ODC 443.3
Research Paper 77
February 1984

ASSESSMENT OF *PHYTOPHTHORA* DISEASE RISK AT THE NANNUP NURSERY

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OF WESTERN AUSTRALIA

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SUMMARY

Concern at a number of deaths in *Pinus radiata* plantations prompted an investigation of planting stock originating from the Nannup nursery.

Three *Pythium* species but no *Phytophthora* species were recovered from *Pinus radiata* stock in samples taken from the nursery.

A glasshouse experiment showed that *Phytophthora cinnamomi* and *P. cryptogea* introduced in plant tissue could infect and kill *P. radiata* seedlings growing in pots of the nursery's soil. The pathogen remained in evidence for the duration of the experiment, and was recovered from live plants at harvest. *Phytophthora cinnamomi* infected soil also caused deaths, but could not be recovered from roots or soil at the end of the experiment.

A soil drench of Ridomil fungicide (CGA 48988 Ciba-Geigy Ltd) was effective in reducing post-emergent mortality, and remained active against *Phytophthora* species for one to three months after application.

The results show that *P. cinnamomi* and *P. cryptogea* are most likely to infect plants in the nursery's soil if they are introduced in plant tissue. Infected soil falling from a vehicle is only likely to cause further infection under highly favourable conditions.

Comprehensive hygiene controls are therefore needed for all traffic to and from the nursery, and should include sample checks of incoming pine "duff" (used to maintain mycorrhizal levels in nursery soil) and departing planting stock.

INTRODUCTION

Phytophthora species are major pathogens in forest and plant nurseries throughout the world (Oxenham and Winks, 1963; Newhook 1972; Linderman and Zeitoun; 1977). In nurseries their most common effect is to increase mortality of susceptible plants, and losses can be severe. A less obvious problem with a *Phytophthora* infected nursery is the distribution of live but infected stock. In this way *Phytophthora* is spread to new, possibly uninfected areas where it can later cause disease in the outplanted stock and possibly infect nearby susceptible species, (Donald and von Broembsen, 1977; Sivasithamparam and Goss, 1980).

The deaths of a number of *Pinus radiata* trees in the Donnybrook Sunkland plantation, south of Busselton, W.A., are associated with infections of *Phytophthora* species, including *P. cinnamomi* and *P. cryptogea* (Chevis and Stukely, 1982). Concern about these deaths resulted in our investigation of the Nannup pine nursery, the supplier of all planting stock for the Sunkland.

This paper reports research carried out to test whether:

(1) *Phytophthora* species were present in the Nannup Nursery;

(2) *Phytophthora* species could be introduced and establish infections in the nursery;

(3) Apparently healthy plants from the nursery could be carriers of infection;

(4) An infection existing in the nursery could be controlled or eliminated by the use of the fungicide Ridomil without detrimental effects to beneficial mycorrhizal fungi.

METHOD

Nursery stock sampling

Dead *Pinus radiata* seedlings, one-year-old planting stock and diseased lupin plants were sampled at the nursery throughout the year (1980). Where isolated deaths occurred, affected plants were dug out, placed in a plastic bag and transported to the laboratory in an insulated container. More extensive sampling of planting stock and diseased lupins was carried out by sampling at 10 m intervals along transects, 20 m apart across the nursery. In this way, 280 pine plants were sampled out of a total of some 3 million and 240 lupin plants were sampled from a 5 ha area.

Isolation of Pythiaceous fungi from nursery stock

Two methods were used to isolate Pythiaceous fungi from the samples.

Small root samples were washed free of adhering soil, surface sterilized in 70% ethanol for 30 seconds and rinsed in running distilled water for 30 seconds. They were then plated onto P_{10} VP(H) medium, which is selective for *Phytophthora* and some *Pythium* species (Tsao and Guy, 1977). Plates were incubated at 25°C and examined after two and four days. Fungal outgrowths were grouped according to morphological characters. Representatives of each fungal type were subcultured onto P_{10} VP(H) medium and then onto cornmeal agar (Difco) and vegetable extract agar (V8) for identification.

Larger numbers of plants and soil samples were tested by baiting with *Lupinus angustifolius* leaves (Palzer, 1977)and *Pinus radiata* needles (Dance *et al*, 1975). Each transect sample of ten root systems was placed in a 6 cm deep plastic tray. The trays were filled with distilled water, upon which five lupin leaves and ten clusters of pine needles were floated, and then incubated in a glasshouse at 20-55°C. After two days the baits were removed, blotted on paper towels and plated onto P_{10} VP(H) medium. Plates were incubated and fungi isolated following the direct plating method. The efficiency of the method was checked using *Phytophthora cinnamomi* pine branch segment inoculation (Shea *et al.* unpublished - see Appendix I).

Pot experiment: to test introduction of infections

Experimental design

As introducing *Phytophthora* to the nursery was out of the question, a glasshouse pot experiment was designed to test the effect of *P. cinnamomi* and *P. cryptogea* on *Pinus radiata* growing in pots of nursery soil. A factorial design tested three inoculation

treatments: *Phytophthora cinnamomi* infected soil (CINS), *P. cinnamomi* pine branch segment inoculum (CINP), and *P. cryptogea* pine branch segment inoculum (CRYP) : three inoculation times; at seeding (O), and one (1) and three (3) months after seedling emergence: and two fungicidal treatments: 0 (-R) and 17 gm^{-2} Ridomil (-R) 25 WP (Ciba- Geigy) applied at seeding. Each treatment was replicated five times.

Five uninoculated control pots were treated with fungicide and five other control pots were not.

Procedure

Granitic red loam soil was collected from the nursery, air dried and sieved through a 5 mm sieve. Nine hundred grams of soil were placed in 10.4 cm diameter trained plastic pots and watered with distilled water.

Pinus radiata seedlot R1 was prepared for sowing following standard nursery procedure: stratification at 4°C for four weeks, followed by drying and dusting with 'Captan' fungicide (83 Lane Ltd).

The following isolates were obtained from Jarrahwood, W.A. and used as inoculum. (Reference cultures are deposited in the DCE collection at Murdoch University, Murdoch, W.A.)

CINS *Phytophthora cinnamomi* DCE 151 was isolated from the root of a dead two-year-old *Pinus radiata*. Four *P. radiata* seedlings growing in a pot of Type 4C grey loamy sand (McCutcheon, 1978) were inoculated with prepared pine branch segment inoculum. Nine to twelve months later, after the death of the seedlings, soil containing dead roots was removed from the pot for use as inoculum. Prior to inoculation the number of *Phytophthora cinnamomi* propagules per gram of soil was estimated by plating 25 g soil and 50 ml distilled water on P_{10} VP(H) medium (Shea *et al*, 1980a).

CINP *Phytophthora cinnamomi* DCE 175 was isolated from the collar of a dead four year old *Pinus radiata*. Pine branch segment inoculum was prepared following the method of Shea *et al* (unpublished) (Appendix 1).

CRYP *Phytophthora cryptogea* M.S. 227a was isolated from a root of a dead three year old *Pinus radiata* and used to prepare inoculum segments (Appendix 1).

Ten seeds were planted 1.5 cm deep around the edge of every pot. CINP(O) and CRYP(O) pots had two inoculum segments pressed, one on top of the other, into the soil in the centre of the pot until the top segment was 1 cm below the soil surface. All pots then had the seeds and, where necessary, inoculum segments covered with soil. CINS(O) pots were inoculated with 5 g of moist infected soil, placed in the centre of the pot and pressed down so that it took up water from the nursery soil below. Fungicide treated pots were watered with 10 ml of Ridomil solution, untreated pots received 10 ml of distilled water. The plots were placed in random blocks in an air-conditioned glasshouse, with air temperature 9-33°C, and kept moist with distilled water to prevent the surface soil drying. Emergence and mortality were assessed every two days.

Two months after seeding, and approximately one month after emergence was completed, the CINS(1), CINP(1) and CRYP(1) treatment pots were inoculated.

The remaining series of treatment pots CINS(3), CINP(3) and CRYP(3) were inoculated two months later.

Samples of emergent seedlings which died during the first month were removed and plated onto selective medium, without surface sterilization. Resulting fungi were later isolated using P_{10} VP(H) medium.

One month after the final inoculation, and five months after seeding, the experiment was harvested. Tops of plants were removed at ground level, dried and weighed. The soil and rootball were removed from each pot, and approximately 200 g of soil and roots taken from the soil at the base of the pot. The remaining soil was washed from the roots, any inoculum plugs recovered and the root systems taken to the laboratory for isolation of fungi.

Isolation of residual fungi from the experimental pots

Approximately thirty 1 cm suberized root pieces, thirty mycorrhizal clusters.

all the unsuberized root tips and the plant collars were taken from each root system collected. They were surface sterilized in 70% alcohol and rinsed in distilled water. Each root type was plated separately onto P_{10} VP (H) medium.

Inoculum plugs were surface sterilized and split lengthwise before plating. The plates were incubated for 36 hours at 25°C, examined for fungal growth and checked again after a further two days' incubation.

Soil and root samples taken from the base of the pots were mixed and 100 g lupin baited (Chee and Newhook, 1965) to see whether the pathogen had spread from the original inoculum.

The proportion of mycorrhizal roots on control pot root systems was estimated by making approximately 120 observations of root tips. The proportion of mycorrhizal roots (dichotomously branched and/or with an obvious fungal mantle), non-mycorrhizal short roots and non-suberized roots were expressed as percentages of the root tips observed.

RESULTS

Nursery stock sampling

Phytophthora species were not isolated from any of the samples tested. *Pythium irregulare* and *Pythium mamillatum* were frequently isolated; *Pythium anandrum* occurred infrequently (Table 1). Other *Pythium* species may have been present, but were not isolated on the P_{10} VP (H) medium. These results only establish that *Phytophthora* species were not present in the samples tested. Only 300 trees were tested from a total of 2 million produced at the Nannup nursery in 1980. A more intensive sampling and baiting programme may have isolated *Phytophthora*.

TABLE 1
Recoveries of Pythiaceous fungi from samples taken from Nannup nursery.

Date	Sample type	No. of Plants tested	Method[1]	Fungi recovered
4.2.80	Dead 6 m. o. *Pinus radiata* seedlings	15	D	*Pythium mamillatum*
	Dead 18 m.o. *P. radiata* seedlings	3	D	nil
29.5.80	Diseased lupin roots on green manure area	20	D	nil
		240	P.L.	nil
26.6.80	Dead 12 m.o. *P. radiata* seedlings	6	D	*Pythium irregulare* *P. mamillatum*
	Soil surrounding dead. *P. radiata* seedling roots		P.L.	nil
	Live 1 y.o. *P. radiata* planting stock	280	P.L.	*P. irregulare* *P. mamillatum* *Pythium anandrum*
3.9.80	Damped off 1 m.o. *P. radiata* seedlings	30	D	nil

1. D - direct plating of surface sterilized roots
P - baiting of sample with pine needles
L - baiting of sample with lupin leaves

Pot experiment: seedling growth and mortality

Seventy-nine per cent of seed emerged within a month of sowing; treatments appeared to have no effect on emergence. Many seedlings in the -R pots died from root disease soon after emergence (Table 2). *Pythium* and *Phytophthora* species were isolated from the dead plants plated at this time. Occasional deaths continued during the remainder of the experiment with CINP -R pots having the highest number (Table 2). However, at harvest, there was no statistically significant (PLO.05) evidence of the effect of inoculation on the inoculum treated plants (Table 3).

Ridomil significantly increased the numbers of surviving plants, but at the igh rate applied caused phytotoxicity symptoms. The tips of old needles yellowed and died. Treated plants were stunted, the mean seedling top weight decreased from 0.170 g in -R pots to 0.114 g per seedling in +R pots. The fungicide also reduced the percentage of root tips forming mycorrhizae from 50% to 25%. Seedlings appeared to recover from the phytotoxicity by the end of the experiment, as new needles were of normal appearance.

TABLE 2

Mortality of *Pinus radiata* seedlings (mean number per pot)

Seed pot treatments	Plants damping off in 1st month after inoculation		Plants dying during remainder of exp.	
	- R	+ R	- R	+ R
CINS 0	1.4	0.2	0	0
1	1.8	0.2	0	0
3	0.8	0	0	0
CINP 0	1.6	0.2	0.4	0
1	2.2	0	0.2	0.2
3	1.6	0	0.2	0
CRYP 0	2.4	1	0	0
1	1.6	0.4	0.2	0
3	2.2	0.2	0	0
CONTROL	1.8	1.6	0.2	0.4

Residual fungal infections

Phytophthora was isolated in roots of plants which had been inoculated with *P. cinnamomi* and *P. cryptogea* segment inoculum (Table 4). Suberized roots were most frequently infected, but unsuberized roots, mycorrhizal roots and collars occasionally yielded the pathogens.

TABLE 3

Mean percentage of *Pinus radiata* seed surviving as seedlings to end of experiment.

Seed pot treatments	- R	+ R
CINS 0	44	64
1	58	82
3	58	74
CINP 0	56	72
1	60	74
3	60	82
CRYP 0	56	70
1	60	80
3	58	74
CONTROL	62	78

Even though the soil inoculum (CINS) added 23 to 38 propagules of *P. cinnamomi* to each pot no residual infections were detected in the roots or soil.

Phytophthora cinnamomi counts were highest in pots where deaths had occurred.

Phytophthora cryptogea was isolated from suberized roots, and the bark of collars of apparently healthy trees. Lupin baiting also isolated *Phytophthora* in samples from pots inoculated with *P. cinnamomi* and *P. cryptogea* segments.

Application of Ridomil caused a failure to isolate *Phytophthora*, excepting three *P. cinnamomi* inoculum segments which remained viable when placed in soil which had been Ridomil treated three months prior to inoculation (see Table 4).

TABLE 4

Recoveries of *Phytophthora* from *Pinus radiata* root systems and soil.

	Root System		Soil/Roots		Inoculum Plugs	
	-R	+R	-R	+R	-R	+R
CINS 0	0	0	0	0	n.a.	n.a.
1	0	0	0	0	n.a.	n.a.
3	0	0	0	0	n.a.	n.a.
CINP 0	2	0	3	0	5	0
1	1	0	0	0	5	0
3	1	0	0	0	5	3
CRYP 0	1	0	4	0	5	0
1	0	0	1	0	4	0
3	3	0	0	0	5	0

n.a. not applicable to this treatment

DISCUSSION

The sampling programme and experimental results indicate that there is a population of pathogenic *Pythium* fungi in the nursery which could, when conditions are suitable, cause considerable losses. Losses are most likely to occur in spring conditions when the soil is warm enough to permit fungal activity.

Under the conditions of our experiment *Phytophthora cinnamomi* and *P. cryptogea* were able to spread from pine branch inoculum and infect *Pinus radiata* seedlings growing in Nannup nursery soil. *P. cinnamomi* was unable to establish an infection or survive when added as infected Sunkland soil.

Infection by both fungi was present also in pots where no deaths had occurred, which means, that if *Phytophthora* became established in the nursery, apparently healthy plants could be carriers of infection.

A high rate of Ridomil fungicide was able to prevent introduction of *P. cryptogea* for a period of 3 months and *P. cinnamomi* for between 1 and 3 months. The fungicide also significantly reduced the incidence of post-emergent damping off, caused by *Pythium* and other *Phytophthora* species. Its effect on the beneficial mycorrhizal was not significant.

Although the soil infected inoculum was ineffective in the experiment, the loss of 5 kg of infected soil from a vehicle could be enough to establish an infection in nursery conditions (Batini and Cameron, 1974). Another possible source of infection is litter used in the nursery as mycorrhizal inoculum.

Once an infection is established in the nursery our results show that it would:

(1) be difficult to detect before considerable losses occurred;

(2) become a base for further infection by live plants which were transported out of the nursery; and

(3) be difficult to eradicate because the infection would remain behind in cut roots and soil after lifting of trees.

These experimental results have important implications for the management of the nursery.

It is recommended that all implements (including boots) be washed down before entering the nursery; that bags used to carry trees to the field be washed in an effective disinfectant and rinsed with treated water before re-entering the nursery (or that disinfected polythene containers be used in place of hessian bags); that treatment of the water used for irrigation be investigated, and that run-off from the car park be diverted to by pass the dam used for irrigation.

It is also recommended that soil samples from the area where the mycorrhizal inoculum is collected be baited for *Phytophthora* species and not used unless the site is proved clean.

ACKNOWLEDGEMENTS

This work was supported by a Rural Credits Development fund grant. The assistance of Dr E. Davison, who identified the fungal isolates, M. Stukely, who provided inoculum and K. Sclater and J. Rynski is gratefully acknowledged.

REFERENCES

Batini, F. and Cameron, J.N. (1974). Some aspects of logging hygiene. Forests Department Western Australia Research Paper No. 4.

Chee, K. and Newhook, F.J. (1965). Improved methods for use in studied on *Phytophthora cinnamomi* Rands and other *Phytophthora* species. New Zealand Journal of Agricultural Research *8*: 88-95.

Chevis, H.W. and Stukely, M.J.C. (1982). Mortalities of young established radiata pine associated with *Phytophthora* spp. in the Donnybrook Sunkland plantations in Western Australia. Australian Forestry 45:193-200.

Dance, M.H., Newhook, F.J. and Cole, J.S. (1975). Bioassay of *Phytophthora* spp. in soil. Plant Disease Reporter *59*: 523-527.

Donald, D.G.M. and von Broembsen, S.L. (1977). The control of *Phytophthora*

cinnamomi Rands in a South African forest nursery. South African Forestry Journal *100*: 50-55.

Linderman, R.G. and Zeitoun, F. (1977). *Phytophthora cinnamomi* causing root rot and wilt of nursery-grown native western azalea and salal. Plant Disease Reporter *61*: 1045-1948.

McCutcheon, G.S., 1978. Broadscale forest site survey techniques used in the Donnybrook Sunkland. Forests Department Western Australia Research Paper 48.

Newhook, F.J. (1972). The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. Annual Review Phytopathology *10*: 299-326.

tenham, G.L. and Winks, B.L. (1963). *Phytophthora* root rot of *Pinus* in Queensland. Queensland Journal Agricultural Science 20: 355-366.

Palzer, C., 1976. Zoospore inoculum potential of *Phytophthora cinnamomi* Doctor of Philosophy thesis. University of Western Australia 222 pp.

Shea, S.R., Gillen, K.J. and Leppard, W.I. (1980a). Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in diseased, freely drained *Eucalyptus marginata* Sm sites in the northern jarrah forest of south-western Australia. Protection Ecology: *2*: 135-156.

Sivasithamparam, K. and Goss, O. (1980). Jarrah dieback - a threat to horticulture. West Australian Department of Agriculture Journal of Agriculture *3*: 88-91.

Tsao, P.H. and Guy, S.O. (1977). Inhibition of *Mortierella* and *Pythium* in a *Phytophthora*-isolation medium containing hymexazol. Phytopathology 67: 796-801.

Appendix 1

Preparation of *Phytophthora cinnamomi* and *P. cryptogea* inoculum.

Pine branch-plug inoculum was prepared for both species by the method of S.R. Shea, T.J. Boughton and B.L. Shearer (unpublished). *Phytophthora cinnamomi* (mating type A2), originally isolated from the roots of a dead *Pinus radiata* in the Sunkland, was incubated at 24-26°C for seven days in 10 ml pots of 10% V8 broth. Live *P. radiata* branches were cut into 2 cm plugs. (1 to 2 cm in diameter). After the bark was removed, the plugs were soaked overnight in distilled water, rinsed and placed in conical flasks (100 plugs per litre of flask capacity).

Sufficient distilled water was added to cover the bottom of the flasks, which were plugged with non-absorbent cotton-wool and autoclaved for 30 minutes at 15 p.s.i. before cooling to room temperature. *Phytophthora cinnamomi* mycelial mats were aseptically lifted from the V8 broths and dropped in with the plugs (one mat per 100 plugs), gently shaken and then incubated at 24-26°C. After 2-3 days when the fungus had started to colonize the plugs, the flasks were again shaken to disperse the inoculum. The plugs were then incubated for a further 2-3 weeks.