Comparison of Three Methods for the Eradication of *Phytophthora cinnamomi* from Deep Leached Sands

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INTRODUCTION

Phytophthora cinnamomi Rands is destroying many indigenous plant communities in south-western Australia (Podger 1968; Newhook and Podger 1972; Malajczuk and Glenn 1981; Shearer and Hill 1989; Hill 1989). The disease affects numerous floristic associations from a wide range of plant formations, including *Eucalyptus marginata* Donn ex Sm. forest, *Banksia* low woodland, Proteaceous thicket, mallee-heath, scrub-heath, heath, and even moss swards (Podger 1968; Havel 1979a; Hart 1983; Brandis *et al.* 1986; Shearer and Hill 1989; Hill 1989). The disease also threatens several endemic taxa of restricted range, including *Banksia brownii* Baxter ex R.Br., a declared rare and highly susceptible species, that faces extinction in the wild - it is known from only six small populations, all of which are infected with *P. cinnamomi* (G.J. Keighery, personal communication). Devastating *P. cinnamomi* infections have been found in scrub-heath vegetation that receive as little as 390 mm of annual rainfall.

In Australia, few attempts have been made to eradicate *Phytophthora cinnamomi* from infected areas of native vegetation. Several techniques and chemicals were tested in Victoria in diseased *Eucalypt* forest and *Banksia* woodland (Weste *et al.* 1973; Weste and Law 1973). Most treatments failed to eradicate or confine the infection, though fumigation with Vapam reduced the fungal population to non-detectable levels for 18 months, on two sites.

In Western Australia, eradication of the fungus was never considered practicable in the jarrah forest (Podger 1972). *P. cinnamomi* was first associated with jarrah dieback in 1964, by which time the disease had already damaged tens of thousands of hectares of forest (Podger 1968). Podger concluded that the combination of widespread infestation, saprophytic ability of the fungus and difficulties of trenching in indurated laterite eliminated any prospect for control. *Phytophthora cinnamomi* has been isolated from where jarrah and *Banksia* roots that penetrate both the laterite and underlying clay horizons (Shea *et al.* 1983). Effective penetration of fungicides below the ironpan would be unlikely. In addition, the disease boundary may be difficult to define, due to the possibility of rapid, yet unpredictable, downhill spread of disease ahead of symptoms in the vegetation (Havel 1979b).

Many of these obstacles would not be encountered in an attempt to eradicate a small infection from a variety of, susceptible plant communities that grow on deep, highly-leached, quartz sands (Uc 2.1 and Uc 2.2)(Northcote 1971). These soil series occur principally within a narrow coastal fringe of aeolian dunes, and to a lesser extent further inland where they are formed on lateritic sandplains by erosional deposition (Beard 1981; Bettenay 1984). Highly susceptible communities include *Banksia attenuata* R. Br. and *Banksia menziesii* R. Br. low woodland, *B. attenuata* scrub-heath, *Banksia coccinea* R. Br. scrub-heath, *Banksia baxteri* R. Br. and *Lambertia inermis* R. Br. scrub-heath, *Banksia speciosa* R. Br. scrub-heath and several mixed coastal heaths.

Firstly, the level of infestation in coastal areas is not as uniform and widespread as in the jarrah forest. Even though the disease occurs throughout 1300 km of dune vegetation, ranging from Eneabba, north of Perth, to Cape Arid, in the Great Australian Bight, levels of infestation vary. Some national parks and reserves have been badly infested, while others appear to have either, entirely escaped infection or contain only one or two diseased sites.

Secondly, many problems associated with effective penetration of fungicides through the duplex soil profile beneath forest areas are alleviated in the deep, well-drained soils that underly much of the woodland and scrub-heath vegetation. In the swales between dunes, however, the prevalence of "coffee rock" ironpan in the vicinity of the water table could be problematic.

Thirdly, *P. cinnamomi* infections in many *Banksia* woodland and scrub heath communities (underlain by deep, well-drained sands) expand slowly and predictably, with sharp and readily defined disease boundaries. Hill *et al.* (forthcoming), from measurements of more than 5 km of disease fronts in Bassendean Dunes at Gnangara, found that the rate of extension of disease fronts in different landscape positions, varied from only 1.0-1.4 m yr^{-1} over a period of 35 years. Rate of extension was almost constant over time, and was little affected by the depth to or movement of the water table, soil profile (Gavin or Jandakot sand), vegetation type, or upslope or downslope direction of movement.

Similar rates of spread of *P.cinnamomi* infections have been reported by other authors in well drained soils or for upslope spread of disease: 1 m yr^{-1} over 14 years in five *Banksia* woodland sites growing on deep aeolian sand (Hart 1983), and a mean of 0.77 m yr^{-1} for upslope extension of disease over 10 years in *E. marginata* forest (Shea and Dillon 1980). However, Podger (1972) recorded a faster rate of downhill spread, 8 m yr^{-1} over five years, on a gently sloping dune of freely drained Gavin sand.

The slow and consistent rate of spread of disease indicates that the fungus is advancing by growing within root systems of susceptible species. Many opportunities exist for cross infection between plants in the interwoven network of lateral roots in the upper soil profile. Shea and Dillon (1980) suggested this mechanism to explain the low rate and irregular pattern of uphill extension in the jarrah forest. Hill *et al.* (forthcoming) observed lateral root invasion leading to collar invasion and death of *B. attenuata* in Bassendean Dunes at Gnangara.

Consequently, *P. cinnamomi* infections in deep, well-drained sands are amenable to control, through their reliance upon growth within susceptible, living root systems for further spread. If the continuous network is upset, by eliminating all vegetation on and around the site, then further extension will cease, and the fungal population will start declining. Use of chemical treatments could hasten the rate of extinguishment, particularly within root material.

This experiment was undertaken to determine the persistence of the fungus in deep sands, and to test the efficacy of a fumigant (formaldehyde) and a fungicide (metalaxyl) at eliminating *P. cinnamomi* to an appreciable depth in deep, leached sand. Formaldehyde, long used as a soil drench/fumigant, was chosen because it was cheap, and because it could easily be applied using existing departmental equipment. Applying moderately heavy rates of formaldehyde, as a 1% solution, would also ensure deep penetration of the soil profile by the fumigant. Metalaxyl was chosen for its proven efficacy at reducing *P. cinnamomi* populations from soil (Sivasithamparam and Hawson 1982; Bruck and Kenerley 1983; Lambe and Wills 1983; Boughton and Crane 1984; Benson 1985). It also inhibits spore formation at very low concentrations (Cohen and Coffey 1986), has long residual activity (Bailey and Coffey 1985), has very low toxicity, and, in granular form, is easy to apply.

The development of a technique to eradicate *P. cinnamomi* from deep sands would enable the treatment of isolated *P. cinnamomi* infections that threaten large remnant areas of disease-free, yet highly susceptible, vegetation.

MATERIALS AND METHODS

Preparation of plots

Plots were established at two sites at Gnangara, 30km north of Perth. The study area lies within the Bassendean Dune System, a geomorphic element consisting of low dunes of highly leached, siliceous sands with intervening swamps in swales, which forms part of the Swan Coastal Plain (McArthur and Bettenay 1960). An unconfined aquifer extends throughout the region (WAWA 1987).

Both sites were underlain by Jandakot Sand, a podzol characterised by a deep (> 5 m), strongly-leached A horizon of quartz sand, darkened slightly in the upper metre by organic matter, and overlying a "coffee rock" B horizon. The B horizon is formed by the redeposition of iron and organic matter in the vicinity of the water table (McArthur and Bettenay 1960; Bettenay 1984; WAWA 1985; Havel 1968). The depth to the winter water table at Site one was 9m, and at Site two 6m. The characteristics of the soils on both sites are presented in Table 1.

Table 1. Characteristics of soils at Sites 1 and 2.

		Percent				
Site	depth (m)	Coarse sand [1]	Fine sand	Silt	Clay	Organic carbon[2]
1	0.3	93.2	4.7	0.3	1.9	0.04
2	0.3	91.1	7.4	0.4	1.1	0.07
1	1.3	90.3	8.0	0.1	1.6	<0.007
2	1.3	83.4	14.7	0.3	1.6	0.007

[1]Coarse sand = 2.0-0.2 mm, fine sand = 0.2-0.02 mm, silt = 0.02-0.002 mm, clay = .002 mm.
[2]analysed by the Walkley-Black method.

Both sites were infected with *Phytophthora cinnamomi* and contained mixed scrub-heath vegetation, the degraded remnants of the original *B. attenuata*, *B. menziesii* low woodland (Plates 2&3)(Beard 1981).

At each site, eight 15 x 15 m plots were pegged in diseased vegetation along the dieback front (Plate 1). Two plots were randomly selected for each treatment, and the two remaining plots were controls. All treatment plots were cleared of vegetation and isolated from the surrounding, diseased soil with a plastic-lined trench (Plates 4,5 and 6). Plots were prepared as follows:

1. *Banksias* were felled and removed.

2. A backhoe removed all stumps and then skimmed the bulk of the vegetation from the surface of the plot. Plots were raked to remove any remaining debris.

3. The backhoe dug a 1.5 m deep trench around the perimeter of each plot. The trench was lined with a continuous sheet of black plastic and then backfilled. Plots to be treated with formaldehyde were bisected by a 0.5 m deep plastic-lined trench.

4. A soil wetting agent (Once-wet; Langley Chemicals) was applied to all treatment plots at four times the recommended rate (0.02 L m^{-2}).

Treatments

The three treatments tested in this experiment were:

1. Herbicide

All vegetation was killed by spraying with glyphosate (Roundup; Monsanto C. Ltd.) at a rate of 7.4 kg a.i. ha^{-1}. Glyphosate was re-applied after 4 and 12 months to maintain absolute bareness on the plots.

2. Herbicide plus metalaxyl

Plots were treated with herbicide as outlined above. One half of each plot was then treated with 5 g a.i. m^{-2} of metalaxyl (Ridomil 5G; Ciba-Geigy Ltd.), and the other half with 15 g a.i. m^{-2}.

3. Herbicide plus formaldehyde

Plots were treated with herbicide as outlined above. One half of each plot was then sprayed with 27 L m^{-2} of 1% formaldehyde, and the other half with 54 L m^{-2}. Plots were covered with black plastic for 2 weeks to ensure thorough fumigation (Plates 8&9).

Treatments were applied in September 1987.

Assessing efficacy of treatments

Prior to the initiation of treatments, *P. cinnamomi* was systematically introduced into the plots by burying pine plugs colonised with the fungus. Plugs were buried at two depths, 0.3 and 1.3 m, and retrieved at varying intervals to assess the impact of treatments. Retrieved plugs were tested for the survival of the fungus by plating them onto selective agar (P_5ARPH)(Jeffers and Martin 1986). Details are given below:

Preparation and burial of colonised pine plugs

Pine plugs were made from young *Pinus radiata* Ait. branches, 0.8-1.5 cm in diameter after being debarked, which were cut into 2.0-3.0 cm lengths. Plugs had an average weight of 3.1 g. After they were autoclaved in conical flasks, the plugs were inoculated with twenty small squares of cornmeal agar colonised with *P. cinnamomi* A2 (IMI 264384) and incubated for 1 month at 26°C. After 3 weeks, aerial mycelium covered the pieces, and, before burial, two pieces from each flask were plated onto selective agar to confirm vigourous colonisation with the fungus.

Colonised pieces were tied to 150 lb breaking-strain nylon line before burial. Each plug was threaded onto a length of line and securely attached (a small hole had been drilled through each piece before autoclaving).

Colonised plugs were buried, in each plot, in a 12 x 12.25 m randomly positioned grid pattern of four lines of eight burial points. Separation between lines was 4 m and between points on a line, 1.75 m. At each point, several deep (1.3 m) or shallow (0.3 m) plugs were buried, 25 cm apart, along an axis running perpendicular to the grid line. There were three or four holes at each point depending on the treatment. Clusters of deeply buried plugs alternated with shallow, both along and across lines.

Plugs were buried by removing a narrow core (2.5 cm diameter) of soil with a Veihmeyer tube and inserting a tethered plug into the soil at the base of each shaft. Shafts were then refilled with dried sand from the same soil horizon, leaving a protruding length of nylon for later retrieval with a winch. Control, herbicide and formaldehyde plots each contained 48 deep and 48 shallow plugs, while metalaxyl plots contained 64 at each depth.

Sampling

Survival of *P. cinnamomi* in buried pine plugs was assessed on three occasions. Subsets of plugs were retrieved in November 1987, June 1988 and May 1989; two, nine and twenty months after the completion of treatments in September 1987. At the first assessment, all plugs from formaldehyde plots were retrieved (24 deep and 24 shallow from each half plot). Due to its volatility, formaldehyde was assumed to have only a short period of activity. At the same assessment, half of all plugs were removed from metalaxyl plots (16 deep and 16 shallow from each half plot) and a third from herbicide and control plots (16 deep and 16 shallow per plot).

At the second assessment, the remaining plugs on metalaxyl plots were retrieved, and a further third of herbicide and control plugs. At the final assessment, the remaining third of herbicide and control plugs were recovered. At least one plug was recovered from every point in the grid of each sampled plot.

Plugs were tested, within 24 h of retrieval, for presence or absence of *P. cinnamomi*. Plugs were washed in DI water and stored overnight at 10°C in moist, sealed plastic bags, with each plug from fungicide and fumigant plots stored separately. Plugs were surface sterilised in 70% alcohol, split in half and plated onto selective agar (a separate agar plate was used for each plug withdrawn from formaldehyde plots). After ten days, all negative plugs were removed and re-tested for *P. cinnamomi* presence by re-splitting each half and plating the newly exposed surface of each quarter piece.

Soil population levels of *P. cinnamomi* were determined for all control, herbicide and metalaxyl treatment plots at intervals during the experiment. Sixteen soil samples were

taken from each sampled plot. Each 1.25 L sample comprised three bulked core samples taken from between 10 and 40 cm depth. Samples were stored in sealed plastic bags, at room temperature, for a maximum of one week before a 100 ml sub-sample was tested for *P. cinnamomi* presence using the cotyledon baiting technique as described by Marks and Kassaby (1974).

Analysis

Results from replicate plots were meaned to give an estimate of *P. cinnamomi* survival on each site, for the various treatments, sample types and depth categories at each assessment date. *Phytophthora cinnamomi* survival was expressed as the proportion of samples that tested positive for fungal presence, and the significance of the difference between proportions was determined using the Fisher's Exact Test (Fisher 1958), applied using the SAS statistical package for personal computers (SAS Institute Inc., North Carolina).

RESULTS

Plating split pine plugs onto selective agar appeared to reliably detect any surviving *P. cinnamomi* inoculum within each piece. The fungus grew from only 1% of around 600 plugs that were re-split, and re-plated, after being initially rated as *P. cinnamomi* negative.

The recovery rate of *P. cinnamomi* from pine plugs was usually comparable between the two sites for corresponding treatments and samples at each assessment date. However, a significant discrepancy existed between sites for results in formaldehyde and metalaxyl treated plots at the first assessment (Figs. 1&2). On this occasion, the recovery rate from deeply buried plugs in chemically treated plots was significantly lower ($p < 0.05$, one tailed Fisher's Exact Test) on site 1 than on site 2. Consequently, pine plug results from each site have been presented separately.

Matching soil sample results on each site and at each assessment were not significantly different ($p > 0.2$), and were combined (Fig. 3).

Herbicide

The elimination of all vegetation on herbicide plots produced apparently ambivalent results for survival of *P. cinnamomi* in pine plugs compared with recoveries from soil.

Phytophthora cinnamomi survival in pine plugs buried at 30 cm depth was significantly greater ($p < 0.01$) in herbicide plots than in controls, in the second and final assessments,

nine and twenty months after treatment. The more rapid attrition rate of the fungus on control plots was likely due to much dryer soil conditions experienced on these plots during summer. Extraction of water by plants on the control plots reduced mean soil moisture contents on the four control plots to between 0.5 and 0.8% of dry soil weight in February 1988 (n = 10 in each plot) at a depth of 30 cm. Corresponding soil moisture contents on herbicide plots were significantly higher, at between 2.6 and 2.8%; moisture loss curbed by a 10-20 cm insulating layer of dry sand at the surface. The mean soil moisture content of soils in control plots in winter was 3.4% (n = 20). After twenty months burial, which included two summers, the recovery rate of *P. cinnamomi* from plugs at 30 cm depth in control plots had fallen to 6 and 22% on sites 1 and 2 respectively, whereas corresponding recovery rates on herbicide plots were 81 and 74%.

Testing only for presence/absence of the fungus overestimated the fungal population surviving in buried plugs. Over the term of the experiment, the strength of the growth from plugs fell, and by the last assessment, the fungus grew abundantly from only a small proportion of plugs, with most producing lobed colonies that reflected survival in one or more discrete pockets. Plugs became discoloured after prolonged burial; some had begun to decay, and others had been partially destroyed by termites.

Lower soil moistures on control plots did not affect survival rates in plugs buried at 1.3 m depth. Recovery rates from both control and herbicide plots fell no lower than 83% for deeply buried plugs over the duration of the experiment (Figs. 1&2). No direct measurements were made of soil moistures at this depth, but Dodd (1984) showed that soil moisture levels, beneath *Banksia* woodland on Bassendean Dunes, fall to below 1% (fresh weight basis) throughout the top 3 m of the soil profile during summer. The death of the *Banksia* overstorey and most of the shrub layer may, however, reduce the intensity of water extraction at depth below diseased areas.

Though survival of the fungus was assisted by killing the vegetation, thereby alleviating summer desiccation, the removal of the food base on herbicide treated plots prevented the seasonal renewal of fungal activity each autumn that follows the return of primary root activity in the moist soil conditions. Consequently, the recovery rate of *P. cinnamomi* from soil samples (always taken in winter or spring) remained relatively constant in control plots, but declined steadily in herbicide treated plots.

Phytophthora cinnamomi recovery rates from soil samples taken in control plots remained at between 17 and 23% (n = 64), throughout the period of the experiment (Fig. 3). Concurrently, recovery rates from soil samples taken in herbicide plots fell to 5% (n = 64) after 18 months. Recovery rates on herbicide plots were significantly lower ($p < 0.05$) than on control plots for samples taken nine and twenty months after herbicide spraying. The final recovery rate of the fungus from soil samples taken in herbicide plots was not significantly higher, at the 10% level, to that in plots treated with 15 g a.i. m^{-2} of metalaxyl (Fig. 3).

Formaldehyde

Plots fumigated with formaldehyde were assessed only once, two months after being treated. Residual activity was short-lived due to the volatility of the chemical combined with the leaching effect of follow up rains.

Formaldehyde efficacy differed greatly between sites. On site 1, both rates (27 and 54 L m^{-2} of 1% formaldehyde) were equally effective against both shallowly and deeply buried plugs (Fig 1&2). Respective shallow and deep recovery rates were 13 and 9% from 27 L m^{-2}, and 4 and 2% from 54 L m^{-2} of formaldehyde. For pooled results from both depth categories, 54 L m^{-2} of 1% formaldehyde disinfected significantly more ($p < 0.05$) plugs than did 27 L m^{-2}. On site 2, the only promising result, a 6% recovery rate, was achieved with shallow plugs in heavily treated plots. Fumigation at depth in heavily treated plots on site 2 was significantly poorer ($p < 0.05$), as was the disinfection of both deep and shallow plugs in plots treated with the lower rate of formaldehyde.

Many deep and shallow plugs, retrieved from both sites, appeared to have wholly escaped contact with the solution or its vapour. Of 74 *P. cinnamomi* positive plugs retrieved from all fumigated plots, only 28 showed some evidence of poisoning, such as sparse colonies or sterilised sectors (Plate 7). The remainder produced strong mycelial growth from the entirety of both plated surfaces.

Metalaxyl

The recovery rate of *P. cinnamomi* fell dramatically between the first and second retrieval of plugs from metalaxyl treated plots (Fig. 1&2). At the first assessment, two months after the application of metalaxyl in granular form, *P. cinnamomi* grew from the majority of plugs. However, many of the colonies, produced by plugs from both depths, were sparse with hyphae that were unusually coralloid, reflecting the residual activity of the fungistat. The recovery rate of *P. cinnamomi* from deep plugs retrieved from site 2 was, as in formaldehyde treated plots, significantly higher than in site 1 ($p < 0.05$).

After a further seven months exposure to the fungistat, the *P. cinnamomi* population had been virtually eliminated in plots treated with the higher metalaxyl rate of 15 g a.i. m^{-2}. The fungus was not recovered from any plugs (deep or shallow) withdrawn from three of the four plots on both sites. In the remaining plot, on site 2, it survived in two deeply buried plugs, but grew from only one point in each. For pooled data from both sites, the impact of 15 g a.i. m^{-2} of metalaxyl was not significantly greater than 54 L m^{-2} of 1% formaldehyde at a depth of 0.3 m but was significantly ($p = 0.005$) more effective at 1.3 m. *Phytophthora cinnamomi* was also not isolated from any of 128 bulked soil samples taken from the same plots (Fig. 3)(two batches of 64, sampled ten and twenty months after treatment). The corresponding *P. cinnamomi* recovery rate in control plots was 26/128.

Five g a.i. m^{-2} of metalaxyl was equally as effective as 15 g a.i. m^{-2} at a depth of 0.3 m nine months after application, but had significantly less impact ($p < 0.01$) at a depth of 1.3 m on both sites. All viable plugs withdrawn from 1.3 m depth produced minimal colonies, suggesting sub-lethal contact with the fungistat.

DISCUSSION

Treatments

One 15 g a.i. m^{-2} application of metalaxyl (Ridomil 5G) dramatically lowered the *P. cinnamomi* population, to a depth of at least 1.3 m, in two 112 m^2 plots in each of two sites. Nine or more months after treatment, *P. cinnamomi* was barely detectable; recovered from 0 and 3% ($n = 64$) of colonised pine plugs at 0.3 and 1.3 m depth respectively, and from 0% ($n = 128$) of soil samples taken from between 0.1-0.4 m depth. Corresponding recovery rates from control plots were 65 and 95% in pine plugs, and from 20% of soil samples.

Similar efficacy of metalaxyl against *P. cinnamomi* in soil has been achieved in pots and landscape beds (Bruck and Kenerley 1983; Lambe and Wills 1983; Boughton and Crane 1984; Benson 1985). Sivasithamparam and Hawson (1982) obtained highly effective suppression in deep, acid grey sands in south-western Australia. The pathogen was not recovered from any of 20, one-year-old avocado trees, sampled eight months after the application of 5 g. a.i. m^{-2} of metalaxyl in granular form.

The eradicant ability of metalaxyl obtained in this experiment is difficult to align with its fungistatic - as opposed to fungicidal - mode of action. Even though growth, and especially spore formation, of *P. cinnamomi* is inhibited by metalaxyl, the fungus is not actually killed by the chemical (Cohen and Coffey 1986). Malajczuk (1983) did, however, find a marked interaction between the soil microflora and metalaxyl, whereby lysis of *P. cinnamomi* hyphae was greatly accelerated in unsterile soil leachates containing the fungicide. Elimination of *P. cinnamomi*, particularly from buried pine plugs, may also have occurred indirectly through the suppression of formation of chlamydospores (Coffey *et al.* 1984), which are essential for extended survival of the fungus within soil (Hwang and Ko 1978; Malajczuck 1983).

Though both rates of metalaxyl were equally effective against shallow plugs (0.3 m depth), increasing the dose threefold reduced the survival rate of the fungus in deep plugs (1.3 m depth) from 31 to 3.5% ($p < 0.01$).

The effective penetration of metalaxyl to a depth of at least 1.3 m was partially due to the heavy rate used (15 g a.i. m^{-2}). Sharom and Edgington (1986) found that metalaxyl, applied at a much lower rate of 0.15 g m^{-2}, penetrated no deeper than 45 cm into a predominantly sandy soil containing 2% organic matter, and subject to field conditions throughout one growing season. In addition, adsorbtion onto organic matter is a major factor limiting the mobility and persistence of the fungistat. Sharom and Edgington (1982) obtained an inverse relationship between metalaxyl mobility and organic matter content of soils. They observed that while 87% of applied metalaxyl was eluted from a 20 cm column of sandy loam (1.7% organic matter) subjected to 30 cm of simulated rainfall, a similarly watered muck soil (68% organic matter) held all applied metalaxyl within the upper 10 cm of the soil column. High leachability of metalaxyl (58 and 92%) through heavily watered, 30 cm long columns was also obtained with two sand types tested by Guth (1976), and Musumeci *et al.* (1982) recorded high mobility of the fungicide in three soils with organic matter contents ranging from 2.0-4.3%. Little impedance to movement would be expected from the Jandakot sands of the study area, which contain a mean of only 0.05% and 0.01% organic carbon at depths of 0.3 and 1.3 m respectively.

The considerable mobility of metalaxyl within percolating water may have been supplemented by an appreciable mobility in the vapour phase. This is inferred from the very low survival rate of *P. cinnamomi* in shallow pine plugs (less than 1%, n = 128). In contrast, the fungus survived in 14% (n = 191) of shallow plugs treated with either 27 or 54 L m^{-2} of 1% formaldehyde (a fumigant). As discussed in the results section, many plugs from formalin treated plots showed no evidence of exposure to the chemical (Plate 7). It is likely that these plugs were buried within non-wetting pockets of soil that insulated them from formaldehyde carried in percolating water. Dry soil pockets were abundant in the upper 35-50 cm of the profile on both sites, even after the application of heavy rates of soil wetting agent followed by more than 200 mm of winter rain. The disinfection of such insulated plugs on metalaxyl plots may have occurred through vapour phase diffusion of the fungicide into non-wetting pockets. Metalaxyl has been found to have considerable activity in the vapour phase (Crute and Jagger 1979, cited by Bruin and Edgington 1983), which was suggested as the mechanism for the redistribution of the chemical in tobacco leaves by Bruin *et al.* (1981, cited by Bruin and Edgington 1983) and used to explain the rapid decline of metalaxyl residues on potato leaflets Milgroom *et al.* (1988).

The persistence of dry soil pockets, and the preferred infiltration pathways so produced, may explain the disparity between results attained with 1% formaldehyde on the two sites. Formaldehyde fumigation gave promising results with both application rates on site 1, but worked poorly against both deep and shallow plugs on site 2. Improved fumigation would be achieved by increasing the concentration (from 1% to 2-5%); possibly by reducing the volume of water applied. Improved infiltration would be achieved by adding soil wetting agent, at a higher rate, to the drench solution itself (the two chemicals do not react). Efficacy of formaldehyde was reviewed by White and Buczacki (1977, cited by Bruin and Edgington 1983) who noted that results with it had been inconsistent, yet it was chosen

from six sporicidal chemicals for broadscale use by Manchee *et al.* (1983) to decontaminate Gruinard island, off Scotland, of anthrax spores.

The extended survival of *P. cinnamomi* in herbicide treated plots, especially in plugs buried at 1.3 m depth, reflects both the saprophytic ability of the fungus and the longevity of its chlamydospores. Similar survival periods have been recorded for a variety of soils and conditions. Zentmyer and Mircetich (1966) isolated *P. cinnamomi* from naturally infested, moist soil after six years storage in the laboratory, while Weste and Marks (1974) observed that a roadside pile of gravel remained highly infectious for at least five years, even though devoid of visible plant growth. Kassaby *et al.* (1977) also recorded extended survival of inoculum for at least 3 years in field soil subject to great temperature extremes. Kuhlman (1964), however, could not re-isolate *P. cinnamomi* from forest soils kept in bottles after 19 months storage and Shea (1977) recorded rapid extinguishment of the fungus from soil after only one month exposure in freely drained jarrah forest sites in summer.

Survival of *P. cinnamomi* in soil follows the order: chlamydospores in roots > free chlamydospores in soil > zoospores and oospores > mycelium (Malajczuk 1983; Hwang and Ko 1978). Free chlamydospores in soil have been shown to survive for a maximum of one year (Hwang and Ko 1978; Weste and Vithanage 1979). The composition and population of soil microflora can also effect survival. Broadbent and Baker (1974) tested sand from beneath jarrah forest and found that it contained low numbers of bacteria, actinomycetes and psuedomonads; conducive to long term survival of the fungus. The Bassendean sands of the study area are likely to be even more lacking in antagonistic microflora than soils of the jarrah forest, especially in the almost pure-white, quartz sand A2 horizon that occurs below 80 cm depth. At this depth on herbicide treated plots, the soil remains moist and cool throughout the year. It seems likely, therefore, that plots treated only to eliminate the vegetation will harbour the fungus for an extended time, particularly at depth in remaining roots and stumps. Survival of the fungus for more than five years is probable.

Management

Metalaxyl and formaldehyde are expensive treatments, and unless highly effective can not be considered. The total cost of applying metalaxyl (excluding trenching) ranged from \$1.90 to \$4.30 m^{-2}, depending on rate, while fumigation with formalin cost from \$2.30 to \$3.70 m^{-2}.

In contrast, the herbicide treatment was very cheap ($0.10m^{-2}$ for two applications). Yearly applications of Roundup, to prevent re-colonisation of the treated area by surrounding vegetation, could be avoided by using a pre-emergent herbicide. Some advantages of the herbicide treatment, over the chemical treatments, are given below:

- Vegetation does not have to be removed (remnant vegetation stabilises the soil).
- Operational disturbance is minimal, reducing the likelihood of further disease spread.
- Pollution of the water table does not have to be considered.
- Dry soil pockets are not of concern.
- The method can be used to treat quite large areas without associated problems of scale.

Use of herbicide alone, though cheap, does have the disadvantage of requiring the maintenance of the treated area for possibly more than ten years. As discussed above, the *P. cinnamomi* population in the soil below denuded sites will decline slowly, with refugial pockets of inoculum surviving at depth and, near the surface, within large roots and stumps. *Phytophthora cinnamomi* has been recovered frequently from soil and roots at a depth of 2 m (Hill *et al.*, forthcoming), and from the groundwater at 3 and 5 m depth by Shearer *et al.* (1989), in *Banksia* woodland. Persistence of the fungus for more than 1-2 years in stumps of dead *Banksias* has been recorded repeatedly (Shearer and Shea (1987); Shea *et al.* (1980); Hill *et al.*, forthcoming). *Banksia* stumps do, however, decay relatively rapidly, decomposing to a friable mass within about five years, and so exposing any surviving inoculum to the soil environment.

If chemical treatments do not penetrate to eliminate the fungus from refugial locations such as stumps, large roots and at depth, then the time taken for the disappearance of *P. cinnamomi* from the site will be the same as on sites treated with herbicide only. Removal of stumps and the attached larger roots requires the use of a backhoe; a "dirty" operation which can greatly enhance the risk of spreading the disease further. Alternatively, stumps could be spot treated, perhaps with formaldehyde at high concentration (5-40%), or with sodium hypochlorite or quaternary ammonium compounds (Noske and Shearer 1985).

Ensuring eradication of *P. cinnamomi* from the deeper soil horizons would be difficult, as well as impossible to assess, yet may not be necessary. Eliminating *P. cinnamomi* from the top 1-2 m of the profile would secure the site from being a risk to further spread of the disease to surrounding areas, and may allow its revegetation with very shallow rooted plants such as grass or small shrubs (Dodd 1984). The site cannot be simply re-cropped with filed resistant species, as these can also harbour the disease (Phillips and Weste 1984). Once secured and rehabilitated with a suitable cover crop, the site could be easily maintained, with minimal input, for a safe period to ensure extinguishment of any residual *P. cinnamomi*.

CONCLUSION

The dramatic efficacy of metalaxyl bodes well for the development of a field technique to eradicate *P. cinnamomi* from isolated infections in the wide range of south-west plant communities that grow on deep, leached sands.

Successful eradication of a small, discrete infection would most likely be achieved by using a combined treatment:

1. The extent of the infected site would be accurately located.
2. The site, plus a safe extra margin, would be sprayed with herbicide to kill all vegetation. Any nearby trees or shrubs with extensive root systems, such as *Nuytsia floribunda, Macrozamia riedlii* or *Adenanthos barbigera*, would also have to be killed.
3. The chosen chemical treatment would be applied. Stumps would either be removed or spot treated.
4. The site would be maintained free of all vegetation for several years, and then cover cropped with shallow rooted species for a suitable period.

The viability (for both economic and practical reasons) of any of the methods is dependant upon early detection of infections. Small spot infections grow exponentially to become dieback sites, from which secondary infections are generated. The key to active protection of susceptible areas lies with quarantining and constant monitoring of disease-free reserves or parks, or of catchments within them, for the appearance of the disease. As spot infections are discovered, a suitable eradication method can be employed.

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REFERENCES

Bailey, A.M. and Coffey, M.D. (1985), Biodegradation of metalaxyl in avocado soils. *Phytopathology* 75(2), 135-37.

Barber,C., Davis G.B. and Farrington P., Sources and sinks for dissolved oxygen in groundwater in an unconfined sand aquifer, Western Australia. *Geochemistry of gaseous Elements and Compounds* (in press).

Beard, J.S. (1981), *Vegetation survey of Western Australia, 1:1,000,000 Series, Sheet 7, Swan.* University of Western Australia Press, Nedlands, Perth.

Benson, D.M. (1985), Fungicides for control of *Phytophthora* root rot of azalea in landscape beds. *Plant Disease* 69(8), 697-99.

Bettenay, E. (1984), Origin and nature of the sandplains. In: Pate, J.S. and Beard, J.S. (eds), *Kwongan, Plant life of the sandplain*. University of Western Australia Press, Nedlands, Perth, Western Australia.

Boughton, T.J. and Crane, C.E. (1984), Assessment of *Phytophthora* disease risk at the Nannup nursery. Forests Department of Western Australia, Research Paper 77.

Brandis, A., Hill, T.C.J., Keighery, G.J. and Tippett, J.T. (1986), Dieback in the Cape Arid National Park and other areas of concern. Department of Conservation and Land Management, Internal Report.

Broadbent, P. and Baker, K.F. (1974), Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. *Australian Journal of Agricultural Research* 25, 121-37.

Bruck, R.I. and Kenerley, C.M. (1983), Effects of metalaxyl on *Phytophthora cinnamomi* root rot of *Abies fraseri*. *Plant Disease* 67(6), 688-90.

Bruin, G.C.A., Ripley, B.D. and Edgington, L.V. (1981), *Tobacco Science* 25, 128-30. (original not seen, see Bruin and Edgington 1983).

Bruin, G.C.A. and Edgington, L.V. (1983), The chemical control of diseases caused by zoosporic fungi. In: Buczacki, S.T. (ed), *Zoosporic Plant Pathogens, A Modern Perspective*. Academic Press, London. ch. 6.

Carlile, M.J. (1983), Motility, taxis and tropism in *Phytophthora*. In: Erwin, D.C., Bartnicki-Garcia, S. and Tsao, P.H. (eds), *Phytophthora*. The American Phytopathological Society, St. Paul, Minnesota. ch. 7.

Coffey, M.D., Klure, L.J. and Bower, L.A. (1984), Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. *Phytopathology* 74(4), 417-422.

Cohen, Y. and Coffey, M.D. (1986), Systemic fungicides and the control of Oomycetes. *Annual Review of Phytopathology* 24, 311-38.

Crute, I.R. and Jagger, B.M. (1979), *Rep. natn. Veg. Res. Stn.* for 1978, pp. 78-79. (original not seen, see Bruin and Edgington 1983).

Dodd, J., Heddle, E.M., Pate, J.S. and Dixon, K.W. (1984), Rooting patterns of sandplain plants and their functional significance. In: Pate, J.S. and Beard, J.S. (eds), *Kwongan, Plant life of the sandplain*. University of Western Australia Press, Nedlands, Perth, Western Australia.

Farrington, P., Greenwood, E.A.N., Bartle, G.A., Beresford, J.D. and Watson, G.D. (1989), Evaporation from *Banksia* woodland on a groundwater mound. *Journal of Hydrology* 105, 173-86.

Fisher, R.A. (1958), *Statistical methods for research workers*, 13th ed. Hafner Publishing Co., Inc., New York.

Guth, J.A. (1976), Leaching model study with the fungicide CGA-48988 in Four Standard Soils. Ciba-Geigy, Project Report 30/76, Switzerland.

Hart, R. (1983), Report on dieback due to *Phytophthora cinnamomi* in Two Peoples Bay Nature Reserve. Department of Fisheries and Wildlife, Perth, Reserve Management Consultant's Report No.3.

Havel, J.J. (1968), The potential of the northern Swan Coastal Plain for *Pinus pinaster* Ait. plantations. Forests Department, Perth, Western Australia, Bulletin No. 76.

Havel, J.J. (1979a), Vegetation: natural factors and human activity. In: Gentilli, J. (ed), *Western Landscapes*. University of Western Australia Press, Perth, Western Australia. pp 122-152.

Havel, J.J. (1979b), Identification of vulnerable communities and prediction of disease spread. In: Old, K.M. (ed), *Phytophthora and forest management in Australia*. CSIRO, Canberra. pp 64-72.

Hill T.C.J. (1989), Dieback disease and other *Phytophthora* species in the northern kwongan. In: Burbidge, A.A., Hopper, S.D. and van Leeuwen, S. (eds), *Nature conservation, landscape and recreation values of the proposed Lesueur National Park*. Department of Conservation and Land Management, Perth, Occasional Paper 3/89, ch.8.

Hill, T.C.J., Tippett, J.T. and Shearer, B.L., *Phytophthora cinnamomi* infection of *Banksia* woodland growing on deep sand. Rate of spread, and invasion of *Banksia attenuata*. (forthcoming).

Hwang, S.C. and Ko, W.H. (1978), Biology of chlamydospores, sporangia, and zoospores of *Phytophthora cinnamomi* in soil. *Phytopathology* 68, 726-31.

Jeffers, S.N. and Martin, S.B. (1986), Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Disease* 70, 1038-43.

Kassaby, F.Y., Fagg, P.C. and Marks, G.C. (1977), Survival in, and colonisation of mountain forest soil by *Phytophthora cinnamomi* Rands in eastern Victoria. *Australian Forest Research* 7,173-83.

Kuhlman, E.G. (1964), Survival and pathogenicity of *Phytophthora cinnamomi* in several western Oregon soils. *Forest Science* 10(2), 151-58.

Lambe, R.C. and Wills, W.H. (1983), Fungicide and Mortierella for the protection of azaleas from *Phytophthora cinnamomi* root rot. *Phytopathology* 73, Abstract 503.

McArthur, W.M. and Bettenay, E. (1960), Development and distribution of soils of the Swan Coastal Plain, Western Australia. CSIRO, Soil Publication No. 16.

Malajczuk, N. and Glenn, A.R. (1981), *Phytophthora cinnamomi* - A threat to the heathlands. In: Goodall, D.W. (ser. ed) and Specht, R.L.(ed), *Ecosystems of the world.* vol. 9: *Heathlands and related shrublands of the world.* B. Analytical studies. Elsevier, Amsterdam.

Malajczuk, N. (1983), Microbial antagonism to *Phytophthora.* In: Erwin, D.C., Bartnicki-Garcia, S. and Tsao, P.H. (eds), *Phytophthora*. The American Phytopathological Society, St. Paul, Minnesota. ch. 16.

Manchee, R.J., Broster, M.G., Anderson, I.S. and Henstridge, R.M. (1983), Decontamination of *Bacillus anthracis* on Gruinard Island. *Nature* 303, 239-240.

Marks, G.C. and Kassaby, F.Y. (1974), Detection of *Phytophthora cinnamomi* in soils. *Australian Forestry* 36, 198-203.

Milgroom, M.G., McCulloch, C.E. and Fry, W.E. (1988), @REFERECE = Distribution and temporal dynamics of metalaxyl in potato foliage. *Phytopathology* 78(5), 555-59.

Musumeci, M.R., Ruegg, E.T. and Campacci, C.A. (1982), Behaviour of ^{14}C-metalaxyl in Brazilian soils. *Turrialba* 32, 496-99.

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

Northcote, K.H. (1971), *A factual key for the recognition of Australian soils*. Rellim Technical Publications. 123pp.

Northcote, K.H., Hubble, G.D., Isbell, R.F., Thompson, C.H. and Bettenay, E. (1975), *A description of Australian soils*. Wilkie and Co. Ltd, Clayton, Victoria, Australia.

Noske, G.L. and Shearer, B.L. (1985), Quaternay ammonium compounds were more effective than a phenolic compound or sodium hypochlorite in inhibiting growth of *Phytophthora cinnamomi* (Rands). *Australasian Plant Pathology* 14(2), 37-40.

Phillips, D. and Weste, G. (1984), Field resistance in three native monocotyledon species that colonise indigenous sclerophyll forest after invasion by *Phytophthora cinnamomi*. *Australian Journal of Botany* 32, 339-52.

Podger, F. D. (1968), *Phytophthora cinnamomi*, Aetiology of jarrah dieback. A disease of dry sclerophyll *Eucalyptus marginata* Sm. forest 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, 972-81.

Sharom, M.S. and Edgington L.V. (1982), The adsorbtion, mobility and persistence of metalaxyl in soil and aqueous systems. *Canadian Journal of Plant Pathology* 4, 334-40

Sharom, M.S. and Edgington L.V. (1986), Mobility and dissipation of metalaxyl in tobacco soils. *Canadian Journal of Plant Science* 66, 761-71.

Shea, S.R. (1977), Environmental factors of the northern jarrah forest in relation to pathogenicity and survival of *Phytophthora cinnamomi*. Forests Department of Western Australia, Perth. Bulletin 85.

Shea, S.R. and Dillon, M.J. (1980), Rate of spread of *Phytophthora cinnamomi* infection in the jarrah (*Eucalyptus marginata*) forest. Forests Department of Western Australia, Research Paper 65.

Shea, S.R., Gillen, K.J. and Leppard, W.I. (1980), Seasonal variation in population levels of *Phytophthora cinnamomi* in soil in diseased, freely-drained *Eucalyptus marginata* sites in the northern jarrah forest of south-western Australia. *Protection Ecology* 2, 135-56.

Shea, S.R., Shearer, B.L., Tippett, J.T. and Deegan, P.M. (1983), Distribution, reproduction, and movement of *Phytophthora cinnamomi* on sites highly conducive to jarrah dieback in south western Australia. *Plant Disease* 67(9), 970-73.

Shearer, B.L. and Shea, S.R. (1987), Variation in seasonal population fluctuations of *Phytophthora cinnamomi* within and between infected *Eucalyptus marginata* sites of south-western Australia. *Forest Ecology* 21, 209-30.

Shearer, B.L. and Hill, T.C.J. (1989), Diseases of *Banksia* woodlands on the Bassendean and Spearwood Dune Systems. *Journal of the Royal Society of Western Australia* 71(4),113-14.

Shearer, B.L., Dillon, M.J. and Buehrig, R.M. (1989), Population dynamics of *Phytophthora cinnamomi* in *Banksia* woodlands and the jarrah forest of southwestern Australia. Transactions of the 7th Australasian Plant Pathology Conference, Brisbane, 1989, p.148.

Sivasithamparam, K. and Hawson, M.G. (1982), Chemical control of *Phytophthora* root rot of newly planted avocados in Western Australia. *Australasian Plant Pathology* September 1982, 30-31.

Water Authority of Western Australia (1985), Investigation of natural groundwater recharge to the unconfined aquifer of the Swan Coastal Plain 1982-1985, Vol. 2. Water Authority of Western Australia, Perth, Western Australia, Report No. WP 4.

Water Authority of Western Australia (1987), Perth urban water balance study. Water Authority of Western Australia, Perth, Western Australia, Vol. 1.

Weste, G. Cooke, D. and Taylor, P. (1973), The invasion of native forest by *Phytophthora cinnamomi*. 2, Post infection vegetation patterns, regeneration, decline in inoculum, and attempted control. *Australian Journal of Botany* 21, 13-29.

Weste, G. and Law, L. (1973), The invasion of native forest by *Phytophthora cinnamomi*. 3, Threat to the national park, Wilson's Promontory, Victoria. *Australian Journal of Botany* 21, 31-51.

Weste, G. and Marks, G.C. (1974), The distribution of *Phytophthora cinnamomi* in Victoria. *Trans. Br. myc. Soc.* 63(3), 559-72.

Weste, G. and Vithanage, K. (1979), Survival of chlamydospores of *Phytophthora cinnamomi* in several non-sterile, host-free forest soils and gravels at different soil water potentials. *Australian Journal of Botany* 27, 1-9.

White, J.G. and Buczacki, S.T. (1977), *Annals of Applied Biology* 85,287-300. (original not seen, see Bruin and Edgington 1983).

Zentmyer, G.A. and Mircetich, S.M. (1966), Saprophytism and persistence in soil by *Phytophthora cinnamomi*. *Phytopathology* 56, 710-12.

APPENDIX

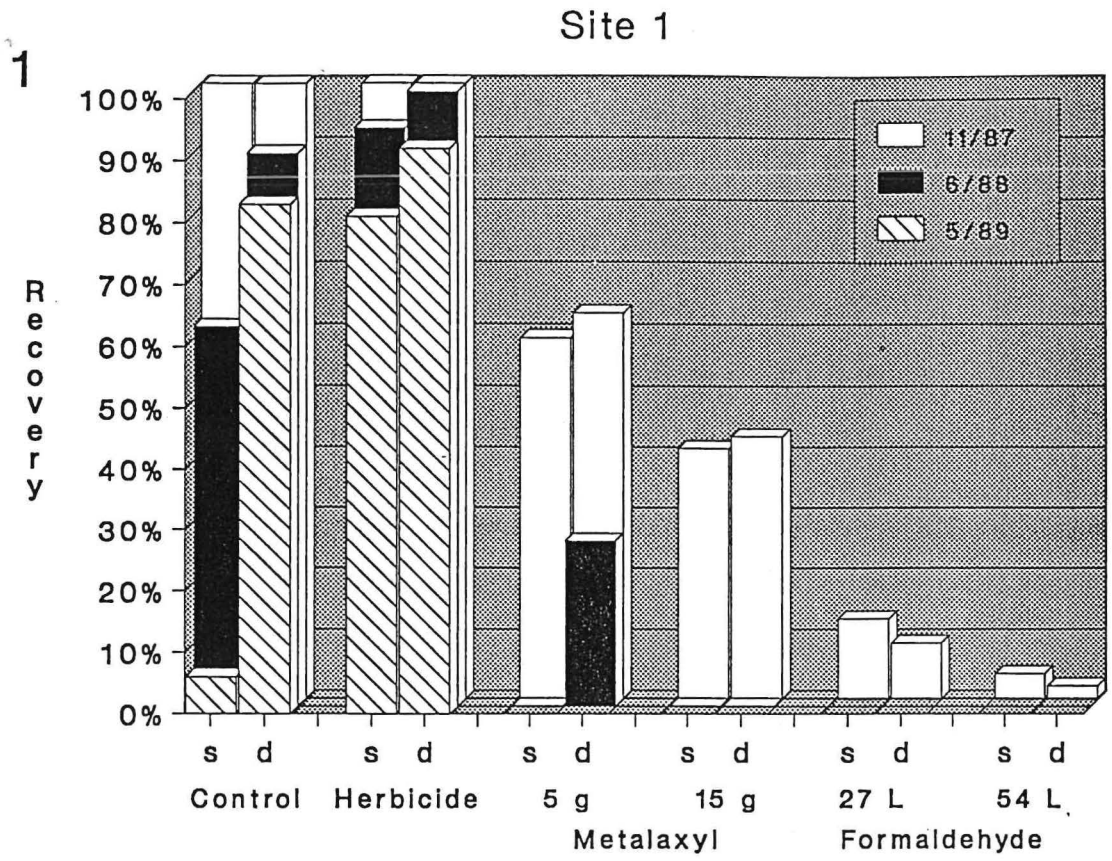
Possible locations for field testing:

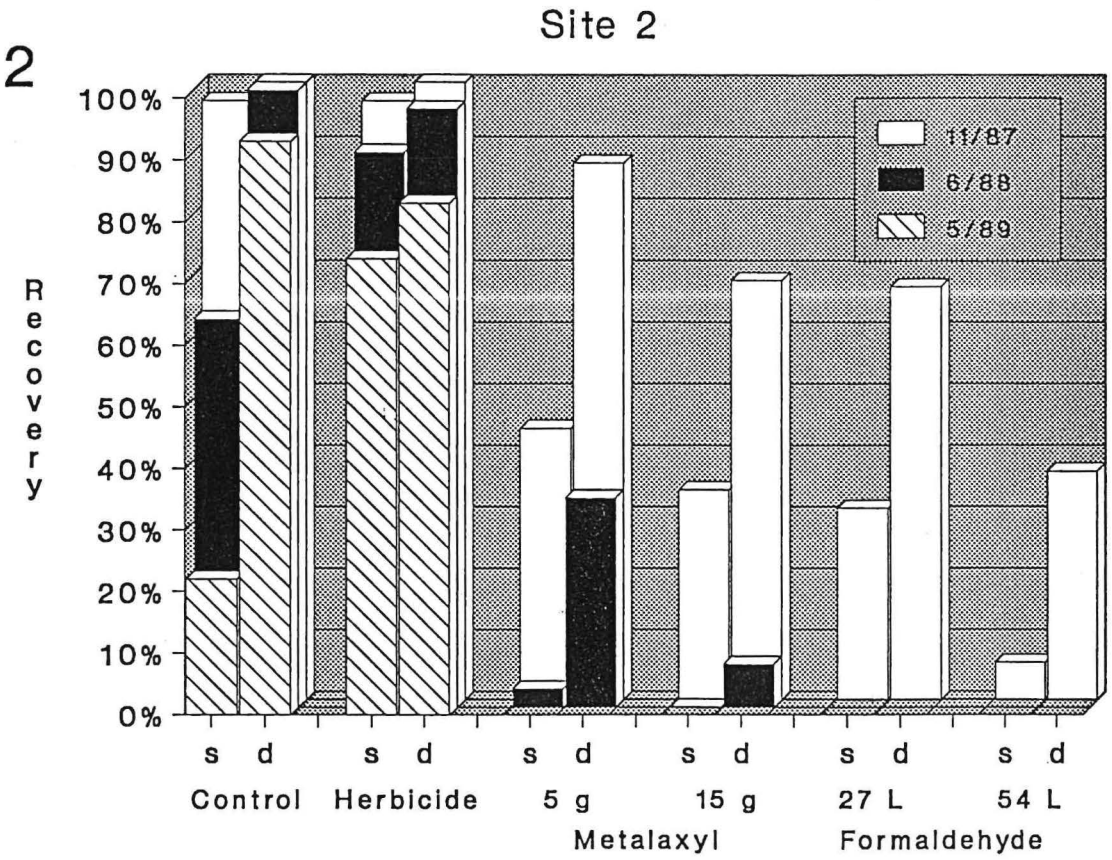
1. Any of a number of discrete infections in the Gnangara or Wanneroo areas, preferably within Melaleuca Park.

2. A portion of the only known *P. cinnamomi* in the Moore River National Park (north-eastern corner).

3. A roadside *Phytophthora* sp. infection in the northern kwongan.

4. A segment of the infection along Bell Tk. F.R.N.P., or any discrete or high priority infection in the South Coast Region.





Figures 1 & 2. Effect of treatments on the recovery rate of *P. cinnamomi* from pine plugs buried at 0.3 m (s) and 1.3 m (d) in site 1 (Fig. 1) and site 2 (Fig. 2). Survival was first assessed in November 1987 (two months after the application of treatments). Rates of chemical treatments are expressed as the quantity applied per square metre. *Phytophthora cinnamomi* survival was not assessed in formaldehyde treated plots after 11/87, nor in metalaxyl treated plots after 6/88.

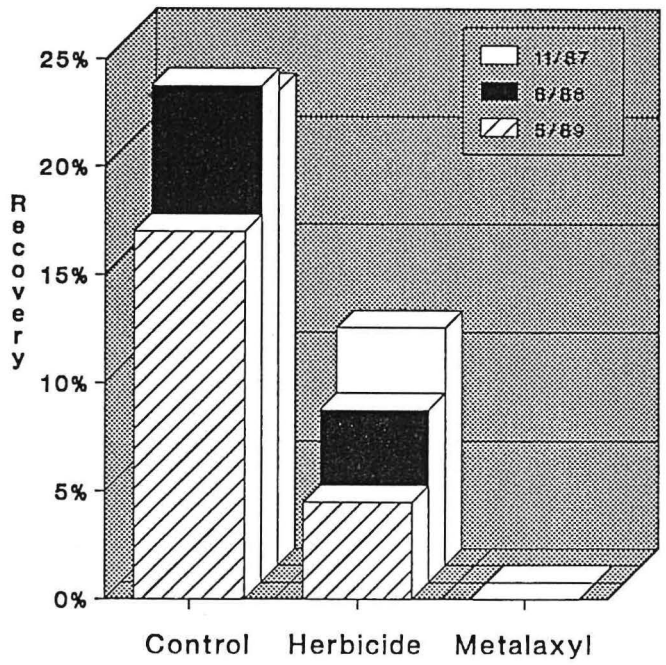


Figure 3. Effect of treatments on the recovery rate of *P. cinnamomi* from soil samples taken from 0.1-0.4 m depth. Results from both sites have been combined. No samples were taken from metalaxyl (15 g a.i. m^{-2}) treated plots on 11/87.

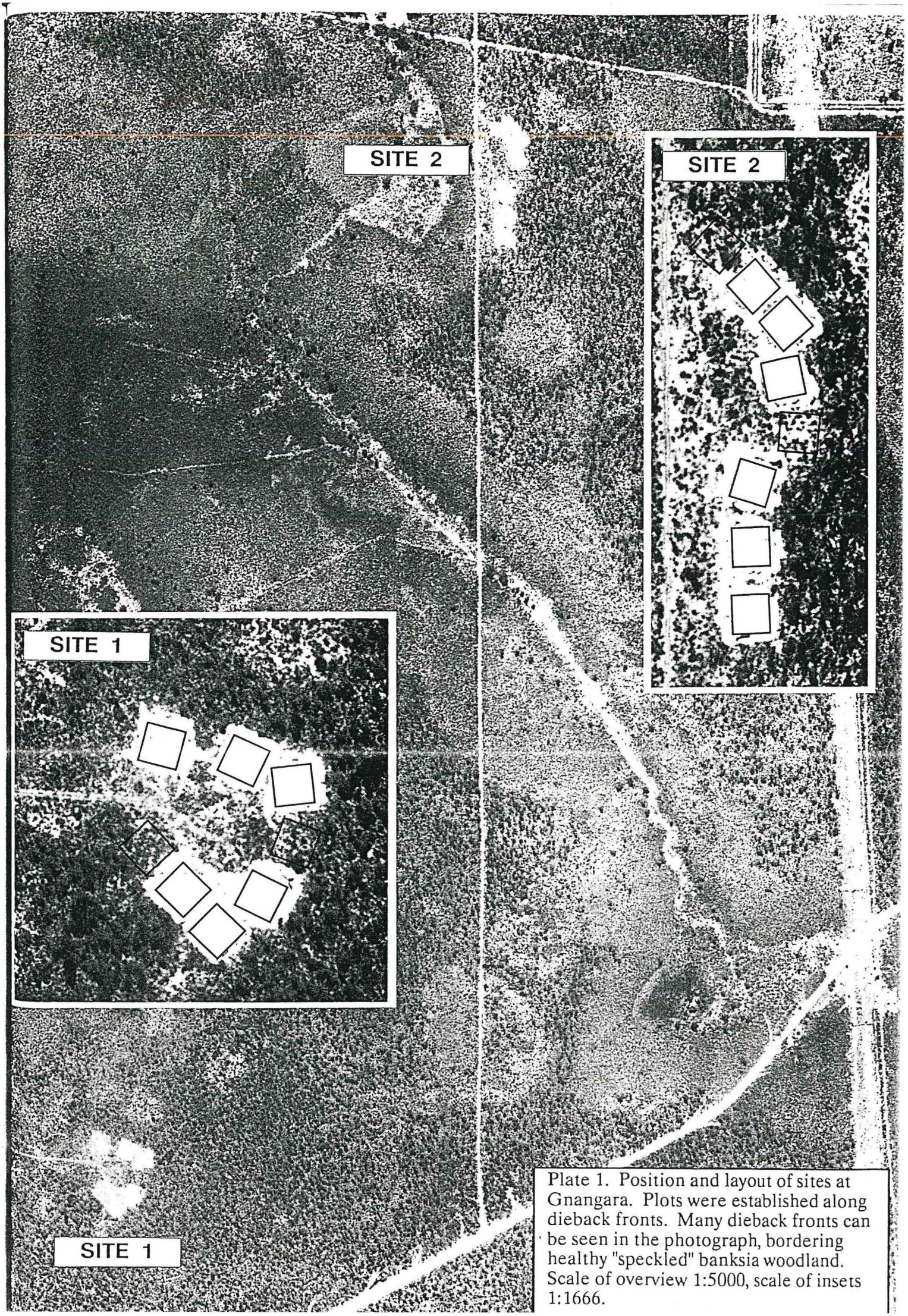


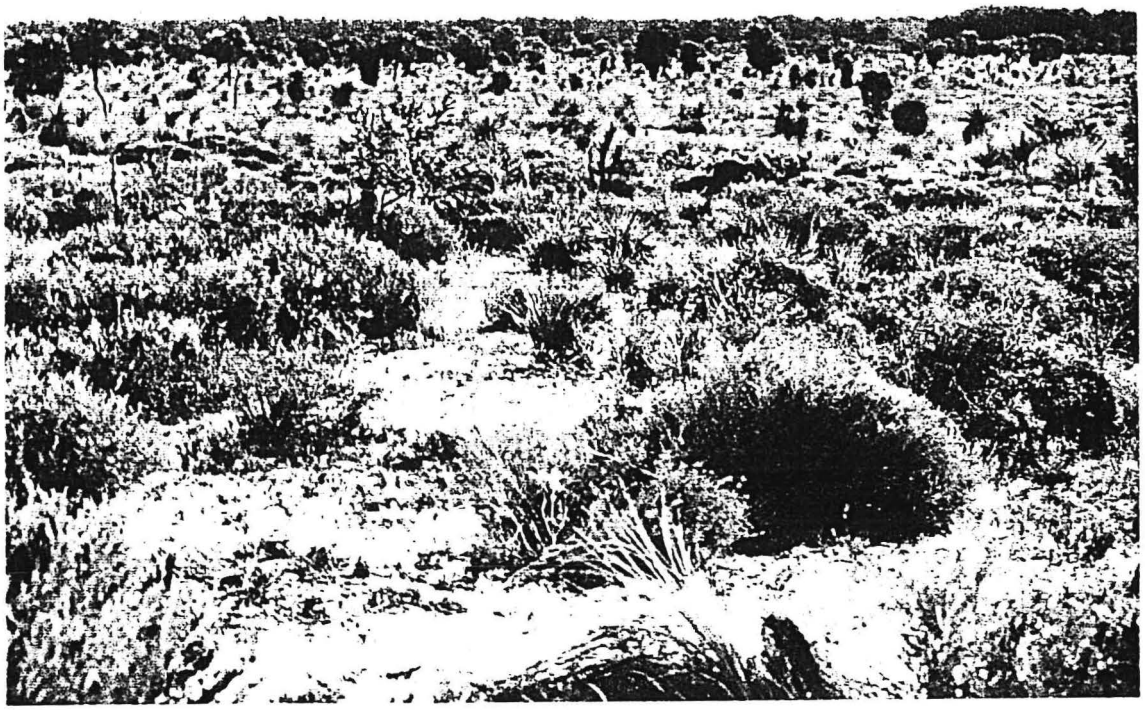
Plate 1. Position and layout of sites at Gnangara. Plots were established along dieback fronts. Many dieback fronts can be seen in the photograph, bordering healthy "speckled" banksia woodland. Scale of overview 1:5000, scale of insets 1:1666.

2

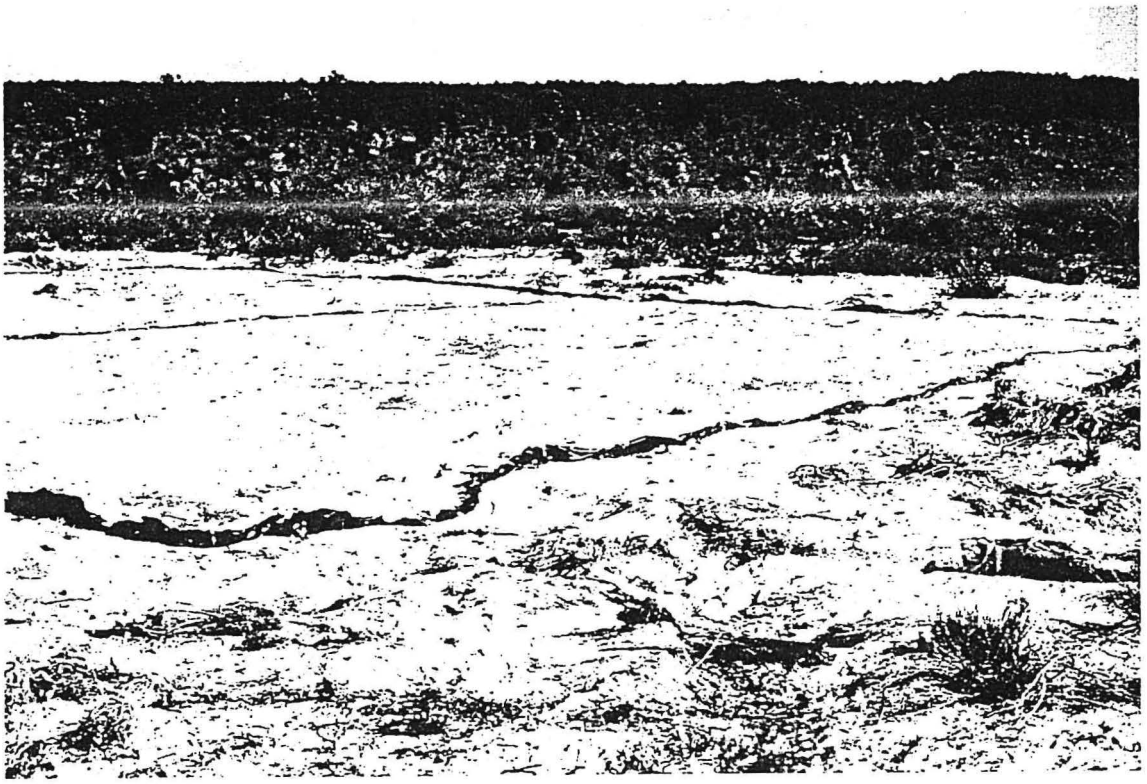
3

Plates 2 & 3. Healthy *Banksia* woodland at Gnangara (Plate 2). Small dieback ulcer (Plate 3). Note recent deaths of *Banksia attenuata* and *Banksia menziesii* along disease front, and reduction in density and species diversity of shrub layer.

4



5



Plates 4 & 5. Before and after photograph of a plot in site 2. Note tagging of stumps (Plate 4) before removal with a backhoe.

6

7

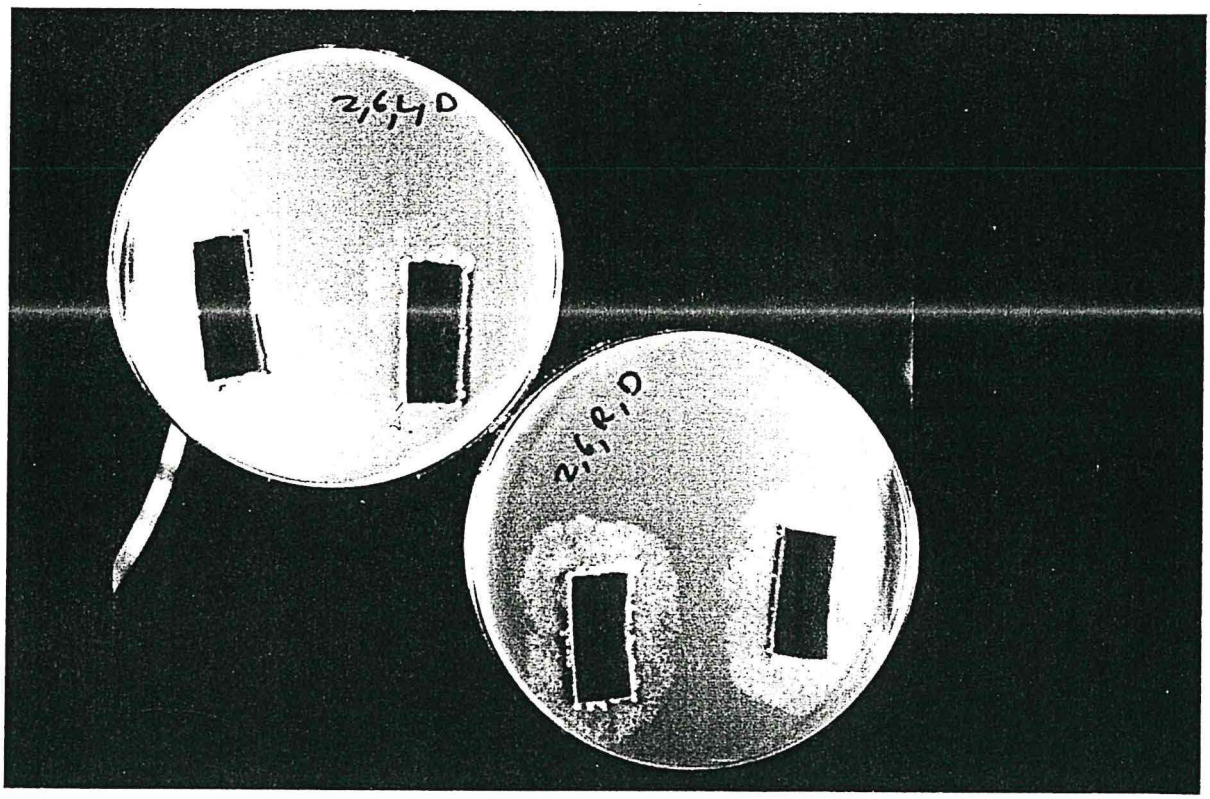


Plate 6. Construction of plastic lined trench around a plot in site 2.

Plate 7. *Phytophthora cinnamomi* growing from colonised pine plugs, split and plated onto selective agar, after burial in formaldehyde treated plots. Note the contrast in the form of growth of the fungus from the two plugs, indicating differing levels of efficacy of the fumigant.

8

9

Plates 8 & 9. Application of formaldehyde to a plot in site 1 (Plate 8), followed by coverage with plastic to assist fumigation (Plate 9).