Final Report for the Australian Flora Foundation

PROJECT TITLE: **Anatomy and ultrastructure of natural and synthesised mycorrhizas of *Leucopogon ericoides* and other members of the Epacridaceae**

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Scientific Findings

The project was extended to include two additional species *Leucopogon parviflorus* and the Western Australian plant Lys*inema ciliatum.* The reasons for this were firstly that a technique for sinking cuttings of *Lysinema ciliatum* had been established by the group at Kings Park led by Kingsley Dixon .and we had been advised by nursery growers in Sydney that *Leucopogon parviflorus* can readily be produced from seed. We were thus assured of adequate plant stocks to work with. Plant stocks of *Lysinema ciliatum* were successfully established from cuttings in the glasshouse and an extensive investigation into the structure and development of the non-mycorrhizal root system was undertaken. The W.A. group at Kings Park has subsequently discovered a method for germinating the seed - by treatment with smoke.

Observations on *Leucopogon ericoides* collected at intervals from La Perouse provided us with basic information on the appearance of the root system in the wild, including the status and appearance of mycorrhizal infection. Production of hair roots and their mycorrhizas appears to be seasonal and coincides in this species with a spring growth period and in *Lysinema ciliatum* with the winter rains in Western Australia. In non-mycorrhizal cuttings grown in fairly moist conditions in the glasshouse we have found that hair roots account for more than 90% of the root system. However they are very fragile and readily break. Because of this, it is very difficult to dig up the root system from plants in the wild without damage and loss of many hair roots. Most previous studies calculating the extent of the root system and the proportion of live hair roots and their activity does not take account of this loss. It also has implications for horticultural practices especially in potting on and transplantation of plant stocks in re-vegetation projects. We found that the best way to recover hair roots was to dig out large clods of soil around the root system and allow this to fall away gently into a large bath of water. We would suspect that successful transplantation would involve transferring large amounts of soil with minimum root disturbance and the use of mycorrhizally infected soil.

The structure of the hair roots is very unusual and this has not been formerly recognised. The structure of the finest hair roots indicates that they are unlikely to be very efficient in water transport. They are very small in diameter with only about 25 cells per cross section. They contain two suberised layers adjacent to one another and no other cells in the cortex. The xylem consists of only a single file of tracheids. All of these features imply low water flow rates along individual roots. We have calculated the likely water flow rates, based on measurements of xylem diameter, and have come to the conclusion

that to move water along individual roots would require generation of very negative pressures in the xylem and we propose that the hair roots are essentially "nutrient gathering roots". When considering the root system as a whole, water flow along individual roots will be at low rates, but there will be many roots drawing water in parallel. Therefore the root system will exploit a large volume of soil, but will remove only small volumes of water from each part of it, thereby conserving water in the rooting region as a whole and avoiding excessive water depletion in any one area. The data also indicate that hair root structure in many species of the closely related Northern Hemisphere family Ericaceae is very similar, but has been misinterpreted in the literature.

Lysinema ciliatum (and to a lesser extent *Leucopogon ericoides)* has an epidermis with unusual cells with thickened walls. These are the cells colonised by mycorrhizal hyphae. In *Lysinema* these cells are sloughed into the soil either individually or in small groups. This occurs by separation at the middle lamella of the radial and inner tangential walls, whether the cells contain fungus or not. Preliminary investigations suggest that they may contain live hyphae. If so, the root cell/fungus complex may serve as a resting propagule for survival of the fungus and promote infection of new roots. We are investigating this further, since, if correct, it is a novel contribution of the plant to survival of the mycorrhizal fungus. To substantiate the hypothesis we need to know the survival rate of fungus in these detached cells and its capacity to grow out and colonise new roots. No members of Ericaceae are reported to have these thick-walled cells and it may be a specific adaptation for survival of fungal propagules in Australian conditions. Field work with *Lysinema ciliatum* in Western Australia enabled us to grasp the cycles of infection and degeneration of hair roots in areas where there is predictable, periodic drought and to check our data with glasshouse grown plants with material from the wild. We found further evidence for our theory that the thick-walled cells in this species protect the fungus and enable it to survive better over dry unfavourable periods so that fungal inoculum is present to infect new hair roots as they emerge.

This is a novel role for the plant in a mycorrhizal symbiosis and one that may be important for survival in the Australian environment. Our studies in Western Australia have indicated that thick-walled cells are preferentially infected with fungus, while the thin-walled cells usually contain no fungus and collapse quite soon. In natural conditions clusters of thick-walled cells containing mycorrhizal hyphal coils remain attached to the root, probably from one year to the next, and these localised centres of fungal activity could act as infection centres when new growth of hair roots occurs. What we now need to determine is the state and activity of the fungus inside these cells at various times of the year and also their capacity to survive long enough to produce new infections.

More recently we have successfully isolated fungi from mycorrhizal roots of *Leucopogon parviflorus.* We have some 30 isolates currently growing in the laboratory. A few of these are very similar in cultural characteristics to isolates obtained from Northern hemisphere Ericaceae and are very similar to *Hymenoscyphus ericae*, an Ascomycete fungus known to form ericoid mycorrhizas in Ericaceae. We intend to use these isolates to inoculate plants of both Epacridaceae and Ericaceae under sterile conditions to see whether there is cross infectivity between these two families. This part of the programme has been held up because of difficulty in germinating sterilised seed of all the epacrids we have tried. We

are now beginning to have some success with germination of *Leucopogon parviflorus* seed. When mycorrhizal synthesis is achieved we will examine whether mycorrhizal plants grow better and have higher nutrient contents than non-mycorrhizal plants and we will also investigate the development of the ericoid mycorrhizas under controlled conditions.

Publications

The work has been presented at 2 conferences:

1. Ashford, A.E., Bullock S.E., Kupsky, L. and Reed M. A. (1992). New Interpretation of the Hair Roots in the Epacridaceae. Australian Conference on Electron Microscopy and Cell Biology, .Perth W.A.

2. Ashford A.E., Bullock S.E.. & Allaway W.G. (1993). Hair roots in Epacridaceae Abstracts, p 71, Ninth North American Conference on Mycorrhizae, Guelph, Canada.

Two manuscripts are in an advanced stage of preparation for publication:

1. Ashford, A.E., Reed, M. A. and Allaway, W.G. (1995) Hair Roots in Epacridaceae 1 : The exodermis and small xylem tracheid system, and the water relations of the root system of *Lysinema ciliatum*
2. Ashford, A.E. and Allaway, W.G. (1995). Hair Roots in Epacridaceae 2 : a novel role for the epidermis in the mycorrhizal association of *Lysinema ciliatum*

Final comments

The money provided by North Shore Society for Growing Australian Plants through the Australian Flora Foundation has provided essential financial support which allowed us to initiate a study of mycorrhizas in the Epacridaceae.
Apart from the intrinsic value of the scientific findings this has led to several other important developments.

- The project was subsequently supported by the Australian Research Council and is continuing on a larger scale.
- A student became interested in the project and is undertaking a PhD in the area.
- Collaborative contacts have been made with Western Australian Scientists working on Epacridaceae.
- A conference on Epacridaceae has been organised by my colleague and collaborator Dr W.G. Allaway to be held in Hobart, Tasmania on Feb. 1-6, 1995. This will cover all aspects of the biology of the Epacridaceae and the closely related Ericaceae and has attracted several overseas experts. We will be presenting some of our data at this conference.