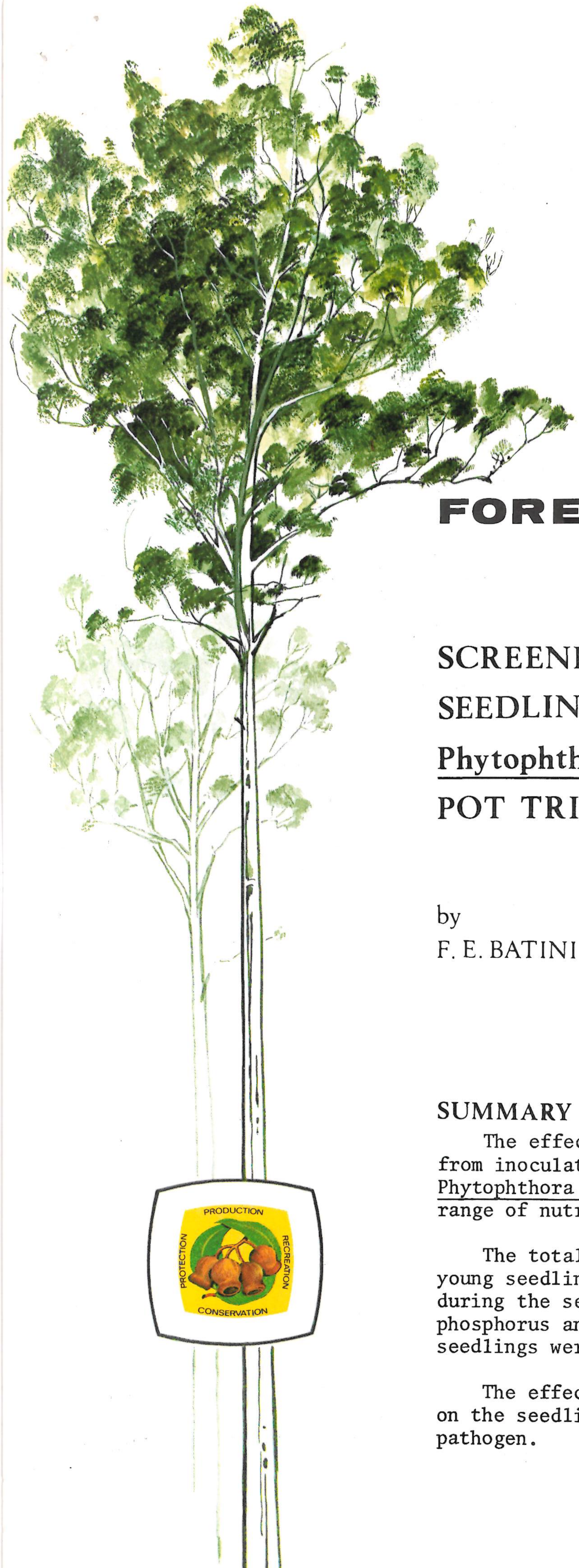

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SCREENING OF Pinus pinaster Ait. SEEDLINGS FOR RESISTANCE TO Phytophthora cinnamomi Rands IN POT TRIALS

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SUMMARY

The effects on plant health and growth resulting from inoculation of Pinus pinaster Ait. seedlings with Phytophthora cinnamomi Rands were investigated under a range of nutritional and watering regimes.

The total height, shoot weight and total weight of young seedlings were depressed and mortality increased during the second growing season, particularly where phosphorus and nitrogen were deficient. Older seedlings were not affected by inoculation.

The effects of the nutritional and watering regimes on the seedlings were greater than those of the pathogen.

INTRODUCTION

Pinus pinaster Ait. is an important timber species in Western Australia and extensive plantations have been established on the sands of the Swan Coastal Plain. This species of pine has also been used to reforest areas of the jarrah (Eucalyptus marginata Sm.) forest infected by the soil pathogen Phytophthora cinnamomi Rands (the cause of jarrah dieback disease). P. pinaster has grown successfully on these sites for 20 years.

P. cinnamomi has, however, been associated with dead and dying mature P. pinaster in shelterbelts on irrigated alluvial soils of the Plain. Trials by Batini and Podger (1968) showed that the root development of seedlings inoculated with the pathogen was poorer than that of seedlings which had not been inoculated. Severe cortical rotting of roots was also observed. Further pot trials were therefore set up to investigate the effects of inoculation on the parameters of plant growth and health under a range of nutritional and watering regimes.

TRIAL 1
METHOD

Species - P. pinaster (Leiria strain), 8 months old at the first inoculation.

Soil - Sand-loam mix (3:1) in 9 l buckets. The mix was sterilised with methyl bromide.

Inoculation - In autumn and then in spring with pure cultures of P. cinnamomi grown on pea sucrose agar (P.S.A.). Control pots received P.S.A. only. The trial was closed 12 months after the autumn inoculation.

Nutrition - From 4 months prior to the autumn inoculation, half the buckets received fortnightly applications of "Aquasol" (see Appendix 1) mixed with water to the recommended concentration. Fertiliser application was discontinued after the spring inoculation.

Watering - The plants received normal amounts of water until the spring. One series was then kept saturated by maintaining a 5 cm water table in the bucket, whilst for the rest of the seedlings, water was gradually reduced until, by early summer, watering consisted of saturating the soil and then allowing it to dry to wilting point. All water was applied from above to encourage sporulation and to allow migration of the zoospores.

Layout - 2 x 2 x 2 factorial, with 6 replicates and 6 seedlings per bucket.

RESULTS

Inoculation with P. cinnamomi did not significantly affect any parameter of plant growth or health. Very few seedlings died during the trial.

The effects of the nutritional and watering regimes on the standard parameters of plant growth were marked. Whereas seedlings responded positively to nutrition under both moisture regimes, high levels of soil moisture induced positive responses only in the fertilised treatments. Results obtained for some parameters are shown in Table 1.

TRIAL 2
METHOD

Species - P. pinaster (Leiria strain), 32 months old at the first inoculation.

Treatments - Soil, inoculation, nutrition and watering were the same as in Trial 1.

Layout - 2 x 2 x 2 factorial, with 6 replicates and 1 seedling per bucket.

RESULTS

Inoculation, watering and nutrition did not significantly affect any parameter of plant growth or health during the trial. None of the seedlings died.

TRIAL 3
METHOD

Species - P. pinaster (Leiria strain), 6 months old at the first inoculation.

Soil - Lateritic gravel which had been collected from healthy forest, screened through a 6 mm mesh, and steam-sterilised. 1800 g of soil was weighed into each 15 cm pot.

TABLE 1

Mean height increment (after 12 months) and mean oven-dry weights of P. pinaster under various nutritional and watering regimes

Treatment	Height increment (cm)	Oven-dry weights Shoots (g)	Roots (g)	Total (g)	Statistical populations ($p < 0.01$)
Fertilised, high soil moisture	63.0	154.0	42.0	196.0	a
Fertilised, fluctuating soil moisture	47.3	97.7	25.8	123.5	b
Unfertilised, fluctuating soil moisture	24.3	38.6	13.7	52.3	c
Unfertilised, high soil moisture	20.4	34.6	13.3	47.9	c

Inoculation - In two successive springs with pure cultures of P. cinnamomi grown on P.S.A. Control pots received P.S.A. only. The trial was closed 18 months after the first inoculation.

Nutrition - Nutrient amendments were begun 3 months prior to inoculation. Nutrients were applied fortnightly as aqueous solutions at the rate of 100 ml per pot.

The 6 major elements (nitrogen N, phosphorus P, potassium K, calcium Ca, magnesium Mg and sulphur S) were incorporated in a subtractive trial based on a full nutrient solution (Meyer and Anderson, 1939), to give 8 treatments ranging from "full" to "nil". The composition of these nutrient solutions is given in Appendix 2.

Watering - The freely drained pots were watered daily after each inoculation to encourage sporulation and to allow zoospore dispersal. During the winter months, water was applied as necessary.

Layout - 2 x 8 factorial, with 4 replicates and 6 seedlings per pot.

RESULTS

Five months after the commencement of nutrient amendments, the seedlings in the "minus phosphorus" and "nil" treatments were significantly smaller than those in all other treatments, and some were dying. Three months later, the level of mortality in these two treatments was significantly higher than in all other treatments.

During the second growing season, 13 months after the commencement of nutrient amendments, the height of seedlings in the "minus nitrogen" treatment was significantly depressed. Mortality increased so that by the end of the trial there were significantly fewer live seedlings in the "minus nitrogen", "minus phosphorus" and "nil" treatments than in the five treatments fertilised with both phosphorus and nitrogen, where nutrient amendments did not significantly affect survival or any parameter of growth.

Inoculation with P. cinnamomi did not affect mortality until the second growing season (Fig. 1). By the end of the trial, inoculation had significantly increased mortality and had depressed total height, shoot weight and total

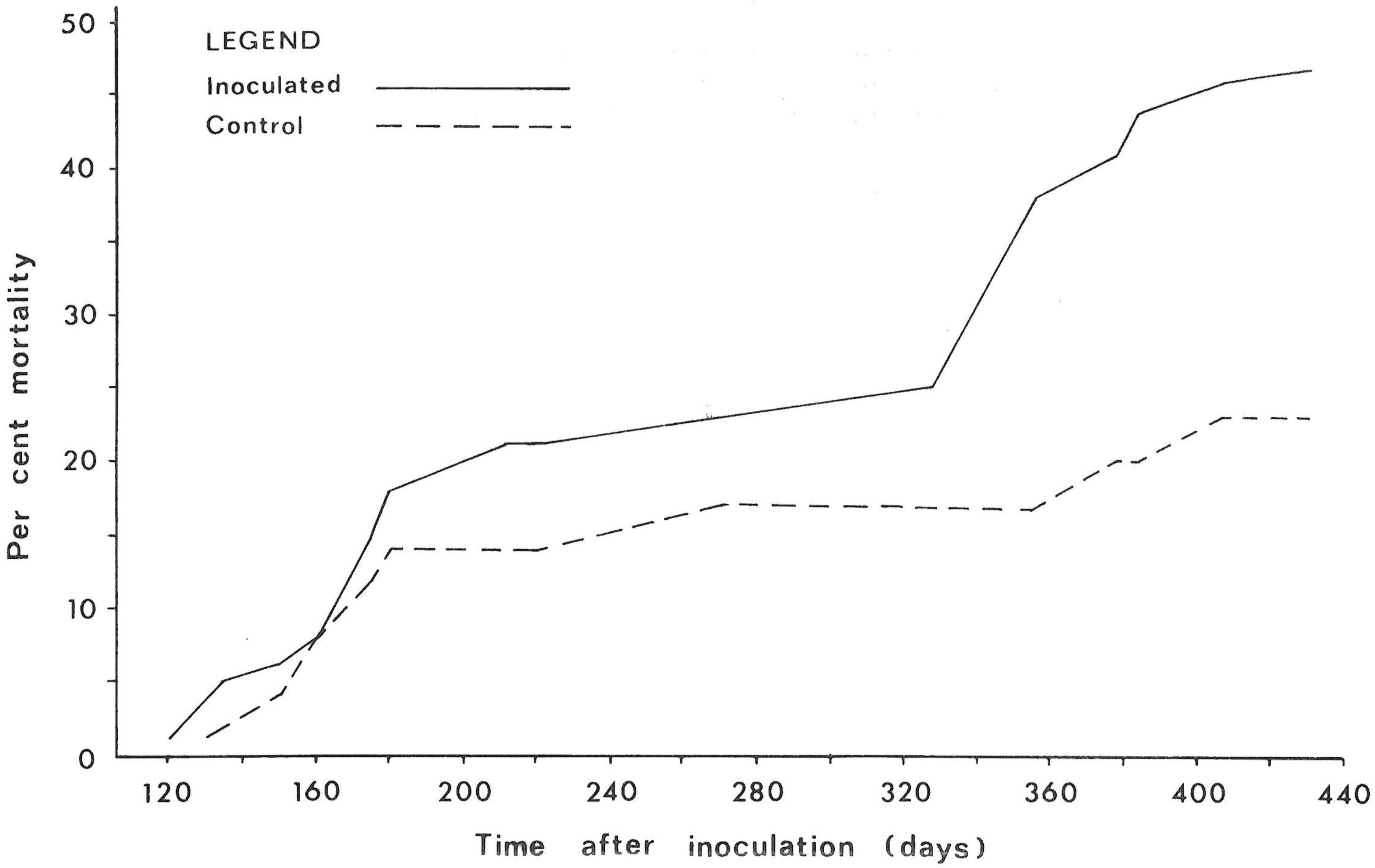

FIGURE 1: Cumulative mortality of inoculated P. pinaster seedlings (Trial 3).

weight. However, root weight and foliar levels of nitrogen, phosphorus, potassium, calcium, magnesium and sulphur were not affected. The effects of inoculation on mortality were less marked in the treatments fertilised with both nitrogen and phosphorus (Fig. 2).

Total soluble salts (per cent) and pH of the soil were not affected by either nutrition or inoculation.

In all three trials, P. cinnamomi was readily isolated when roots from inoculated seedlings were plated onto 3P agar (Eckert and Tsao, 1962); no isolations were obtained from the controls.

DISCUSSION

In Trial 1 (the fertiliser x water interaction), the seedlings responded favourably to the fertiliser, and in the series where soil moisture was not limiting, positive growth response was even more marked.

A nitrogen x phosphorus interaction for both height and mortality was observed in Trial 3; of the two, phosphorus deficiency was more detrimental. Clearly, the lateritic gravel soil was unable to supply either of these elements in quantities sufficient for satisfactory growth of P. pinaster. In contrast, however, it supplied calcium, potassium, magnesium and sulphur in quantities sufficient for the plants' growth.

In those nutrient treatments where pine growth was poor, mortality was high in both the inoculated and the control pots. Plant decline was first observed as a scorch to the tops of the seedlings and the tips of the needles. After a considerable period, these seedlings died. They were distinctly purplish in colour.

Where seedling growth was more satisfactory owing to fertilisation with nitrogen and phosphorus, mortality in

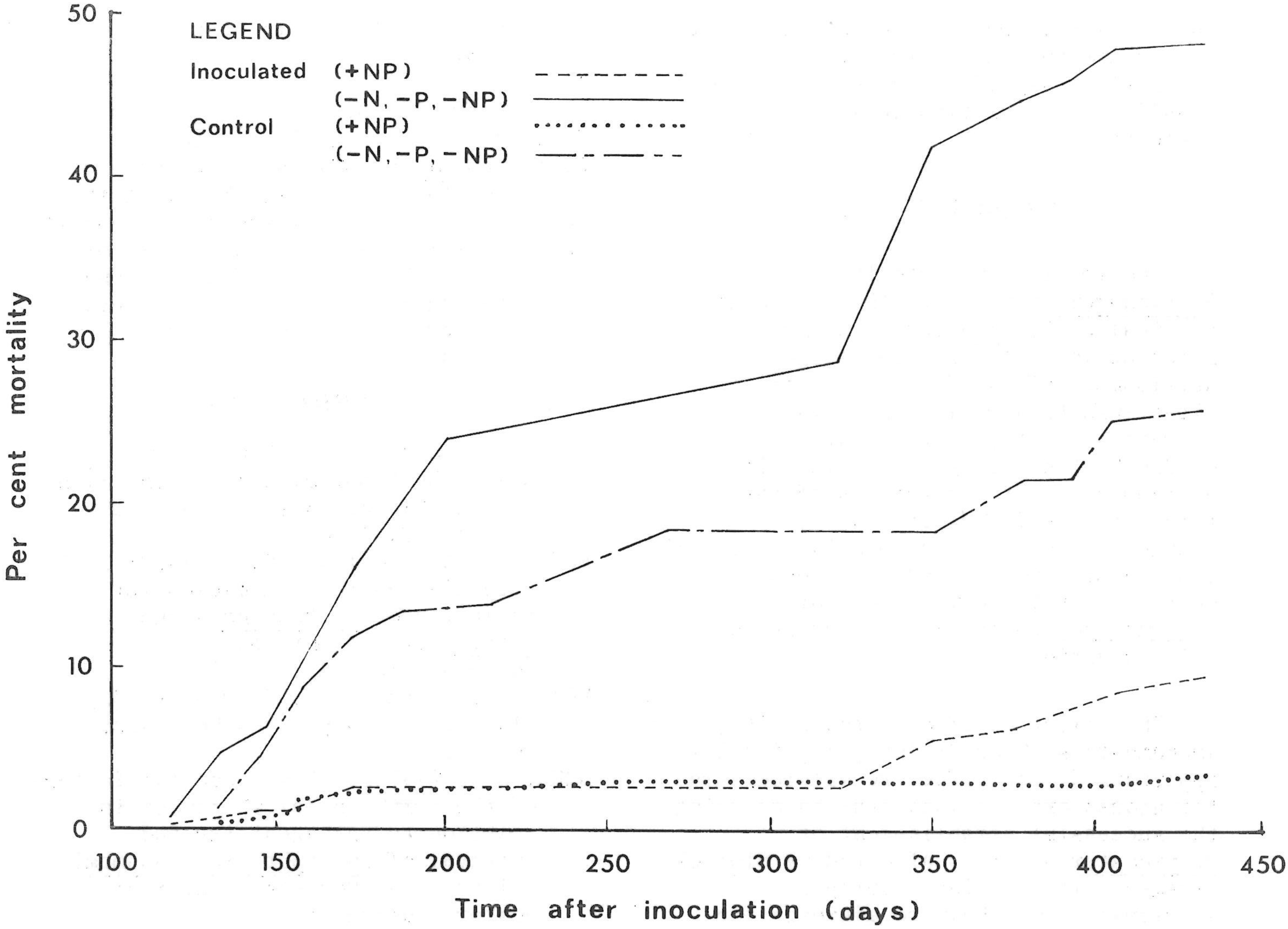

FIGURE 3: Cumulative mortality of inoculated P. pinaster seedlings under various nutritional regimes (Trial 3)

both the inoculated and the control treatments was much lower.

However, the death of these seedlings was more rapid. Dead tops and wilt were common symptoms, and were followed by death. Since total soluble salts and pH were not significantly affected by nutrient amendments, death cannot be ascribed to a change in either of these factors.

The symptoms suggest that physiological drought caused by poor soil aeration contributed to seedling mortality. The pots were watered frequently after each inoculation, and moisture sampling indicated that the soil was maintained consistently above field capacity; furthermore, the poor growth in some treatments prolonged the period during which the soil remained moist.

No significant effects of inoculation were observed until the second growing season (Trial 3). At this stage, mortality occurring as a result of inoculation was greatest in those nutrient treatments where plant growth was poor. With older seedlings, however, no significant effects of inoculation were observed during the year following inoculation. In Trials 1 and 2, where height growth, shoot weight and total weight were slightly depressed by inoculation, significant differences between the two inoculation treatments and their controls may have become evident if the trials had been continued for a further growing season. It appears that a single seedling per bucket (Trial 2) was not enough to provide for statistical significance owing to the considerable variation in seedling response.

CONCLUSIONS

The results indicate that P. cinnamomi may affect the growth and survival of P. pinaster seedlings on sites ecologically suited to the development of the fungus. On these sites infection could result in decreased growth, little-leaf disease or death of seedlings. However, in any extrapolation of the results of pot trials to the field, it must be recognised that in pine plantations, soil temperature and moisture are less suitable for the development of P. cinnamomi (Shea, 1975) than are pot soil conditions.

The selection of a suitable site, site amendments such as draining and fertiliser application, and effective silvicultural techniques are all important in assuring the successful establishment of P. pinaster on sites affected by dieback disease. In addition, appropriate silviculture may help to minimize the effects of the fungus in highly susceptible areas.

Observation of P. pinaster planted on dieback sites has indicated that P. cinnamomi is only one of a range of environmental factors which affect the survival and growth of the pines, and it is interesting to note that in two of the trials, the effects of nutritional and watering regimes on the seedlings were greater than the effects of inoculation.

REFERENCES

BATINI, F. and PODGER, F.D. (1968). Shelterbelt mortalities on the Swan Coastal Plain. Australian Forest Research 3, 39-45.

ECKERT, J.W. and TSAO, P.H. (1962). A selective antibiotic medium for isolation of Phytophthora and Pithium from plant roots. Phytopathology 52, 771-777.

MEYER, B.S. and ANDERSON, D.B. (1939). Plant Physiology. Van Nostrand, New York.

SHEA, S.R. (1975). Environmental factors of the northern jarrah forest in relation to pathogenicity and survival of Phytophthora cinnamomi. Bulletin 85, Forests Department of Western Australia.

APPENDIX 1

"Aquasol" is a water-soluble complete fertiliser. Its composition is as follows:

<u>Analysis</u>: N.P.K. ratio 20:5:18

Nitrogen (N) as mono-ammonium phosphate	2.0%
Nitrogen (N) as potassium nitrate	6.0%
Nitrogen (N) as urea	12.0%
Total nitrogen (N)	20.0%
Total phosphorus (P), water-soluble as mono-ammonium phosphate	5.0%
Total potassium (K) as potassium nitrate	18.0%
Zinc (Zn) as zinc sulphate	0.05%
Copper (Cu) as copper sulphate	0.06%
Molybdenum (Mo) as sodium molybdate	0.0015%
Sulphur (S) as sulphates	0.40%
Manganese (Mn) as manganese sulphate	0.15%
Iron (Fe) as sodium ferric E.D.T.A.	0.12%
Boron (B) as sodium borate	0.012%
Magnesium (Mg) as magnesium sulphate	0.18%

APPENDIX 2

The composition of the full nutrient solution used in the pot trials follows that of the "three salt" solution which was originally proposed by Shive and which is discussed in Meyer and Anderson (1939), Chapter 25:

1M $MgSO_4 \cdot 7H_2O$	2.3 $ml \cdot l^{-1}$
1M $Ca(NO_3)_2 \cdot 4H_2O$	4.5 $ml \cdot l^{-1}$
1M KH_2PO_4	2.3 $ml \cdot l^{-1}$

Nutrient amendments were made as follows:

Minus nitrogen: $Ca(NO_3)_2$ replaced by 1M $CaCl_2$

Minus phosphorus: KH_2PO_4 replaced by 1M KCl

Minus potassium: KH_2PO_4 replaced by 1M $NaH_2PO_4 \cdot 2H_2O$

Minus magnesium: $MgSO_4$ replaced by 1M Na_2SO_4

Minus sulphur: $MgSO_4$ replaced by 1M $MgCl_2 \cdot 6H_2O$

Minus calcium: $Ca(NO_3)_2$ replaced by 1M $NaNO_3$