Containment and Eradication of *Phytophthora cinnamomi* in Native Vegetation in South-Western Australia and Tasmania[1]

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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.

In the WA experiment, *P. cinnamomi* was not recovered (by soil baiting) from plots after treatment with Ridomil and metham-sodium. In the TAS experiment, similar results were achieved with combined treatments (vegetation removal + Ridomil + metham sodium) but in plots treated with Ridomil alone, recoveries of *P. cinnamomi* increased after initially showing a significant reduction in recoveries.

Introduction

Phytophthora cinnamomi, and disease caused by it, is listed as one of five key threatening processes affecting biodiversity in Australia. The area of native vegetation affected by *P. cinnamomi* exceeds many hundreds of thousands of hectares in Western Australia (> 635 000

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ha; Department of Environment & Conservation 2006), Victoria and Tasmania, and tens of thousands of hectares in South Australia (Environment Australia 2001). Although infestations within the conservation estate are widespread and frequently extensive, large areas remain free of the pathogen and may be protectable. 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 and others 2001). Apart from the application of control measures designed to prevent human vectoring of the pathogen, there has been little testing and operational use of control measures in native vegetation apart from the use of phosphite (Hardy and others 2001). Phosphite has been shown to slow, but not stop, the autonomous spread of P. cinnamomi in native vegetation (Shearer and others 2004).

The aims of the experiments were to contain and eradicate *P. cinnamomi* within natural infestations in native vegetation. To achieve the aims a combination of methods were applied sequentially to experimental plots within sites naturally infested with *P. cinnamomi*. Methods and the rationale for their employment included: 1) localised destruction of vegetation to deny food resources to the pathogen, 2) physical root barriers, to prevent root intrusion into pathogen infested soil, particularly by plants with extensive lateral root systems, and 3) direct attack on the pathogen with fungicides and fumigant/s, starting with oomycete specific compounds and progressing to more robust treatments. The vulnerable or endangered grass-tree *Xanthorrhoea braceata* occurs within the site experimental site in Tasmania. A fungicide + root barriers only treatment was included in the experiment with the aim of assessing its potential as a non-destructive method of protecting individual plants at risk of destruction by *P. cinnamomi.*

Methods and Material

Experimental Sites

Tasmania (TAS)

The experimental site (146.62ºE, 41.16ºS) is located within Narawntapu National Park on the north central coast of Tasmania. The soil is an Aeric (or Semiaquic) Podosol (after the Australian soil classification of Isbell 2002), derived from Quaternary sands that are greater than 3 m deep. The vegetation is classified as *Eucalyptus amygdalina* coastal forest and woodland (Harris and Kitchener 2005), with scattered *Eucalyptus viminalis* and an understorey of *Banksia marginata* and scattered to dense *Xanthorrhoea australis* in heath comprised principally of species within the families epacridaceae and papilionaceae. The climate is temperate. Estimates for the mean maximum in the hottest month is ca. 21ºC, mean minimum in the coldest month ca. 5ºC, and mean annual rainfall is ca. 800 mm.

Western Australia (WA)

The experimental site (118.72ºE, 34.57ºS) is located near Cape Riche. The soil is a Rudosol, derived from fine (< 0.8 mm) aeolian Quaternary sands that are greater than 2.5 m deep. The vegetation is a scrub-heath dominated by *Banksia* spp., principally *B. attenuata* and *B. baxteri*, with an understorey that includes a diverse range of other woody shrubs from species within the families myrtaceae, proteaceae, epacridaceae and papilionaceae. Mean annual rainfall is ca. 490 mm.

Isolates of *P. cinnamomi* from both sites were all of the A_2 mating type.

Site treatments

Western Australia

Treatment and control plots (*n* = 7 each) were set-up along an active disease front, with each plot covering 10 m of disease front, and separated from adjacent plots by a buffer of not less than 5 m. Treatments, applied sequentially and in combination, included: 1) Destruction of the largest plants (principally *Banksia* spp.) to a distance of 10 m on the disease free side of the disease front, by felling and application of glyphosate to stumps (May 2006). 2) Removal of all plants to a distance of 4 m from the disease front, by slashing and applications of herbicides (triclopyr and glyphosate (Aug-Sep 2006). 3) Installation of HDPE root barriers (90 cm deep x 1 mm thick) to ca. 80 cm depth and, subsurface irrigation for the application of fungicide/s (Mar 2007). 4) Surface applications of Terrazole (triadiazole, 10 g/m^2 a.i.; Jun and Aug 2006). Applications of Ridomil 25G (metalaxyl-M, 2.5 g/m^2 a.i.; Jun and Aug 2007). 5) Surface injection, at 15 x 25 cm injection point spacings and to ca. 20 cm depth with Metham-sodium (90 g/m^2; Jun 2007). 6) Deep (± 1 m) treatments with Metham-sodium, 0.5l/m^2 applied via 40 mm PVC vertical tubes installed at 1 m centres, between the disease front and root barrier (Sep 2007).

Tasmania

Experimental plots (5 m x 5 m) were selected from areas showing signs of recent pathogen activity within an extensive area of infestations. The experiment was an unbalanced design, with four plots receiving all treatments (vegetation removal + fungicide + fumigation), four plots were treated with fungicide only (after minor clearance of organic litter and woody debris), and seven plots were untreated. Root barriers (120 cm deep x 1 mm thick) were installed at 80-90 cm deep around all treated plots (complete treatments and fungicide only) with the aim of preventing reinfestation of plots by root borne *P. cinnamomi.* Six of the 11 larger species of terrestrial mammals known to occur within the park could be considered as potential vectors of *P. cinnamomi* because of their digging habits. Given the large number of animals present, and the significant amount of soil disturbance caused by them, exclusion fencing was installed around each plot. Vegetation removal, barrier and fence installation, fungicide application, and injection with metham-sodium, were completed in Apr 2007. Initial treatments were followed by a further fungicide treatment (Aug 2007) and deep application of metham-sodium (Sep 2007). Ridomil 25G (2.5 g/kg Metalaxyl-M) was applied at 100 g/ m^2. Surface injection with Metham-sodium (90 g/m^2) to 15- 20 cm deep was at 150 cm x 250 cm spacings. Deep treatment to ± 1 m with Metham-sodium, (0.75 l/m^2) was applied via 40 mm PVC vertical tubes installed at 1 m spacings. The calculated potential mass of methylisothiocyanate (MITC; the active product from the hydrolysis of metham-sodium) applied to the plots was 180 g/m^2.

Assessment

In the WA experiment, soil sampling was systematic and stratified in two ways: (1) soil depth; sampled at 0-25 cm, 25-50 cm, 80-100 cm and 1.5 m, and (2) distance from disease front (as surveyed in Feb 2006); sampled at 0.5 m and 2.5 m from the disease front, with three sample points at each distance. In the TAS experiment, plots

were sampled systematically at five points, and at three depths per sampling point (0-25 cm, 25-50 cm, and 80-100 cm). Soil samples with roots (120-150 g wet wt.) were flooded and baited with *Lupinus angustifolius* seedlings. Seedling radicles were plated onto NARPH medium (Hüberli 2000), and *P. cinnamomi* was identified by colony form and micro morphology.

Results

Recoveries of *P. cinnamomi* from the WA experiment are shown in Figure 1. A trend in increasing recoveries of *P. cinnamomi* in untreated plots, at 2.5 m from the original disease front (figs 1A-D), showed that the pathogen was active and the infestation was expanding. Surface treatments with terrazole had no significant effect on recoveries of *P. cinnamomi* (recoveries at 0.5 m in treated plots, figs 1A and B). There were no recoveries of *P. cinnamomi* from any of the treated plots after ridomil treatments and surface injection with metham-sodium (fig 1C), or after the additional deep treatment with metham-sodium (fig 1D). There were no recoveries from the 0.5 m mark in treated plots in the last two assessments (figs 1C and 1D), in contrast to the previous harvest where recoveries of *P. cinnamomi* at the same distance in treated plots were similar to untreated controls (fig 1B).

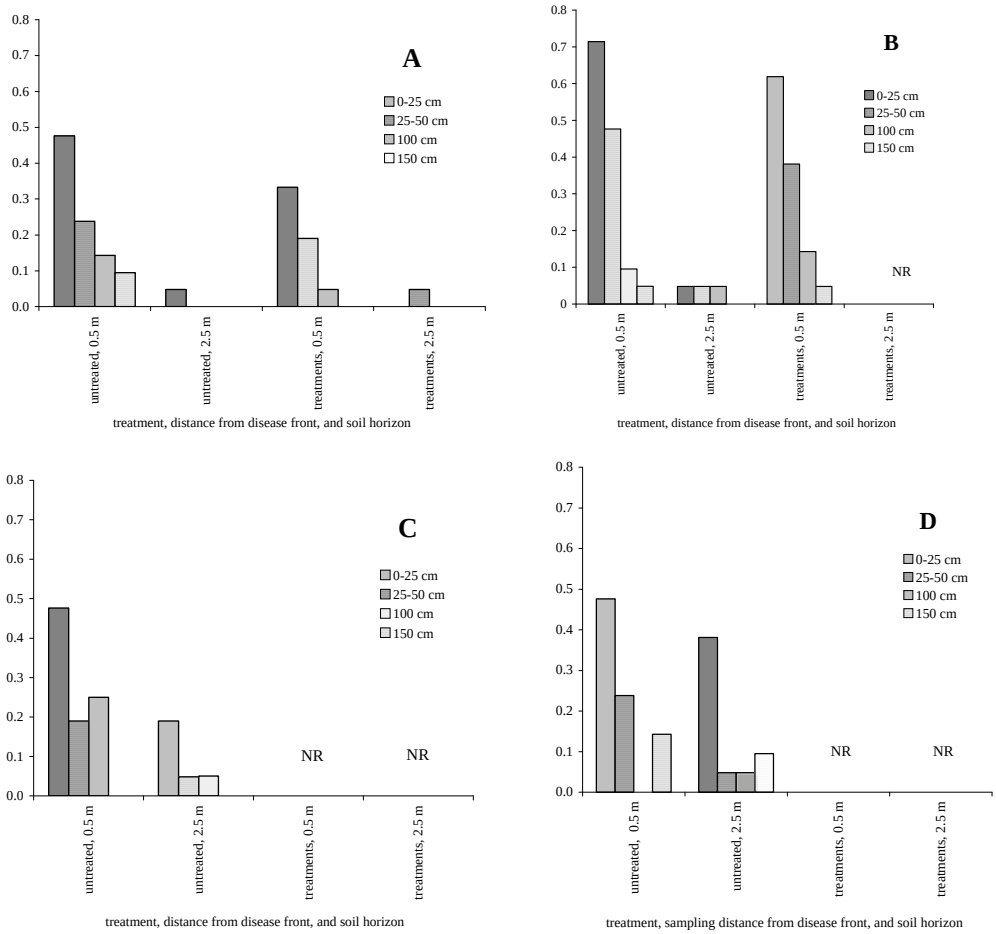

Figure 1—Effect of treatment on recovery of *Phytophthora cinnamomi*, Cape Riche, Western Australia. Untreated plots (controls, + *P. cinnamomi*) *n* = 7, treatments (+ vegetation clearance + fungicide + fumigation) *n* = 7. *n* = 21 for each histogram bar. A. After first Terrazole application and partial vegetation clearance (Jul 2007). B. After second Terrazole application and completion of vegetation clearance (Dec 2006). C. After ridomil treatments and surface injection with metham-sodium. (July 2007). D. After deep treatment with metham-sodium (Nov 2007). Distances (m) are from the active disease front (surveyed Feb 2006). NR = no recovery of *P. cinnamomi.*

Results from four assessments from the TAS experiment are shown in Figure 2. Recoveries of *P. cinnamomi* in the pre-treatment assessment showed a similar distribution of the pathogen between plots and within the soil profile to 1 m (fig 2A). In plots treated with Ridomil alone, recoveries of *P. cinnamomi* were reduced significantly ($p < 0.001$), with a trend for increased recoveries with soil depth (fig 2B). In fungicide only treated plots, *P. cinnamomi* was recovered at all assessments and post-treatment deaths of *X. australis* had also occurred. *Phytophthora cinnamomi* was recovered at low frequency at 1 m in treated plots after the first treatment (ridomil + metham-sodium surface injection; fig 2B) but no further recoveries were made from any of the complete treatment plots after further application of ridomil and deep treatment with metham sodium (figs 2C-D).

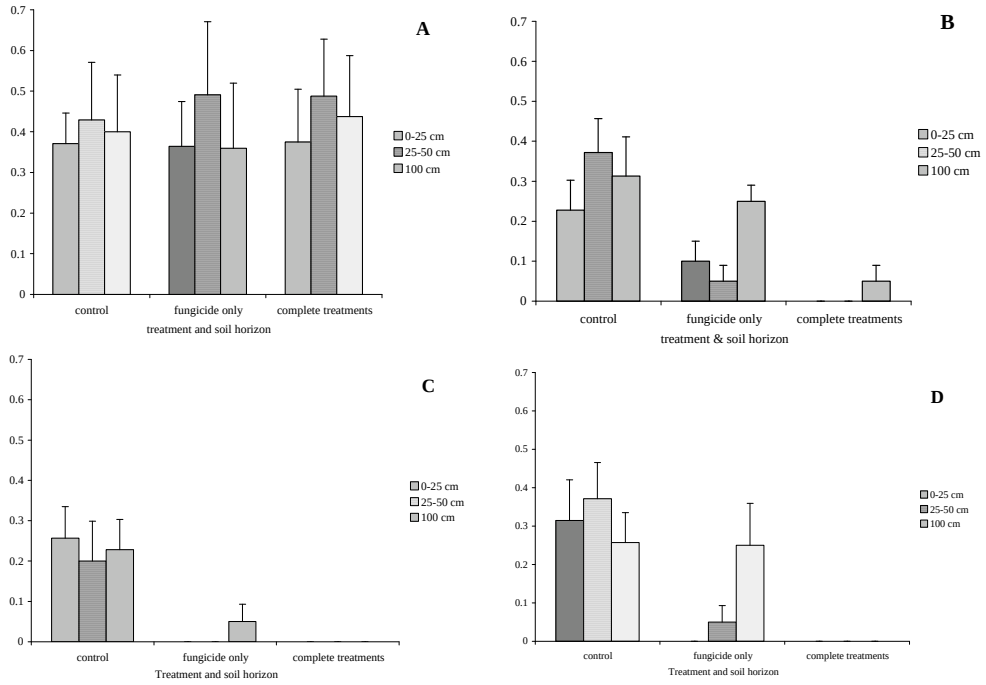

Figure 2—Effect of treatment on recovery of *Phytophthora cinnamomi*, Narawntapu National Park, Tasmania. Control (untreated) plots *n* = 7, fungicide only *n* = 4, complete treatments *n* = *4.* *n* = 20 for each histogram bar in fungicide only and complete treatments, and *n* = 35 in each histogram bar in controls, Error bars are one standard error from 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.

Discussion

Physical root barriers at both sites remain untested. From the results in the WA experiment so far, the pathogen has been stopped short of the barriers by the combination of other treatments. The rate of progress of disease over the last 4-5 years is estimated to be 1-2 m/yr, too slow to expect deaths of susceptible plants beyond root barriers, if the system has failed. A separate experiment is in progress, designed to test the efficacy of root barriers alone. The WA experiment was not a fair test of the efficacy of terrazole because there was almost certainly inadequate rainfall after both applications of the fungicide to enable its infiltration into the soil profile. Rainfall at the site over any 24 h period within the critical period after either application of Terrazole was not more than 6 mm. In the WA experiment, incidental observations on unintended treatment effects included: 1) A decline in plant health, including deaths, of individual plants in some understorey species where large plants had been removed, that was probably caused by increased exposure. 2) In treated plots, an increase in soil moisture within the zone where vegetation was completely removed. Increased soil moisture is likely to be more favourable to the survival of chlamydospores (Weste and Vithanage 1979) but, given the soil type, would be unlikely to enhance lateral movement of the pathogen within the soil profile. 3) Soil erosion by wind and water within the 'dead zone' of treated plots has been minimal (after > 1.5 yr), but could be a problem in the longer term.

Based on recoveries of the pathogen in both experiments, a combination of vegetation destruction, ridomil and metham-sodium applications shows promise as a method to at least contain, and possibly eliminate, the pathogen within small infestations. In neither experiment was fumigation with metham-sodium alone used as a treatment. Therefore, we cannot make any conclusions about the efficacy of metham alone, or metham x metalaxyl interactions. Metham-sodium was used alone in an attempt to eradicate *P. cinnamomi* from plots within infested eucalypt forest by Weste and others (1973), with some success, where *Phytophthora cinnamomi* was not recovered from two of three sites for at least 18 months after treatment, but reinfestation from adjacent infested forest occurred at one site. Vegetation removal and metalaxyl was used, among other treatments, in an eradication experiment in *Banksia* woodland by Hill and others (1995). Very large reductions in recoveries of *P. cinnamomi* were achieved at 0.1-0.4 m soil depth for up to at least 20 months after treatment, however the pathogen was always recovered at later assessments (up to 20 months). Persistence of metalaxyl (half-life 10-82 d; Davison and McKay 1999) and MITC (2 percent of applied amount after 24 d; Zhang and Wang (2002)in agricultural soils is low, but further assessments will be required to determine whether the pathogen has been eliminated or has only been suppressed by residual fungicide/s. From long-term monitoring of *P. cinnamomi* in native vegetation (Victoria, Australia), Weste (2003) did not recover the pathogen in four of six sites after 30 years, and observed regeneration of highly susceptible species. If the autonomous spread of the pathogen can be arrested, and adequate measures to prevent vectoring of the pathogen off-site by animals and people can be implemented, then there is some prospect for limiting damage and recovery of vegetation to its original state. In both experiments we chose sites where a combination of characteristics that included topography, soil type, vegetation (species composition and structure), and climate, were favourable to achieving positive results. However, extensive areas within south-western Australia, south-eastern Australia and Tasmania have similar site

characteristics to those used in both experiments, therefore the methods described could be applied on a broader scale, with modification to suite individual sites.

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