Frank Bay

Frank Bay

INOCULATION OF FOREST TREE ROOTS *In-situ* WITH AXENICALLY CULTURED ZOOSPORES OF *Phytophthora cinnamomi*.

L.R. McGann.

CSIRO, Division of Forest Research,
W.A. Regional Station,
P.O. Box 144, Kelmscott, W.A. 6111.

SUMMARY

A technique for inoculating fine roots of forest trees *in-situ* with pure cultured zoospores of *Phytophthora cinnamomi* Rands is described.

Practical applications of the method have indicated that high levels of primary infection of unsuberised roots can be achieved.

INTRODUCTION

The soil-borne fungal pathogen, *P.cinnamomi*, the cause of "Jarrah dieback" disease in the *Eucalyptus marginata* Donn. ex Sm. forests of Western Australia (Podger 1968, 1972) has the capacity to rapidly produce large numbers of zoospores when soil conditions are suitable (Shea et al 1978).

This motile stage in the life cycle of *P.cinnamomi* causes damage to plants by attacking their root systems and is probably a major cause of rapid intensification of the disease, particularly in low-land areas of the forest.

To better understand their role in the root infection process, motile and encysted zoospores in axenic suspension may be used to infect forest tree roots *in-situ* .

METHODS

The production of axenic sporangia is based on the method of Chen and Zentmyer (1970), whilst plant root inoculations are outlined by Palzer (1976). These two methods form the basis for the technique described in this paper.

1. Preparation of mycelium.

   Disposable plastic petri dishes (9cm diameter) containing 15 mls of cornmeal agar (Difco) are seeded with three 5mm diameter plugs of *P.cinnamomi* and incubated at 26°C in the dark. After two days the mycelium grows sufficiently to provide a large, actively growing edge. From the growing edge of these young colonies, 200-300 mycelial pieces are cut with a fine wire mesh and then dispersed into 9cm petri dishes containing pea broth. After 24 hours incubation at 26°C in the dark, the inoculum pieces develop into small individual colonies which nearly merge to form a large mycelial mat.

2. Production of axenic sporangia.

   The young mycelial mats are washed six times at approximately one hour intervals with 20 ml of an autoclaved mineral salts solution (calcium nitrate 0·01M, potassium nitrate 0·005M, magnesium sulphate 0·004M, deionised water to make 1 litre), supplemented with a sterilised chelated iron solution (EDTA 13·05g, KOH 7·5g, $FeSO_4 \cdot 7H_2O$ 24·9g, deionised water to make 1 litre). Each washing consists of gently swirling the mats in the solution for approximately one minute and then thoroughly draining to remove nutrients as completely as possible.

After washing, the mats are incubated in the same salts plus chelated iron solution, at 26°C under continuous light from a 15 watt fluorescent tube (colour temperature 4,300K) for 24 hours. The number of sporangia produced after this time usually ranges between 8-15 sporangia per mm^{2} of mycelial mat. These numbers are sufficient to give ample zoospores for root inoculation studies.

The axenic condition of the cultures can be confirmed by placing small quantities of mycelium and solution into nutrient broth and incubating at 26°C for six days.

3. Collection of zoospores.

For the release of zoospores, the sporangia bearing mycelium is transferred to 5cm diameter Pyrex glass dishes, rinsed four times in sterile deionised water, chilled for 30 minutes at approximately 4°C and then returned to 26°C. Spore release normally occurs within 30-60 minutes.

If motile zoospores in distilled water are required for inoculation studies it is necessary to release zoospores into specially washed Pyrex glassware, as outlined by Palzer (1976). Motility of zoospores can be drastically reduced in standard laboratory-washed Pyred glassware. Motile zoospores in suspension can be encysted by shaking vigorously in a stoppered test tube. After separating zoospores from sporangia and mycelia by filtering suspension through four layers of fine muslin, the number of zoospores present in suspension can be determined. Pipetting 1 μl droplets onto glass slides and counting spore numbers under the microscope gives fast, accurate counts. The concentration of zoospores can be adjusted with sterile distilled water to a suitable density for inoculation.

To prolong motility of spores, or to ensure that encysted spores do not prematurely germinate, the suspension is decanted into a test tube, stoppered and immersed in water at 4°C inside a vacuum flask for transport to the field.

4. Inoculation of roots in the field.

At the field inoculation site, the covers are removed from root tray boxes (Rockel 1977) used to promote the formation of rootlets on selected trees.

To facilitate inoculation of individual, unsuberised roots within the root tray boxes, toothpicks are placed as supports under each selected root. A 1 μl drop of zoospore suspension is pipetted from a microsyringe onto the upper surface of each rootlet, 1 to 2mm from the tip.

Cover should be provided to shade roots during inoculation and roots must remain undisturbed for 15-30 minutes after inoculation to allow time for the zoospores to settle on the root surface.

Finally, the toothpicks are carefully removed and the covers replaced on the root boxes.

To verify that infection has taken place, a small number of the inoculated roots can be removed after four days. By plating the surface-sterilised root pieces on selective antibiotic agar the presence of *P. cinnamomi* may be established. If the inoculation is successful, the remaining roots can be left for further observations.

REFERENCES

Chen, D.W. & Zentmyer, G.A. (1970). Production of Sporangia by *Phytophthora cinnamomi* in Axenic Culture. Mycologia. 62, 397-402.

Palzer, C. (1976). Zoospore inoculum potential of *Phytophthora cinnamomi*. Ph.D. Thesis, University of Western Australia.

Podger, F.D. (1968). Aetiology of jarrah dieback, a disease of dry sclerophyll *Eucalyptus marginata* Sm. forests in Western Australia. M.Sc. Thesis, University of Melbourne.

Podger, F.D. (1972). *Phytophthora cinnamomi*, a cause of lethal disease in indigenous plant communities in Western Australia. Phytopathology. 62, 972-981.

Rockel, B.A. (1977). How to make growing roots of a tree accessible. Aust. For. Res. 7, 269-270.

Shea, S.R., Gillen, K.J. & Kitt, R.J. (1978). Variation in sporangia production of *Phytophthora cinnamomi* Rands on jarrah (*E.marginata* Sm.) forest sites with different understorey compositions. Aust. For. Res. 8, No. 3/4. 219-226.

Written as a handout for the *Phytophthora cinnamomi* Workshop held as part of the A.P.P.S. Conference in Perth, W.A., May, 1980.