INTERIM FOUNDATION FOR DIEBACK RESEARCH

Application of cell, tissue and organ culture to *Eucalyptus* improvement, particularly in relation to resistance to *Phytophthora cinnamomi* toxins

Annual Report July 1981 - June 1982

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The work during the past year has concentrated on three aspects: the host (*Eucalyptus marginata*), the pathogen (*Phytophthora cinnamomi*), and the interaction between the two.

1. The host.

Before tissue cultured plantlets can be screened for resistance to *P. cinnamomi* they must be shown to be similar to seedling material, and so the morphology and anatomy of plantlet and seedling roots is being compared.

The roots of plantlets produced in agar have distorted root hairs and swollen epidermal cells compared with seedlings grown in the greenhouse. This distortion is thought to be due to the agar medium not to an inherent difference in the plantlets as it also occurs to a lesser extent in aseptically produced seedlings in agar.

As young roots are easily distorted by fixatives, a comparison of fixatives is underway. This must be done before the anatomy of the seedling and plantlet roots can be compared.

A pot trial to compare the growth of plantlets with seedlings has been started. The plantlets have been very difficult to handle and many failed to establish when transferred to the greenhouse. A different potting mixture is now being used and a repeat experiment will be started as soon as plantlets are available.

2. The fungus

Aseptically produced zoospores are used to inoculate the host as these are the smallest quantifiable fungal propagule.

Two methods of zoospore production have been compared for reliability and ease of handling, and four *P. cinnamomi* isolates have been compared for consistant production of high zoospore numbers. A high-yielding, recent isolate from jarrah is being used in the inoculation work.

3. The interaction between the host and the pathogen

Much of the preliminary work has been done using seedlings which are easier to obtain than tissue cultured plants.

Techniques for staining the fungus within whole, cleared roots have been tested, and some work well with *P. cinnamomi* in jarrah roots in the early infection stages. Several methods for staining fungal and plant nuclei have been tried, but none have been completely successful. Shrinkage and distortion of roots due to fixation have prevented sectioning of young roots. As mentioned earlier, a comparison of different fixatives is underway.

An approximate timing of the events leading up to and following penetration of seedling roots by the pathogen has been established. Infection of tissue culture plantlet roots growing on agar occurs more rapidly than infection of seedling roots.

Unfortunately the project has been rather slow to develop. Mrs. E. Carter, who was appointed in July, 1981, left in January, 1982, to take a position as plant pathologist in the W.A. Department of Agriculture. Her replacement, Mrs. C. Tonkin, who started in March, 1982, has had to repeat all the previous work in order to learn the techniques necessary for the project.

Tissue culture

The research on the tissue culture aspects of this project has been supported by the Reserve Bank up to June 1982. A copy of the report submitted to them for the period January - June 1982 is included.

REPORT TO : Rural Credits Development Fund

PROJECT : Application of cell, tissue and organ culture to *Eucalyptus* improvement, particularly in relation to resistance to *Phytophthora cinnamomi*.

GRANT : $4,442 (part-time graduate research assistant and maintenance)

SUPERVISOR : Dr. J. A. McComb

DATE : January - June 1982

During this period the micropropagation methods for jarrah were improved.

Sterilization of shoots from mature trees for initiation of cultures remains a problem but we have found 1% zephiran in 10% alcohol to be superior to sodium hypochlorite as it damages the plant tissue less. The difficulty of obtaining cultures varies with the type of tree (see below). It seems reasonable

Selection criteria	No. of trees attempted	Successful cultures
Fast growing trees	10	2
Dieback 'resistant' trees	35	5
Leaf miner 'resistant' trees	5	5

to conclude that whatever confers resistance to leaf miner either also makes the plant tissues more tolerant of sterilizing solutions, or it makes the parent trees resistant to attack by various insects and the consequent freedom from insect damages makes the plants easier to sterilize.

Shoot multiplication Shoots from mature trees consistently show a slower multiplication rate in culture than shoots from seedlings. In fact some trees show no multiplication for a period of 6-10 months after culture initiation, then over a period of 2-3 months the multiplication rate increases to a maximum of x5.

Rooting. This remains a problem for material from mature trees. Various alterations to mineral levels and hormones in the media have not improved the percentage of rooting but have improved the general health of the plants and the likelyhood of plantlet survival after transplanting. The greatest influence on rooting percentage is the genotype of the parent tree and the length of time shoots have been in culture. Shoots show an initially low percentage of rooting and then after some 12 months in culture rooting may rise to 90-100%. Not all cultures however have shown this desirable rise in rooting frequency after 12 months.

Transplanting. Plantlets are transplanted into 3:1 sand:peat mixture and placed under mist in the glasshouse. Humidity is gradually reduced over a period of four weeks when the plants are individually potted and transferred to normal glasshouse conditions. Survival of plantlets from mature trees is 50%.

Stage of project in relation to the mycological work funded by the W.A. Dieback Foundation.

We are at the stage of comparing the root morphology and anatomy of seedlings and tissue cultured plantlets, and about to start producing tissue cultured lines for pathogenicity testing with *Phytophthora cinnamomi*. The tissue culture work will be funded by the W.A. Dieback Foundation as from July 1982.

Publications

Bennett, I. and McComb, J. (1982). Propagation of jarrah (*Eucalyptus marginata*) by organ and tissue culture (Aust. For. Res. 12 in press)

Bennett, I. and McComb, J. (1982). Vegetative propagation of *Eucalyptus* using tissue culture and its application to forest improvement in Western Australia. Vth International Congress of Plant Tissue and Cell Culture. Tokyo (July 1982) (in press)