May 2008

Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia

Cape Riche, Western Australia

Photos: WA Dunstan

Narawntapu National Park, Tasmania

Eradication of *Phytophthora cinnamomi* from spot infections in native plant communities in Western Australia and Tasmania

Murdoch LINK

Australian Government
Department of the Environment and Heritage

CPSM
Centre for *Phytophthora* Science and Management

Project Period and Period Covered by this Report
25 May 2005 – 2 May 2008

Project Manager Details
Associate Professor Giles Hardy
Forest Pathology
Rm 3.037 Biological Sciences Building
Murdoch University, South Street
MURDOCH WA 6150
Ph: 08 0360 6272
Email: G.Hardy@murdoch.edu.au

Report compiled by:
Dr Kay Howard
Email: K.Howard@murdoch.edu.au

Sub-Project Manager Details
Dr Bill Dunstan
Research Associate
Rm 3.011 Biological Sciences Building
Ph: 08 0360 2400
E-mail: W.Dunstan@murdoch.edu.au

Other sub-Project Staff
Sub-Project Co-ordinators (CPSM) - Dr Emer O'Gara– (2005-2007), Dr Kay Howard (2008)
Project Advisors: Associate Professor Bernie Dell (CPSM), Emeritus Professor Jen McComb CPSM), Bryan Shearer (DEC), Colin Crane (DEC), Mr Tim Rudman (DPIWE, Tasmania)
Associate Professor Mike Calver (Murdoch University) – Statistician
Ms Nicole Moore – Graduate Research Assistant (2005-2007)
Dr Trudy Paap (2007-2008)
Mr Rodney Armistead – Graduate Research Assistant (2006-2008)
Tim Wardlaw (Tasforests, Hobart)
Ms Brigid Morrison – Field and laboratory assistant (UTAS, Launceston)

Ackowledgements
Daniel Huberli, Nari Williams, Janet Box (CPSM)
John Watson, Sarah Barrett, Greg Freebury, Deon Utber, Damion Kuret (DEC, Albany)
Joanna Young (SCRIPT, Denmark)
P Leighton, John Moir (Wellstead), E Nichols (Waterhouse Homestead)
M Ikolowski (Tasmania), A Timmerman, G Sutton, J Westbrook, A Hrasky (Parks & Wildlife Service, Narawntapu NP), A Ford, M Hedditch, D Widger (Mersey NRM, Latrobe)
C Yinglong, N Long, M Dobrowolski, D White, J Gilovitz, G Scott, P Scott, C Davis, J Davis, P Hollick, S Jackson, B Palmer (Murdoch University)

How to cite this report:

Dunstan WA, Rudman T, Shearer BL, Moore NA, Paap T, Calver MC, Armistead R, Dobrowolski MP, Morrison B, Howard K, O'Gara E, Crane C, Dell B, O¹Brien P, McComb JA, Hardy GEStJ (2008) Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia. Eradication of *Phytophthora cinnamomi* from spot infections in native plant communities in Western Australia and Tasmania. Prepared by the Centre for Phytophthora Science and Management for the Australian Government Department of the Environment, Water, Heritage and the Arts.

Contents

EXECUTIVE SUMMARY

The area of Australian native vegetation in temperate and tropical Australia affected by *Phytophthora cinnamomi* exceeds many hundreds of thousands of hectares, and continues to increase. In Western Australia alone, greater than 6000 km^2 are now infested and 41% of the approximately 6000 plant species in the South West Botanical Province are susceptible. *P. cinnamomi* and the disease caused by it is a 'key threatening process to Australia's Biodiversity'. While the pathogen is widespread and large areas are now infested, many areas of high conservation value remain free of the pathogen. Pathogen free areas could remain so, given effective hygiene and quarantine measures are applied, and if effective methods can be developed to eliminate incursions of the pathogen. To date, there are no robust methods available to eradicate *P. cinnamomi* from spot infestations or to contain the spread of the pathogen along an active disease front. The need to eradicate or contain the pathogen is now paramount to ensure threatened flora or threatened ecological communities are protected for the long-term. The aim of this study was to develop protocols that can be used to contain and eradicate spot infestations of *P. cinnamomi* that, if untreated, are likely to threaten extensive areas of native vegetation or areas of high conservation value. Treatment regimes were guided by two assumptions: 1) within the selected sites transmission of the pathogen is by root-to-root contact; and 2) the pathogen is a weakly competitive saprotroph. In Cape Riche, Western Australia, treatment and control plots were set up along an active disease front within scrub-heath vegetation dominated by *Banksia* spp. Treatments applied sequentially and in combination, included:

1 destruction of the largest plants within disease free vegetation forward of the disease front;
2 destruction of all plants to create a fallow or 'dead zone';
3 installation of physical root barriers and subsurface irrigation for the application of fungicide/s;
4 surface applications of fungicides selective against Oomycetes (triadiazole and Metalaxyl-M) and;
5 surface injection and deep (± 1 m) treatments with the soil fumigant metham-sodium.

In a separate experiment in Narawntapu National Park (NP), Tasmania, two treatment regimes were applied to experimental heath plots with active disease centres within a *Eucalyptus-Banksia* woodland. Treatments were:

1 a combined treatment including vegetation removal, Metalaxyl-M and metham-sodium and root barriers and;
2 with Metalaxyl-M and root barriers alone.

Standard baiting techniques were used to recover *P. cinnamomi* from combined soil and root samples, down to 1.5 m deep at Cape Riche, and to 1 m at Narawntapu NP.

Findings

This study has clearly shown that *P. cinnamomi* can potentially be eradicated from key sites where there is a need to protect threatened flora or threatened plant communities. In both experiments, *P. cinnamomi* was not recovered from soil and root material from plots in three consecutive assessments over six months (Narawntapu NP) and nine months (Cape Riche) after the application of the complete regime of treatments (vegetation destruction + fungicide + fumigation). At Narawntapu NP, recovery of *P. cinnamomi* was reduced in plots treated with Metalaxyl-M alone, but the pathogen was always recovered. In contrast to treated plots, the overall mean recovery rates in untreated control plots (across all soil horizons and assessments) were 16.7% at Cape Riche, and 30.1% at Narawntapu NP.

At Cape Riche, recovery of *P. cinnamomi* was significantly affected by time (time after treatment; $p < 0.0001$) and soil depth ($p < 0.00001$). There was also a significant time x soil depth interaction ($p < 0.001$). At Narawntapu NP, recovery of the pathogen was affected by time (time after treatment; $p < 0.000001$), and there was also a significant treatment x time interaction ($p < 0.05$). Based on the probability of recovery across control plots, the probability for *P. cinnamomi* not being detected in treated plots (and therefore, plots being free of the pathogen) were estimated for the assessment times where there was no recovery of *P. cinnamomi.* At both Cape Riche and Narawntapu NP, estimates of the probability that *P. cinnamomi* was present in treated plots were very low ($p < 0.000001$ and $p < 0.003$, respectively). Even if the limitations in assessment methods (baiting) are taken into account, the results remain extremely robust.

The treatment regimes applied at both sites (located 2500 km apart) appear to be extremely effective at eliminating the pathogen from the treated areas. These treatment regimes, or elements of them, are most likely to be effective in arresting the progression of the pathogen and the disease it causes, from other sites with similar characteristics (soil, vegetation and topography). However, it is critical funding is found to monitor both sites comprehensively at least twice more (in approximately 12 and 24 months) to ensure the treatments have been effective in the medium to long-term. This is necessary as the pathogen may be present in a dormant form in large root material at depth and hence survived the chemical treatments.

RECOMMENDATIONS

Future research recommendations

1 Recovery rates of *P. cinnamomi* in this study, using standard baiting techniques, were at least equal to other published results. Molecular genetic methods, theoretically more sensitive in detection of *P. cinnamomi* than baiting, were compared with baiting results in at least one assessment for each site (data not provided). However, there were a large proportion of false negatives in the results using molecular genetic detection methods. There is a

need for further development of reliable molecular genetic methods, particularly to detect dormant resting stages of *P. cinnamomi* that may not be detected by baiting, and to reduce the costs (labour, materials, space and time) associated with baiting methods.

2 There have been casual observations that native mammals are vectors for *P. cinnamomi* dispersal. To be able to justify the high cost of exclusion fencing around infested sites, it is critical to determine if any native or feral animals are causing spread of *P. cinnamomi* in natural vegetation. If this is the case, then analysis of the risk associated with particular species and site characteristics should also be undertaken.

3 Effects of host removal, fungicides, or fumigation were not tested in isolation, with the exception of fungicide alone at Narawntapu NP, therefore, future studies should consider these treatments in isolation of each other to determine if the pathogen could be eliminated from sites. Given the slow rate of disease progression over the life of the experiment, and apparent effectiveness of treatments, the efficacy of root barriers still has to be thoroughly tested. Future studies should be implemented to determine the effectiveness of root barriers at a range of sites.

4 In both experiments, metham-sodium was used as the fumigant of choice, partly on the grounds of availability, cost and comparative handling risk. Alternative fumigants may be much more efficient at killing *P. cinnamomi*, and need to be compared alongside metham-sodium, at least on a small scale, under similar field conditions. Some of the alternatives (iodomethane, chloropicrin, ethanedinitrile, propargyl bromide and 1-3 dichloropropene) are much more expensive, present significant handling risks (particularly in the kind of field situations likely to be encountered), and may not be readily available (they may be restricted substances). However, future studies must be conducted to examine the effectiveness of alternative fumigants. Consideration should be given to using blends of these chemicals, or using sequential treatments over a short period of time.

5 Future research should also be conducted to determine if the root barrier methods with and without the installation of sub-surface irrigation systems for herbicide and fumigant application is an effective method to stop the movement of disease fronts in key susceptible, but threatened plant communities, both up- and down-slope of disease fronts. Such studies should examine a range of vegetation and soil types across the continent and in Tasmania.

6 Soil fumigation does result in a partial ‘biological vacuum’, therefore future studies should examine ways to ‘seed’ treated areas with beneficial microflora to ensure treated sites rapidly recover their biodiversity values with respect to microbial populations. High microbial populations are known to be competitors

and/or antagonists to *P. cinnamomi* and if any viable pathogen propagules remain after chemical treatments it less likely that these remaining propagules will survive. 'Seeding' would include the introduction of wood decay fungi to rapidly degrade coarse woody debris that may harbour dormant or resistant propagules of the pathogen.

Management recommendations

1 The approach to containment and eradication described in this report should be effective in areas that have similar site conditions (particularly soil types, vegetation and site topography) to the sites described in the present study, and where autonomous spread of the pathogen is by root-to-root contact. While the site characteristics chosen for these experiments provided highly favourable conditions for us to achieve containment and eradication, there are very large areas with similar characteristics on the northern sand plains and south coast of Western Australia, and the coast of eastern Australia including Tasmania.

2 The approach we have described is likely to have some application in stopping up-slope progression of disease in some other soil types, such as duplex soils and shallow organosols that have an impeding layer at shallow depth.

3 It is critical to remove living host material from a site from which *P. cinnamomi* is to be 'eradicated'. *P. cinnamomi* is a poor saprotroph, therefore, if the kind of operation described in this report is to be attempted, then it must be accepted that some vegetation will have be sacrificed in order to protect the whole. A ruthless approach to the problem from the start is much more likely to lead to a successful outcome and to reduce the need for further follow-up treatment(s).

4 Both experiments were conducted in vegetation where the size of individual plants was small in comparison to plant species within some other susceptible vegetation types (including root diameters, and the extent of root systems). In other vegetation types on similar soil types, the size of fallowed barriers and the depth of application of fumigants would have to be scaled in proportion to the size of the root architecture of the largest plant species within the site.

5 To prevent the movement of *P. cinnamomi* on sites containing threatened plant species and/or plant communities, consideration should also be given to using the high-density polyethylene root barriers in combination with the placement of an irrigation delivery system at the base of the barrier and at the soil surface alongside the barrier. Metham-sodium and herbicides could be applied through the irrigation system periodically when conditions are warm and wet. Such a system has the potential to prevent the movement of the pathogen by root-to-root spread and soil water movement of zoospores, this is especially true on deep sandy soils and sites where flooding is unlikely.

6 Before commencing a treatment regime, a comprehensive soil sampling and testing program should be undertaken, as detailed as resources (particularly time), allow. The aims of soil sampling and testing are: a) confirm the presence of *P. cinnamomi,* as similar damage may be caused by other pathogens and abiotic stresses; b) validate aboveground surveys of the infestation, as interpreted by vegetation health; and c) enable the size of a buffer around the infestation to be calculated. Consideration must be giving to managing native or introduced fauna on the site to be treated. Until demonstrated otherwise, fauna should be treated as vectors of the pathogen.

7 As molecular diagnostic tools become more robust and sensitive, it will be appropriate to consider using these tools alongside or in combination with baiting when surveying a site for the presence of the pathogen and when determining if chemical treatments have been effective.

8 If herbicide treatments are to be used and large areas of bare soil are exposed, it is recommended that a soil stabiliser is used to reduce the incidence of wind erosion.

9 In the initial project proposal an estimate of 200 m^2 was given as a limit to the area of recently established spot infestations from which *P. cinnamomi* could be eradicated. The results at Cape Riche and Narawntapu NP suggest that it may be possible to at least contain much more extensive and well-established infestations in similar types of vegetation, provided that the spread of the pathogen off-site by animal vectors can be prevented.

INTRODUCTION

The importance of the areas studied

Phytophthora cinnamomi is present in all states and territories of Australia where it causes disease in an extremely diverse range of native, ornamental, forestry and horticultural plants. *P. cinnamomi* results in the loss of millions of dollars from Australia's horticultural industry. Horticultural crops, including apricot, peach, grapevines, kiwi fruit, pineapple, avocado, chestnut, walnut and macadamia nut, are all susceptible to *P. cinnamomi* (Cahill 1993, Erwin and Ribeiro 1996) and a number of flower crops, most notably Proteas, are also susceptible (Cahill 1993). In Australia, there are over one million hectares of plantation production forests, nearly 41 million hectares of native forests and 112 million hectares of woodland as well as conservation reserves and urban landscapes (Gadgil 2000), much of which is under threat from *P. cinnamomi*. The area of native vegetation affected by *P. cinnamomi* exceeds many hundreds of thousands of hectares in Western Australia, Victoria and Tasmania, and tens of thousands of hectares in South Australia (Environment Australia 2001).

Western Australia (WA) suffers the greatest impact from this pathogen, due to the favourable environmental conditions and the large number of susceptible species that are killed (DWG 2000). In general, *P. cinnamomi* is restricted to areas in the southwest of WA, where many of the plant species often have highly localised distributions with small population sizes (Barrett *et al*. 2003). A conservative estimate of area infested by *P. cinnamomi* includes: 15 - 20% of the jarrah forest; around 60% of the montane shrublands, *Banksia* and mallee woodlands of the 116,000 ha Stirling Range National Park; and 70% of the seasonally inundated *Banksia* woodlands in the Shannon and D'Entrecasteaux National Parks (CALM 2004). Due mainly to restricted vehicular access, less than 0.1% of the 328,000 ha Fitzgerald River National Park is infested with *P. cinnamomi*, even though a large part of it receives more than 400 mm annual average rainfall (CALM 2004). Banksia woodlands in the vicinity of Perth, particularly those on the Bassendean dune system, are severely impacted and the disease is widespread in the metropolitan area of Perth (Colquhoun and Dunne 2004).

The area of Tasmania vulnerable to *P. cinnamomi* is climatically restricted to where the mean annual temperature is greater than 7.5ºC and the average annual rainfall is greater than 600 mm. Elevations above about 700 m and the dry midlands of Tasmania are therefore not at risk from disease (Podger *et al*. 1990). Additionally, its distribution is further restricted by cool soil temperatures under dense forest canopies. The actual area affected by *P. cinnamomi* is likely to be many tens of thousands of hectares (T Rudman *pers. comm*.). There are 12 National Parks in Tasmania infested with *P. cinnamomi*, of which 6 are extensively infested. Schahinger *et al*. (2003) shows that approximately one third of Tasmanian plants that are susceptible to *P. cinnamomi* are listed under the *Threatened Species Protection Act 1995* and/or the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

The barrier system developed in this study is most applicable where deep sandy soils occur, as these sites are well drained with little lateral water movement and flooding is

highly unlikely to occur. Sandy soils with less than about 10% clay content cover approximately one third of Australia (Carter *et al*. 2004); more than 310 M hectares, and occur in each state (Table 1). The occurrence of *P. cinnamomi* is limited by rainfall and temperature, and the mapped distribution of *P. cinnamomi* in non-agricultural land in Australia shows the pathogen fringing the coastline in areas which generally receive more than 600 mm of rainfall per annum (Figure 1a). Areas with deep sandy soils (such as Podosols, Rudosols and Tenosols) that occur within the climatic envelop suitable for *P. cinnamomi* are shown (Figure 1b). It is in these areas where the barrier system and associated chemical treatments can be installed and implemented. Consequently, there are substantial areas in Australia where *P. cinnamomi* is present and where these treatments have potential to be effective.

Table 1 Area of Australia (hectares) that comprise deep sandy soils of Podosol, Rudosol or Tenosol groups. From: http://www.anra.gov.au/topics/soils/overview/index.html

	WA	**SA**	**Tas**	**NSW**	**Vic**	**Qld**	**NT**
Podosol	1 028 300	150 100	235 100	156 000	524 200	610 800	
Rudosol	34 158 700	22 098 900	112 100	1 058 700	174 400	13 558 900	36 030 400
Tenosol	113 960 800	10 495 200	1 389 900	4 141 100	1 557 000	21 434 100	48 840 700
Total	149 147 800	32 744 200	1 737 100	5 355 800	2 255 600	35 603 800	84 871 100

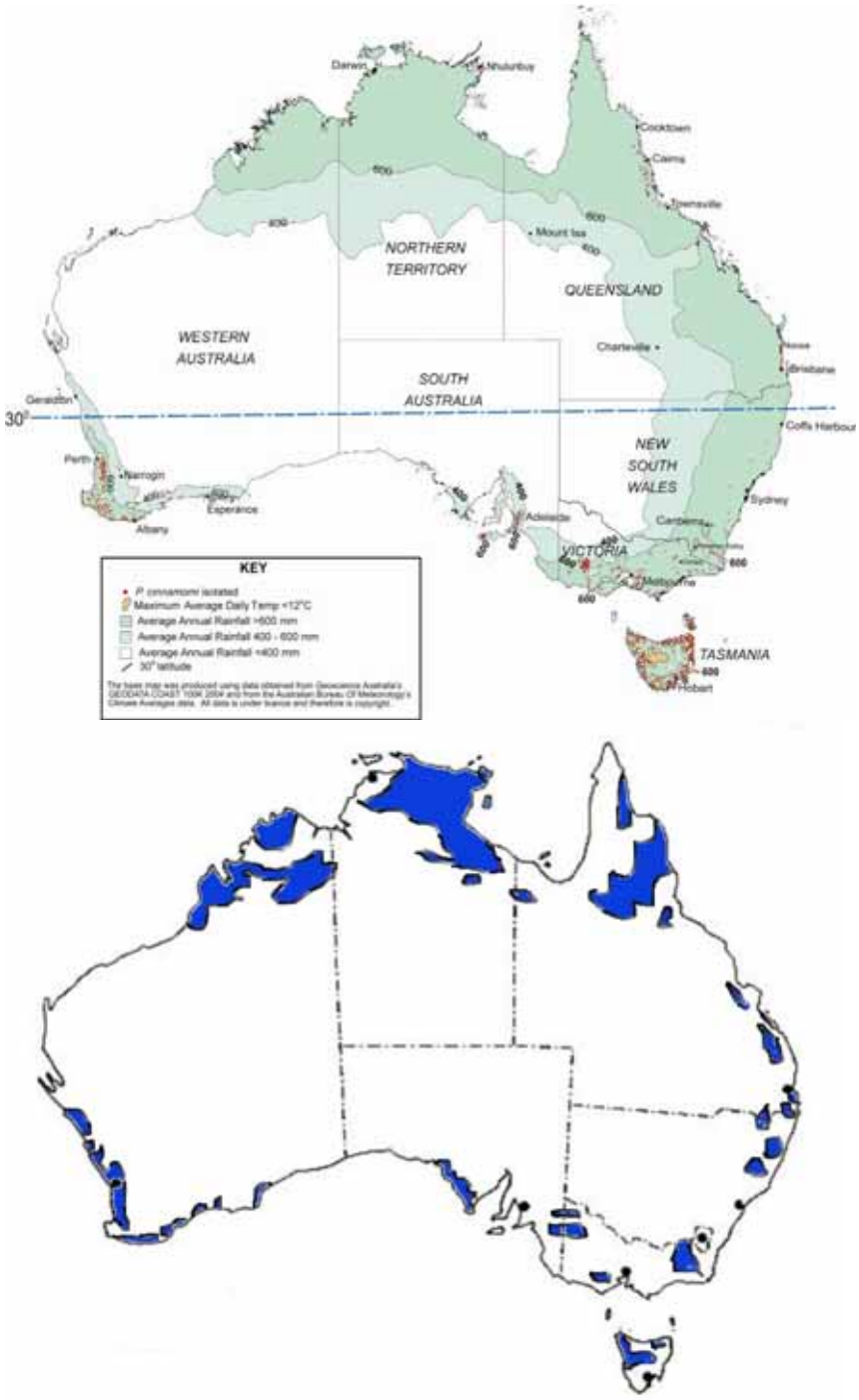


Figure 1 **a**) Broad climatic envelope of *Phytophthora cinnamomi* in Australia based on current rainfall and temperature requirements. Red spots indicate non-agricultural sites from which *P. cinnamomi* has been isolated. From: O'Gara *et al*. (2005). **b**) Areas within the climatic envelope of *P. cinnamomi* which contain deep sands (including Podosols, Rudosols and Tenosols; shown in blue) where the barrier system and associated chemical treatments can be installed and implemented, given other site characteristics are suitable.

Spread of *Phytophthora cinnamomi*

While *P. cinnamomi* is mainly spread by vectors, it spreads in undisturbed areas by autonomous or passive means. Rates of disease extension vary between different communities and ecosystems (Strelein *et al*. 2006). Autonomous spread of the pathogen occurs via motile propagules or via the transfer of the pathogen from one host to another (root to root contact), while passive spread results due to movement of infective propagules in subsurface and surface water flow. Passive movement of the pathogen is restricted in the environment by soil type and rainfall events. Passive spread has been reported to exceed 4 m/year in Banksia woodland on the Bassendean Dune System (Shearer *et al.* 2004). However, it is believed that due to the free draining nature of the soils (e.g. gravely soils, deep sandy soils and sandy duplexes) of the southwest of WA that disease spread by root to root contact between infected and non-infected plants is a major contribution to spread and is the cause of movement upslope when other vectors are not present. Spread of *P. cinnamomi* by root to root contact has been measured at 1.01 - 1.13 m/year in *Banksia* woodland growing on deep sand on the Swan coastal plain (Hill *et al.* 1994) and at 2 - 3 m/year in a similar community in the Fitzgerald River National Park (CALM 1999). In the jarrah forest, disease fronts mapped between 1986 and 1998 had varied rates of movement but overall upslope movement was 1.5 m/year with incised creeks and watercourses showing the greatest rate. The rate of up-slope movement in the wetter Blackwood Plateau (2.15 m/year) was different from the Darling Plateau to the east (0.37 m/year). The higher rate in the Blackwood Plateau was predicted to be related to a clay layer, perched water table and poor drainage (Strelein *et al.* 2006).

P. cinnamomi is a poor soil saprophyte (Kuhlman 1964, McCarren 2005) and is only able to survive in moist soils for extended periods of time (Nesbitt *et al.* 1979). *P. cinnamomi* inhabits plant roots and once the host plant material breaks down, it competes poorly with antagonistic soil flora (Zentmyer and Mircetich 1966). With forecasts for a drier climate, the spread of *P. cinnamomi* in the future is more likely to be caused by root-to-root contact as opportunities (suitable conditions) for sporulation decrease.

Treatment of existing infestations/stopping autonomous spread

The control and management of *P. cinnamomi* in natural ecosystems raises considerable challenges in terms of managing the impact of the pathogen in diverse plant communities (Hardy *et al.* 2001) and there has been little testing of control measures in native vegetation. Investigations have included applications of fertilisers, organic matter and maintenance of ground litter, and the effectiveness of fire for the control of *P. cinnamomi* has also been considered but has not been proven (Environment Australia 2001).

Chemical and biological methods have been successfully applied to control *P. cinnamomi* in agricultural and horticultural situations (Hardy *et al*. 1994, Erwin and Ribeiro 1996), but these have not proven appropriate for control of the pathogen in native plant communities (Shearer and Tippett 1989). Options for the control of *P. cinnamomi* in native plant communities cannot simply be extrapolated from agriculture and horticulture as their application to natural ecosystems involve significant specific problems. Control using broad-spectrum chemicals on a broad scale in native communities is prohibitive on basis

of cost and ecological and conservation grounds (Shearer and Tippett 1989). Many broad-spectrum chemicals used against *P. cinnamomi* in agriculture and horticulture not only have deleterious effects on host plants, but also on non-target organisms with associated significant ecological and conservation consequences. However, broad-spectrum chemicals have the potential for use to eradicate *P. cinnamomi* in small areas of vegetation when the pathogen has established as a spot or localised infestation, and where damage to other taxa is relatively minor compared to the advantage of eliminating the pathogen.

In contrast to broad-spectrum chemicals, the *Phytophthora* specific and systemic chemical phosphite has been successfully used to protect targeted host populations from *P. cinnamomi* infestation, such as threatened flora (Barrett *et al*. 2003), and other native species (Pilbeam *et al*. 2000, Hardy *et al*. 2001, Wilkinson *et al*. 2001, Shearer *et al.* 2006, Shearer and Fairman 2007). Phosphite exhibits a complex mode of action, acting directly on the pathogen and indirectly in stimulating host defence responses to ultimately inhibit pathogen growth (Guest and Grant 1991). Although phosphite is effective against *P. cinnamomi* in the host, it does not kill the pathogen or prevent sporulation (Wilkinson *et al*. 2001) and has minimal residual action against the pathogen in the soil.

Biological control options have been successfully used in some agriculture and horticulture situations (Hardy *et al*. 1994, Erwin and Ribeiro 1996). However, two factors very much constrain the potential for application of biological control to *P. cinnamomi* in native plant communities (Shearer and Tippett 1989). Firstly, most of the biological activity occurs in the organically rich surface soil, but *P. cinnamomi* exploits moist horizons down to 5 m below the soil surface (Shearer and Shea 1987, Shearer and Hill 1989). Manipulation of the environment of surface soils to favour antagonists and disfavour the pathogen is difficult enough, but is likely to be impossible at depth within the soil profile. Secondly, when conditions are favourable for *P. cinnamomi*, the pathogen can quickly produce large numbers of infectious spores. Antagonistic microflora cannot generally increase fast enough to counteract the pathogen during the periods favourable for pathogen increase and spread.

Membrane barriers have been used in agriculture and horticulture situations, either to contain infection or prevent infection entering an area (Mann 2004). However, such a control option has been tested only once in native plant communities (Hill *et al*. 1995). Eradication of inoculum using fallow or alternate cropping is a well-established option for the control of *P. cinnamomi* in agriculture and horticulture. Hill *et al*. (1995) imposed a fallow-like option on a *P. cinnamomi* disease centre by removing all the vegetation by an herbicide treatment. They concluded that confinement or eradication of the pathogen may be achieved by keeping sites completely bare for many years (Hill *et al*. 1995). While such drastic action has considerable practical and conservation concerns, it would have the potential to eradicate *P. cinnamomi* in small areas when the pathogen initially establishes as a spot or small, localised infection.

Previous research has shown that *P. cinnamomi* could not be eradicated from 0.02 ha plots in infected *Banksia* woodland on deep infertile sand on the Northern Swan Coastal

Plain (Hill *et al*. 1995). This was despite the fact that extreme treatments such as completely killing off the vegetation with herbicide and a combination of herbicide and broad-spectrum fungicides were used. It was concluded that eradication was not possible because:

- in a *P. cinnamomi* disease centre the inoculum was well established in large woody roots up to 2 m below the soil surface (Hill *et al*. 1994, 1995);
- *P. cinnamomi* inoculum was well established in moist horizons up to 5 m below the soil surface (Shearer and Shea 1987; Shearer and Hill 1989);
- the chance of a treated area being reinfected was high (Hill *et al*. 1995), especially since treatments leave a "biological vacuum" (Shearer and Tippett 1989); and
- fungicide effectiveness and longevity (with the compounds employed) was substantially reduced, due to binding by soil colloids and minerals.

The only practical way to eradicate infection may be to treat infected areas when they are at the "spot infection" stage of a few dead plants following initial establishment of the pathogen as a small, localised infestation. Eradication of spot infestations has already been identified as a high research priority (Shearer and Tippett 1989), but methods of eradication have yet to be tested.

In this project we aimed to contain the pathogen near the disease front, and then to reduce the population of the pathogen to extinction. Four strategies that were applied to contain the pathogen, reduce potential sources of nutrition for the pathogen and to directly attack the pathogen were:

- the destruction of *Banksia* and other plant species with large lateral root systems to reduce the potential for comparatively long distance transmission of the pathogen by contact between diseased roots within infested soil and uninfected roots intruding from pathogen free soil;
- a non-selective herbicide treatment to kill remaining vegetation (fallow treatment) to reduce available nutritional resources for the pathogen. The literature strongly suggests that the pathogen is a poor saprotroph, consequently removal of living food sources will mean the pathogen can only survive as resting spores. There is limited information on which native plant species are fully resistant or tolerant to the pathogen. In tolerant species, development of the pathogen is not restricted but damage to the plant is minimal and the plant survives. The pathogen survives and is able to maintain its life cycle. To be certain of removing all potential nutrient sources, we chose to kill all plants.
- the use of fungicide and fumigant treatments, to kill the pathogen, comprised of alternate applications of two fungicides effective against *P. cinnamomi* and a soil fumigant; and

- the establishment of physical barriers made of high-density polyethylene with a sub-surface irrigation system to enable application of fungicide and/or soil

fumigant below the effective depth of physical barriers. Barrier installation will greatly reduce the potential for root-to-root transmission of *P. cinnamomi.*

This was undertaken in Cape Riche, Western Australia and Narawntapu National Park (NP), Tasmania. Cape Riche is at the eastern part of the Albany subregion which is very gently inclined and has a fairly uniform range of soil types including yellowish brown sandy and gravely duplex soils underlain by yellow to grey siltstone, silty sandstone and spongeolite Eocene sediments, laterite and deep sands. Narawntapu NP stretches from the low coastal ranges to the shores of the Bass Strait from the Port Sorell estuary in the west to the mouth of the Tamar River in the east between Devonport and Low Head on the central north coast of Tasmania. The vegetation at this site is open eucalypt woodland with a heathy understorey on a Podosol soil.

The following two sections describe in detail the eradication of *P. cinnamomi* from a site at Cape Riche, Western Australia, and a site at Narawntapu NP, Tasmania.

Eradication of *Phytophthora cinnamomi* from spot infections in a native plant community in Western Australia

Eradication of *Phytophthora cinnamomi* from spot infections in CAPE RICHE, WESTERN AUSTRALIA

INTRODUCTION

Cape Riche is located on the south coast of Western Australia in an area rich in plant and animal diversity. Throughout this region *P. cinnamomi* is causing a continuing decline in biodiversity. The vegetation of Cape Riche is scrub-heath (Pate and Beard 1984) containing plants and trees up to 1 m in height, with *Banksia* and *Eucalyptus* as the dominant components (Figure 2). The soil type, climate and general vegetation type within the experimental site are representative of conditions across the southwest of Western Australia where many infestations of *P. cinnamomi* occur, and where the control methods described could be applied.

AIM

Experiments at the field sites had the following aims:

- to contain the pathogen to the existing *P. cinnamomi* infestation; and
- to eliminate the pathogen from infested plots, using repeated applications of chemical control agents.

Figure 2

Destruction of vegetation by *P. cinnamomi* at Cape Riche experimental site. Photographs are taken from the same point on the disease front during April 2007.

➢ Intact and disease-free scrub-heath dominated by *Banksia* species.

➢ The trail of death, showing elimination of banksias and many other susceptible species. A few plants of a susceptible species (arrows) have regenerated from seed, but they are also being eliminated by the pathogen. Note the dominance of rushes and sedges.

Photos: WA Dunstan

METHODS

Site description

Location

The experimental site is located 7 km NW of Cape Riche, Western Australia (Figure 3). The centre of the experimental site is at 118° 43' 00" E, 34° 33' 36" S. The site lies between 35 and 40 m above mean sea level (AMSL), and the disease front runs approximately NNW-SSE for approximately 285 m parallel to an intermittent streamline, and is the western edge of a *P. cinnamomi* infestation > 4 ha in extent. A succession of tasks was undertaken, which required movement across, and activity within, an old diseased area, an active disease front and disease free vegetation. To reduce the risk of spreading the pathogen into disease free vegetation, most tasks were completed without the need for movement of vehicles onto the site.

Geology and soils

Soil within the site is comprised principally of a fine textured white siliceous sand with a poorly developed A horizon, classified as a strongly acid Arenic Rudosol in the Australian soil classification (Isbell 2002). Sampling at selected points along the entire disease front shows that the soil profile is greater than 2.5 m deep (Figure 4). The deep sand probably overlies Eocene spongeolite of the Plantagenet group (map unit Tp in Thom and Chin 1984), that outcrops approximately 200 m west of the experimental site. In drainage lines to the northwest and southeast of the site, soils change to a silty clay loam, where seasonal waterlogging may occur (indicated by the plant species present). Selected soil physical and chemical characteristics are given in Table 2. Soil within the site is highly deficient in all macro- and micro-nutrients, but has slightly higher organic matter and carbon in the A horizons than many other soil types at the local and regional scale. In this soil, there are no impediments to internal drainage and there would be little or no lateral movement of soil water above the water table. From soil sampling at depth (February 2006), soils were saturated from 2.0 - 2.2 m below the soil surface. Given the type of soil, *P. cinnamomi* is highly unlikely to spread by the dispersion of zoospores within the site.

Figure 3

The experimental site (yellow arrow) at Cape Riche, Western Australia (red arrow).

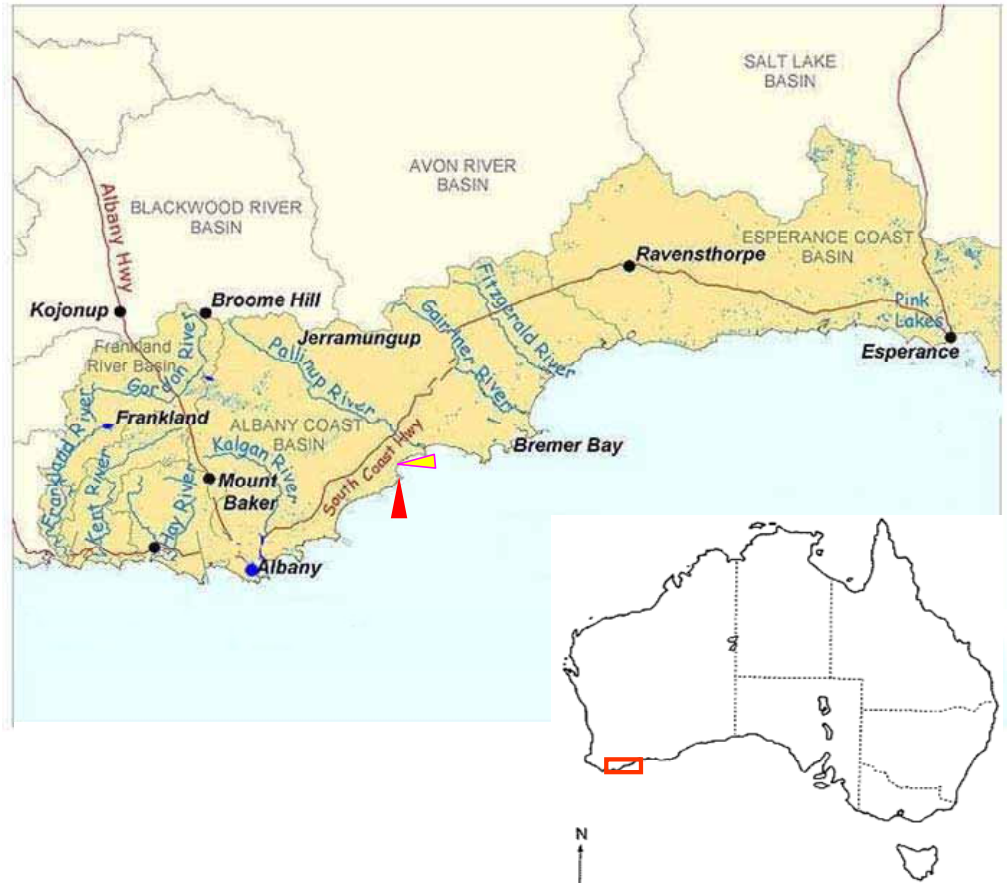


Figure 4

The soil at Cape Riche, Western Australia is a fine textured white siliceous sand more than 2.5 m deep, with a poorly developed organic A horizon (0 - 30 cm).

Photo: WA Dunstan

Table 2 Soil characteristics of the *Phytophthora cinnamomi* containment and eradication study site, at Cape Riche, Western Australia.

		(Horizon) / depth (cm)		
	Unit	**(A_1) 0-10**	**(A_2) 10-20**	**(B) 100**
NITRATE N	mg/kg	1	1	1
AMMONIUM N	mg/kg	1	1	1
P COLWELL	mg/kg	2	2	2
P OLSEN	mg/kg	0	0	0
TOTAL P	mg/kg	9	6	23
POTASSIUM	mg/kg	40	22	11
SULPHUR	mg/kg	5.3	2.4	1.3
OM	%	15	8.6	-
ORG CARBON	%	2.94	1.62	0.48
IRON	mg/kg	51	37	28
CONDUCTIVITY	dS/m	0.02	0.023	0.017
pH $CaCl_2$	pH	3.9	3.9	4.4
pH H_2O	pH	5.3	5.1	5.2
DTPA Cu	mg/kg	0.1	0.11	1.2
DTPA Zn	mg/kg	0.09	0.06	2.27
DTPA Mn	mg/kg	0.36	0.13	0.18
DTPA Fe	mg/kg	9.82	7.24	3.94
P RETEN	index	0	0	0
EXC Ca	meq/100 g	2.21	0.88	0.23
EXC Mg	meq/100 g	0.99	0.45	0.16
EXC Na	meq/100 g	0.18	0.12	0.05
EXC K	meq/100 g	0.09	0.05	0.02
Al $CaCl_2$	mg/kg	3.3	3.7	1.7
B HOT WATER	mg/kg	0	0	0
EDTA Cu	mg/kg	0	0	0
EDTA Zn	mg/kg	0	0	0
EDTA Mn	mg/kg	0	0	0
EDTA Fe	mg/kg	0	0	0
EXC Al	meq/100 g	0.07	0.03	0.02
CHLORIDE	mg/kg	0	0	0
PBI	index	0	0	0
COLOUR		GREY1/3/N	GREY1/4/N	2.5YR/8/1
180-700 µM	%WT	48	52	-
125-180 µM	%WT	27	28	-
<125 µM	%WT	10	12	-

Vegetation

Disease free areas of the experimental site contain scrub-heath (Pate and Beard 1984) comprised of a mixture of Proteaceae, Epacridaceae and Myrtaceae less than 1 m in height, with a dwarf form of *Banksia attenuata* and *Banksia nutans* the dominant components. The upper taller layer is comprised of discrete patches of *Banksia baxteri* and scattered *Banksia coccinea* with a few scattered *Eucalyptus staeri, Eucalyptus decipiens* (mallee form) and *Lambertia inermis.* A partial list of species is included in Appendix III.

Sixty-three percent of the larger and/or most common species present at the site have been negatively impacted on by the presence of *P. cinnamomi* (Appendix III). Diseased areas have been reduced to sedgeland, dominated by Restionaceae (*Anarthria* spp. and a *Hypolaena* sp.) with some *Phytophthora* resistant Myrtaceae (*Melaleuca striata*) and 'survivor' *Adenanthos cuneatus* (a susceptible species). Some reseeder species, specifically *B. coccinea* and *L. inermis* (Figure 5), have germinated and become established in older parts of the diseased area. However, it is likely these will be killed by the pathogen in the near future, as was observed with similar recruits in the near vicinity. To the north, east and west of the experimental site the vegetation grades from scrub-heath into mallee-heath dominated by *Eucalyptus pleurocarpa* (syn. *E. tetragona*).

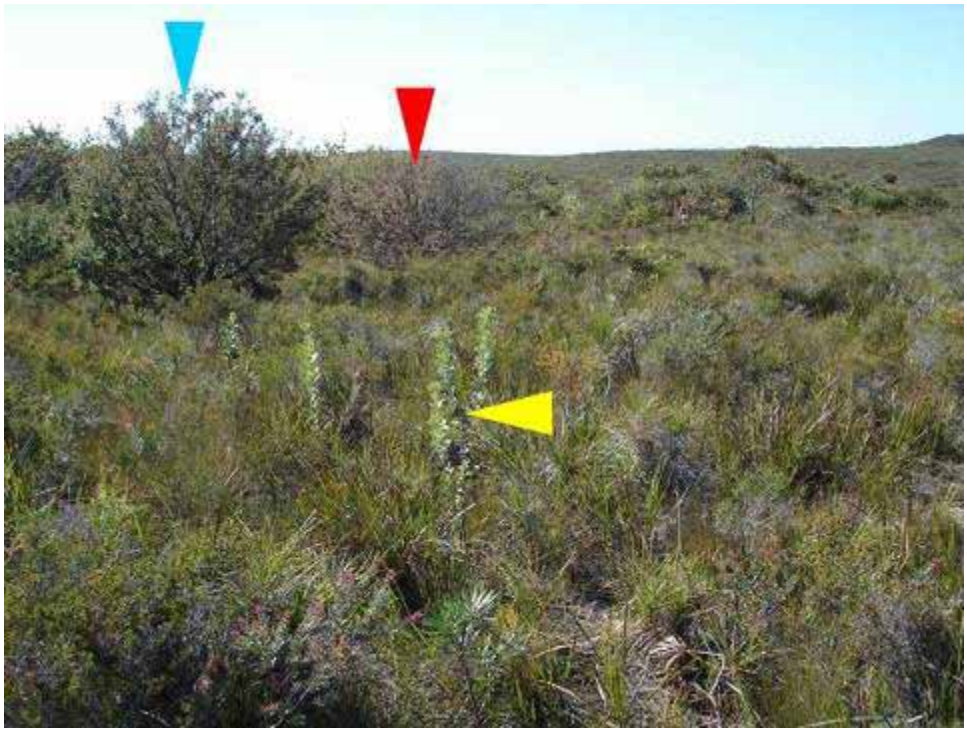


Figure 5

Reseeded *Banksia coccinea* (yellow arrow) and *Lambertia inermis* (blue arrow) on old diseased ground adjacent to the experimental site at Cape Riche, Western Australia. These plants will eventually be killed by *Phytophthora cinnamomi* from inoculum surviving in the soil, as is already occurring with a *Lambertia* (red arrow).
Photo: WA Dunstan

Climate

Summaries of mean monthly minimum and maximum air temperatures, and mean monthly rainfall for Wellstead (34° 29.30′ S, 118° 37.09 ′E; years 1998 - 2007) are shown in Figure 6, and annual rainfall between 1998 and 2007 are shown in Figure 7.

Figure 6

Mean monthly minimum (□) and maximum (■) air temperatures, and monthly rainfall (▭) for Wellstead, Western Australia (34° 29.30′ S, 118° 37.09 ′E) for the years 1998 - 2007.

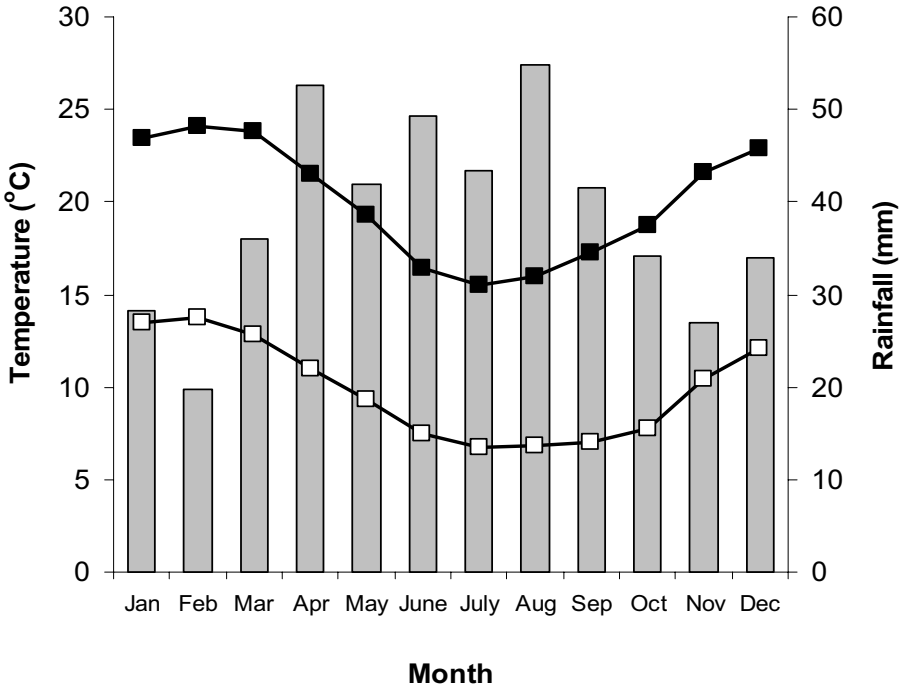
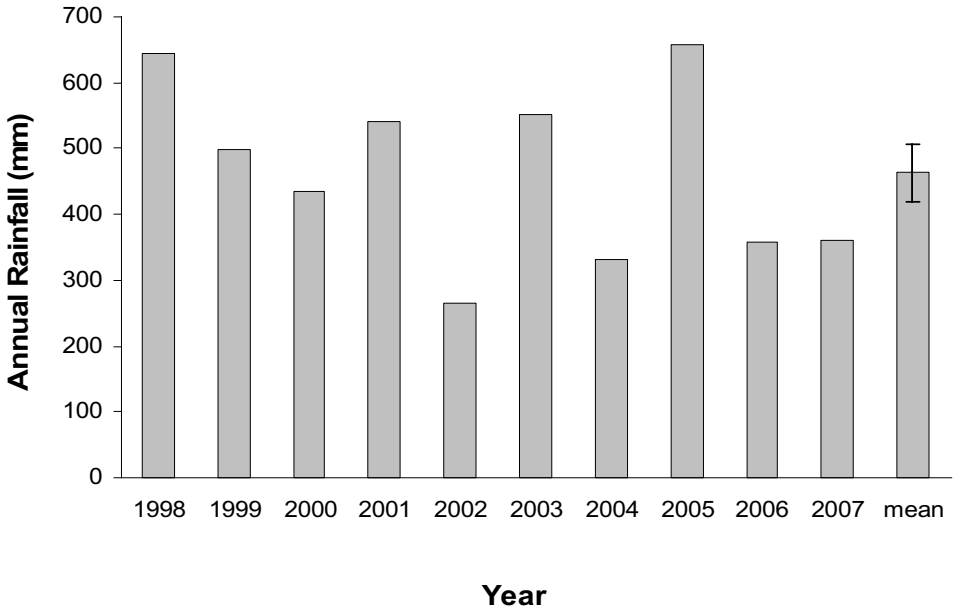


Figure 7

Annual rainfall for Wellstead, Western Australia (34° 29.30′ S, 118° 37.09′ E) for the years 1998 - 2007. Vertical bar represents two standard errors of the mean.



In some years, the summer months had unusual rainfall events where the rainfall was more than 200% above the monthly average (1997-2006); February 1997 (65.6 mm, mean usually 25.6 mm), January 2000 (105.6 mm, mean usually 26.5 mm), December 2001 (199.2 mm, mean usually 31.0 mm), February 2003 (90.2 mm, mean usually 21.6 mm), January 2004 (54.8 mm, mean usually 26.5 mm) and January 2006 (71.0 mm, mean usually 26.5 mm).

A permanent weather station (HOBO®, Onset Computer Corp.) was established in May 2006, at a central point within the site. Data monitored included:

- ambient air and rainfall (Figure 8)
- soil temperatures at depths of 15, 50 and 150 cm (Figure 9); and
- soil moisture at 15, 50 and 150 cm (Figure 10) using capacitance type sensors (ECH_2O® Dielectric Aquameter probes, Decagon Devices Inc., as modified by Onset Computer Corp.).

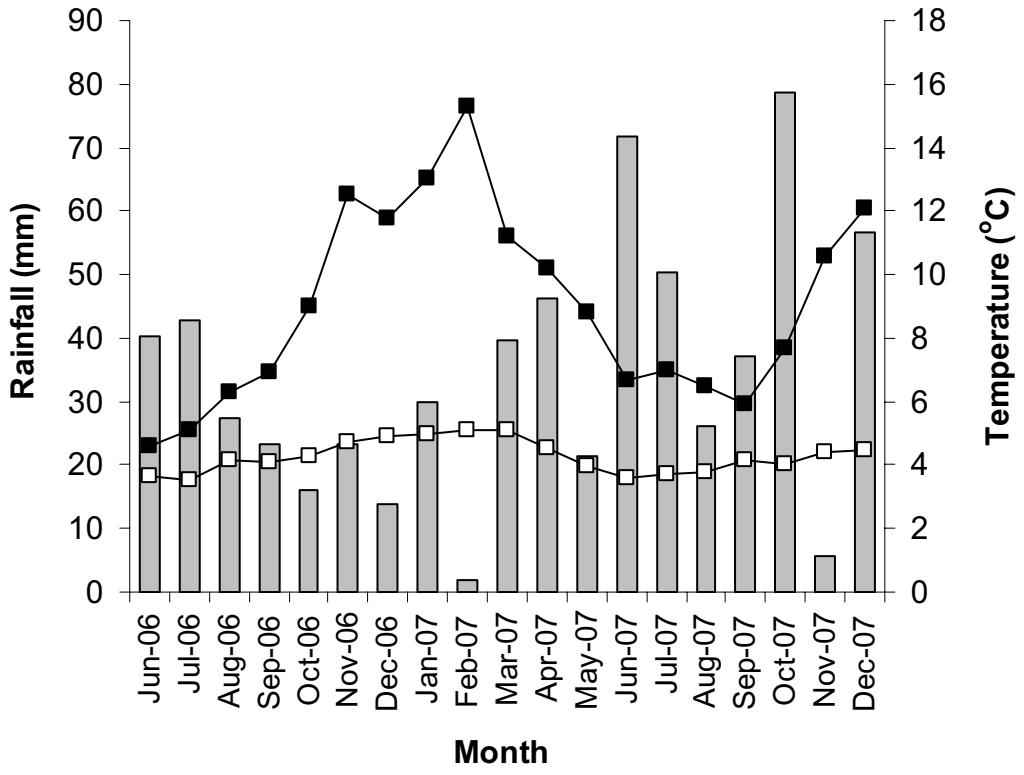


Figure 8 Mean daily minimum (□) and maximum (■) air temperatures, and mean monthly rainfall (▭) for Cape Riche experimental site, Western Australia, for June 2006 to Jan 2008.

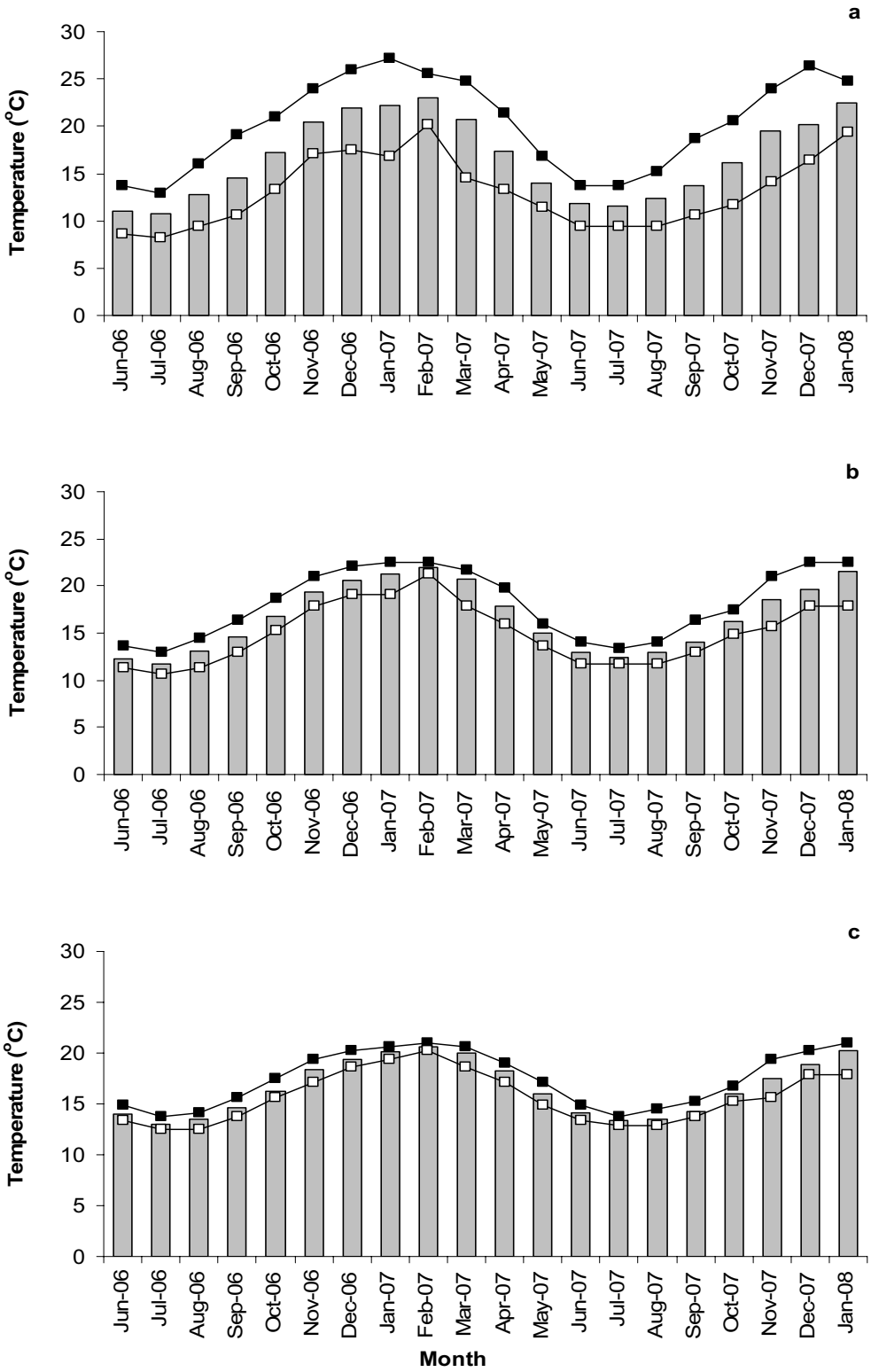


Figure 9 Mean monthly minimum (□), maximum (■) and average (▒) soil temperatures at **a**) 15 cm, **b**) 50 cm and **c**) 100 cm, for Cape Riche experimental site, Western Australia, for June 2006 to Jan 2008.

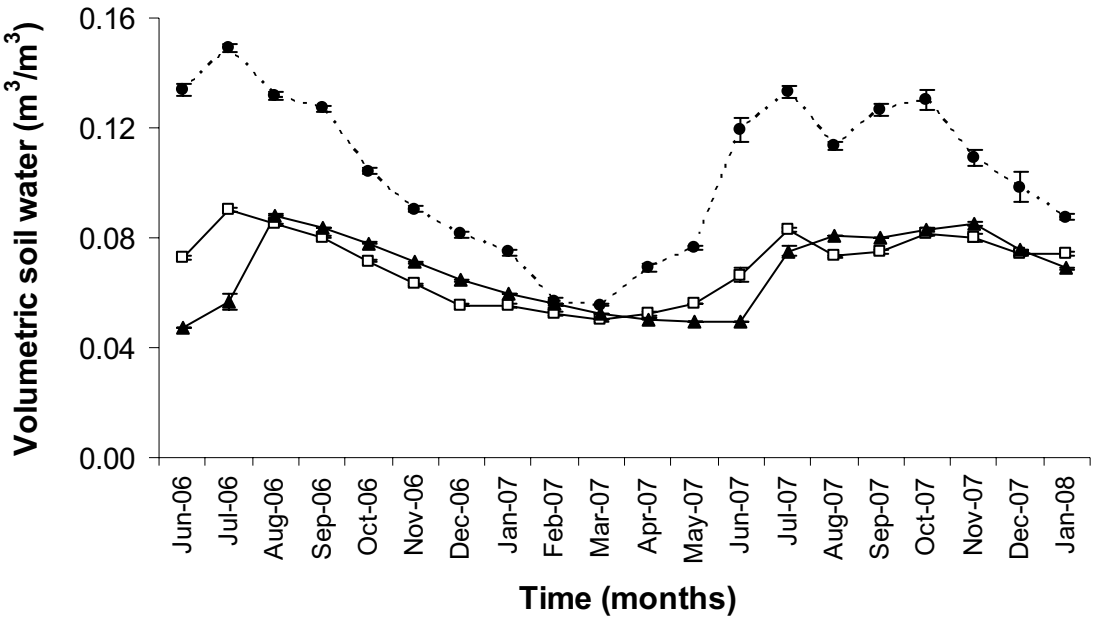
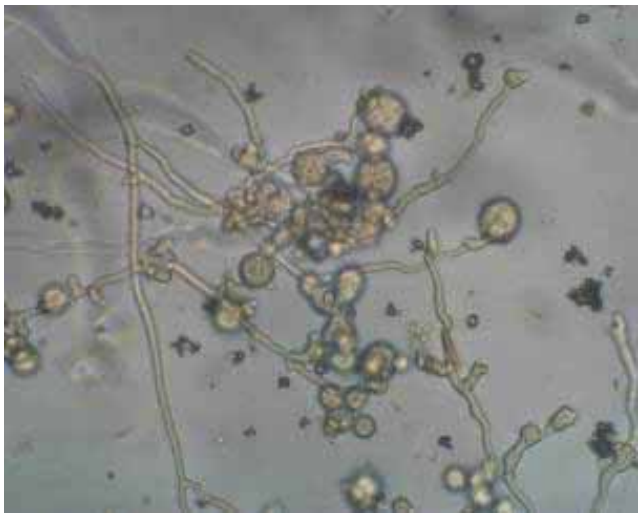


Figure 10 Average monthly volumetric soil water content at 15 (●), 50 (□) and 100 cm (▲) in Cape Riche experimental site, Western Australia, for June 2006 to Jan 2008.

The pathogen

A selection of 10 *P. cinnamomi* isolates, recovered from across the length of the active disease front, were tested to determine their mating type by using known A1 and A2 mating types (isolates DCE25 (A1) and DCE60 (A2); supplied by Vegetation Health Services, DEC). Oospores were produced in all tests with the A1 tester (Figure 11), while none were produced with the A2 mating type; therefore, all the isolates from the site were of the A2 mating type. Across the southwest of Western Australia in general, most isolates recovered from diseased plants and soil are A2, whereas A1 types are mostly confined to the south coast. Recently it has been determined that the majority of isolates from the Bell Track infestation in Fitzgerald National Park are A1 mating type isolates (M Stukely *pers. comm.*).

Figure 11



Primary isolate of *Phytophthora cinnamomi* from Cape Riche, Western Australia, showing typical coralloid hyphae.

Oospore produced from a cross between a *Phytophthora cinnamomi* isolate (CR25) from Cape Riche and an A1 tester isolate (DCE25).

Photos: WA Dunstan

Experimental design and plot layout

The disease front was mapped in February and March 2006 using *Phytophthora* susceptible indicator species; principally *Banksia attenuata, B. baxteri, B. coccinea, B. nutans, Adenanthos cuneatus, Petrophile teretifolia, Andersonia simplex* and *Leucopogon* spp. *Isopogon trilobus* was used with caution as an indicator species as some deaths putatively caused by an unidentified stem canker pathogen could be confused with disease caused by *P. cinnamomi*. The disease front was permanently marked at 5 - 10 m intervals with steel posts (Figure 12).

Photo: WA Dunstan

Figure 12 The disease front caused by *Phytophthora cinnamomi* at Cape Riche, Western Australia. Arrows highlight the disease front, with healthy vegetation on the right.

Treatment and control plots were marked out at right angles across 10 m of the visible disease front. Plots were 10 x 15 m, with 5 m within diseased ground and 10 m extending into apparently disease free ground. Plot selection was based on the following criteria:

- sufficient numbers of indicator plants within the plot;
- linearity in visible disease front and to the flanks;
- a high level of activity of *P. cinnamomi* (indicated by recent plant deaths); and
- an allowance of at least 5 m buffer between plots.

A large plot size was chosen to reduce the effects of trampling. That is, to reduce damage or the destruction of vegetation (particularly pathogen hosts) that may affect the natural progression of disease (particularly in control plots). The large plot size also allowed for sufficient distance between sampling points to ensure independence between samplings.

Treatment for the first subject (plot) was selected randomly and control and treatment plots were alternated along the disease front to avoid problems that may have been associated with complete randomisation, such as having adjacent plots with the same treatment covering ground with abnormal pathogen activity.

To further define the extent of disease, surface soil within the putatively disease-free section of the plots to be treated were grid sampled at 2 m intervals, in a 3 x 5 point array starting at the visible disease front and extending 8 m into the disease free section of the plot. Soil was sampled to a depth of 20 cm. Soil samples were flooded and baited with petals *(Rosa* sp. and *Hibbertia* spp.) to detect *P. cinnamomi*. After 2 - 3 days, water soaked petals were plated onto NARPH selective medium (Hüberli *et al*. 2000) and incubated for a further 2 - 3 days at 23°C before plates were examined for the presence of *P. cinnamomi.* Suspect colonies were identified by colony morphology followed by confirmation by microscopic examination.

Plot treatments

The aims of plot treatments were to contain the pathogen near the disease front and then reduce the population of the pathogen to extinction. The four strategies tested to contain the pathogen, reduce potential food sources and directly attack the pathogen were:

- removal and/or the destruction of *Banksia* species with large lateral root systems. This was done to reduce the potential for comparatively long distance transmission of the pathogen;
- vegetation removal by herbicide treatment, designed to reduce the potential for root-to-root transmission of the pathogen, and to remove nutrient supply from the pathogen;
- surface and subsurface applications of fungicides aimed at destroying the pathogen, comprised of alternate applications of Terrazole (Triadiazole; Crompton Specialities P/L, Australia) and Metalaxyl-M (Syngenta, Australia), and the fumigant Metham Sodium (metham, Nufarm P/L, Australia); and
- physical barriers (high-density polyethylene, HDPE) with a sub-surface fungicide injection system to prevent root-to-root transmission of *P. cinnamomi* in soil horizons below the physical barrier.

Figure 13 shows the general outline of the control strategy.

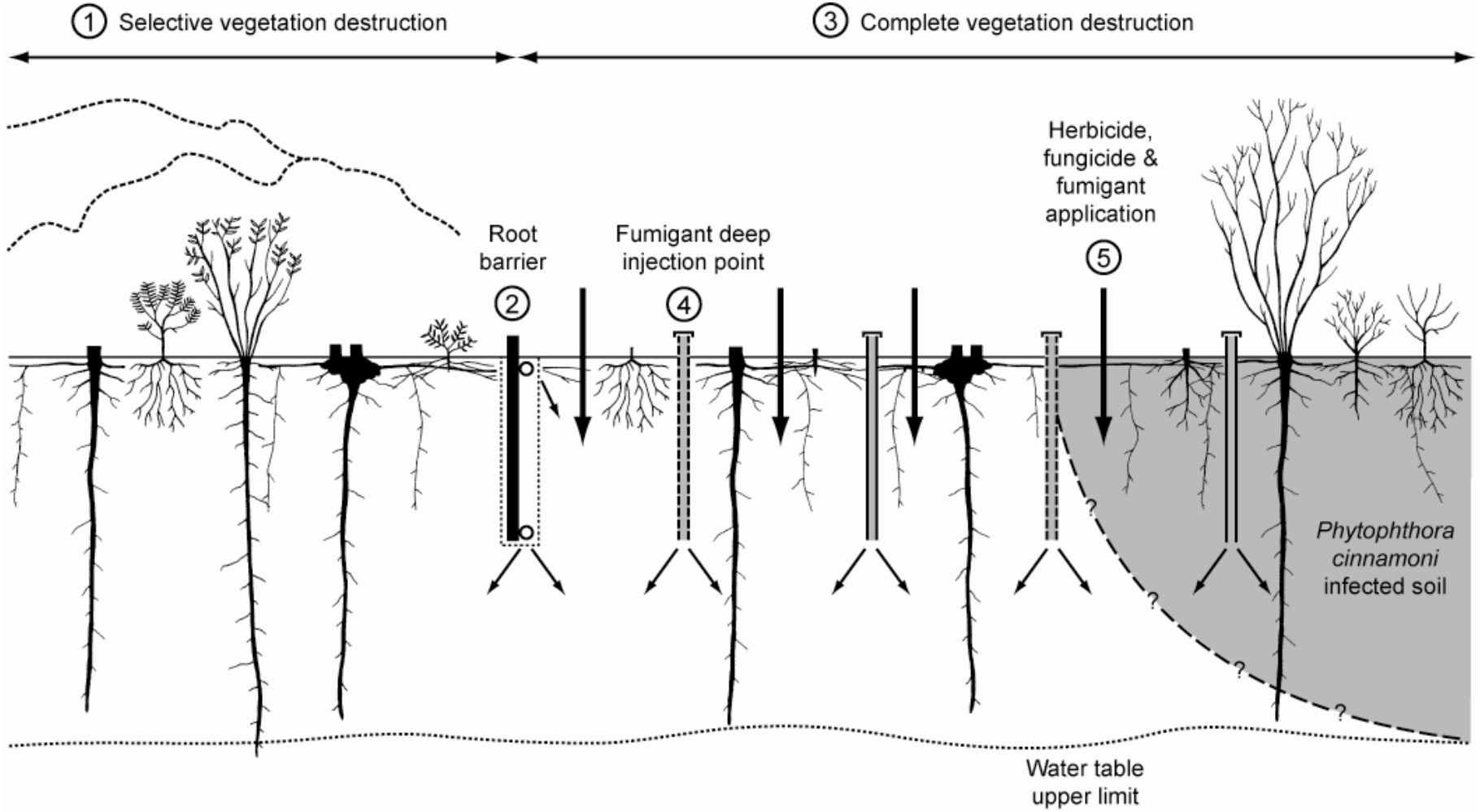


Figure 13 Schematic diagram of treatments used to contain and eradicate *Phytophthora cinnamomi* at Cape Riche, Western Australia. 1 Selective destruction of large plants with extensive lateral root systems. 2 Physical barrier and sub-surface irrigation system to disrupt lateral root systems. 3 Complete vegetation destruction, to deny living hosts and nutrition sources to the pathogen. 4 Deep 'injection' of fumigant to kill the pathogen and roots. 5 Herbicide, fungicide and shank injection of fumigant, to kill hosts and the pathogen.

Vegetation removal and herbicide treatment
It is known that *P. cinnamomi* is a poor saprotroph; therefore, when living vegetation is removed, the pathogen is most likely to remain in a dormant state. In addition, this provides the opportunity for other saprotrophic microorganisms to parasitise the resting stages of *P. cinnamomi*. Therefore, treatments were aimed at denying living host material to the pathogen.

Within disease-free sections of the treatment plots, all large shrubs within 10 m of the disease front, principally *Banksia* spp., were cut down to within approximately 10 cm of ground level. The stumps were treated with a 1:1 mixture of water and 360 g/L glyphosate (180 g/L ai) containing 1 mL/L Redye marking dye and 500 μL/L BS1000 alcohol alkoxylate surfactant (100% alcohol alkoxylate; Crop Care Australasia, Murarrie, Qld). The stumps were sprayed to run-off and all cut plant material was removed from the plots. The number of plants treated per plot ranged from 19 to 40 (mean = 31). In August 2006, the remaining vegetation within 4 m of the disease front in treatment plots was slashed to less than ca. 5 cm of ground level. Trash was raked clear (into *Phytophthora* infested part of the plots) and the cleared portion of the plots sprayed to runoff with glyphosate (3.4 g/L ai) containing 130 μL/L BS1000 surfactant.

In March 2007, individual plants that had survived were spot sprayed with Garlon^{TM} 600 herbicide (Dow AgroSciences LLC; ai triclopyr butoxyethyl ester, CAS # 064700-56-7), in a 1:60 mixture with diesel fuel oil, according to the manufacturer's instructions. On average, approximately 30 - 40 mL of garlon-diesel mixture was used per m^2 of treatment plot.

Fungicide and fumigant treatments
Fungicide was applied across the disease front (10 m) and 4 m from the disease front into putatively disease-free ground. For ease of treatment, and to aid uniformity in application of fungicide, the treatment plots were subdivided into four sub-plots of 2 x 5 m, which were treated individually. In the first two treatments, Terrazole WP 350 (ai Triadiazole 350 g/kg, Crompton Specialities P/L, Adelaide) was applied at 10 g/m^2 in a volume of 0.5 L/m^2, using a watering can. No wetting agents were used. Terrazole treatments were applied in June and August 2006. Fumigant (metham-sodium) was applied by shank injection at a rate equivalent to 380 kg/ha ai as a 1:10 aqueous solution in early June 2007. Surface applications of fungicide (2 x Ridomil 25G; 2.5 g/m^2 Metalaxyl-M) were completed in late May 2007 and early August 2007. Deep 'injection' of metham-sodium (500 mL/m^2; equivalent to 2115 kg ai/ha) at 1 m depth was completed in late September 2007.

Chemical applications undertaken during the conduct of this experiment comply with the requirements of Australian Pesticides and Veterinary Medicines Authority (APVMA) permit number PER 7250 (Permit to allow the conduct of small-scale trials with agvet chemicals; http://permits.apvma.gov.au/PER7250.PDF)

Physical barriers
For the most effective placement of root barriers two important criteria had to be satisfied. The barriers had to be:

- placed sufficiently close to the disease front to give a fair probability that given the possible maximum likely spread of the pathogen, the barriers

would be tested within the time frame of the experiment; and
- positioned sufficiently far from the disease front to ensure that *Phytophthora* infested soil was not excavated.

Preliminary grid sampling of treatment plots, followed by two rounds of systematic sampling to depth, indicated that *P. cinnamomi* was present in soil up to 2 m from where disease was expressed by dead susceptible plants. Therefore, given that rate of spread was calculated at up to 2 m/yr, the barriers were installed 3 m from the original disease front (as surveyed in February 2006). Where the disease front encroached on the flanks of treatment plots (plots 4, 10, and 12), the length of the frontage covered by barriers was reduced by 2 m, to further provide a buffer against digging into infested soil.

Barriers were installed in March 2007, when the soil in all horizons to 1 m was at, or close to, it's driest over the duration of the experiment (Figure 10), and activity of *P. cinnamomi* was likely to be at a low point for the year. The dry soils reduced the risk of transferring infested soil between plots and spread was further minimised by keeping machinery clean. A 'Bobcat' digging machine was used for excavations. Prior to entering the site the machine was cleaned down with water using a high pressure hose, inspected for residual soil and sprayed down with Phytoclean™ (ai benzalkonium chloride; Bold Scape P/L, Belgrave, Vic) using a 1: 20 dilution with water. Entry to, and movement within, the site was always over putatively disease free ground, SW of the disease front. The angle of approach to, and exit from, each plot that was to be trenched was at as close as possible to 90°.

Trenches dug for the barriers were in the form of "U", (Figure 14a, b) 8 or 10 m parallel to the disease front, with two arms of 5 m at each end at right angles to the main cut. Trenches were excavated to approximately 90 cm deep and 25 cm wide, and were excavated in three stages:
- the first flanking arm (5 m), cut towards the disease front;
- the main trench, cut from SE to NW; and
- a second flanking arm (5 m), cut away from the disease front.

A "U" shape for installation of the root barriers was chosen in order to protect vegetation within the 'D' from root intrusion from infested soil on the flanks of the treated ground, and therefore extend the effective life of the experiment.

The order in which trenches were cut was planned to ensure that most spoil was deposited outside the trench, closest to the disease front.

Figure 14
Root barriers constructed adjacent to disease fronts caused by *Phytophthora cinnamomi* at seven plots in the Cape Riche, Western Australia project site. Note dead vegetation on right of trench, previously killed with herbicide treatments.

a Trenches were dug to 90 cm depth with the majority of the spoil piled outside the area to be protected.

c

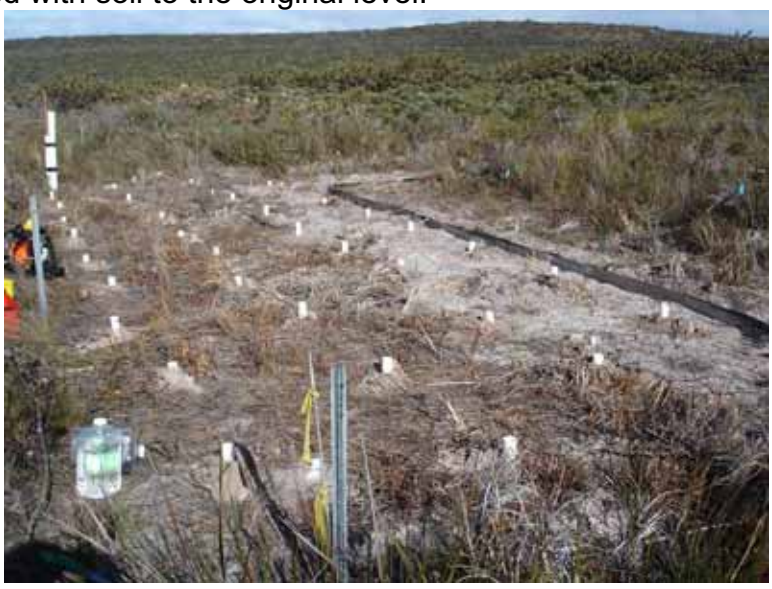
The trenches were filled manually to within 10 cm of original level and a second length of micro irrigation hose was installed and covered with soil to the original level.

b

A continuous 20 m length of 90 cm deep root barrier (high-density polyethylene) was inserted into the trench and pinned to the inside wall of the trench. A continuous length of micro irrigation hose (17 mm dia.) was installed at the base of the trench.

d

The completed plot root barrier treatment. The area inside the "U" shaped barrier is disease-free.



e

To enable deep application of metham-sodium, holes were dug and lined with PVC tube at 1 m centres, to greater than 1 m in depth. Four rows of application tubes were dug between the root barrier and disease front to deliver fungicide to depth.

Care was taken to minimise the disturbance at each site. Note healthy vegetation in the background on the ridge.

Photos: WA Dunstan

After excavation, a single 20 m length of 90 cm wide HDPE root barrier (Treemax P/L, Laverton, Vic) was inserted into the trench and fixed to the inside wall of the trench with steel pickets, nearest to the disease free section of the experimental plot. A continuous length of 17 mm diameter polythene micro-irrigation hose (Netafim, Uniram 17) was placed in the bottom of the trench, clear of the root barrier and the trench was filled to within 10 - 15 cm of the natural ground level with, as far as possible, clean sand from the excavation. An additional length of micro-irrigation hose was laid at the depth of 10 - 15 cm, and covered with spoil to natural ground level (Figure 14c, d). To enable deep application of metham-sodium, holes were dug and lined with PVC tube at 1 m centres, to greater than 1 m in depth. Four rows of application tubes were dug between the root barrier and disease front to deliver fungicide to depth (Figure 14e). As a hygiene measure, tools and footwear were drenched with methylated spirits between each treatment plot.

This method of barrier placement was achieved at a rate of approximately 2 m/min for digging time. This time could be further reduced if right angles were not used in the barrier. The machine used had a width of 1.7 m and had a fairly low impact on surrounding scrub. This impact would also be reduced if the right angles on the barriers were removed. The time taken to complete each 20 m section of barrier insertion to completion was 2 h which could be reduced if a better method of spoil handling (i.e. lay down sheeting to prevent mixing of vegetation and spoil) were adopted.

Assessment of *P. cinnamomi* infestation

Soil sampling

Samples were taken at approximately 0.5 and 2.5 m from the expressed disease front, at 3 points across the plots (6 sampling points for each treatment and control plot) with samples taken furtherest from the disease front first. Holes were dug using a Dormer™ 80 mm sand auger and a sub-sample (200 - 600 g) of soil for each sample were collected at four depths per sampling point: 0 - 25, 25 - 50, 100 and 150 cm. Roots were hand picked from soil at each horizon and included in the retained soil samples. Between each sample collected (including the 4 from each hole) the auger and extension bars were cleaned free of soil by hand (brushed where necessary). At the end of each line of three sampling points within plots (0.5 and 2.5 m), and between plots, augers and other equipment were surface sterilised by total immersion in methylated spirits and then air-dried. Samples, were mixed by hand in plastic zip lock bags and stored at air temperature during transport and at 4 - 6ºC during storage prior to processing, which was commenced within 5 - 7 days of collection. To reduce the probability of introducing *Phytophthora* from upper horizons into lower soil horizons during sampling, holes were refilled after sampling in the reverse order of their extraction.

Recovery of *P. cinnamomi*

Methods used to recover *P. cinnamomi* from samples were modified between some of the initial and later assessments. In this experiment, although we hoped to demonstrate that we had eradicated *P. cinnamomi*, we also needed to be able to measure any decline in the pathogen. Therefore, in the initial assessments, we chose to use methods designed to provide a quantitative value of the amount of *P. cinnamomi* in each sample. For the last four assessments we used methods that produce absence/presence data only. In total, four recovery methods were used.

P. cinnamomi *recovery method 1 – direct plating*
A measure of *P. cinnamomi* population density index (PDI) for each soil sample was attempted by direct plating onto selective media after the method described by D'Souza *et al.* (2004) and then using a soil serial dilution method (after Marks and Kassaby 1974) using a combined sample of soils from each horizon by plot (four samples per plot).

Direct plating was discontinued as a method of assessment after the first attempt because of the confounding effects of the very large numbers of propagules of soil fungi other than *P. cinnamomi* that were present in soil samples from the site.

P. cinnamomi *recovery method 2 – serial dilution*
To gain qualitative and quantitative data, the serial dilution method (after Marks and Kassaby 1974) was used to recover *P. cinnamomi*. Briefly, a series of 7 sub samples of 1, 2, 4, 8, 16, 32 and 64 g of soil were taken from each sample and submerged in 100 mL of water in a polystyrene cup (*n* = 2354 sub-samples per soil sampling and harvest). To each cup, 3 - 4 petals of *Rosa* sp. and/or *Hibbertia* spp. petals were floated on the water. When the petals became water soaked (a sign that they were potentially infected with *Phytophthora*), the petals were rinsed in deionised water, blotted dry and plated onto NARPH medium, and incubated at 23°C and checked after 48 - 72 h and again at 6 days for growth of *P. cinnamomi.*

P. cinnamomi *recovery method 3 – soil baiting 1*
With the aim of increasing the probability of detecting the presence of *P. cinnamomi*, and reducing the total number of samples handled, the baiting strategy was modified. Firstly, duplicate sub-samples of 128 g each, from each primary soil sample were submerged in deionised or distilled water and baited with petals in 500 mL plastic containers. Where baiting recovered *P. cinnamomi* from either one or both sub-samples, the remainder of the original soil sample was serially diluted (1 - 64 g sub samples) and baited to obtain an estimate of inoculum potential, as described for recovery method 2.

P. cinnamomi *recovery method 4 – soil baiting 2*
For each sample, 130 - 150 g of soil including roots was placed in a plastic container with ± 300 mL distilled water. Five *Lupinus angustifolius* seedlings (2 - 4 days after germination), were floated on the surface on a perforated plastic raft. Between 4 and 5 days after baiting, symptomatic and asymptomatic *Lupinus* seedlings were removed from each container/sample, blotted dry, cut into sections and plated onto NARPH selective medium, and incubated at room temperature. The temperature was maintained at 18 – 28°C for the duration of baiting and plating. Isolation plates were examined periodically for up to 7 days for the presence of *P. cinnamomi* using a compound microscope, and *P. cinnamomi* was identified by micromorphology. Primary isolates of *P. cinnamomi* were selected from each plot for the recovery of pure cultures of the pathogen. Every negative sample was rebaited with lupin or *Eucalyptus sieberi* cotyledons, or juvenile *Quercus ilex* leaves 7 days after the first round of baiting. A modification of the methods described by Chee and Newhook (1965) was used for assessments 3 to 5 (July and November 2007, mMrch 2008).

Complete usable sets of recovery data from untreated control and treatment plots were

compiled for September 2006, December 2006, and after all treatments had been applied, in July and November 2007, and March 2008.

Statistical analysis

For each subject (plot) and for each assessment, the numbers of recoveries of *P. cinnamomi* (dependant variable) were summed for each soil depth and sampling distance from the disease front within each plot (possible values 0, 1, 2, 3, 4 or 5).

Data were analysed using analysis of variance. The design for the experiment involved four factors (treatment, distance from disease front, soil sampling depth and time (harvest)) corresponding to a repeated-measures design. The spatial component of the design was partly nested, with distance from disease front (0.5 and 2.5 m) and soil sampling depth (0 - 25, 25 - 50, 100 and 150 cm) within subjects (plots) as factors.

Data analysis was performed using Statistica (version 5 for PC; Statsoft Inc., Tulsa, OK, USA). For all analyses, the assumption of normality was examined by examining residuals by probability plots.

Within the raw data there were too many null values (0) to satisfy the assumptions for analysis of variance. To reduce the proportion of null values (0), adjacent control and treatment plots were treated as pairs (i.e. 1 - 2, 3 - 4, etc.). Data were transformed by subtracting the numbers of recoveries for each line and soil horizon at each harvest within treated plots from the values for the corresponding points within control plots.

Using the methods described by Davison and Tay (2005), estimates for the probabilities that *P. cinnamomi* was not detected in treatment plots (and that the treatment plots were pathogen free) were calculated for assessments 3, 4 and 5, where *P. cinnamomi* was not recovered. The mean frequency of isolation from untreated control plots, within assessments was used to calculate the probability (P) of all samples within treated plots being negative using the formula:

$$P = (1-r)^n$$

where r is the proportion of samples from which *P. cinnamomi* was isolated (in untreated control plots), and n is the sample size (number of samples taken in treated plots, including both distances from the disease front and soil horizons.

RESULTS AND DISCUSSION

Disease progression

The mean rate of disease progression within the site was estimated over the last 4 - 5 years (2001/2 - 2006) to be 2.2 m/year (SD = 0.6 m; *n* transects = 6). This was calculated from node numbers on dead *Banksia attenuata* and *B. baxteri* (Wills 2003). A comparison of the distance between sites of deaths throughout 2006 and 2007 strongly suggests that the rate of disease progression was less than 1 m/year since the beginning of 2006, and this may be related to the below average rainfall for 2006, and/or the pattern of rainfall distribution.

A plan of the disease front was derived from the location of dead plants of susceptible species (Figure 15).

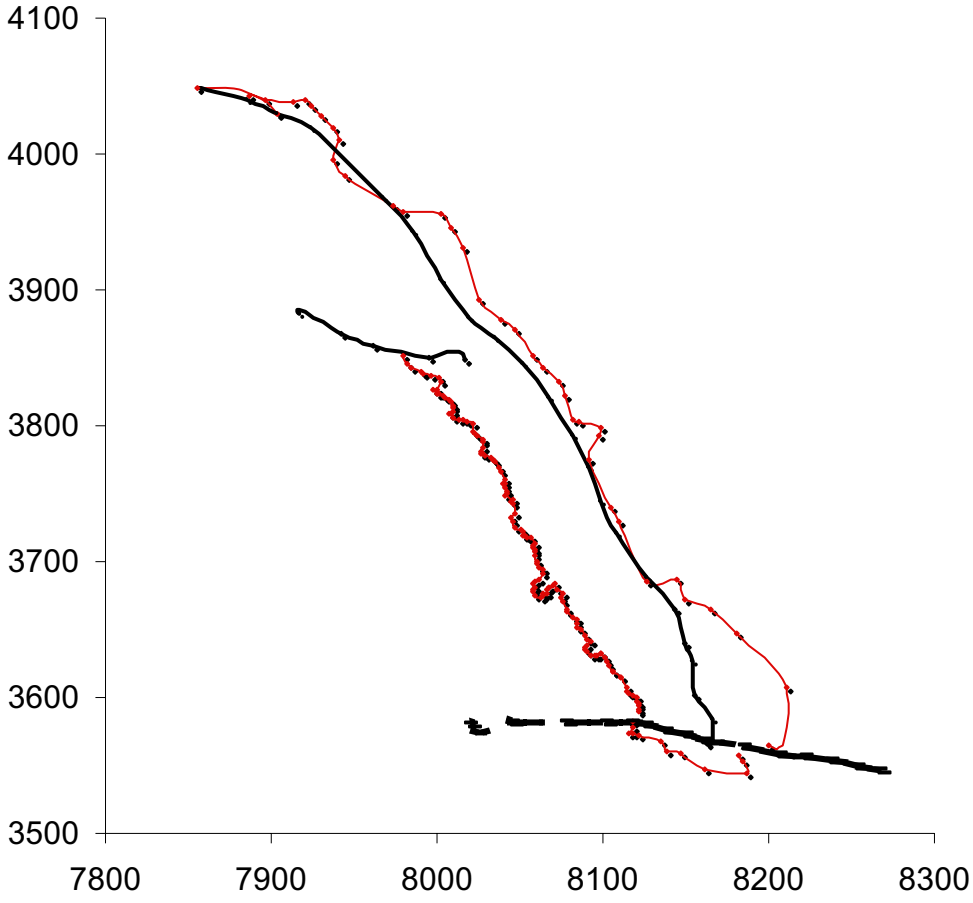


Figure 15 Plan of *Phytophthora cinnamomi* infestation at Cape Riche, Western Australia experimental site as at February 2006. Scale of x and y axes are in m. Points surveyed by GPS to a resolution of 0.6 - 0.8 m (experimental site) and < 5 m for remainder of the site. Black = tracks; red = disease front. Grid scale in metres and values correspond to Geocentric Datum of Australia 1994 (GDA94).

Recovery of *Phytophthora cinnamomi*

Using the direct plating method (D'Souza *et al*. 2004), only 5 recoveries (2%) of *P. cinnamomi* were made from 288 samples. For assessing the presence/absence of *P. cinnamomi* and assessing inoculum potential, direct plating proved to be ineffective in gaining any useful measure of PDI from the experimental site, or even qualitative (absence/presence) data. Where *P. cinnamomi* was recovered, counting colonies was difficult because of the presence of abundant colonies of fungi (and sometimes bacteria) other than *P. cinnamomi.* Large numbers of contaminants were usually recovered from the two upper horizons sampled. Other media that include pimaricin, more selective for *P. cinnamomi* (PARPH; Erwin and Ribeiro 1996), could be tested if the additional expense of some components (particularly pimaricin) was not important. The method was not used in further assessments of soil from Cape Riche, and only standard baiting techniques (previously described) were used thereafter.

Recovery of *P. cinnamomi* from the WA experiment (July 2006 - March 2008) is shown in Figure 16. The pathogen has not been recovered from any of the plots that received the complete treatment regime of fungicide + fumigation + vegetation removal in the last three assessments between July 2007 and March 2008 (*n* pathogen free samples = 504). In addition, in treated plots at 2.5 m, no recoveries of the pathogen were made at the previous harvest (December 2006) after vegetation clearance had been completed and two applications of Terrazole (Figure 16B).

A trend in increasing recovery of *P. cinnamomi* in untreated plots, at 2.5 m from the original disease front (Figures 16A - E), showed that the pathogen was active and the infestation was expanding in untreated plots. Surface treatments with Terrazole had no significant effect on recoveries of *P. cinnamomi* (recoveries at 0.5 m in treated plots, Figures 16A and B). There were no recoveries of *P. cinnamomi* from any of the treated plots after Ridomil treatments and surface injection with metham-sodium (Figure 16C), or after the additional deep treatment with metham-sodium (Figure 16D). There were no recoveries from the 0.5 m mark in treated plots in the last three assessments (Figures 16C, D and E), in contrast to the previous harvest where recoveries of *P. cinnamomi* at the same distance in treated plots were similar to untreated controls (Figure 16B).

The pattern of a rapid decline in the frequency of recoveries of *P. cinnamomi* with increasing soil depth in control plots (Figure 16A - E) is similar to the pattern found by Hill *et al*. (1995) in a Banksia woodland.

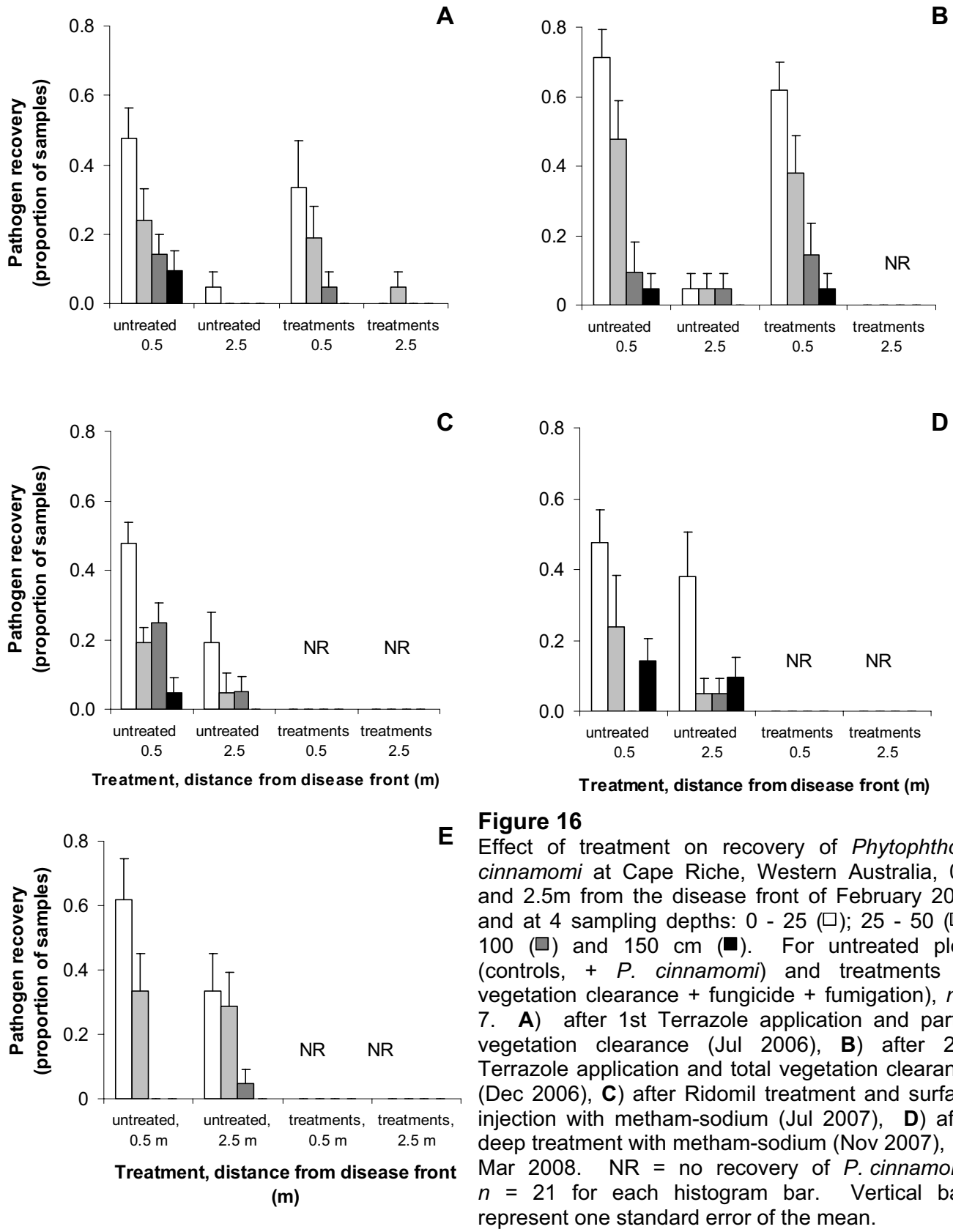


Figure 16
Effect of treatment on recovery of *Phytophthora cinnamomi* at Cape Riche, Western Australia, 0.5 and 2.5m from the disease front of February 2006 and at 4 sampling depths: 0 - 25 (□); 25 - 50 (▒); 100 (▓) and 150 cm (■). For untreated plots (controls, + *P. cinnamomi*) and treatments (+ vegetation clearance + fungicide + fumigation), *n* = 7. **A**) after 1st Terrazole application and partial vegetation clearance (Jul 2006), **B**) after 2nd Terrazole application and total vegetation clearance (Dec 2006), **C**) after Ridomil treatment and surface injection with metham-sodium (Jul 2007), **D**) after deep treatment with metham-sodium (Nov 2007), **E**) Mar 2008. NR = no recovery of *P. cinnamomi.* *n* = 21 for each histogram bar. Vertical bars represent one standard error of the mean.

In the July 2006 harvest, 34 samples across all plots yielded *P. cinnamomi* (9%), in contrast to 56 (15.3%) recovered from the November - December harvest. No direct comparison between results can be made because of the differences between methods. However, the following observations were made:

1. There was a trend at both assessments for less recovery of *P. cinnamomi* from treated plots, but the difference is probably not statistically significant, and the trend is for an increase in the proportion of recovery in the treated plots from harvest two to harvest three (in comparison with the control plots). The effect of fungicide application may have been transitory, with suppression of the pathogen rather than destruction occurring. In order to minimise soil disturbance, the fungicide (Terrazole) was applied to the soil surface only, and was reliant on rainfall to ensure infiltration. Between the first application of fungicide and the first sampling, there was 130 mm of rainfall (at Wellstead, the nearest weather station) with a maximum of 14 mm in a 24 h period, and only 3 days where rainfall exceeded 10 mm. Rainfall data are unavailable for the site, but if the Wellstead figures are an indication, then conditions were not favourable for maximising the effectiveness of the fungicide.

2. In the third assessment, only 50% (28/56) of the samples that were positive for *P. cinnamomi* using double baiting (*P. cinnamomi* recovery method 4) with larger samples were positive in a following serial dilution of the same samples aimed at estimating inoculum potential. To demonstrate eradication of *P. cinnamomi,* assessment must maximise the probability of recovering *P. cinnamomi*. The same information was gained for less effort using double baiting with the larger samples. Also the value of estimates of PDI must be critically reviewed as in future assessments the number of samples to be handled could approach 3300 at each harvest necessitating a 9-fold increase in material resources and people (ca. 700 h labour).

3. In the treated plots, in assessments two and three, only one sample of 336 taken from the line of sampling points 2.5 m from the disease front was positive for *P. cinnamomi.* Given this result and the low rainfall at the site between November 2006 and March 2007, the placement of the HDPE barriers at approximately 4 m from the visible disease front (as surveyed in February 2006) was correct in so far as placement of barriers was into clean (uninfested) soil.

4. Across all assessments there was a consistent trend for a decline in recovery of *P. cinnamomi* with increasing depth in the soil profile (Figure 16). There are two possible explanations for these observations: 1) in close proximity to the disease front *P. cinnamomi* is confined to the upper soil horizon/s, where most of the fine roots occur; or 2) the soil sample size used in baiting is not sufficiently large to detect the presence of *Phytophthora* at depth where there may be little inoculum present, or where root density is low and distribution of the pathogen is more heterogeneous. By ensuring that good numbers of roots were included within each sample, we increased the probability of recovering the pathogen, and the observed trend is probably a real effect.

5 There does not appear to have been any cross contamination between samples collected from upper horizons and those collected lower in the soil profile. Soil sampling procedures appear to have been sound. The soil type (well-drained sands) greatly simplified hygiene procedures.

   From the initial grid sampling of treatment plots, 16 of 105 samples yielded *P. cinnamomi* by baiting. Fourteen of 16 recoveries were from the disease front (as indicated by dead plants) and two isolations were made at points (2 and 4 m) forward of the front in asymptomatic vegetation.

6 From assessments one and two where soil dilution was used to gain estimates of PDI for *P. cinnamomi* recoveries of *P. cinnamomi* were made from nine samples comprising 1 g of soil. This reinforces the requirement for compliance with hygiene measures when operating within the site.

7 In three consecutive assessments, July and November 2007, and March 2008, no recovery of *P. cinnamomi* was made from treated plots. In contrast, there was a trend for increasing numbers of recoveries in the control plots.

Analysis of variance showed highly significant effects of time (time after treatment) and soil depth on recoveries of *P. cinnamomi* (Table 3). There was also a highly significant time x soil depth interaction in effect on recoveries at different soil horizons, with the difference between control and treatment plots being greatest in the upper two horizons. The difference is probably due to the much fewer recoveries at the lower soil sampling depths (1 m and 1.5 m) across all assessments (Figures 16A - E).

Table 3 ANOVA summary of all effects: differences in recoveries of *Phytophthora cinnamomi* between untreated and treated plots, Cape Riche. Distance = distance from disease front, Time = time of harvest, Depth = soil depth.

	Effect	Effect	Error	Error	F	p-level
	df	MS	df	MS		
Distance	1	3.432143	12	1.295238	2.64982	0.129515
Time	4	3.625000	48	0.470833	7.69912	**0.000071**
Depth	3	8.146428	36	0.523810	15.55227	**0.000001**
Distance, Time	4	0.450000	48	0.470833	0.95575	0.440356
Distance, Depth	3	0.584524	36	0.523810	1.11591	0.355411
Time, Depth	12	1.301190	144	0.366071	3.55447	**0.000122**
Distance,Time,Depth	12	0.197619	144	0.366071	0.53984	0.885624

Estimates of pathogen free status

For a single assessment of treatment plots, the probability of not detecting *P. cinnamomi* in treatment plots at any of assessments 3, 4 or 5 in the upper soil horizon ranged from 1.6 x 10^{-12} - 8.7x10^{-9}. Consequently, the probability that we failed to detect the pathogen was extremely unlikely.

Operational matters

Baiting efficiency

Lupin (*Lupinus angustifolius*) was used in most assessments. One advantage of using lupins as the bait is that they are easily obtained and seedlings are produced rapidly. Suitable plant material, germinated seeds with radicles 1.5 - 3 cm long, could be produced in 48 - 72 h, and seed germination was always close to 100%. Another advantage is the baits can be produced under semi-sterile conditions, and have a short turn around time (compared with, for example, *Eucalyptus sieberi* cotyledons which require 3 - 4 weeks before suitable plant material is available for use), meaning that the potential for contamination with other microorganisms that could interfere with recovery of *P. cinnamomi* was greatly reduced.

In contrast to other baits (petals, leaves and cotyledons), visual symptoms of infection by *P. cinnamomi* in lupin roots were often less obvious and the pathogen was frequently recovered from asymptomatic roots. However, in contrast to other baits, recoveries of Eumycota and Oomycetes other than *P. cinnamomi* (principally *Pythium*) were markedly reduced in lupin.

Marks and Kassaby (1974) and Greenhalgh (1978) achieved better recovery of *P. cinnamomi* using *Eucalyptus sieberi* cotyledons than lupin radicles. In our assessments we did not do a direct comparison between the two, but where soil samples were rebaited, alternate baits (including *E. sieberi* cotyledons, *Rosa* sp. petals, juvenile leaves of *Pittosporum* sp. and *Quercus ilex*) yielded not more than 10 - 12% in additional recoveries of *P. cinnamomi* above those in the first round of baiting using lupins only. Further, Davison and Tay (2005) using *E. sieberi* cotyledons as baits, concluded that their results were similar to those obtained by others in the 1960's and 70's using lupin baits. At Cape Riche, the overall mean recovery rate in untreated control plots, from all soil horizons and all five main assessments, was 16.8% and ranged from 55% in soil horizon 0 - 25 cm (all assessments) to 6.7% in 1.5 m depth (all assessments). Within individual assessments, at assessments 4 and 5 there were no recoveries of *P. cinnamomi* at the 1.5 m and/or 1 m soil horizons. At Narawntapu NP, the overall recovery rate in control plots was 30.1%, ranging from 27.4 - 33.7% across the three sampling horizons. Within assessments, the lowest mean recovery rate was 17.1%, and the highest 42.9%

In both experiments, at Cape Riche and Narawntapu NP the recovery rates compared very favourably with published results from other studies. Davison and Tay (2005) achieved a mean recovery rate of 7.2% (jarrah forest; systematic sampling within 5 m of dieback margins). Podger (1968) had a recovery rate of 10.5%, and McDougall (1996) 25%, both under similar vegetation and soil conditions to Davison and Tay (2005). Higher rates of recovery have been reported by D'Souza *et al*. (2001) and Davison and Tay (2005), but sampling in their studies was much more targeted (symptomatic plants and associated soil) than the sampling in this study.

Treatment with Terrazole was ineffective in controlling *P. cinnamomi.* The method of application of the fungicide was chosen in preference to other methods that would result in more soil disturbance in order to reduce the risk of spreading the pathogen. Rainfall post-treatment was highly unfavourable to infiltration of the fungicide, and the results are not a fair test of its potential. A progressively more severe regime of treatments was applied after the Terrazole applications. In addition to further applications of fungicide, shank injection of fumigant, and deep 'injection' of fumigant (metham-sodium) to ca. 1 m were completed in 2007.

As part of plot treatments, vegetation removal may have had some unintended consequences. Removal of larger shrubs appears to have had a detrimental impact on the survival of plants adjoining the cleared strips, possibly by increasing wind exposure. Vegetation removal also had some impact on soil water. There is a strong trend for gravimetric water content within soil horizons in treated plots to be greater than water content in similar horizons within untreated plots (Figure 17).

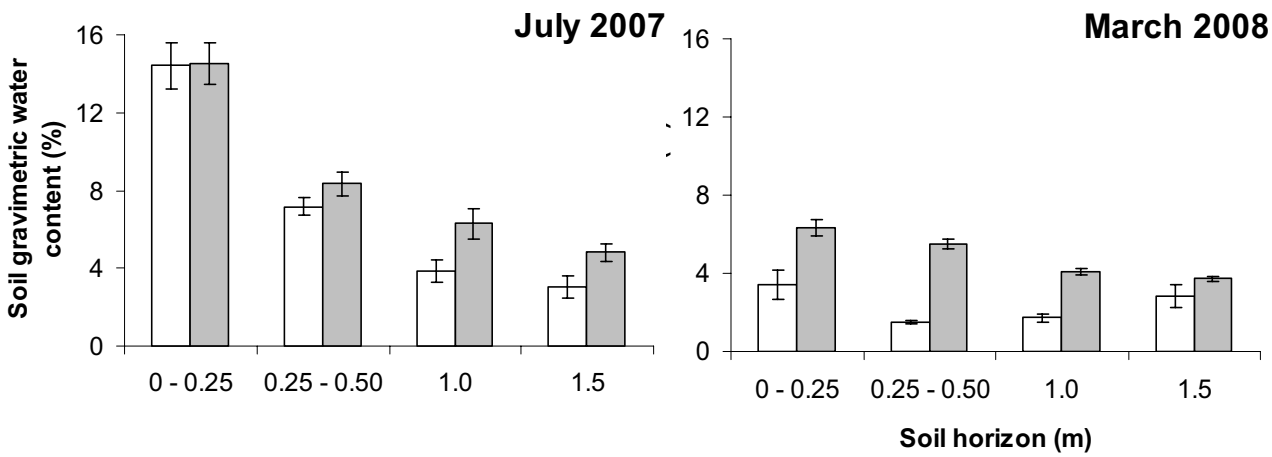


Figure 17 Soil water content in July 2007 and March 2008 at Cape Riche, Western Australia, of control plots (□) and treatment plots(▯).

Increased soil water may make conditions more favourable for *P. cinnamomi*, however it is just as likely that conditions are equally favourable to potential competitors and antagonists of the pathogen. Further, in addition to increased soil water, treatments with herbicides, fungicides and fumigants added macronutrients to the soil in organic form.

Estimates of elemental additions from chemical applications, not including those in wetting agents and carriers, were: C, 42 g/m^2; N, 34 g/m^2; P, 1.2 g/m^2, and S, 143 g/m^2. Although no analyses of nutrients in soil were done post treatment, given the low levels in soils before treatment (Table 2) the additions are probably very significant.

Increased soil water may be advantageous in some circumstances, specifically where seasonal application of fungicides or fumigants may be brought forward because it takes a

shorter time for soils to wet up.

The risk of wind erosion has also been increased, although there was no measurable effect at 18 months after site clearance. Given that most pathogen recovery was made from the topsoil, there is an unknown risk of transport of pathogen inoculum off site in wind borne fine particulate matter if wind erosion were to occur. Application of surface stabilisation agents of some kind may have to be considered in the future, and would certainly be necessary if the site was burnt. In addition, there is some evidence (tracks and scats) of increased animal (kangaroo) activity within and adjacent to treated plots, that may have become more attractive for loafing and/or grazing animals. If animal activity increases further, exclusion fencing may be required to deny access across the disease front within treatment plots.

Residue tests

Residual fungicides and/or fumigant in soils could interfere with detection of the pathogen when using baiting, by suppressing the pathogen or by interference with the infection process. A qualitative seed germination test, using garden cress (*Lepidium sativum* cv. 'curly leaf') that is highly sensitive to MITC, was used to test for the presence of fumigant residues in soil samples (0.5 m and 1 m depths) from both control and treated soils from the last harvest at Cape Riche (*n* = 10 samples per treatment). Results from the germination test indicated that there were no MITC residues in treated soils.

To further test for interference by chemical residues in the recovery of *P. cinnamomi* when using baiting methods, a sub-sample of treated and untreated soils from horizons 25 – 50 cm and 100 cm (Cape Riche) were rebaited with lupin and were also inoculated with an isolate of *P. cinnamomi* that had been previously recovered from the site (isolate CR30). There was no difference in recovery rates of *P. cinnamomi* between treated and untreated soils (> 95% in both), but recovery of the pathogen appears to have been delayed or slightly inhibited in treated soils. It is unlikely that the observed effect is directly related to either fungicide or fumigant residues because the average half-life of Metalaxyl-M in soil is fairly short ($t_{1/2}$ = 77 days, range 27 - 296 days, http://www.ars.usda.gov). Furthermore, the half-life of MITC (the active breakdown product of metham-sodium) under similar conditions is also short ($t_{1/2}$ range 3.5 - 35 days, Dungan and Yates 2003, Zhang *et al.* 2005).

In the current experiment, the natural progression of *P. cinnamomi* appears to have been stopped and the pathogen has apparently been eliminated, at least close to the disease front. In contrast to Hill *et al*. (1995), who used only one chemical treatment (Metalaxyl-M or formaldehyde) in combination with herbicide treatment, we used multiple treatments of a fumigant in addition to other fungicide treatments. Furthermore, the largest individual plants in their study site (including root systems) were very much larger than those in this study. Additionally, in a previous experiment where a precursor to MITC was used by other researchers in an attempt to control *P. cinnamomi* (Weste *et al*. 1973), certain site characteristics and events eventually conspired to render the treatment ineffective.

In conclusion, a combination of vegetation removal, herbicide use to ensure *P. cinnamomi* has no living host material to invade, fungicide and fumigant treatments were effective in eradicating the pathogen from the Cape Riche treatment plots for the duration of the

study. The installation of the heavy-duty root barriers will further facilitate keeping these sites disease-free in the future. These results are extremely promising in terms of protecting key plant species and plant communities from *P. cinnamomi.* However, it is critical that the Cape Riche plots are reassessed at least twice more (at 12 and 24 months) to confirm that the pathogen has been completely eradicated.

Sub Project 19.3.a(ii)
Eradication of *Phytophthora cinnamomi* from spot infections in native plant communities in Tasmania

Sub Project 19.3.a(ii)
Eradication of *Phytophthora cinnamomi* from spot infections in native plant communities in Tasmania

INTRODUCTION

Narawntapu NP stretches from the low coastal ranges to the shores of the Bass Strait from the Port Sorell estuary in the west to the mouth of the Tamar River in the east between Devonport and Low Head on the central north coast of Tasmania. The vegetation at this site is open eucalypt woodland with a heathy understorey on a Podosol soil.

Active *Phytophthora cinnamomi* infestations are widespread within the National Park. Based on the extent of individual infestations, the pathogen has been present for some time.

Aims

In this project we aim to contain the pathogen within active disease centres in a larger complex of disease fronts, and then to reduce the population of the pathogen to extinction. Four strategies that were tested to contain the pathogen, reduce potential food sources and directly attack the pathogen were:

1. The removal and/or destruction of plant species with large lateral root systems. This will reduce the potential for comparatively long distance transmission of the pathogen.

2. Herbicide treatment to kill remaining vegetation. This will reduce available food sources for the pathogen. We know the pathogen is a poor saprophyte, consequently we will be removing a living food source and the pathogen will have to rely on survival as resting spores.

3. Use of fungicide treatments, to the kill pathogen, comprised of alternate applications of two fungicides effective against *P. cinnamomi* and a fumigant.

4. Establishment of physical barriers made of high-density polyethylene with a sub-surface irrigation system to enable application of fungicide and/or soil fumigant below the effective depth of physical barriers. This will greatly reduce the potential for root-to-root transmission of *P. cinnamomi.*

METHODS

Site selection

The following is a summary of potential experimental sites that were visited, site characteristics and the preliminary works undertaken:

Dark Creek, in the northwest of Tasmania, is 8 km southwest of the junction of the Frankland and Arthur Rivers, and about 40 km southwest of Smithton. The site was located in moorland surrounded by wet eucalypt forest, in an area of high rainfall (1300 mm/yr). The *Phytophthora* infestation was active and fairly well defined, and appeared to have been initiated as a result of road works by the transfer of material from

an old infestation in an adjacent watershed. The site was eliminated for three reasons:

- given the site conditions, the rapid rate of disease progression was likely to make planning and set-up of experiments difficult;
- the site was remote from services; and
- there was a likelihood of seasonal difficulties in access.

Rocky Cape National Park is on the northwest coast and contains extensive and active infestations of *Phytophthora* in heathland. Steep terrain combined with the soil type would have made operations hazardous and difficult to set up experiments.

Hunter Island lies 6 km off the north western tip of Tasmania and is 7330 ha. Inspection showed it to be unsuitable for experimental sites. Several new infestations of *P. cinnamomi*, in addition to most of the ones previously reported, were visited. Infestations were active, but were much larger than had been previously indicated. The slipway at Cave Bay was in a derelict condition, there was an absence of suitable landing points near the road head, the main airstrip was in poor condition and tracks were overgrown. This would mean that most of the materials and machinery, required to set up any experiment, would have to be to be slung, and transported by, rotary wing aircraft, directly to the experimental site. In addition, depending on the time of year, up to a week would have to be allowed either side of transit time (by sea or air) to allow for bad weather. *P. cinnamomi* was recovered from samples taken from infested sites, and some initial plant surveys were also undertaken.

Waterhouse Conservation Area is located in the north east of Tasmania and comprises 6953 ha of coastal heaths, marrams and forests. The eastern part of the area was visited on 6 occasions during 2006. *P. cinnamomi* had been recovered from various locations within the Area in the past (Tim Rudman, *pers. comm.*). Some small infestations of *P. cinnamomi* were identified but had to be discounted because of poor site security and/or proximity to wetlands, which would have precluded the use of some chemical treatments. Two sites, where recent deaths of *Xanthorrhoea* spp. and other signs indicated the presence of *P. cinnamomi,* were systematically sampled in an attempt to confirm the presence of the pathogen. Potential experimental plots were surveyed and marked out at both sites. In addition, plant surveys of each plot (at both sites) were also completed, along with most of the process of gaining approvals for research activities within the area. *P. cinnamomi* was never recovered from either site and, eventually a *Fusarium* sp. was identified as the probable cause of the deaths in *Xanthorrhoea* spp. The site was abandoned in October 2006.

Two surveys of a potential site were carried out in late 2006 and early 2007 in Narawntapu National Park (formerly Asbestos Ranges National Park). Further work at the site was not undertaken until *P. cinnamomi* was confirmed to be present in most of the potential experimental plots. Site characteristics, good site security, accessibility and proximity to services made the site suitable for our requirements.

Site description - Narawntapu National Park

Narawntapu National Park (NP) stretches from the low coastal ranges to the shores of the Bass Strait from the Port Sorell estuary in the west to the mouth of the Tamar River in the

east between Devonport and Low Head on the central north coast of Tasmania.

In Narawntapu NP, *P. cinnamomi* is causing a continuing decline of *Xanthorrhoea bracteata*, which is listed as Endangered by the Australian *Environment Protection and Biodiversity Conservation Act 1999*, and vulnerable under the Tasmanian *Threatened Species Protection Act 1995*. A National recovery plan for threatened Tasmanian Grasstrees (2006 - 2010) has been implemented to address recovery objectives and actions necessary to ensure their long-term survival (Threatened Species Section 2006). The Park includes coastal heathlands and wetlands rising to forested hills and ranges inland. In much of the Park, biodiversity is high and in a relatively natural state (Parks and Wildlife 2000).

The soil type, climate and general vegetation type within the experimental site are representative of conditions across the north eastern and eastern coast of Tasmania where many infestations of *P. cinnamomi* occur, and where the control methods described could be applied.

Location
The experimental site is located within Narawntapu NP, on the north central coast of Tasmania (Figure 18 and 19), approximately 25 km east of Devonport and 20 km west of the mouth of the Tamar River (Port Dalrymple).

Figure 18

The location of *Phytophthora* eradication site, Narawntapu National Park, Tasmania. Area marked by red arrow.

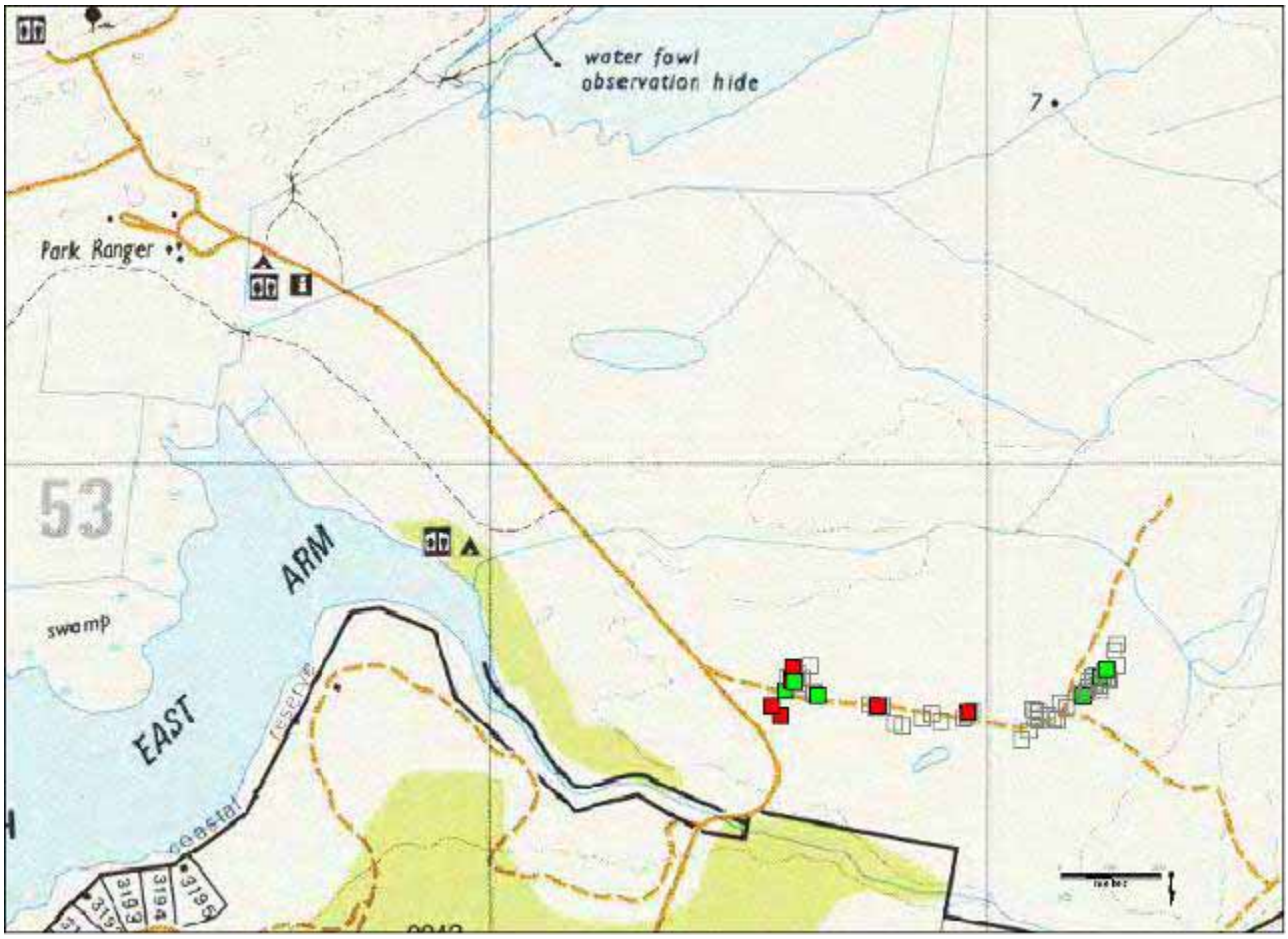


Figure 19 Location of the *Phytophthora* eradication site, Narawntapu National Park, Tasmania. Red and green squares indicate recoveries of *P. cinnamomi* in initial surveys of the site. Open squares indicate vegetation showing signs of infestation with *Phytophthora* (T Rudman). Map: Port Sorell 1:25 000 sheet 4644 Edition 2, Tasmap, Hobart, Tasmania.

Geology and soils

The study site lies within a unit of Quaternary deposits of (possibly aeolian) quartz sands (map unit Ts, on the 1:250 000 Launceston sheet; Tasmanian Geological Survey, 1974), that is bounded by more recent Quaternary alluvial deposits to the north and south, and the Mesoproterozoic sedimentary rocks of the Dazzler Range to the East. The experimental plots are located on the berm of an ancient dune system that has an east-west trend that is bordered by areas of seasonally inundated ground to the north and south. The altitudinal range between plots is not more than 3 - 5 m (approximately 12 - 15 m AMSL) rising from west to east.

Soils across the study site, determined from soil sampling within experimental plots (for recovery of *P. cinnamomi*) and soil pits, can be classified as Aeric (or Semiaquic) Podosol with a pipey B horizon, after the Australian soil classification (McKenzie *et al*. 1999, Isbell 2002). The pipey Bh horizon intersected 70 cm (60 - 80 cm) from the soil surface. In 4 of 15 potential experimental plots initially surveyed, lacustrine gravel to cobble sized quartz fragments were recovered within 70 - 80 cm from the soil surface. A soil profile (Figure 20) and selected soil physical and chemical characteristics (Table 4) are provided.

Figure 20

Typical soil profile, *Phytophthora cinnamomi* control site, Narawntapu National Park, Tasmania.

Depth of profile shown is 60 cm, showing tongues of variably developed 'coffee rock' within the B horizon.

Photo: WA Dunstan

Table 4 Soil characteristics of *Phytophthora cinnamomi* containment and eradication study site, Narawntapu National Park, Tasmania. Values are for bulked samples from across the site (n = 6 for A_1 and A_2 horizons and n = 4 for Bh horizon).

		(Horizon) / depth (cm)		
	Unit	**A_1**	**A_2**	**Bh**
NITRATE N	mg/kg	1	1	1
AMMONIUM N	mg/kg	1	1	1
TOTAL N	%	0.17	0.13	0.75
P COLWELL	mg/kg	2	2	2
P OLSEN	mg/kg	1.8	0.7	0.6
TOTAL P	mg/kg	34	37	29
POTASSIUM	mg/kg	25	26	44
SULPHUR	mg/kg	2.4	2.2	4.5
ORG CARBON	%	1.45	0.54	0.83
IRON	mg/kg	59	46	2712
CONDUCTIVITY	dS/m	0.027	0.011	0.017
pH $CaCl_2$	pH	3.6	3.8	4.4
pH H_2O	pH	5	5.1	5.3
DTPA Cu	mg/kg	0.19	0.2	0.1
DTPA Zn	mg/kg	0.18	0.15	0.14
DTPA Mn	mg/kg	7.47	1.38	1.31
DTPA Fe	mg/kg	24.69	13.86	56.34
P RETEN	index	0.5	1.1	140.1
EXC Ca	meq/100 g	1.38	0.37	0.23
EXC Mg	meq/100 g	0.42	0.17	0.16
EXC Na	meq/100 g	0.07	0.06	0.06
EXC K	meq/100 g	0.1	0.07	0.08
ALUM $CaCl_2$	mg/kg	1.3	1.7	13.8
B HOT WATER	mg/kg	0.5	0.3	0.3
EXC Al	meq/100 g	0.17	0.13	0.75
CHLORIDE	mg/kg	13	19	15
COLOUR		grey	grey	brown
Coarse sand	200-2000μm	12.8	19.4	7.8
Fine sand	20-200μm	81.6	74.4	80.5
Silt	>2-<20μm	1.9	4.7	5.1
Clay	<2μm	3.8	1.5	6.6
sand	20-2000μm	94.4	93.8	88.3

Soils at the site are deficient in macro- and micro-nutrients, except for the high concentration of iron in the Bh horizon. In this soil, there are no impediments to internal drainage and there would be little or no lateral movement of soil water above the water table. Given the type of soil, *P. cinnamomi* is unlikely to spread by the dispersion of zoospores within the A and upper B horizon, but the potential for this to occur at depth is unknown. Sampling in the vicinity of plots H and N, (mid and eastern extremity of the site) showed that the sandy podosolised profile continues to depth. The water table was reached at 3 m (plot H) and approximately 2 m (plot N).

Vegetation
The vegetation within the experimental site is Low (M) eucalypt woodland (after the classification system of Gillison and Walker 1981), that is dominated by *Eucalyptus amygdalina* with scattered *E. viminalis* of poor form as a co-dominant. The understorey is comprised of scattered mature *Banksia marginata* and scattered to dense *Xanthorrhoea australis* in heath. This vegetation type is classified as *E. amygdalina* coastal forest and woodland (mapping unit DAC; Tasveg) in Harris and Kitchener (2005).

Prior to 1974, when land tenure was changed to National Park, the area was used as rough grazing for livestock, and common bracken (*Pteridium esculentum*) has probably invaded and out-competed many other native shrubs and herbs. The area appears not to have been burnt by controlled fuel reduction burn or wildfire since at least before 1974 (A Timmerman, *pers. comm*.). A more complete list of plant species recorded from within individual experimental plots is included within the vegetation report for the experimental site (Table IIIa, Appendix III). Approximately 29% of plant species listed in Table IIIa are susceptible to *P. cinnamomi.*

Climate
The site lies within the temperate zone, with a warm summer and cool winter. Until recently (2006), records of rainfall and air temperature have not been available for Narawntapu NP. Figure 21 shows mean monthly rainfall and air temperature data for the two closest Bureau of Meteorology stations to Narawntapu; Devonport Airport (22 km west) and Low Head (18 km north east), both at similar elevation and in close proximity to the moderating influence of Bass Strait.

Rainfall data for Narawntapu NP has only been recorded since September 2006, at a location 1.5 km northwest of the experimental site. A comparison with rainfall at the closest Bureau of Meteorology stations and available data from Narawntapu NP show similar results (Table 5), suggesting that historical records for either or both stations are likely to be a reasonable indication of past rainfall and air temperatures at Narawntapu NP.

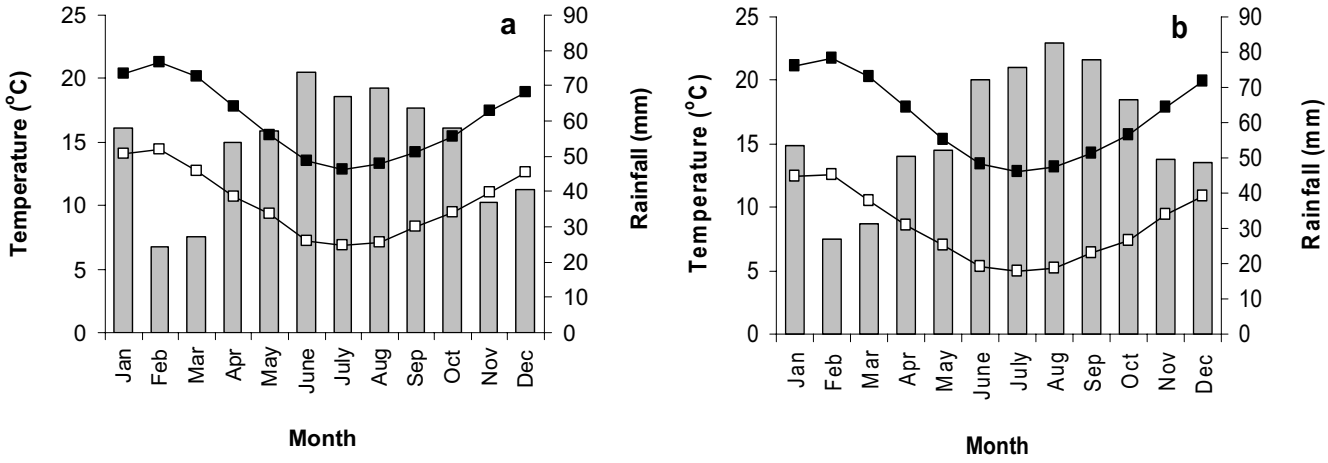


Figure 21 Mean monthly minimum (□) and maximum (■) air temperatures, and mean monthly rainfall (▒) for **a**) Low Head (41°03.20'S, 146°47.08'E) and **b**) Devonport (41°10.15'S, 146°25.36'E) for the years 1998 - 2007.

Table 5 Monthly rainfall: Devonport, Narawntapu National Park and Low Head, Tasmania for July 2006 to Feb 2008. May 2007 data for Narawntapu NP is incomplete – rainfall for 01 - 09 May 2007 only. nd = no data.

	Rainfall (mm) July 2006 – June 2007											
Location	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
Devonport	54.2	18.4	44.7	5.8	10.8	18.1	45.5	16.5	28.3	13.8	20.4	nd
Narawntapu NP	nd	nd	47.2	6.0	nd	21.7	41.5	26.8	28.3	15.2	29.9	nd
Low Head	41.6	19.0	42.4	6.8	12.0	16.6	43.4	25.6	28.3	13.8	20.4	13.2

	Rainfall (mm) July 2008 – Feb 2008							
Location	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**
Devonport	57.7	84.0	63.9	46.0	12.4	89.9	14.8	43.7
Narawntapu NP	53.0	100.8	53.2	32.4	13.2	69.4	21.8	36.9
Low Head	42.2	87.2	63.8	32.6	15.2	57.8	18.0	37.2

The pathogen

Infestations of *P. cinnamomi* within the experimental site and adjacent areas range from fairly well defined 'fronts' that may have originated from vehicle traffic along permanent access tracks and firebreaks, to infestations that are small, discrete and not obviously associated with any disease front. The smaller discrete infestations are most likely to have been initiated by soil disturbance and transport of infested soil by native animals, particularly wombats, as has been observed elsewhere in Tasmania (Podger *et al*. 1990).

Given the soil type and the way disease fronts have developed, the principal means of movement of the pathogen appears to be by root-to-root contact. However, unlike the Cape Riche site in Western Australia, extensive digging by native mammals and the consequent transport of soil from infested to un-infested ground appears to be important in the establishment of new infestations within the site (Figure 22). Narawntapu NP is renowned for its abundance of native mammals. Six of the 11 larger species of terrestrial mammals known to occur within the park (Watts 2002, A Timmerman, *pers. comm*.) could be considered as potential vectors of *P. cinnamomi* because of their digging habits (Figure 22).

A selection of 10 isolates, from most plots across the experimental site, was tested against A1 and A2 mating types (DCE25 and DCE60; supplied by DEC, Western Australia). Oospores were produced only in Narawntapu NP isolates x A1 tests, showing that the Narawntapu NP isolates are of the A2 mating type.

In two preliminary surveys of the site for selection of experimental plots, roots and associated soil from dead and dying *Xanthorrhoea australis* were sampled and tested for the presence of *P. cinnamomi* by baiting with lupin (*Lupinus angustifolius*) and recovery on P_{10}ARPH selective medium (Jeffers and Martin 1986) for the presence of *P. cinnamomi.* Where recovered, fungi could not be positively identified as *P. cinnamomi* by micro-morphology alone. Therefore, roots of bait plants and mycelium from isolation plates were subjected to molecular genetic detection methods (Anderson 2007). Overall, in the preliminary survey, the presence of *P. cinnamomi* was confirmed in 13 of 15 plots surveyed.

Experimental design
After selecting and marking out plots, an assessment of *P. cinnamomi* infestation of each plot was determined by systematic sampling. The plots were treated and the first assessment of the efficacy of treatments (soil baiting and selective isolation of *Phytophthora*) was undertaken 7 weeks after treatment (June 2007). A second application of Metalaxyl-M was made 15 weeks after the first application (August 2007) and a deep treatment with metham sodium to 1 m was completed 19 weeks after the first fumigation treatment (September 2007). Further spot treatments of herbicide were used to destroy remaining vegetation in complete treatment plots, as required.

The experiment was set up as a randomised complete block design with 4 subjects (experimental plots) per treatment. There were 3 treatments:

- positive control - *P. cinnamomi* present, no *Phytophthora* control treatment/s applied;
- fungicide treatment - *P. cinnamomi* present, to be subjected to a regime of fungicide treatments; and
- complete treatments - *P. cinnamomi* present, treated with a combination of fungicide/s, fumigation and herbicide.

A treatment regime of fungicides without the use of fumigation and herbicide, was included as rare or endangered flora are present within an infested area and a completely destructive combination of fungicide, herbicide and fumigation treatments was considered undesirable. A fungicide + herbicide treatment, previously planned for inclusion in

Figure 22

Foraging disturbance by native marsupials, including wombats, at *Phytophthora* control site, Narawntapu National Park, Tasmania.

Burrowing common wombat (*Vombatus ursinus*).

Soil encrusted rear foot of the same animal (in dry conditions), showing the potential of these animals as vectors of *Phytophthora.*

Photos: WD Dunstan

this experiment, was deleted because the combined treatment regime was considered likely to achieve similar results. Plots were 5 m^2, with 4 replicates per treatment (treated plots) and 7 replicates for control plots. A plan of plot locations is shown in Figure 23 with the GIS data for plot locations in Appendix III (Table IIIb).

Plot layout
At Narawntapu NP, disease fronts are markedly less definable than at Cape Riche, Western Australia. Therefore, in this experiment root barriers were used to prevent reinfestation of treated experimental sites by *P. cinnamomi*, rather than as a test of methods to contain a disease front.

Selection of individual plots was partially based on the level of disease expression (presence of recent deaths of indicator plants, principally *X. australis*) and the presence of living plants of *P. cinnamomi* susceptible species, which were likely to sustain a high level of activity by the pathogen over the life of the experiment.

Assessment of *P. cinnamomi* infestation
Prior to the application of treatments, soil in all plots were sampled systematically and assessed for the presence of *P. cinnamomi* by baiting and recovery on selective medium.

Soil sampling
Soil and fine roots were taken from each plot at 3 depths and at 5 points within each plot (*n* samples = 225). Locations of the 5 points were the centre of the plot, and as close to the centre of each quarter of the plot as was possible, depending on the location of large *Xanthorrhoea*. Soil samples were collected by hand using a 76 mm diameter Dormer sand auger (Dormer Engineering, Murwillumbah, NSW). Soil was sampled at depths between 0 - 25, 25 - 50 and at 80 - 100 cm. Some samples were taken from less than 80 - 100 cm where gravel and cobbles prevented deeper excavation using the auger. Due to the extreme dryness of the soil most holes were wet up with a small volume of tank water or dam water (determined to be free of *P. cinnamomi* by baiting) to enable samples below 30 cm to be collected. The soil from each sample (approximately 750 g) was bagged in a plastic zip lock bag, thoroughly mixed by hand and then a sub-sample of 50 - 100 g was retained, along with fine roots picked from the bulk sample. Auger holes were refilled with the remainder of soil samples in order of excavation (Figure 24). Augers and hand tools were cleaned by brushing and immersion in 100% methylated spirit after sampling each plot, and by brushing between sampling points within plots where necessary. Soil samples were stored at air temperature in the shade and at room temperature, and were used for baiting within 2 days after collection.

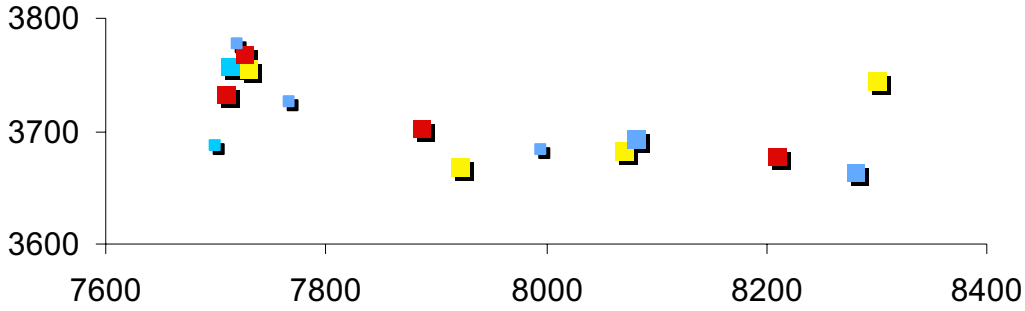


Figure 23 Plan of experimental plots, *Phytophthora* eradication experiment, Narawntapu National Park, Tasmania. Both axes in m; these grid numbers correspond to the GDA94 grid in map Port Sorell, sheet 4644. Plots given (■) complete treatment (vegetation clearance, fungicide and fumigation), (■) fungicide treatment only, and (■) no treatment (positive control with *Phytophthora*).

Figure 24

➢ Representative sample of soil sampling profile from *Phytophthora* eradication experiment, Narawntapu National Park, Tasmania.

Photo: WA Dunstan

Soil baiting and recovery of P. cinnamomi

A soil baiting method, modified from Chee and Newhook (1965) was used to assess the presence of *P. cinnamomi*. For each sample, 50 g of soil including fine roots was placed in a plastic container with 300 mL distilled water. Five *Lupinus angustifolius* seedlings (2 - 4 days after germination), were floated on the surface on a perforated plastic raft (Figure 25). Between 4 and 5 days after baiting, *Lupinus* seedlings were removed from the

containers, blotted dry, cut into sections and plated onto 1 or 2 plates of NARPH selective medium, and incubated in the dark at room temperature. The temperature was maintained at 22 - 27°C for the duration of baiting and plating. Isolation plates were examined for the presence of *P. cinnamomi* using a compound microscope for up to 7 days, and *P. cinnamomi* was identified by micromorphology. Primary isolates of *P. cinnamomi* were selected from each plot for the recovery of pure cultures of the pathogen.

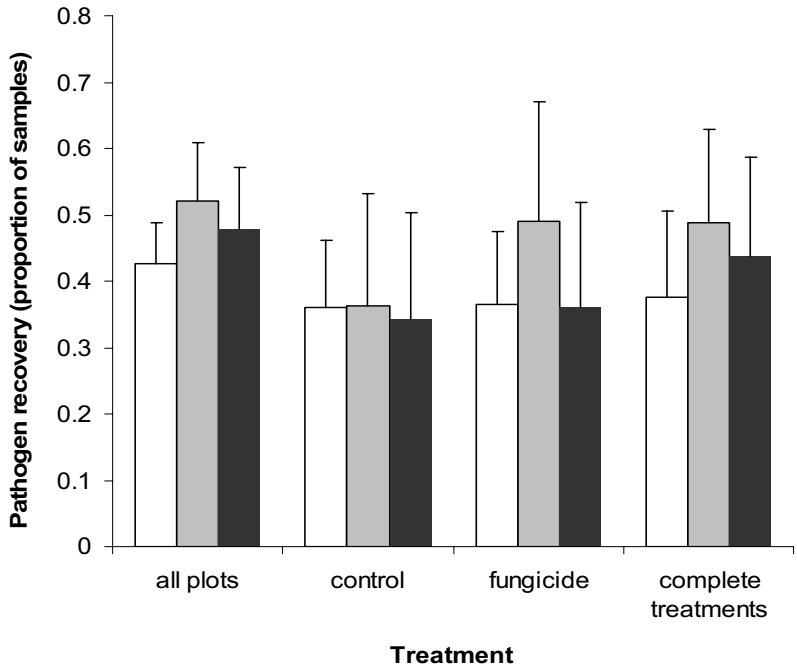
Photo: WA Dunstan

Figure 25 Soil baiting for recovery of *Phytophthora cinnamomi*, Narawntapu National Park, Tasmania. Bait plants are lupin seedlings suspended in water over potentially *Phytophthora* infested soil.

From the initial systematic survey, *P. cinnamomi* was recovered from 48% of all samples, and ranged from 1 isolation/plot (of a possible 25; 4% recovery) up to 14 isolations (56%). Given the drought conditions at the time of sampling and the non-targeted nature of sampling, this high recovery rate was not expected. A breakdown of recoveries by plot allocation and soil horizons is shown in Figure 26.

Figure 26

Mean proportion of recovery of *Phytophthora cinnamomi* from experimental plots at Narawntapu National Park, Tasmania before treatment application. 'All plots' shows mean pathogen recovery from all 15 plots surveyed before allocation of treatments. Treatments 'control' *n*=7, 'fungicide' *n*=4, and 'complete treatments' *n*=4 show mean recoveries from the plots allocated to the treatment. Profile depth 0 - 25 (□; approximately A_1 horizon), 25 - 50 (▫; approx. A_2 horizon) and 80 - 100 cm (■; equivalent to Bh horizon). Vertical bars represent one standard error of the mean.



Experimental plot treatments

Control plots

After soil sampling, assessment for the presence of *P. cinnamomi* and final marking out, control plots were marked permanently with steel posts at each corner.

Site preparation

In plots to be treated with fungicide only, vegetation disturbance was restricted to removal of just enough plant material to enable even and complete coverage of the soil surface with a granular formulation of fungicide. In practice, clearance was restricted to the removal of the dead skirts of *Xanthorrhoea,* and less frequently, the removal of litter from the plots. For plots that were to be treated with fungicides and fumigated, plots were slashed as close to ground level as possible with a motorised brush cutter and the waste plant material was raked clear of the plot (Figure 27).

Figure 27
Experimental plot in Narawntapu National Park, Tasmania, selected for complete treatment in *Phytophthora* eradication experiment.

➢ Natural state before clearing. Note dead *Xanthorrhoea.*

➢ Same area after slashing and removal of vegetation and deep litter.

Photos:
WA Dunstan

Root barrier installation

Trenching for the installation of root barriers was completed using a wheeled 16.6 kW Dingo Digger with a 20 cm wide cutting chain. Trenches were dug around the complete perimeter of the 5 x 5 m plots that were to be treated with fungicide only, and in the complete treatment plots (*n* = 8, in total). They were dug to 80 - 90 cm, unless impeded by large cobbles, where the depth of parts of the trench was reduced to 70 cm. This occurred in only a few plots. Spoil from excavations was deposited to the outside of the plots. Excavations were trimmed and cleaned out by hand.

HDPE sheeting (120 cm; Treemax P/L, Laverton, Vic) was installed in trenches on the inside wall and pinned with steel pickets. A single line of 17 mm micro-irrigation pipe (Netafim, Uniram 17) was laid in the bottom of the trench and the hole was backfilled with spoil, leaving 30 - 50 cm of barrier projecting above the natural soil level. The micro-irrigation line was fitted with 19 mm polyethylene risers to 50 cm above soil level, with an air release valve and ball valve fitted to each end of the line. In compliance with the conditions of the Grant of Authority for this project an Aboriginal Heritage Officer was present when excavation at each plot was in progress. The digging parts of the excavator were brushed clean between plots and the entire excavator was washed with water using a fire unit before exiting the site. Stages in barrier installation are shown in Figure 28.

Figure 28

Experimental plot in Narawntapu National Park, Tasmania, in *Phytophthora* eradication experiment.

➢ Trench excavation with a Dingo Digger.

Root barrier installed, prior to being filled in. ➘

Photos: WA Dunstan

Fencing to restrict animal access

Reinfestation of treated plots with *P. cinnamomi* by native mammals was considered to be a high risk. To prevent animal access, each plot was fenced to a standard considered adequate to deny access to all terrestrial mammals larger than a rat or juvenile bandicoot up to the size of wallabies and wombats. Plots were surrounded by a 7 x 7 m uninterrupted fence of 50 mm galvanised wire mesh supported by 1.8 m steel posts at 3.5 m intervals that were driven 0.8 m into the ground (1.0 m standing above ground). Netting was stapled on the outside of posts, to 4 fixed plain wires. Wire netting (0.4 - 0.5 m) was left overhanging at the top of the fence (away from the plot) to prevent animals climbing over the top of the fence. At the base of the fence, 0.4 - 0.5 m of netting was turned out and fixed to a separate plain wire attached to short steel pickets at 2 m intervals 0.5 m outside the fence line (Figure 29). After attachment of the netting, the short pickets were driven in to ground level. Fence wires and diagonal corner stay wires were tensioned with Gripple™ devices. Given the small size of the fenced plots, animals are more likely to go around the fence rather than attempt to break through. Animals small enough to fit through the wire mesh are likely to be prevented access to the treated plots by the continuous barrier of 40 cm high HDPE sheeting that projects above ground level.

Figure 29

Native animal exclusion fence around treatment plot in Narawntapu National Park, Tasmania, in *Phytophthora* eradication experiment.

Photo: WA Dunstan

Fungicide treatment

Surface applications of Terrazole (triadiazole) were used in initial treatments at the Cape Riche site. However, at least in the short term, treatment with Terrazole showed no effect on recoveries of *P. cinnamomi* at Cape Riche. Treatment with Ridomil (Metalaxyl-M), at high rates of application, has been claimed to be effective in the eradication of *P. cinnamomi* from a disturbed site elsewhere in Tasmania (T Wardlaw, *pers. comm*.), therefore Metalaxyl-M was selected as a first choice among fungicide treatments for

the site. To enable even application and complete coverage of plots with fungicide, plots were marked out into quarters (nominally 6.25 m^2 each). To each quarter of the plot, 625 g of Ridomil® Gold 25G (Syngenta Australia; 25 g/kg active ingredient), a granular formulation of the fungicide, was applied to the soil surface with a mechanical hand spreader. The rate of application applied in this experiment is equal to 1000 kg/ha of formulation, or 25 kg/ha of the active ingredient.

Fumigation
Soils need to be moist to ensure that the fumigant, metham-sodium (Metham, Nufarm Australia Ltd, Laverton VIC.; 423 g/l metham as the sodium salt), is hydrolysed to the active ingredient, methylisothiocyanate. Due to the very dry soil, plots that were to be fumigated were watered with 1000 L of dam water, in 2 applications of 500 L the day prior to fumigant application. The volume of water applied was equivalent to 40 mm of rainfall, and approximately 150 - 200 mm of soil water. As the dry soil was highly water repellent, 2 L of wetting agent (Furexpan-S, class A foam concentrate, Angus Fire Armour Australia P/L, Dingley, Vic) was applied to each plot with the water to aid infiltration.

Metham-sodium was applied to experimental plots at a rate equivalent to 500 L/ha. The fumigant was injected every 12 cm to a depth of 20 cm using a purpose built probe (Figure 30) attached to a motorised spray unit, with an output pressure of 275 kPa (40 psi). Immediately after fumigant application, each plot was watered with an additional 250 L of dam water (10 mm rainfall equivalent) to seal the injection holes and retain the methylisothiocyanate vapour within the soil. Application of fumigant is shown in Figure 31.

Metalaxyl-M (as Ridomil 25G) was reapplied at the rate of 100 g/m^2 to fungicide and fungicide and fumigation treatment plots 15 weeks after the initial treatment (with Metalaxyl). However, a single recovery of *P. cinnamomi* was made from fungicide and fumigation treated plots, so a further application of metham-sodium was applied to all plots. For this application, 40 mm diameter holes were dug with a powered auger to a depth of 1 m within a 1 m x 1 m array across the fungicide and fumigation plots. In each of the 25 holes per plot, a 1 m section of 40 mm class 6 PVC tubing (40 mm internal and 44 mm external diameter) was inserted to a depth of 90 cm after the hole had been cleared of soil to a depth of 1 m (Figure 32). Tubes were left undisturbed for 20 days to allow the soil to settle and for a reasonable contact between soil and tube to form. After 20 days, 750 mL of metham-sodium was poured into each tube. Individual tubes were sealed immediately after fumigant application with PE wrap (Figure 32) and a PVC end cap.

The calculated potential mass and volume of methylisothiocyanate (MITC; the active product from the hydrolysis of metham-sodium) applied to the plots amounted to 180 g/m^2 and 60 L/m^2, respectively.

Chemical applications undertaken during this study comply with the requirements of Australian Pesticides and Veterinary Medicines Authority (APVMA) permit number PER 7250 (Permit to allow the conduct of small-scale trials with agvet chemicals; http://permits.apvma.gov.au/PER7250.PDF).

Assessment of *P. cinnamomi* infestation after treatment

The first post treatment assessment commenced in June 2007. The same sampling and assessment methods as for previous assessments were used (described in Assessment of *P. cinnamomi* infestation).

The fourth assessment of *P. cinnamomi* recovery commenced 40 days after the deep application of metham-sodium (December 2007) and a final (fifth) assessment was completed in March 2008).

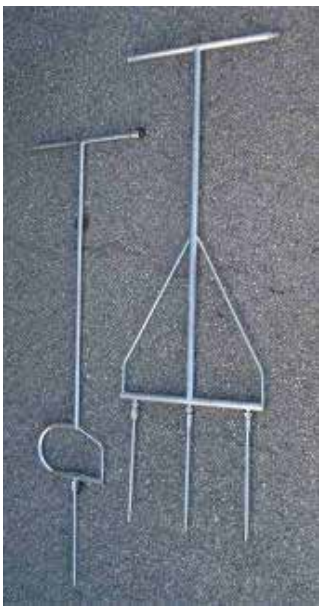


Figure 30

Specially designed shank injection probes, used to apply metham-sodium in treatment plots in Narawntapu National Park, Tasmania, in *Phytophthora* eradication experiment. Standard commercial probe (left), in comparison with custom probe (right) used for fumigation at Narawntapu NP.

Figure 31

Fumigation of *Phytophthora cinnamomi* infested ground with metham-sodium in fenced treatment plot in Narawntapu National Park, Tasmania, in *Phytophthora* eradication experiment.

Photos: WA Dunstan

Figure 32
Deep application of metham-sodium.

Site preparation and installation of PVC tubes to 1 m depth in the soil across the disease front.
➢

Application of the soil fumigant, metham-sodium.
➢

➢
Tubes were sealed immediately after fumigant application with PE wrap followed by a PVC end cap.

Photos: WA Dunstan

Statistical analysis

Statistical analysis was similar to that used for the Cape Riche experiment. For each subject (plot) and for each assessment, the numbers of recoveries of *P. cinnamomi* (dependant variable) were summed for each soil depth within each plot (possible values 0, 1, 2, or 3).

Data were analysed by analysis of variance. The design for the experiment involved three factors (treatment, soil sampling depth and time) in a repeated-measures design. The spatial component of the design was partly nested, with soil sampling depth (0 - 25, 25 - 50 and 100 cm) within subjects (plots) as factors.

Data analysis was done using Statistica (version 5 for PC; Statsoft Inc., Tulsa, OK, USA). For all analyses, the assumption of normality was examined by examining residuals by probability plots. Assumptions of normality and independence of errors were satisfied and data was analysed without transformation.

Probability estimates that treatment plots were pathogen free (at assessments where *P. cinnamomi* was not recovered) were estimated according to the methods described for the Cape Riche site (after Davison and Tay 2005).

RESULTS

***Phytophthora* recovery**

Results for five assessments, one before application of treatments (April 2007) and four after treatment (June, October and December 2007, and March 2008) are shown in Figure 33. These showed:

- at the pre-treatment assessment, recoveries of *P. cinnamomi* between the randomly allocated treatments were essentially similar, although there was a trend for fewer recoveries in the control group (Figure 33A);
- low recoveries of *P. cinnamomi* were made from fungicide only treated plots, with a mean of 3% of samples yielding the pathogen at the second assessment (Figure 33C) after treatment (range, 0 - 8%), but higher at later assessments. Over the life of the experiment *P. cinnamomi* was always recovered from fungicide only treated plots.; and
- no recoveries of *P. cinnamomi* were obtained from the plots given the complete treatment (fungicide + fumigation + vegetation removal) at each of the last three assessments (October 2007 - March 2008; Figures 33C, D and E).

In contrast to *Phytophthora* recoveries from the Cape Riche site in Western Australia, the recovery of *P. cinnamomi* from Narawntapu NP was distributed more evenly throughout the soil profile. This could be attributed to the nature of each site; Cape Riche was sampled at the disease front in the early stages of development of the infestation, whereas sampling at Narawntapu NP was (principally) from within well established infestations where the pathogen has been present for a number of years.

A comparison between recovery of *P. cinnamomi* from untreated control plots pre- and post treatment (Figures 33A and B) shows an average decline in recovery of 24% between harvests 1 and 2, and rates of recovery in control plots remained fairly constant thereafter.

In comparison with untreated controls, recovery of *P. cinnamomi* in the upper two horizons (0 - 25 and 25 - 50 cm) within fungicide treated plots were reduced by 56 and 87%, respectively. In contrast, there was only a 20% reduction in recoveries of the pathogen from samples taken from 1 m (Figures 33A and B).

In contrast to the fungicide only treatment, *P. cinnamomi* was not recovered from the upper two horizons in any plots treated with both Metalaxyl-M and surface injection of metham sodium (Figure 33B). A single recovery of the pathogen was made at 1 m in one plot that had both treatments (Figure 33B).

Summary statistics for analysis of variance (ANOVA) of *P. cinnamomi* recovery between all assessments are shown in Table 6.

Table 6 Repeated measures ANOVA with factors of treatment and repeated measures factors of soil depth and time: *Phytophthora cinnamomi* recovery, Narawntapu National Park. Significant results indicated in bold.

	Effect	Effect	Error	Error	F	p-level
	df	MS	df	MS		
Treatment	2	21.70952	12	9.437302	2.30040	0.142664
Time	4	29.75377	48	1.037996	28.66463	**0.000000**
Depth	2	0.94087	24	0.567659	1.65746	0.211707
Treatment, Time	8	4.15536	48	1.037996	4.00325	**0.001059**
Treatment, Depth	4	0.90071	24	0.567659	1.58672	0.210107
Time, Depth	8	0.21270	96	0.358085	0.59399	0.780688
Treatment, Time, Depth	16	0.52482	96	0.358085	1.46563	0.128933

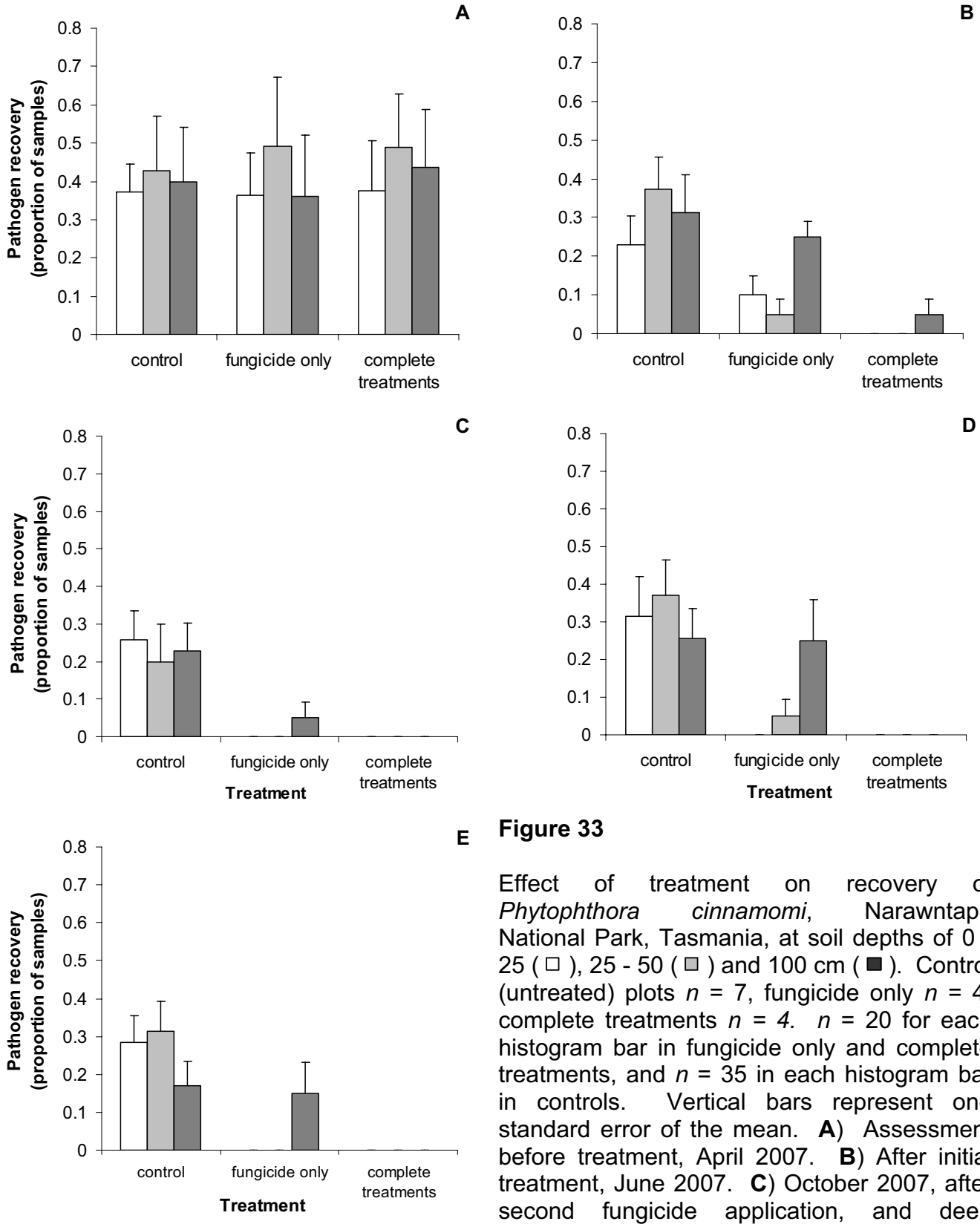


Figure 33

Effect of treatment on recovery of *Phytophthora cinnamomi*, Narawntapu National Park, Tasmania, at soil depths of 0 - 25 (□), 25 - 50 (▒) and 100 cm (■). Control (untreated) plots *n* = 7, fungicide only *n* = 4, complete treatments *n* = *4.* *n* = 20 for each histogram bar in fungicide only and complete treatments, and *n* = 35 in each histogram bar in controls. Vertical bars represent one standard error of the mean. **A**) Assessment before treatment, April 2007. **B**) After initial treatment, June 2007. **C**) October 2007, after second fungicide application, and deep treatment with metham-sodium. **D**) December 2007. **E**) March 2008.

The most significant effect on recovery of *P. cinnamomi* was time (highly significant; p = 0.000000), and there was also a very significant interaction between treatment and time ($p < 0.05$).

Estimates of pathogen free status in treatment plots
For a single assessment of treatment plots, estimates of the probability (*P*) of not detecting *P. cinnamomi* in treatment plots at any of assessments 3, 4 or 5 in the upper soil horizon ranged from 0.0005 - <0.003, much less significant than estimates for the Cape Riche site. The higher probability of recovering the pathogen in untreated control plots (high value for *r*) than at Cape Riche, was counteracted by the smaller combined sample size in treated plots (*n* = 20, compared to *n* = 42 at Cape Riche).

DISCUSSION

The results from plots treated with combined Metalaxyl-M and metham-sodium treatments are highly promising and are comparable with the results achieved with similar treatments in the experiment established at Cape Riche, Western Australia. Apart from a single isolation of the pathogen from one plot at the first assessment post treatment, the pathogen was not recovered from any of the plots that received the complete treatment regime of fungicide + fumigation + vegetation removal (four assessments over 11 months). Treatment with Metalaxyl-M alone is less promising, and further alternate treatments of Terrazole and Metalaxyl-M, at higher rates are probably required to achieve the desired effect.

In contrast to the experiment at Cape Riche, where treatments were confined to the vicinity of a recently established disease front, treatments in this experiment were applied to plots where the pathogen had been established for at least a few years, and were apparently also effective. This is significant, as our initial premise was that eradication of *P. cinnamomi* would only be effective on recently established 'spot' infestations or along active disease fronts.

While the number of replicate plots within treatments is small, reduction in *P. cinnamomi* recovery between control plots and plots receiving the complete treatments are significant. Given the high rates of application of Metalaxyl-M and its half-life of 70 - 82 days (Kookana *et al.* 1995, Davison and McKay 1999), we cannot discount the possibility that suppression of the pathogen by residual fungicide and fumigant is being observed, rather than eradication. However, no residual effects of fungicide or fumigant were detected after the last assessment at Cape Riche, from plots that had been treated in a similar way.

The decline in recovery of 24% between the first and second assessments in untreated control plots is consistent with the seasonal pattern of recoveries of the pathogen from similar soil in field sites elsewhere (for example, Weste and Vithanage 1977), and may also be partly attributed to below average rainfall throughout the study period. Across the complete study, recovery rates in control plots between all assessments were not significantly different (p = 0.129).

In this experiment there was a minimum of replication in treatment and control plots. The power of the experiment was increased by including plots that had been surveyed and

sampled but not included in the experimental plots with the control group, changing the design to an unbalanced randomised complete block.

As for the Cape Riche experiment, treatments with herbicides, fungicides and fumigants added macronutrients to the soil in organic form. Estimates of elemental additions from chemical applications, not including those in wetting agents and carriers, were: C, 91 g/m^2; N, 38 g/m^2; P, 1.3 g/m^2; and S, 168 g/m^2. Like the Cape Riche site, chemical treatments are likely to have increased soil fertility.

Experimental set-up: operational matters

Logistics

A better study site could not have been chosen. Located within the National Park and within 1.5 km from the ranger station, access to the site is controlled and security is excellent. We have been able to access workshop facilities, tools and other equipment, storage space, space for laboratory work and accommodation at short notice within the park. Materials and services for setting up the experiment have been sourced locally at Latrobe and Devonport, within a 30 - 45 min drive from the site. Laboratory supplies and equipment have been sourced through the Launceston campus of the University of Tasmania.

Hygiene

Operations to set up the experiment were undertaken during a period of prolonged drought in autumn with conditions providing the least risk of spreading *Phytophthora*. While the riskiest operations have been completed, further fumigation treatments may require vehicular access across infested vegetation when conditions will make the risk of spread much higher. In addition, the access track intersects and adjoins *Phytophthora* infestations and should therefore be treated as infested. Before future operations at the site, a simple wash down point should be established at the beginning of the access track.

Site preparation

The experimental site had not been burnt for approximately 30 years. In the preparation of plots that were to receive complete treatments, significant amounts of live and dead plant material, some of it probably infested with *P. cinnamomi*, were removed. If larger infestations are to be treated then safe disposal of potentially contaminated material within the infested site will pose an additional problem.

Barrier installation

At Narawntapu NP, a 'Dingo Digger' (Dingo Mini Diggers, Dalby, Qld) was used in trenching, whereas a Bobcat machine (Ingersol-Rand) was used at Cape Riche. However, directional control in trenching was poor with the Dingo Digger (16.9 kW/650 kg), it had difficulty in dealing with trenching in plots with gravel and cobbles in the B horizon, and it was less stable on uneven ground. The Dingo Digger has characteristics that may be advantageous for carrying out similar operations elsewhere:

- smaller size (width 1.05 m, compared with 1.8 m for the Bobcat) allowing it to be used in more restricted spaces (forest),
- causes less damage to vegetation and is much easier to clean down; and
- smaller mass, therefore potentially air portable, for operations in remote areas.

In the process of installing barriers most of the time and physical effort was used in cleaning out trenches and filling them back in. In future operations, the amount of effort in these tasks could be reduced significantly by using a narrower cutter (available in 10 and 15 cm widths). This would reduce the amount of excavated spoil, and trenching to a depth greater than the depth of the barrier material, with the aim of eliminating the need to undertake the laborious task of cleaning out trenches. The Rootwall barrier used in this experiment is strong and durable enough to stop practically any root and last for decades in the soil. However, it is quite heavy (20 m roll = ~20 kg) and difficult to roll out and install, even under the favourable conditions at Narawntapu NP. For operations in areas more difficult to access, by terrain or vegetation, a lighter weight material would have to be sourced and used.

Fumigation
Fumigation of plots generally proceeded well with the equipment used. The injection points bent to some extent, but did not become unusable. Welding in the probe failed and it had to be re-welded onsite. In soft soil the injection probe system failed, and must be rebuilt to a much more robust design. The three probe design of the injection system proved useful in that the application time for the fumigant was reduced to 1.2 - 1.8 min/m^2, faster than expected.

In conclusion, similar results were achieved in this experiment as those at Cape Riche. A combination of vegetation removal, herbicide use to ensure *P. cinnamomi* has no living host material to invade, fungicide and fumigant treatments were effective in eradicating the pathogen from the Narawntapu NP treatment plots for the duration of the study. The fungicide treatments alone were ineffective, and additional treatments with other fungicides in addition to Metalaxyl-M, and at higher application rates, need to be applied to the site and their efficacy assessed. The installation of the heavy-duty root barriers will further facilitate keeping these sites disease-free in the future, by preventing reinfestation of the site from adjacent diseased vegetation. These results are extremely promising in terms of protecting key plant species and plant communities from *P. cinnamomi.* However, it is critical that the Narawntapu NP plots are reassessed at least twice more (e.g. after a further 12 and 24 months) to confirm that the pathogen has been completely eradicated.

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APPENDIX I

Notes on chemicals used in *Phytophthora cinnamomi* control operations.

Triadiazole ('Terrazole')
IUPAC: ethyl3-trichloromethyl-1,2,4-tyhiadiazoyl ether, $C_5H_5C_{13}N_2OS$, MW 247.5. CAS number 2593-15-9.
This is selectively effective against Oomycetes. There is a low risk of development of resistance in target organisms, with repeated use.

Metham-Sodium ('Metam', 'Metham')
IUPAC: sodium methylaminomethanedithioate, $C_2H_4NNaS_2$, MW 129.2
CAS number: 137-42-8
Decomposes slowly on contact with water to produce the active compound, methyl isothiocyanate (CH_3NCS; MITC; CAS number 556-61-6), it inactivates sulfhydryl groups in enzymes. It is active against soil organisms in general and is phytotoxic.

Metalaxyl-M ('Ridomil')
IUPAC: methyl 2-[(2,6-dimethylphenyl)-(2-methoxyacetyl)amino] propanoate, a phenylamide. $C_{15}H_{21}NO_4$, MW 279.3.
CAS number: 57837-19-1
This fungicide interferes with RNA synthesis. There is high risk of development of resistance in target organisms with repeated use, therefore its use is alternated with fungicides of a different class, such as Terrazole. It is selectively effective against Oomycetes.

Triclopyr ('Garlon')
IUPAC: 2-butoxyethyl 2-(3,5,6-trichloropyridin-2-yl)oxyacetate, $C_{13}H_{16}C_{l3}NO_4$, MW 356.6.
CAS number: 55335-06-3
A systemic herbicide which induces auxin-like responses in most broad-leaved plants, but not in Gramineae, at normal rates of application.

Sodium glyphosate ('Glyphosate', 'Roundup')
IUPAC: sodium 2-[(hydroxy-oxido-phosphoryl)methylamino]acetic acid, $C_3H_7NNaO_5P$, MW 191.1.
CAS number: 1071-83-6
This is a broad spectrum systemic herbicide.

APPENDIX II

Table II Experimental plot locations, *Phytophthora* eradication experiment, Narawntapu National Park, Tasmania.

Plot	GDA94 E	GDA94 N	Treatment
A	467708	5443733	complete
B	467729	5443755	fungicide
C	467726	5443768	complete
D	467720	5443777	control
E	467712	5443758	control
F	467766	5443725	control
G	467700	5443686	control
H	467886	5443703	complete
I	467921	5443669	fungicide
J	467995	5443684	control
K	468080	5443694	control
L	468069	5443684	fungicide
M	468301	5443745	fungicide
N	468280	5443664	control
O	468209	5443678	complete

APPENDIX III

Phytophthora Eradication Research, Narawntapu National Park, Tasmania. Vegetation Report, 2007. Prepared by Tim Rudman DPIW, Tasmania.

PART 1: IMPACT ASSESSMENT

Threatened plant species recorded within the proposed research site

The area has been surveyed generally for threatened plant species and also a number of plots surveyed around potential research plots. No threatened plant species were recorded from within the proposed research area on the Natural Values Atlas. However, a single plant with affinities to *Xanthorrhoea bracteata* was located within the area, though the Tasmanian Herbarium is not yet able to put a name on the collected specimen. As very little of the overall research area will be affected by the 5 x 5 m plots they will be placed to avoid the plant and any other *X. bracteata* encountered. This is not expected to be an issue as the area has been reasonable well examined and no other similar plants were found. *X. bracteata* is not recorded from this area of the Park.

All the threatened species occurring within 5000 m of the site (see natural values report) were assessed for their potential to be present. In particular the threatened orchids were of concern. I am satisfied that the habitat in the research area is a low risk for those threatened plants to be present but unobserved. The orchid potential was further discussed with Peter Tonelli (Latrobe University) who advised that there are no threatened orchids in that area and they are unlikely to be so.

Vegetation communities

No listed vegetation communities will be affected by the proposed research. The potential plot sites occur in coastal *E. amygdalina* forest and regenerating cleared land alongside the slashed firebreak. In this area the vegetation is dominated by bracken with relatively few native species present. These two communities along with adjacent heath and scrub communities integrate in a complex mosaic in the general area. The TASVEG mapping is inaccurate. No impacts of conservation significance will occur at the community level.

Research plot impacts

The small scale and widely distributed nature of the treated plots suggests that the impact of localised plant destruction will be minor and of no significance to the overall conservation value of the Park or those communities within the Park.

For some plots the vegetation will be destroyed to remove the food source for *P. cinnamomi*. The impact of destroying the vegetation is likely to be readily reversible for those species that are not affected by *P. cinnamomi*. Those species are also destined to be lost from the plots if no action is taken in the short term as *P. cinnamomi* is already active within each plot. If the research fails they will also be lost from the plots. In the

case the research is successful regeneration of the susceptible species on the plots is anticipated.

The treatments will be transitory in impact and small plot size and their dispersed siting will aid recolonisation by any flora affected by the research. The fencing will be maintained for an additional period to ensure protection of regeneration from grazing prior to removal. As the sites are small it is proposed natural regeneration be left to occur and that this be monitored in early summer by DPIW. If regeneration needs assistance an action plan appropriate to the monitoring results will be developed. DPIW will hold a trust account for this monitoring, rehabilitation and fence removal.

Species recorded in the research area
The following species were recorded in the research site (Table IIIa). Some species were not seen in later visits due to the drought conditions prevalent during the establishment of the site or due to life form e.g. geophytes.

PART 2: PLOT VEGETATION DATA

Plot vegetation data were collected from 8 to 10 January 2007 during drought conditions. The general health of the vegetation was poor due to a combination of the drought and grazing pressure and the presence of *P. cinnamomi*. The plots also had a very high level of soil disturbance by wildlife. It is probable that other species would be present in more favourable conditions.

All plots were located in vegetation classified as Coastal *Eucalyptus amygdalina* forest (Harris and Kitchener 2005) or disturbed remnant of coastal *E. amygdalina* forest in the case of plots N and O. Species nomenclature follows Buchanan (2005).

General plot data is presented in Table IIIb. Table IIIc details species cover records for each plot.

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Table IIIa *Phytophthora* eradication site plant species records. * susceptible to *P. cinnamomi*

DICOTYLEDONAE	MONCOTYLEDONAE
Acrotriche serrulata *	*Aira elegantissima*
Acetosella vulgaris	*Austrodanthonia* sp.
Allocasuarina monilifera	*Caladenia alata?*
Allocasuarina verticillata	*Dianella revoluta*
Aotus ericoides *	*Diuris sulphurea*
Astroloma humifusum *	*Ehrharta distichophylla*
Banksia marginata *	*Ehrharta tasmanica*
Bossiaea cinerea *	*Glossodia major*
Bossiaea prostrata *	*Hypolaena fastigiata*
Cassytha glabella	*Lepidosperma concavum*
Centaurium erythraea	*Lepidosperma longitudinale*
Drosera sp.	*Leptocarpus tenax*
Epacris impressa *	*Lomandra longifolia*
Eucalyptus amygdalina	*Patersonia fragilis*
Eucalyptus viminalis	*Poaceae* sp 1
Euryomyrtus ramosissima	*Pteridium esculentum*
Euchiton sp.	*Selaginella* sp.
Gonocarpus tetragynus	*Thelymitra ixioides*
Goodenia lanata	*Thelymitra juncifolia*
Helichrysum scorpioides	*Xanthorrhoea australis* *
Hibbertia acicularis *	
Hibbertia prostrata *	
Hibbertia sericea *	
Hypochoeris radicata	
Kennedia prostrata	
Leptospermum scoparium	
Leucopogon ericoides *	
Leucopogon virgatus *	
Persoonia juniperina	
Pimelea linifolia	
Platylobium triangular *	
Stylidium graminifolium *	
Wahlenbergia sp.	

There is some confusion in the *Thelymitra* spp. present and this is put down to hybridisation in the *T. ixioides – T. juncifolia* complex (Peter Tonelli, *pers. comm.*).

Table IIIb Plot data for potential *Phytophthora* eradication plots, Narawntapu National Park, Tasmania, November 2006 - April 2007.

Plot	GDA E	GDA N	Slope	Aspect (°)	Rock (%)	Litter (%)	Bare ground (%)	Moss/lichen (%)
A	468210	5443680	3	0	0	40	8	0
B	467692	5443684	2	225	0	0	3	0
C	467725	5443724	2	280	0	25	60	0
D	467718	5443750	1	270	0	28	32	0
E	467723	5443775	0	0	0	27	10	0
F	467712	5443753	0	0	0	35	25	0
G	467771	5443726	1	120	0	70	4	0
H	467694	5443686	0	0	0	35	5	0
I	467885	5443701	1	0	0	56	0	0
J	467920	5443667	0	0	0	60	10	0
K	467996	5443687	0	0	0	50	15	0
L	468083	5443694	1	270	0	30	24	0
M	468068	5443694	2	310	0	35	8	0
N	468298	5443745	0	0	0	50	9	0
O	468276	5443665	0	0	0	40	6	0

Table IIIc Percentage cover of plants in each plot for potential *Phytophthora* eradication plots, Narawntapu National Park, Tasmania, November 2006 - April 2007.

Plot	Species name	Cover (%)	Plot	Species name	Cover (%)
A	*Allocasuarina monilifera*	6.0	D	*Lepidosperma concavum*	5.0
A	*Lepidosperma concavum*	7.0	D	*Pteridium esculentum*	4.0
A	*Pteridium esculentum*	5.0	D	*Hibbertia acicularis*	0.1
A	*Hibbertia acicularis*	0.1	D	*Hibbertia prostrata*	0.1
A	*Astroloma humifusum*	0.1	D	*Epacris impressa*	5.0
A	*Epacris impressa*	2.0	D	*Brachyloma ciliatum*	0.1
A	*Brachyloma ciliatum*	2.0	D	*Leucopogon ericoides*	5.0
A	*Leucopogon ericoides*	3.0	D	*Leucopogon virgatus*	0.1
A	*Leucopogon virgatus*	0.1	D	*Bossiaea cinerea*	0.1
A	*Bossiaea cinerea*	0.1	D	*Bossiaea prostrata*	0.1
A	*Bossiaea prostrata*	0.1	D	*Dillwynia glaberrima*	0.1
A	*Platylobium triangulare*	0.1	D	*Goodenia lanata*	0.1
A	*Gonocarpus tetragynus*	0.1	D	*Gonocarpus tetragynus*	0.1
A	*Poa sp.*	0.1	D	*Austrodanthonia sp.*	0.1
A	*Banksia marginata*	0.1	D	*Ehrharta tasmanica*	0.1
A	*Lomandra longifolia*	2.0	D	*Poa sp.*	0.1
A	*Xanthorrhoea australis*	20.0	D	*Banksia marginata*	0.1
B	*Lepidosperma concavum*	10.0	D	*Hypolaena fastigiata*	0.1
B	*Pteridium esculentum*	0.1	D	*Stylidium graminifolium*	0.1
B	*Hibbertia prostrata*	0.1	D	*Pimelea linifolia*	0.1
B	*Astroloma humifusum*	0.1	D	*Lomandra longifolia*	0.1
B	*Epacris impressa*	0.1	D	*Xanthorrhoea australis*	40.0
B	*Leucopogon ericoides*	2.0	E	*Lepidosperma concavum*	5.0
B	*Leucopogon virgatus*	0.1	E	*Pteridium esculentum*	2.0
B	*Bossiaea cinerea*	0.1	E	*Hibbertia acicularis*	0.1
B	*Austrodanthonia setacea*	0.1	E	*Hibbertia prostrata*	0.1
B	*Poa sp.*	0.1	E	*Acrotriche serrulata*	3.0
B	*Banksia marginata*	0.1	E	*Astroloma humifusum*	0.1
B	*Xanthorrhoea australis*	12.0	E	*Epacris impressa*	0.1
C	*Allocasuarina monilifera*	0.1	E	*Brachyloma ciliatum*	3.0
C	*Lepidosperma concavum*	8.0	E	*Leucopogon ericoides*	4.0
C	*Schoenus* sp.	0.1	E	*Leucopogon virgatus*	0.1
C	*Pteridium esculentum*	4.0	E	*Bossiaea cinerea*	0.1
C	*Epacris impressa*	4.0	E	*Dillwynia glaberrima*	0.1
C	*Leucopogon ericoides*	2.0	E	*Platylobium triangulare*	0.1
C	*Leucopogon virgatus*	0.1	E	*Goodenia lanata*	0.1
C	*Bossiaea cinerea*	0.1	E	*Austrodanthonia sp.*	0.1
C	*Bossiaea prostrata*	0.1	E	*Banksia marginata*	0.1
C	*Austrodanthonia sp.*	0.1	E	*Persoonia juniperina*	0.1
C	*Ehrharta tasmanica*	0.1	E	*Stylidium graminifolium*	0.1
C	*Banksia marginata*	0.1	E	*Pimelea linifolia*	0.1
C	*Hypolaena fastigiata*	0.1	E	*Xanthorrhoea australis*	20.0
C	*Lomandra longifolia*	0.1	F	*Helichrysum scorpioides*	0.1
C	*Xanthorrhoea australis*	28.0	F	*Allocasuarina monilifera*	8.0

Plot	Species name	Cover (%)	Plot	Species name	Cover (%)
F	*Lepidosperma concavum*	0.1	H	*Goodenia lanata*	0.1
F	*Pteridium esculentum*	3.0	H	*Gonocarpus tetragynus*	0.1
F	*Hibbertia acicularis*	0.1	H	*Cassytha glabella*	0.1
F	*Epacris impressa*	0.1	H	*Leptospermum scoparium*	2.0
F	*Brachyloma ciliatum*	0.1	H	*Banksia marginata*	0.1
F	*Leucopogon ericoides*	6.0	H	*Hypolaena fastigiata*	0.1
F	*Bossiaea cinerea*	0.1	H	*Pimelea linifolia*	0.1
F	*Bossiaea prostrata*	0.1	H	*Xanthorrhoea australis*	40.0
F	*Dillwynia glaberrima*	0.1	I	*Lepidosperma longitudinale*	0.1
F	*Leptospermum scoparium*	5.0	I	*Lepidosperma concavum*	10.0
F	*Austrodanthonia* sp.	0.1	I	*Pteridium esculentum*	6.0
F	*Ehrharta tasmanica*	0.1	I	*Epacris impressa*	0.1
F	*Poa* sp.	0.1	I	*Brachyloma ciliatum*	0.1
F	*Distichlis distichophylla*	0.1	I	*Leucopogon ericoides*	0.1
F	*Dichelachne rara*	0.1	I	*Leucopogon ericoides*	0.1
F	*Banksia marginata*	0.1	I	*Bossiaea cinerea*	2.0
F	*Hypolaena fastigiata*	0.1	I	*Bossiaea prostrata*	0.1
F	*Schizaea bifida*	0.1	I	*Gompholobium huegelii*	0.1
F	*Stylidium graminifolium*	0.1	I	*Platylobium triangulare*	0.1
F	*Pimelea linifolia*	0.1	I	*Eucalyptus amygdalina*	0.1
F	*Xanthorrhoea australis*	3.0	I	*Leptospermum scoparium*	1.0
G	*Lepidosperma concavum*	4.0	I	*Ehrharta tasmanica*	0.1
G	*Pteridium esculentum*	6.0	I	*Banksia marginata*	0.1
G	*Hibbertia prostrata*	0.1	I	*Selaginella* sp.	0.1
G	*Astroloma humifusum*	0.1	I	*Xanthorrhoea australis*	12.0
G	*Epacris impressa*	0.1	J	*Allocasuarina monilifera*	2.0
G	*Brachyloma ciliatum*	0.1	J	*Lepidosperma longitudinale*	0.1
G	*Leucopogon ericoides*	1.0	J	*Lepidosperma concavum*	0.1
G	*Leucopogon virgatus*	0.1	J	*Pteridium esculentum*	4.0
G	*Bossiaea cinerea*	0.1	J	*Epacris impressa*	0.1
G	*Bossiaea prostrata*	0.1	J	*Brachyloma ciliatum*	0.1
G	*Dillwynia glaberrima*	0.1	J	*Leucopogon ericoides*	3.0
G	*Platylobium triangulare*	0.1	J	*Leucopogon virgatus*	0.1
G	*Leptospermum scoparium*	5.0	J	*Bossiaea cinerea*	0.1
G	*Ehrharta tasmanica*	0.1	J	*Goodenia lanata*	0.1
G	*Poa* sp.	0.1	J	*Gonocarpus tetragynus*	0.1
G	*Hypolaena fastigiata*	0.1	J	*Austrodanthonia* sp.	0.1
G	*Stylidium graminifolium*	0.1	J	*Poa* sp.	0.1
G	*Lomandra longifolia*	0.1	J	*Banksia marginata*	0.1
G	*Xanthorrhoea australis*	40.0	J	*Hypolaena fastigiata*	1.0
H	*Pteridium esculentum*	3.0	J	*Pimelea linifolia*	0.1
H	*Epacris impressa*	4.0	J	*Xanthorrhoea australis*	25.0
H	*Leucopogon ericoides*	3.0	K	*Lepidosperma concavum*	3.0
H	*Leucopogon virgatus*	0.1	K	*Pteridium esculentum*	2.0
H	*Bossiaea cinerea*	0.1	K	*Hibbertia acicularis*	0.1
H	*Bossiaea prostrata*	0.1	K	*Hibbertia prostrata*	0.1
H	*Platylobium triangulare*	0.1	K	*Hibbertia sericea*	0.1

Plot	Species name	Cover (%)	Plot	Species name	Cover (%)
K	*Epacris impressa*	0.1	M	*Pteridium esculentum*	10.0
K	*Brachyloma ciliatum*	0.1	M	*Astroloma humifusum*	0.1
K	*Leucopogon ericoides*	0.1	M	*Bossiaea cinerea*	0.1
K	*Leucopogon virgatus*	0.1	M	*Leptospermum scoparium*	0.1
K	*Aotus ericoides*	0.1	M	*Acetosella vulgaris*	1.0
K	*Bossiaea cinerea*	0.1	M	*Xanthorrhoea australis*	30.0
K	*Bossiaea prostrata*	0.1	N	*Euchiton* sp.	0.1
K	*Goodenia lanata*	0.1	N	*Wahlenbergia* sp.	0.1
K	*Gonocarpus tetragynus*	0.1	N	*Allocasuarina monilifera*	8.0
K	*Patersonia fragilis*	0.1	N	*Lepidosperma concavum*	0.1
K	*Cassytha glabella*	0.1	N	*Pteridium esculentum*	6.0
K	*Caesia* sp.	0.1	N	*Hibbertia prostrata*	0.1
K	*Acacia verticillata*	0.1	N	*Epacris impressa*	0.1
K	*Leptospermum scoparium*	2.0	N	*Brachyloma ciliatum*	0.1
K	*Distichlis distichophylla*	0.1	N	*Leucopogon virgatus*	0.1
K	*Banksia marginata*	0.1	N	*Bossiaea prostrata*	0.1
K	*Persoonia juniperina*	0.1	N	*Kennedia prostrata*	0.1
K	*Hypolaena fastigiata*	0.1	N	*Centaurium erythraea*	0.1
K	*Xanthorrhoea australis*	45.0	N	*Goodenia lanata*	0.1
K	*Xyris gracilis*	0.1	N	*Gonocarpus tetragynus*	0.1
L	*Lepidosperma concavum*	5.0	N	*Leptospermum scoparium*	0.1
L	*Pteridium esculentum*	0.1	N	*Distichlis distichophylla*	0.1
L	*Hibbertia sericea*	0.1	N	*Banksia marginata*	0.1
L	*Astroloma humifusum*	0.1	N	*Pimelea linifolia*	0.1
L	*Epacris impressa*	0.1	N	*Xanthorrhoea australis*	40.0
L	*Leucopogon ericoides*	1.0	O	*Lepidosperma concavum*	1.0
L	*Leucopogon virgatus*	1.0	O	*Pteridium esculentum*	8.0
L	*Aotus ericoides*	5.0	O	*Hibbertia sericea*	0.1
L	*Bossiaea cinerea*	0.1	O	*Epacris impressa*	0.1
L	*Bossiaea prostrata*	0.1	O	*Brachyloma ciliatum*	0.1
L	*Dillwynia glaberrima*	0.1	O	*Leucopogon ericoides*	2.0
L	*Goodenia lanata*	0.1	O	*Leucopogon virgatus*	0.1
L	*Patersonia fragilis*	2.0	O	*Bossiaea cinerea*	0.1
L	*Leptospermum scoparium*	2.0	O	*Platylobium triangulare*	0.1
L	*Austrodanthonia* sp.	0.1	O	*Gonocarpus tetragynus*	0.1
L	*Poa* sp.	0.1	O	*Leptospermum scoparium*	0.1
L	*Banksia marginata*	0.1	O	*Distichlis distichophylla*	0.1
L	*Hypolaena fastigiata*	0.1	O	*Banksia marginata*	0.1
L	*Leptocarpus tenax*	1.0	O	*Persoonia juniperina*	0.1
L	*Pimelea linifolia*	0.1	O	*Hypolaena fastigiata*	0.1
L	*Xanthorrhoea australis*	50.0	O	*Stylidium graminifolium*	0.1
L	*Xyris marginata*	0.1	O	*Xanthorrhoea australis*	35.0
M	*Lepidosperma concavum*	0.1			

APPENDIX IV

Publications arising from this research

Abstract for: The 4th meeting of the International Union of Forest Research Organisations (IUFRO) Working Party 7.02.09. Phytophthoras in Forests and Natural Ecosystems. Asilomar Conference Center, Monterey, CA, USA. August 26-31, 2007.

CONTAINMENT AND ERADICATION OF *PHYTOPHTHORA CINNAMOMI* IN NATIVE VEGETATION IN SOUTH-WESTERN AUSTRALIA AND TASMANIA

William A Dunstan[1], Timothy Rudman[2], Bryan L Shearer[3], Nicole A Moore[1,4], Bernard Dell[1], Colin Crane[3], Sarah Barrett[4], Giles E St J Hardy[1]
1Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA 6150, Australia
2Biodiversity Conservation Branch, Resource Management & Conservation, Department of Primary Industries & Water, 134 Macquarie St., Hobart, TAS 7001, Australia
3Science Division, Dept of Environment & Conservation, 50 Hayman Rd, Como WA 6152, Australia
4Nature Conservation Section, Dept of Environment & Conservation, 120 Albany Highway, Albany, WA 6330, Australia

Phytophthora cinnamomi, and disease caused by it, is listed as one of five key threatening processes affecting biodiversity in Australia. Although infestations within the conservation estate are widespread and frequently extensive, large areas remain free of the pathogen and may be protectable, particularly if new, small spot infestations can be contained. The aim of our experiments was to develop protocols that can be used to contain and eradicate spot infestations of *P. cinnamomi,* nominally < 0.02 ha in extent. Treatment regimes were guided by two assumptions: 1. Within the selected sites, transmission of the pathogen is by root-root contact; and 2. The pathogen is a weak saprotroph. In Western Australia, treatment and control plots were set up along an active disease front within scrub-heath vegetation dominated by *Banksia* spp. Treatments, applied in combination, included: (1) removal of the largest plants to a distance of 10 m in front of the disease front; (2) removal of all plants to a distance of 4 m; (3) installation of root barriers to ca. 80 cm depth and subsurface irrigation for application of fungicide; and (4) surface applications of Terrazole (triadiazole) fungicide.

In the Western Australia experiment, recoveries of *P. cinnamomi* by soil baiting have shown no significant difference in numbers of recoveries, and in estimates of inoculum potential, between treated and control plots. However, recoveries of *P. cinnamomi* indicate no extension in the disease front in treated plots. Rainfall over the time of the experiment (one year) has been highly unfavourable for activity by *P. cinnamomi.* Further treatments, fungicides and fumigation, will be applied in the second season. An additional experiment will be set-up in Tasmania on a similar site, with the aim of assessing the efficacy of similar treatments, alone and in combination.

Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia.
Tender Number: 19/2005DEH Sub Projects 19.3.a(i and ii) 30 May 2008

Extended abstract accepted December 2007 for: Progress in Research on Phytophthora Diseases of Forest Trees. Proceedings of the 4th International IUFRO Working Party S07.02.09 Meeting in Monterey, California, 26-21 August 2007. Eds E Goheen, S Frankel, K Palmieri. USDA, Albany, California, US. *In press*.

Containment and Eradication of *Phytophthora cinnamomi* in Native Vegetation in South-Western Australia and Tasmania

William A. Dunstan[1], Timothy Rudman[2], Giles E. StJ. Hardy[1], Bryan L. Shearer[3], Nicole A. Moore[1,4], Bernard Dell[1], Colin Crane[3], Sarah Barrett[4]

[1]Centre for Phytophthora Science & Management, School of Biological Sciences & Biotechnology, DSE, Murdoch University, Perth 6150, WA, Australia
[2]Biodiversity Conservation Branch, Resource Management & Conservation, Department of Primary Industries & Water, 134 Macquarie St, Hobart 7001, Tasmania, Australia
[3]Science Division, Department of Environment & Conservation, 50 Hayman Rd, Como 6152, WA, Australia
[4]Nature Conservation Section, Department of Environment & Conservation, 120 Albany Highway, Albany 6330, WA, Australia
Corresponding author: w.dunstan@murdoch.edu.au

Abstract
The aim of our experiments was to develop protocols that can be used to contain and eradicate spot infestations of *P. cinnamomi* that, if untreated, are likely to threaten extensive areas of native vegetation or areas of high conservation value. Treatment regimes were guided by two assumptions: 1. within the selected sites, transmission of the pathogen is by root-to-root contact; and 2. The pathogen is a weakly competitive saprotroph. In Western Australia (WA), treatment and control plots were set-up along an active disease front within scrub-heath vegetation dominated by *Banksia* spp.. Treatments, applied sequentially and in combination, included: (1) destruction of the largest plants within disease free vegetation forward of the disease front; (2) destruction of all plants to create a 'dead zone'; (3) installation of physical root barriers and subsurface irrigation for the application of fungicide/s; (4) surface applications of fungicides selective against oomycetes (triadiazole and Metalaxyl-M) and; (5) surface injection and deep (± 1 m) treatments with Metham-sodium. In a separate experiment in Tasmania (TAS), combined treatments including vegetation removal, Ridomil and Metham-sodium and root barriers, or Ridomil and root barriers alone, were applied to experimental plots within active disease centres in *Eucalyptus-Banksia* woodland.

Abstract for manuscript submitted to Nature May 2008

Containment and eradication of *Phytophthora cinnamomi,* an introduced plant pathogen

W.A. Dunstan[1], T. Rudman[2], N.A. Moore[1,3], T. Paap[1], M.C. Calver[1], B.L. Shearer[4], G.E. StJ. Hardy[1]

[1]Centre for Phytophthora Science & Management, School of Biological Sciences and Biotechnology, DSE, Murdoch University, Perth 6150, Western Australia, Australia

[2]Biodiversity Conservation Branch, Resource Management & Conservation, Department of Primary Industries & Water, 134 Macquarie St., Hobart 7001, Tasmania, Australia

[3]Nature Conservation Section, Department of Environment & Conservation, 120 Albany Highway, Albany 6330, Western Australia, Australia

[4]Science Division, Department of Environment & Conservation, 50 Hayman Rd, Como 6152, Western Australia, Australia

Phytophthora cinnamomi Rands (Stramenopile, Peronosporomycetes), an aggressive root pathogen, has been introduced from its putative natural range in New Guinea-Sulawesi to most continents where it has become a major cause of death to many important species of forest trees. In the biodiversity hotspot of the south-west botanical province of Western Australia *P. cinnamomi* and the disease caused by it is a major threatening process where greater than 6000 km^2 is infested, and more than 3000 of the 5700 described plant species are susceptible.

The first line of defence against new incursions of *P. cinnamomi* are quarantine and hygiene protocols, but to protect remaining areas of high floristic biodiversity from *P. cinnamomi*, emergency response measures are required that will enable recently established infestations to be eliminated.

Here we show a successful approach to the containment and eradication of spot infestations of *P. cinnamomi* in two native plant communities.

Repeated applications of phosphonates (neutralised phosphorous acid) can protect individual plants and have been shown to slow, but not stop, disease progression in native vegetation. At two *P. cinnamomi* infested sites, we applied a sequence of treatments including vegetation (host) destruction, fungicides, fumigation, and physical (root) barriers, after which no *P. cinnamomi* was recovered at repeated assessments. In previous studies, where fewer treatments were used, alone or in combination, *P. cinnamomi* was suppressed but never eradicated, or the infestation was not contained.