Final Report on the Australian Flora Foundation funded project

Development of mycorrhizas of Thysanotus

P. McGee, School of Biological Sciences, The University of Sydney

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The research on *Thysanotus* has had two goals. In the first instance, we have aimed to determine how the association developed. The purpose was to elucidate what happened to the fungus following infection of the root. I believed that following penetration of the root, contents of hyphae were removed and the association stoped functioning. Successful formation of the mycorrhiza required continual reinfection from hyphae outside the root. In typical mycorrhiza the fungus spreads down the root from the mycorrhiza and outside infection is unnecessary for spread of infection. We attempted to use transmission electron microscopy to follow infection. TEM was inappropriate because of the number of specimens which had to be processed-and examined. Further, sand particles in the soil medium made sectioning difficult. Thus we examined the use of Confocal microscopy and tissue culture.

Experiments with confocal microscopy resulted in highly variable information. Initially, we had difficulty visualising the fungi in roots, we have just found that we had examined our specimens at the incorrect pH. By raising the pH, we could visualise the hyphae within the roots using a flourescent dye that stained the lipids within the hyphae. We chose a lipid dye because it would tell us when and where the cytoplasm was being extracted from hyphae, as lipid is the main constituent of fungal cytoplasm when the fungus is viable. These investigations are the basis of a M.Sc. project to be commenced by Mr F. Chee of the Electron Microscope Unit at The University of Sydney.

Experimental plants are usually raised in sterile soil and inoculated with the appropriate fungus for mycorrhiza formation. The system was inappropriate for TEM and Confocal microscopy. Thus we investigated the possible use of tissue culture to produce infected seedlings of *Thysanotus*. We have established a simple system to grow the seedlings in tissue culture. However, the fungus, when inoculated into the media, invaded the roots and destroyed them. This pathogenic response was largely due to the high concentration of sugars in the media. We are now attempting a system where we raise the seedlings normally, place them in high light and then transfer the plantlets into media with no sucrose. The plants will then be inoculated with the mycorrhizal fungus. This system may provide us with clean mycorrhizas and the development of infection can be followed using confocal microscopy. However, the system may have to be adapted to include a companion plant or maybe split to have the fungus supplied with sucrose when the plant is without. This experiment is progressing.

The second goal arose as we have became aware of the commercial development of *Thysanotus multiflorus*. Pots of the species are sold through Merricks Nursery of Victoria. They use a sterile soil mix so mycorrhizal fungi are absent.

The genus *Thysanotus* has three sections in it: tuberous plants with no perennial shoots, rhizomatous plants with perennating flower scapes and fibrous plants with perennating leaves. *T. multiflorus* belongs with the latter group, with a student from University of Technology, Ms C. Newman, an experiment has been established where we are examining the need for mycorrhizas, a companion plant and nutrients in a representative from each of the groups. We have had difficulties obtaining sufficient viable seed and only the tuberous plants have been established so far. This work will continue with outplanting experiments later. We will need to establish reliable sources of seed for further experiments. Should mycorrhizas prove necessary for one or more of the groups, we may examine the methods most likely to establish the mycorrhizal fungus in pot culture.

The research has lead us to think that the genus *Thysanotus* contains a complex of biological groups. At least three discrete subgenera exist based on morphological criteria. The tissue culture media used for culture of plantlets differs and methods needed to grow the plants seem to differ.

Funds from the Australian Flora Foundation will be spent by about the end of March this year'. The project will continue because of the involvement of a M.Sc. student and a possible Honours student in 1993. The students will be funded from internal monies. I have applied unsuccessfully for funding to ARC in 1991. An application to ARC has been submitted for funding in 1993. Approaches to commercial organisations for assistance have been unsuccessful so far. The research is still viewed as too uncertain and theoretical. I expect that if our inoculation experiments are successful, we would approach the Horticultural Industries Research and Development Council for assistance. The research has links to another project in this laboratory entitled "Rehabilitation of Bushland using VA mycorrhizal Fungi" and funded by the Environmental Trust.

The research commenced under the auspices of the Australian Flora Foundation has developed several different strands, some of which have immediate practical implications, some of which will lead us to better understand the details of the development of mycorrhizas, with more practical implications at that point. I am extremely grateful to the Foundation for the support it has provided for it has allowed the project to become established, even though at this stage further support is lacking.