrazole and the nematicide Fenamiphos. Each of these pesticides has been reported elsewhere to cause significant delays in the initiation of VAM in other plants. We found that though initiation of infection might be delayed, the effect of all pesticides on seedling growth and amount of infection at 6 weeks was insignificant, provided that the pesticides were applied at recommended rates.

IMPLICATIONS

In most soils used to grow cotton, more than adequate quantities of VAM fungi are present for maximum rates of production of cotton. The density of VAM fungi appears to decline rapidly down the soil profile. Any factors which reduce fungal growth and survival in the surface soils is likely to lead to a serious decline in fungal densities and rates of establishment of VAM in cotton. These factors are thought to lead to Long Fallow Decline, but the causes of LFD are as yet unclear. They may include anoxia associated with flooding in heavy clay soils, or microbial interactions suppressing VAM fungi.

QUESTIONS REMAINING

Still to be clarified are: the relationship between lint formation and VAM, the development of infection down the root system and how this impacts on nutrient acquisition and growth of the cotton plant, the effect of reduced oxygen, especially in heavy clay soils, on germination of fungi, growth through soil and the spread of VAM in roots.

MANAGEMENT OF VA MYCORRHIZAL FUNGI FOR SUSTAINABLE PRODUCTION OF COTTON.

US 21C

ABSTRACT

The research program examined how common agricultural factors influenced the population of VAM fungi in soil. In soils from Warren, Narrabri and Dalby, VAM fungi were common in the upper soil profile. Densities of the fungi appear to decline very rapidly with depth. The fungi were not seriously depleted by fallows up to 18 months, cultivation, and use of some pesticides. Rainfall events caused some fungi to germinate and the initiation of VAM was slowed, though production is probably not significantly affected. Still to be clarified are: the relationship between lint formation and VAM, the development of infection down the root system, and the effect of reduced oxygen, especially in flooded or heavy clay soils, on the spread of VAM in roots.