Biol Invasions (2010) 12:913–925
DOI 10.1007/s10530-009-9512-6

ORIGINAL PAPER

Containment and spot eradication of a highly destructive, invasive plant pathogen (*Phytophthora cinnamomi*) in natural ecosystems

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Received: 8 January 2009 / Accepted: 25 June 2009 / Published online: 10 July 2009

Abstract The invasive plant pathogen *Phytophthora cinnamomi* (Stramenopila, Oomycota) has been introduced into 15 of the 25 global biodiversity hotspots, threatening susceptible rare flora and degrading plant communities with severe consequences for fauna. We developed protocols to contain or eradicate *P. cinnamomi* from spot infestations in threatened ecosystems based on two assumptions: in the absence of living hosts, *P. cinnamomi* is a weakly competitive saprotroph; and in the ecosystems we treated, the transmission of the pathogen occurs mainly by root-to-root contact. At two *P. cinnamomi*-infested sites differing in climate and vegetation types, we applied increasingly robust treatments including vegetation (host) destruction, fungicides, fumigation and physical root barriers. *P. cinnamomi* was not recovered at three assessments of treated plots 6–9 months after treatments. Given the high rates of recovery of *P. cinnamomi* from untreated infested soil and the sampling frequency, the probability of failing to detect *P. cinnamomi* in treated soil was <0.0003. The methods described have application in containing large infestations, eradicating small infestations and protecting remnant populations of threatened species.

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Keywords Biodiversity protection · Plant pathogen · Eradication · Fumigation · Fungicide · Invasive species · Root barriers · Vegetation destruction

Introduction

Phytophthora cinnamomi Rands (Stramenopila, Oomycota) is an invasive root pathogen that has been introduced from its putative natural range in New Guinea-Sulawesi (Zentmyer 1988; Hardham 2005) to most continents, including 76 countries (Zentmyer 1980; EPPO 2006) and at least 15 of the 25 global biodiversity hotspots (Myers et al. 2000). The pathogen can be present but cause limited or no apparent damage to vegetation (von Broembsen and Kruger 1985; Balci and Halmschlager 2003; Balci et al. 2007). In contrast, where there are susceptible hosts and favourable environmental conditions, *P. cinnamomi* can destroy populations of plant species that are rare or restricted in range (for example *Arctostaphylos myrtifolia*; Swiecki et al. 2003) and kill dominant species on an extensive scale, such as *Quercus* spp. in Iberia (Brasier 1992), Mexico (Tainter et al. 2000) and California (Garbelotto et al. 2006), *Castanea* species in south-eastern USA (Crandall et al. 1945), and *Eucalyptus* spp. in Australia (Davison and Shearer 1989). Climate change modelling by Brasier (1996) and Bergot et al. (2004) suggest that, depending on the level of increase in temperatures, *P. cinnamomi* could expand its range in Europe, and Podger et al. (1990) predicted increased activity and range of *P. cinnamomi* in Tasmania (Australia) if ambient and soil temperatures increase.

In south-western Australia (SWA), a global biodiversity hotspot (Myers et al. 2000), nearly 1,000,000 ha are infested with *P. cinnamomi* (DEC 2006). Shearer et al. (2004a) estimate that 3,000+ of 5,700 indigenous plant species in SWA are susceptible to *P. cinnamomi* and irreversible damage is being caused to some plant communities (Shearer et al. 2007). In eastern Australia the pathogen also threatens populations of endemic flora (Peters and Weste 1997; Reiter et al. 2004), including the 'living fossil' *Wollemia nobilis* within one of its three known occurrences (Cahill et al. 2008). The threat posed by *P. cinnamomi* in Australia is at least equivalent to that of the 'transformer' class of invasive plants as defined by Richardson et al. (2000), especially when flow-on impacts to fauna are also considered (Garkaklis et al. 2004). The total impacts are sufficient for disease caused by *P. cinnamomi* to be designated as a 'Key Threatening Process to Australia's Biodiversity' in the Australian Commonwealth's Environment Protection and Biodiversity Conservation Act, 1999.

Although *P. cinnamomi* has been widely dispersed around the globe, significant areas of vegetation with high floristic diversity remain free of the pathogen. Strict hygiene controls and the application of phosphonic acid (phosphite, a mixture of HPO_3^{2-} and $H_2PO_3^{-}$) are currently the key management tools available (Hardy et al. 2001; Dell et al. 2005). Phosphite can protect individual plants (Hardy et al. 2001) but cannot halt disease progression in native vegetation (Shearer et al. 2004b). Therefore, in order to protect remaining areas of high floristic diversity from *P. cinnamomi*, innovative responses are required to eliminate recently established infestations.

Attempts to eradicate *P. cinnamomi* have been made previously in both horticultural and natural settings. For example, Zentmyer and Ohr (1978) described several approaches for eradication or containment of *P. cinnamomi* in avocado orchards, including host destruction and soil fumigation or chemical barriers, depending on the size of the infestation. In an unreplicated experiment in eucalypt woodland, Weste et al. (1973) used trenching and a chemical barrier treatment, combined with fumigation, in an attempt to contain an extensive infestation of *P. cinnamomi*. The experiment showed promise but ultimately failed (Weste et al. 1973; Weste and Marks 1987), probably because of a combination of unfavourable site conditions (soil type and topography), and unusually high rainfall. Hill et al. (1995) used physical root barriers and vegetation destruction, combined with chemical treatments (formaldehyde or metalaxyl), in eradication experiments in *Banksia* woodland. They were unable to eradicate *P. cinnamomi* with formaldehyde. Using metalaxyl (15 g/m^2), they did not recover *P. cinnamomi* from artificial inoculum at one of two sites (to a depth of 1.3 m), nor from naturally infested soil at two sites (0.1–0.4 m depth).

Here we extend the previous studies by identifying a successful approach to the containment and

eradication of existing infestations of *P. cinnamomi* in natural ecosystems. To establish the widespread applicability of our approach, we assessed two sites differing in climate and vegetation. We chose to work on sandy sites of low relief because of the biodiversity threat from *P. cinnamomi* in such widely distributed soils in southern Australia where *P. cinnamomi* has a high impact. On these soils, rainfall infiltration rates are high, surface water movement is rare and the pathogen moves slowly by root to root contact (Hill et al. 1994). Management options developed for these sites will not apply to landscapes where zoospores have been shown to be transported 50–70 cm/d in subsurface water (Shea et al. 1983), and where propagules of *P. cinnamomi* can be transported in surface or sub-surface water and cause expansion of disease foci at rates of up to 400 m/year (Weste et al. 1973).

Methods

Study areas

Site 1 was in *Banksia*-dominated shrubland (Cape Riche, Western Australia; 118.72°E, 34.57°S), and site 2 in *Xanthorrhoea* (grass-tree) dominated heath in *Eucalyptus* woodland (Narawntapu National Park, Tasmania; 146.62°E, 41.16°S). Climate at site 1 is temperate Mediterranean, and at site 2, maritime temperate (Peel et al. 2007). Soils at both sites were highly infertile (Table 1) deep apedal sands within soil orders Rudosol and Podosol, respectively (Isbell 2002). They are representative of many *P. cinnamomi*-infested locations across southern mainland Australia and Tasmania, including areas of high conservation value. Sites were disease active (Dell et al. 2005) and (a) large enough to allow adequate replication of treatments, (b) with indicator plants remaining to allow for accurate mapping of the disease fronts prior to soil baiting for the pathogen, and (c) secure against vandalism.

Using characteristics of disease expression defined by Ristaino and Gumpertz (2000), namely linearity of disease fronts, slow rates of advance of disease fronts and similar rates of advance in disease fronts up and down slope, we concluded that the spread of *P. cinnamomi* at these sites was by root-to-root contact rather than water transport. Furthermore, soil particle size distribution of soils from both sites (99% less than 0.5 mm and 22% within 20–200 μm at site 1; 80% within 20–200 μm at site 2) were not likely to be conducive to *Phytophthora* zoospore movement in soil water (Duniway 1976; Hill et al. 1994). The only incidence of dispersal by a means other than root to root contact was at site 2, where discrete disease foci occurred at some distance from the disease front. We attributed this to activities of native mammals, particularly *Vombatus ursinus* (common wombat). Basing estimations on current rates of disease spread, the sites had been infested for 20 years or longer. Disease impact was very high at both sites, with 63 and 29% of the larger and/or common plant species present at sites 1 and 2, respectively, killed by *P. cinnamomi*. Mating tests (Erwin and Ribeiro 1996) with isolates of known mating type showed that isolates of *P. cinnamomi* from both sites ($n = 10$ per site) were A_2 mating type, the common type in Australia (Weste and Marks 1987) and globally (Erwin and Ribeiro 1996).

Treatments

The treatment regimes we applied were guided by two assumptions: (1) At both sites, the development of well-defined linear disease fronts indicated that

Table 1 Soil nutrient analyses, *Phytophthora* containment and eradication sites

Location	Element or soil characteristic[a]											
	N[b]	P[b]	K[c]	S[b]	Ca[c]	Mg[c]	Fe[d]	Mn[d]	Cu[d]	B[b]	C[e]	pH[f]
Cape Riche	2 (nd)	2 (12.7)	0.1	3	1.1	0.5	7	0.2	0.5	nd	1.7	4.1
Narawntapu	2 (0.07)	2 (33.3)	0.1	3	0.7	0.3	31.6	3.4	0.2	0.4	0.9	3.9

nd not determined or below detection limit

[a] Values represent the mean of three soil horizons to 1 m at each site; [b] mg/kg, nitrate + ammonium N, extractable P and (total, %N or mg/kg P); [c] cmol/kg;[d] mg/kg, DTPA extractable; [e] organic carbon, %; [f] in 0.01 M $CaCl_2$

autonomous spread of the pathogen was by root-to-root contact (see justification above); and (2) In the absence of host plants, the pathogen is a weakly competitive saprotroph (Marks et al. 1975; Malajczuk 1983).

Site 1: Western Australia

The approach we took to contain and eradicate *P. cinnamomi* at site 1 is shown in Fig. 1. Alternating treated and untreated control plots ($n = 7$ each, each 10 m long and extending 4 m away from the disease front and separated by at least 5 m) were established along 300 m of an active disease front. The estimated mean rate of disease progression was 2.2 m/year over the 4–5 years prior to establishment of the experiment. The location of the disease front was determined from deaths of susceptible species of plants and confirmed by recoveries of *P. cinnamomi* from baited surface soil samples taken along the front and up to 8 m into healthy vegetation.

Treatments we applied sequentially were: (1) Destruction of the largest plants (principally *Banksia* spp.) to a distance of 10 m forward of the disease front, by felling and spot treatment with glyphosate herbicide (180 g/l a.i.) to stumps (May 2006). The aim was to reduce the probability of infection of extensive lateral roots from plants in non-infested soil by roots from infested plants and soil. (2) Destruction of all plants to a distance of 4 m forward of the

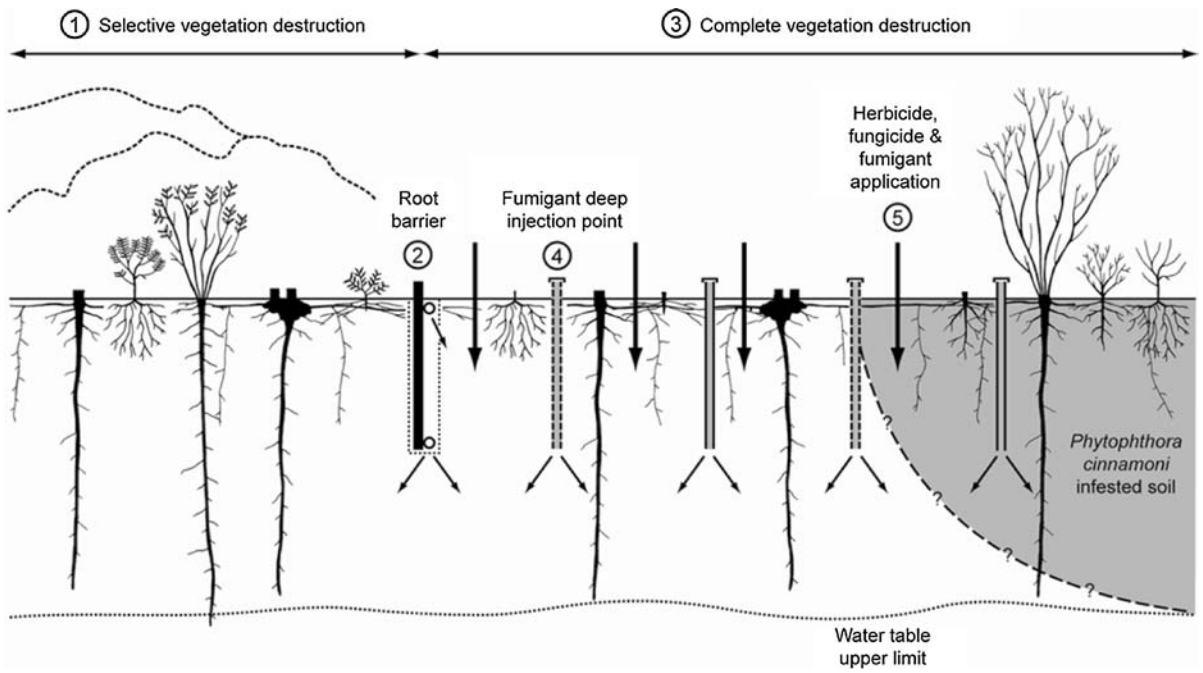

Fig. 1 Approach to containment and eradication of *Phytophthora cinnamomi* at site 1 (Cape Riche, Western Australia). Progression of the pathogen and disease caused by it was from *right* to *left*. Treatments: (*1*) Selective vegetation destruction. Felling and herbicide treatment of larger plant species, that may have lateral roots intruding into *P. cinnamomi* infested soil. (*2*) Root barrier. High density polyethylene (HDPE) sheeting, installed vertically to ca. 1 m combined with irrigation tubing for the application of fumigant or root retardant below the physical barrier. The aim was to prevent lateral root intrusion into, or out of, infested soil. (*3*) Complete vegetation destruction, within infested soil, and into disease free vegetation. Vegetation was removed by slashing to ground level, followed by herbicide treatments (area treatment followed by spot treatments). The aim was to remove sources of nutrition for the pathogen. (*4*) Deep injection of fumigant. Fumigant (metham-sodium) was applied to 1 m deep in the soil profile via permanently installed tubing. The aim was to kill the pathogen and remaining living roots below the level treated by shank injected fumigant and fungicides. (*5*) Herbicide, fungicide and fumigant treatments (by shank injection). The aim of herbicide treatments was to kill remaining plants and remove sources of nutrition for the pathogen. Application of fungicides with a high specificity for oomycetes (triadiazole and metalaxyl) was followed by shank injection of fumigant (metham-sodium). Figure proportions are approximately to scale

disease front, by slashing vegetation to ground level and spot application of triclopyr (10 g/l a.i.) and area application of glyphosate (1.1 g/m^2 a.i.) in August–September 2006. The aim was to deny the pathogen any living host plants, and therefore sources of nutrition. (3) Physical root barriers and subsurface irrigation for the application of fungicide or fumigant. We installed high-density polyethylene (HDPE) root barriers (90 cm deep × 1 mm thick) to ca. 80 cm depth and 4 m forward of, and parallel to, the disease front. Two lines of subsurface irrigation (17 mm diameter polyethylene), for the application of fumigants/herbicides, were installed at depths of 10 and 80 cm on the side of the root barriers facing infested soil (March 2007). As for treatment (1), the aim of the barrier was to disrupt root-to-root contact between non-infected plants and infested roots and soil. (4) Surface applications of selective fungicides, and surface and deep application of a soil fumigant, that were aimed at killing the pathogen and hosts. A granular formulation of triadiazole (CAS no. 2593-15-9) was applied at the rate of 10 g/m^2 a.i. at each of two applications in June and August 2006, and metalaxyl-M (CAS no. 57837-19-1) (2.5 g/m^2 a.i., wettable powder) was applied in June and August 2007. These two compounds were chosen because they have specificity for Oomycota (Worthing and Walker 1983) and have been used for control of *Phytophthora* in horticultural and other crops (Erwin and Ribeiro 1996; Sastry and Hegde 1992). The fumigant metham-sodium (CAS no. 137-42-8) was applied to the soil at 90 g/m^2 a.i. by shank injection at 15 × 25 cm spacing and to ca. 20 cm depth (June 2007). Metham-sodium was also applied at 212 g/m^2 a.i. and to a depth of ±1 m via 40 mm polyvinyl chloride (PVC) tubes that were installed vertically at 1 m centres, between the disease front and root barrier (September 2007).

Site 2: Tasmania

Treatments at site 2 were similar to those at site 1. The complete treatment regime was applied to *P. cinnamomi*-infested ground within a larger complex of infestations. Experimental plots (5 × 5 m) were selected from areas showing a chronology of pathogen activity over several years that included old death, stumps and recently dead plants of susceptible species. The progression of disease fronts (mean 1.2 m/year) indicated that the plots had been infested for ca. 4 years at the commencement of the experiment. Sites were fenced to exclude wombats.

The experiment was an unbalanced design, with four plots receiving all treatments (barriers + vegetation destruction + fencing + fungicide + fumigation), four plots were treated with fungicide only (after root barrier installation, fencing, and minor clearance of litter and woody debris), and seven plots were untreated. HDPE root barriers (120 cm deep × 1 mm thick) were installed at 80–90 cm deep around all treated plots (complete treatments and fungicide only) with the aim of preventing re-infestation of plots by root-borne *P. cinnamomi*. Subsurface irrigation tubing, similar to site 1, was installed at the same time as the root barriers. Vegetation removal, spot herbicide treatment (glyphosate, 180 g/l a.i.) barrier and fence installation, fungicide application and injection with metham-sodium were completed in April 2007. Initial treatments were followed by a further fungicide treatment at repeat rates (August 2007). Metalaxyl-M was applied at 2.5 g/m^2 a.i. per treatment, and surface shank injection with metham-sodium was at 90 g/m^2 a.i., to 15–20 cm deep and at 15 × 25 cm spacing. A deep treatment to ± 1 m with metham-sodium (317 g/m^2 a.i.) was applied via 40 mm PVC vertical tubes installed at 1 m centres (September 2007).

Assessment

In site 1, soil sampling was systematic and stratified in two ways: (1) In order to detect progression of disease into pathogen-free vegetation over time, within each plot we sampled soil in three transects at points 0.5 and 2.5 m forward of the disease front. (2) At each of the six points we sampled soil at depths of 0–25, 25–50, 80–100 and 130–150 cm, using an 8 cm hand auger. Site 1 was sampled in September and December 2006, July and November 2007, and March 2008. Therefore, at site 1 we aimed to contain and eradicate the pathogen in a 4 m wide corridor along the disease front, not from the entire infestation.

At site 2, we sampled each plot systematically at five points, within 1 m from the centre and from each quarter of the plot, and at three depths per sampling point (0–25, 25–50 and 80–100 cm). At both sites, roots were hand-picked from soil at each sampling

depth and included in the samples retained for recovery of *P. cinnamomi*. Site 2 was sampled in April, June, October and December 2007, and March 2008. At site 2 we aimed to eradicate the pathogen from each of the treatment plots.

We recovered *P. cinnamomi* by soil baiting (Chee and Newhook 1965; Marks and Kassaby 1974) using 2–3 d old *Lupinus angustifolius* cv. 'Mandelup' seedlings that were floated in distilled water over 120–150 g (wet wt.) for samples from Cape Riche, and 50–60 g (wet wt.) for samples from Narawntapu N.P. After 2–5 days we plated lupin root baits from every sample, both symptomatic and asymptomatic of infection by *P. cinnamomi*, onto NARPH selective medium (Hüberli et al. 2000). We repeat baited each sample from site 1.

Phytophthora cinnamomi was identified by macro- and micro-morphology of primary isolations (Stamps et al. 1990). To validate our identifications we sequenced the internal transcribed spacers (ITS) of the ribosomal DNA (rDNA) from selected isolates recovered from both experimental sites. Briefly, the *Phytophthora* isolates were grown on half-strength potato dextrose agar (PDA) at 20°C for 2 weeks and genomic DNA was extracted from mycelium according to Andjic et al. (2007). The region spanning the internal transcribed spacer region (ITS1-5.8S-ITS2) of the ribosomal DNA was amplified using primers ITS-6 (Cooke et al. 2000) and ITS-4 (White et al. 1990). The PCR reaction mixture, PCR conditions, the clean-up of products and sequencing were as described by Andjic et al. (2007).

Statistical methods

Both experiments were repeated-measures ANOVA designs with fixed factors of: (1) treatment (complete treatments and untreated controls in both experiments, and additional fungicide only treatment in experiment 2), (2) sampling depth ($n = 4$, experiment 1; $n = 3$, experiment 2), and (3) distance from disease front at site 1. For each subject (plot) and for each assessment, the number of recoveries of *P. cinnamomi* (dependent variable) was scored for each sampling depth within each plot (Site 1, possible values 0–3, and 0–5 from site 2). Greenhouse-Geisser epsilons (ε) were used to modify the degrees of freedom in all main effects and interactions involving the repeated-measures factor to protect against possible violation of the sphericity assumption (von Ende 2001).

Untransformed data from site 2 fulfilled assumptions of normality and independence of errors. To reduce the proportion of null values (0) in data from site 1, the difference in recovery values was calculated for each adjacent pair of control and treated plots, for each distance, depth and assessment time. Statistica (version 5 for PC; Statsoft, Tulsa, OK, USA) was used for analysis of variance.

For assessments 3–5 (July, November 2007, March 2008 at site 1; October, December 2007, March 2008 at site 2), where *P. cinnamomi* was not recovered in treated plots from either site, we calculated the probability that we failed to detect *P. cinnamomi* if it were present in treated plots (P), after the methods described by Davison and Tay (2005) where $P = (1-r)^n$, and r was the proportion of samples within control plots yielding *P. cinnamomi* (at depth 0–25 cm) and n was the number of samples taken at similar soil depth and position within treated plots (6 per plot, site1; or 5 per plot, site 2).

Results

Site 1, Cape Riche, Western Australia

Within untreated plots, recoveries of *P. cinnamomi* ranged from 0–71.4%, depending on assessment time and sampling depth (Fig. 2a, b). In close proximity to where plant deaths had occurred in untreated plots, the mean recovery of *P. cinnamomi* across all assessments was 55% at 0–25 cm sampling depth and recoveries declined with increasing soil depth to a mean of 7% at 1.3–1.5 m. The roots of most plant species were concentrated within the upper 40 cm of the soil profile. There was a trend of increasing frequency in recoveries of *P. cinnamomi* with time in untreated plots at 2.5 m forward of the disease front (Fig. 2b), consistent with progression of the pathogen into disease-free vegetation, and recoveries from soil were mirrored by a succession of plant deaths further into disease free vegetation over the length of the disease front, outside the treated plots.

With regard to the difference in recovery between treated and untreated plots, repeated measures ANOVA revealed significant main effects of distance ($F_{1,48} = 4.7890$, $P < 0.05$), depth

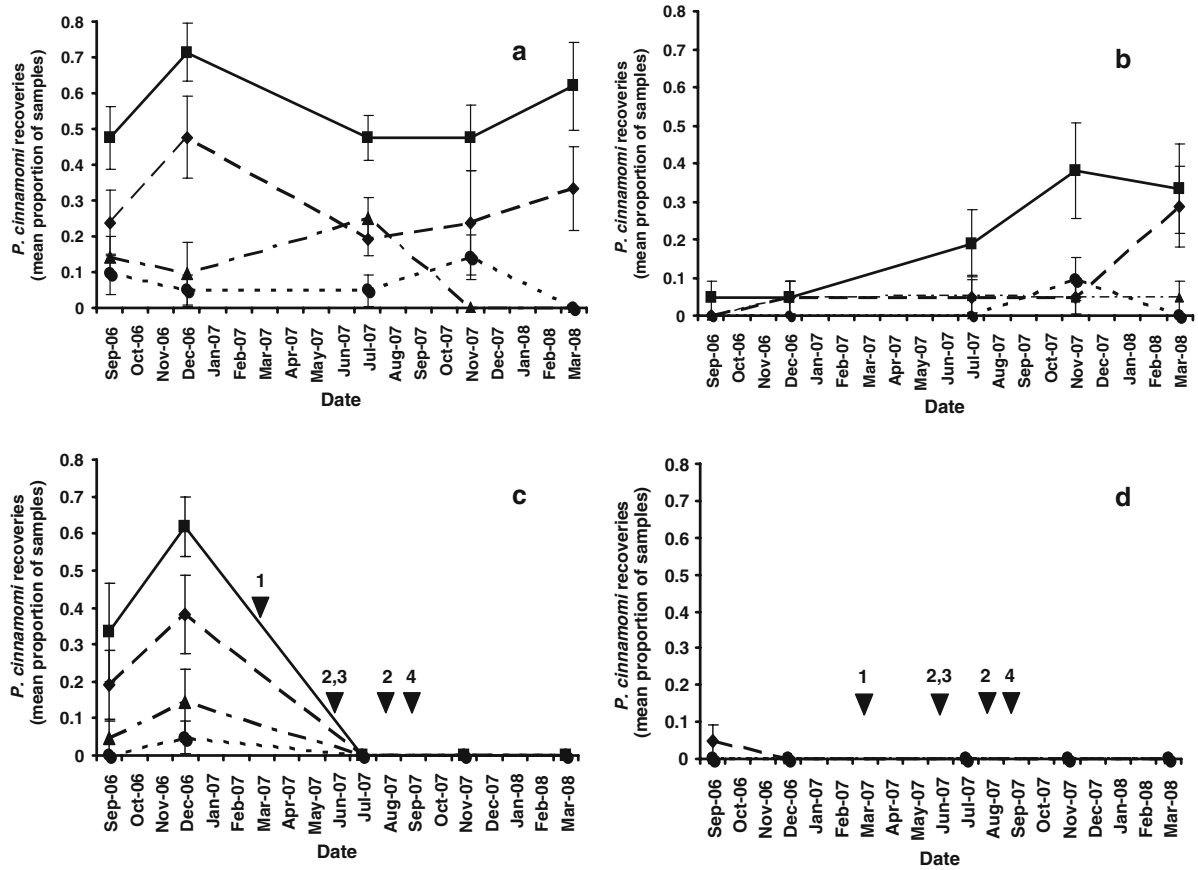

Fig. 2 Recoveries of *Phytophthora cinnamomi*, site 1, Cape Riche, Western Australia, stratified by distance from the disease front (0.5 or 2.5 m; as surveyed in February 2006), and soil sampling depth (■ 0–25 cm; ◆ 25–50 cm; ▲100 cm; ● 150 cm). **a** Untreated control plots, 0.5 m from the disease front. **b** Untreated control plots, 2.5 m from the disease front. **c** Treated plots, 0.5 m from the disease front. **d** Treated plots, 2.5 m from the disease front. Treatment timings are indicated by arrows: *1* root barrier installation; *2* fungicide (metalaxyl) application; *3* fumigant (metham-sodium) shank injection; *4* fumigant (metham-sodium) deep treatment. Vegetation destruction and triadiazole (fungicide) treatment predates the first assessment shown. Values are means ± 1 SE, $n = 7$ plots per treatment, with three samples per plot at each soil sampling depth and distance from the disease front

($F_{3,48} = 11.3671$, $P < 0.00001$) and time of harvest ($F_{4,192} = 9.2413$, $P < 0.00001$ after Greenhouse-Geisser correction $\varepsilon = 0.78$), as well as a significant interaction between depth and time of harvest ($F_{4,192} = 3.3172$, $P < 0.0005$ after Greenhouse-Geisser correction). Over time the differences in *P. cinnamomi* recoveries between treated and untreated plots were greater closer to the initial disease front, indicating that treatments were successful in reducing recoveries of *P. cinnamomi*. There were no recoveries of *P. cinnamomi* from treated plots after the second assessment (December 2006), where plots had been treated with metalaxyl and by shank injection with metham-sodium between assessments 2 and 3 (December 2006 an July 2007; Fig. 2c, d). Deep application of metham-sodium (between assessments in July and November 2007) may have been unnecessary to eradicate *P. cinnamomi* up to and including a depth of 1.5 m within the soil profile (Fig. 2c), but was applied as an additional precaution, with the aim of fumigating soil below 1.5 m down to the upper limit of the water table at ca. 2.5 m. Differences in recoveries were also greater at depths up to 50 cm, showing that treatments killed *P. cinnamomi* recoveries at these depths. At greater depths recoveries were low overall, so there was little difference between treated and untreated plots. The difference in recoveries of *P. cinnamomi* between

treated and untreated plots increased over time. The interaction between depth and harvest showed that the difference in *P. cinnamomi* recoveries between treated and untreated plots was initially low at all depths, but over time increased markedly at depths up to 50 cm (Fig. 2). Triadiazole treatments (applied before the first assessment in September 2006) were ineffective, as the recovery of *P. cinnamomi* at all sampling depths increased between assessments in September and December 2006 (Fig. 2c). At the final assessment, indirect bioassays of soil samples from treated plots showed no evidence for residual fumigant or fungicides.

Site 2, Narawntapu National Park, Tasmania

In treated plots, recoveries of *P. cinnamomi* decreased significantly with time ($F_{2,72} = 63.3825$, $P < 0.0001$ after Greenhouse-Geisser correction $\varepsilon = 0.84$) and there was also a significant time × treatment interaction ($F_{4,72} = 8.2745$, $P < 0.0001$, after Greenhouse-Geisser correction). Sampling depth was not significant as a main effect or in any interaction. Across all fungicide plus fumigation treatment plots, the recovery of *P. cinnamomi* was reduced from a mean of 43% at the pre-treatment assessment to none at the third assessment in October 2007 and in subsequent assessments (Fig. 3c). Unlike site 1, in site 2 *P. cinnamomi* was recovered infrequently at 1 m depth after the first application of metalaxyl and shank injection of metham-sodium, but was not recovered at all after the second application of metalaxyl and deep application of metham-sodium (Fig. 3c).

In all fungicide-only treated plots, mean recoveries before treatment were 41% and post-treatment ranged between 2–14% (Fig. 3b). At any given assessment and soil sampling depth, recoveries were less in fungicide-only treated plots than in untreated plots, but the pathogen was always recovered from at least one of the plots treated with fungicide only (Fig. 3b).

In the untreated controls, little change was evident (Fig. 3). Recoveries of *P. cinnamomi* ranged from 17 to 43% with a mean of 30% across all assessments and sampling depths. There was a general trend for declining recoveries with time in untreated plots (Fig. 3a), that may have been attributable to a prolonged local drought. Unlike site 1, there were no significant differences in recoveries between sampling horizons in untreated plots (Figs. 2a, 3a).

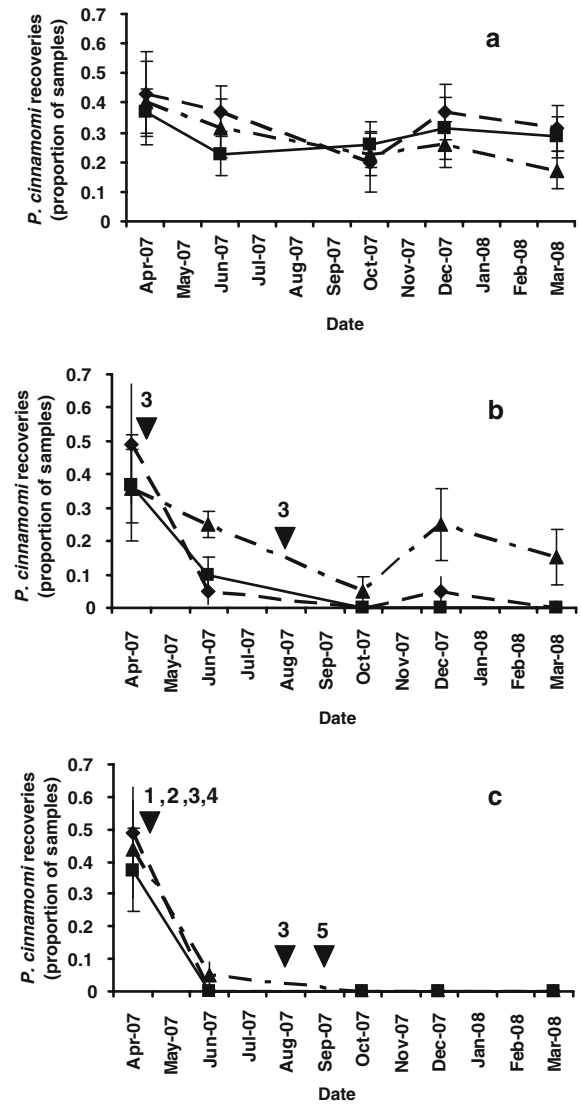

Fig. 3 Recoveries of *Phytophthora cinnamomi*, site 2, Narawntapu National Park, Tasmania. Soil sampling depths: ■ 0–25 cm; ◆ 25–50 cm; ▲ 100 cm. **a** untreated controls. **b** Fungicide treatment only. **c** Complete treatments (vegetation destruction + fungicide + fumigation). Treatment timings indicated by arrows: *1* Vegetation destruction; *2* root barrier installation; *3* fungicide (metalaxyl) application; *4* fumigant (metham-sodium) shank injection; *5* fumigant (metham-sodium) deep treatment. Values are means ± 1 SE; $n = 7$ control plots, $n = 4$ each for both fungicide treated plots and complete treatments plots, with five samples per plot at each soil sampling depth

At sites 1 and 2, pathogen recovery rates (in untreated plots) were high, and the number of samples taken at each assessment was sufficiently large to show that the probability of not detecting the

pathogen in treated plots was very low, where P values for estimates at any one assessment ranged from 1.6×10^{-12} to 8.7×10^{-9} at site 1, and from 0.0005 to <0.003 at site 2.

Discussion

Controlling the pathogen in field trials

In this study, we show for the first time, and with high probability, that it is possible to both contain and to eradicate *P. cinnamomi* from spot infestations in native vegetation growing in sand with low relief. We demonstrated that we could eradicate *P. cinnamomi* at site 2 (NP, Tasmania) and contain and eradicate *P. cinnamomi* along the disease front at site 1 (Cape Riche, Western Australia). While we cannot make conclusions regarding the relative importance of any treatment on its own, or the significance of interactions between treatments, vegetation destruction and fumigation were probably the key treatments in the process. It is likely that vegetation destruction removes living host material for further colonisation by the pathogen, a weak saprotroph, whilst the fumigation treatment killed the vegetative and survival structures in the treated area. Consideration of treatments that address the different aspects of the life cycle of the pathogen and its local modes of spread are considered critical to implementing eradication programs.

We chose to apply a sequence of treatments because of the need to achieve adequate replication of treatment and control plots within existing infestations, and the high probability of confounding results by disturbance caused from repeated sampling if plot sizes were too small. Within the time frame of the experiments at both sites, it is unlikely that the physical root barriers have been robustly tested as roots would not have had time to grow back and challenge the barriers. However, at site 1 vegetation removal, metalaxyl and fumigation treatments halted movement of the pathogen from infested soil. Barriers are likely to be important in the long-term, reducing the probability of root intrusion from disease-free vegetation into untreated soil. We plan on-going monitoring of the barrier's effectiveness. In metalaxyl treated plots at site 2, like Hill et al. (1995), we did not recover the pathogen from the upper soil horizon (0–25 cm), but we recovered the pathogen in up to 25% of samples at 1 m depth at the last three assessments. Therefore, metalaxyl may be useful as an initial response whilst other more effective treatments are being established.

Fungicide applications only suppressed *P. cinnamomi* at site 2, whilst at site 1 the initial fungicide application (triadiazole) was ineffective, indicating the importance of fumigation. At site 1, rainfall over the period when triadiazole was applied was not conducive to satisfactory infiltration of the fungicide: rainfalls in the month after each fungicide treatment were 40 and 37 mm, respectively, with not more than 11 mm in any 24 h period.

Since the 1990s, phosphite has been applied to natural ecosystems to maintain populations of some critically endangered flora under threat from *P. cinnamomi* (Barrett 2003; Shearer et al. 2004b, 2007). Although phosphite can protect some species we chose not to apply phosphite because of variable responses between plant species in both uptake of phosphite and duration of its protective effect (Tynan et al. 2001), and it does not stop disease progression in native vegetation (Shearer et al. 2004b). Furthermore, phosphite inhibits, but does not kill, *Phytophthora* spp. (Barchietto 1992; Wilkinson et al. 2001; Daniel and Guest 2006). There is also uncertainty about its ability to provide protection to some common species (including some present at site 1; Shearer and Fairman 2007; Shearer et al. 2007), and phosphite protected plants mask the presence of the pathogen while still producing infective stages (Shearer and Fairman 2007). Isolates of *P. cinnamomi* from sites with prolonged phosphite use can be less sensitive to phosphite *in planta* (Dobrowolski et al. 2008). Therefore, the use of phosphite should not be relied on to contain the spread of *P. cinnamomi* and it should only be used as a temporary management tool, particularly where vegetation is already infested, until more long lasting and robust methods of control can be implemented.

Complete vegetation destruction was used at both sites because, for the plant species that survive challenge by *P. cinnamomi*, we had no information on their level of resistance or tolerance to *P. cinnamomi*. Retention of *P. cinnamomi*-tolerant species would enable survival of the pathogen, as has been shown to be possible by Websdane et al. (1994), build-up of inoculum and in the long term, episodes

of disease in susceptible plants recolonizing treated areas, and further spread.

At sites 1 and 2 the overall mean recovery rates of *P. cinnamomi* within untreated plots, across all assessments and soil horizons, were 16.8 and 30.1%, respectively. Results from both sites were similar to results from other studies where recovery rates ranged from 7.2–25% in eucalypt forest (Podger 1968; McDougall 1996; Davison and Tay 2005), even given that *P. cinnamomi* was not expected to be recovered from a large proportion of samples from site 1. Recovery of the pathogen by soil baiting is dependant on production of zoospores that are produced from sporangia arising from somatic hyphae, or resting stages of the pathogen (oospores or chlamydospores; Erwin and Ribeiro 1996). Therefore, in the absence of mycelium, detection is dependant on stimulation of germination in oospores and/or chlamydospores, if they are present. At both sites we found only the A_2 mating type, therefore oospores were unlikely to be present, and even where both A_1 and A_2 mating types are present, sexual reproduction has been shown to be infrequent in nature, if it occurs at all (Hardham 2005).

Although we have no direct evidence, chlamydospores were potentially the long-term survival life stage of the pathogen present at both sites, in soil and in dead plant tissue. Chlamydospores survive for up to 12 months in non-sterile field soils (Weste and Vithanage 1979) and are observed in dead roots for up to 6 years (Zentmyer and Mircetich 1966), although McCarren et al. (2005) have questioned the results of most survival studies, stating that chlamydospores were not conclusively identified. Oospores and/or chlamydospores are likely to be present in other ecosystems, and their presence could compromise detection of the pathogen (by baiting) and eradication.

Although metham-sodium was effective against *P. cinnamomi* at both study sites, we did not identify the life-stages of the pathogen at either site, therefore its efficacy against resting stages of the pathogen needs to be confirmed.

In this study we had adequate negative controls, to compare with treatments and assess their efficacy, that would be absent if the approach described in this study were applied to an entire discrete infestation. A selective sampling program, targeted at root systems, combined with detection by baiting and a PCR based method (for example, Williams et al. 2009) would be a more sensitive and economical approach to assessment of treated infestations in the longer term, in contrast to the systematic but less targeted approach used in this study. Both sites in this study will be maintained, with spot applications of herbicides, and monitored for at least 2 years after the last fumigation treatments.

Management implications

In 2007, some of the elements of our experimental approach to containment and eradication of *P. cinnamomi* were adopted by the Western Australian Department of Environment and Conservation to contain a large (ca. 250 ha) 35-year-old infestation that threatens the integrity of the Fitzgerald River National Park, a 3,300 km^2 World Biosphere Reserve. Some soil types within the infestation are conducive to dispersal of *P. cinnamomi* by animals, therefore the site was surrounded by exclusion fencing and macropods (kangaroos and wallabies) were removed. Vegetation was mechanically removed in a 6 m wide belt to enable access for fencing and trenching. Detailed elevation maps to 20 cm resolution were drawn of the site and data used to build drains to move surface water into naturally occurring sumps within the infested areas, and to reduce surface water movement from disease-free areas into the infested area. The engineering works were designed to allow for extreme 1 in 100 year rainfall events to ensure excessive surface water movement across the treated infested areas would not occur. Some 3 km of HDPE barriers were installed in part of the infestation boundary with sub-surface tubing to allow application of fumigants to prevent *P. cinnamomi* from crossing watershed boundaries.

The approach used in this study could be applied more widely to contain or eradicate any soil-borne fungal pathogen on sites of similar topography, soils and climate. The approach described is likely to be ineffective where soil type and/or site topography permit movement of propagules of the pathogen such as zoospores in water (surface or sub-surface), unless some engineering approaches to contain or redistribute water are included. Most of the operations, apart from root barrier installation, were deliberately undertaken using equipment that could be carried in one or two person loads that would enable treatment

of small infestations in remote or difficult terrain. The trench excavator used at site 2 weighed 650 kg, and can be carried by air. The same approach may also be applied strategically for aerially dispersed *Phytophthora* species that also produce inoculum in soil (such as *Phytophthora ramorum*; Fichtner et al. 2007). Destruction of host plants is the main method used to contain new infestations of *P. ramorum* (Hansen 2008), a pathogen with an extensive and expanding range in forests and woodlands in Europe and western North America. Therefore, the approach we have taken in the current study has potential for application in other natural ecosystems and against other *Phytophthora* species, especially where there are high value biodiversity assets under threat.

Fumigants such as iodomethane, propargyl bromide, ethanedinitrile, 1,3-dichloropropene, alternatives to methyl bromide, are potentially more effective than metham-sodium (Ruzo 2006), and their efficiency against *Phytophthora* species needs to be tested. However, they are expensive and likely to be significantly more hazardous to handle than metham sodium, particularly under conditions likely to be encountered in remote locations.

In conclusion, this study has demonstrated the potential of eradicating *P. cinnamomi* and other similar soil-borne plant pathogens through vegetation destruction, fungicide and fumigant treatments together with containment barriers to protect threatened vegetation.

Acknowledgments Funding was provided by the Department of Environment, Water, Heritage and the Arts (Commonwealth of Australia). We thank R. Armistead, B. Morrison, M. P. Dobrowolski, K. Howard, C. E. Crane, Y. Chen, and S. Barrett as principal contributors in the execution of field experiments. The Department of Environment and Conservation (Western Australia), and the Parks and Wildlife Service (Tasmania) gave material support. We thank, without implication, two anonymous reviewers for thorough and perceptive feedback on the first submission of this paper.

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