Phytophthora in Forests and Natural Ecosystems

2nd International IUFRO Working Party 7.02.09 Meeting
Albany, W. Australia 30th Sept.- 5th Oct 2001

Editors

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CONTENTS

i

iv

IUFRO Working Party 7.02.09 *Phytophthora* Diseases of Forest Trees

The second meeting of Working Group 7.02.09, "*Phytophthora* Diseases of Forest Trees," under the auspices of the International Union of Forestry Research Organizations, bought together 83 scientists from 9 countries in Albany, Western Australia. These proceedings provide an overview of the extensive range of active *Phytophthora* research and its management that is being conducted worldwide on forest trees and natural ecosystems.

The meeting was sponsored by Murdoch University, Alcoa World Alumina, the Department of Conservation and Land Management of Western Australia, CSIRO and the Australasian Plant Pathology Society. We appreciate the support of all our sponsors. The management committee included Giles Hardy, Jen McComb, Inez Tommerup, Ian Colquhoun, Bryan Shearer, and Phil O'Brien. However, many students and colleagues made significant contributions to the running and success of the meeting. Special thanks to Daniel Huberli for designing the web page, the Blue Wren Logo and the Meeting handbook front-page. Many thanks must also go to Nola D'Souza and Colin Crane for organizing the food on the 2-day field trip. Also, thanks to Chris Dunne and Colin Crane for organizing the caps and mugs with the logo design imprinted on them.

The meeting started with a two-day field trip to Albany. On the way we visited a number of jarrah forest and *Banksia* woodland and heathland sites the have been severely impacted on by *Phytophthora cinnamomi*. Many people contributed to the discussions at each of these field sites and the delegates had excellent opportunity to get to know one another on an easy going and casual basis, which then set the scene for the remainder of the meeting. This interaction was further facilitated by an overnight stop at the very scenic Karri Valley Lakeside Resort in Pemberton, which is set in the wonderful tall karri (*Eucalyptus diversicolor*) forest. The next day we continued to Albany, again stopping at a number of different *P. cinnamomi* impacted sites. The last stop was at the "Treetop Walk" set in the Tingle Forest. The delegates were able to walk in the canopy some 25m above the ground, followed by a stroll through the forest. This two-day field trip before the start of the meeting allowed delegates to fully appreciate the huge impact *P. cinnamomi* has on the diversity of flora in the south west of Western Australia and set the scene for the more formal part of the meeting.

The meeting began with three regional (Europe and Africa, Americas, and Australasia) overviews which presented a broad outline of how our knowledge of *Phytophthora* had progressed since the inaugural meeting in Grants Pass, Oregon. It certainly became clear that significant progress and unexpected developments had occurred. For example, our increased knowledge on the hybrid alder Phytophthoras and their spread and evolution, and the considerable array of new *Phytophthora* taxa that are now being characterized in Europe, including the *P. ramorum* story on rhododendrons in Europe and the link with Sudden Oak Death in California. The latter has since proved to be a dramatic new disease of oaks and many other woody hosts.

The next session examined pathogenicity of various *Phytophthora* species including *P. cinnamomi* in jarrah, identification of pathogenicity genes in *P. nicotianae* and variation, distribution and pathogenicity of the hybrid alder Phytophthoras. The second session looked at molecular and developmental studies to include diagnostic methods and the effects of phosphite on meiosis and sexual reproduction in plants. The second day concentrated on the impact of *Phytophthora* in Australia and covered a range of ecosystems from throughout the country. This was followed by talks on the use of the fungicide phosphite in natural ecosystems, and covered aerial applications through to uptake of phosphite, phytotoxicity and control. These two morning sessions set the scene for the afternoon field trip which allowed delegates to study the impact of *P. cinnamomi* on coastal heath ecosystems and the use of phosphite to reduce the impact and spread of *P. cinnamomi*.

The third day began with more discussions on the impact of *Phytophthora* in Australia and New Zealand and included foliar disease of eucalypts by *Phytophthora* spp. and the impact of *Phytophthora* on fauna. The impact of *Phytophthora* diseases on vertebrate and invertebrate fauna is a new research aspect that is now being studied in earnest and is really the first attempt to look seriously at ecosystem function and health as threatened by a plant pathogen. The next session then looked closely at different management

1

techniques used on *Phytophthora* in natural ecosystems and brought together a number of different perspectives with much lively discussion between the delegates. This discussion was continued into the evening at the 'Management Symposium' and the value of the meeting was very much emphasized by discussions and input from managers, scientists and '*Phytophthora* dieback' interpreters. It was at this session that the idea of more informal information groups should be established to ensure continued dissemination between all parties involved in the Science and Management of *Phytophthora*. In Western Australia, we now have a 'Dieback Information Group' or 'DIG', this group has now met four times, with some 120 people attending the last DIG meeting. Certainly a successful outcome of the Albany meeting and something that is going from strength to strength in Western Australia.

The last day started with discussions on the impact of *Phytophthora* in Europe, followed by a series of talks on the biology of *Phytophthora* in a range of different environments and hosts. The final session examined *Phytophthora* impact in America and covered susceptibility of different oaks to four species of *Phytophthora*, the transmission and survival of *P. ramorum*, and *P. palmivora* in *Ochroma pyramidalis* in Ecuador.

The meeting concluded with a business meeting and votes of thanks. The next meeting will be held from the 12th – 17th September 2004 and will be hosted in Germany by Thomas Jung and Clive Brasier. We all look forward to this meeting and we have no doubt that it will be a stimulating meeting, but more importantly it will be a great opportunity to catch up with old friends and to make new ones.

Lastly, many thanks must be given to Jen McComb for ensuring that the Proceedings went to press. All papers were refereed, but not the abstracts and poster papers. We sincerely thank our anonymous referees.

Giles Hardy,
Convenor
Second IUFRO Meeting on '*Phytophthora* in Forests and Natural Ecosystems'.

Regional Overviews
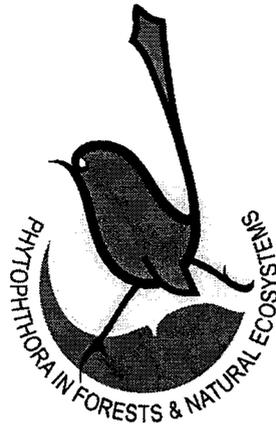
.Progress in understanding *Phytophthora* diseases of trees in Europe

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Abstract. Significant progress and unexpected developments since the 1999 meeting will be reviewed. Topics covered will include the following. Understanding the role of Phytophthoras in European oak declines; continuing spread and evolution of the new hybrid alder Phytophthoras; status of Phytophthoras involved in chestnut mortality; the remarkable array of new *Phytophthora* taxa now being characterised in Europe; emerging evidence of the role of nurseries in the evolution and spread of *Phytopthora* diseases; and the connection between a new *Phytophthora* on rhododendrons in Europe and Sudden Oak Death *Phytophthora* in California.

Introduction

Much new information has accumulated about the role of *Phytophthora* pathogens in European forests and natural ecosystems since the first IUFRO meeting on *Phytophthora* disease of trees in 1999 (see Brasier 2000). This includes the identification of yet more new *Phytophthora* taxa, progress in constructing a *Phytophthora* molecular phylogeny, advances in understanding the role of Phytophthoras in oak declines, and new information on the biology and pathology of the hybrid alder Phytophthoras. Some remarkable new developments have occurred, including the identification of the Californian 'Sudden Oak Death' *Phytophthora* in Europe. There is also increasing evidence that infested nursery stock is involved in *Phytophthora* evolution and spread. This paper outlines these developments and considers some of their implications.

Many new *Phytophthora* pathogens on trees

It was apparent several years ago that the current investigations into European forests and semi-natural ecosystems were leading to the discovery of new *Phytophthora* species (Brasier 2000; Hansen 2000). This process has continued. Table 1 lists many of the new taxa that have been identified. Some have recently been formally described as species, others are still awaiting formal description. A significant proportion, including *P. quercina, P. europaea, P. psychrophila, P. pseudosyringae* and *P. uliginosa* spp. nov., have come from recent studies of European oak forests (Table 1). Several others, such as the alder Phytophthoras, *P. inundata* and P.taxon Pgchlamydo, come from studies of riparian ecosystems or from trees on temporarily flooded sites.

It remains unclear for many of these taxa whether they are endemic to Europe or introduced. For some, further work is required before their nomenclatural status can be clarified. The alder Phytopthoras, for example, need highly detailed characterisation because of the challenge of formally designating the elements of a hybrid swarm. Other taxa show close morphological similarities with already described species. Thus, according to a RAPDs analysis *P.pseudosyringae* may actually comprise four subtaxa. (T Jung, J Nechwatal & DEL Cookeunpublished data). All four are virtually morphologically indistinguishable from *P. syringae*. However, on ITS sequences they are not naturally related to *P. syringae* at all, but to *P. ilicis* and *P. psychrophila* (Jung *et al*. 2003). *P.* taxon Riversoil remains poorly characterised because it grows <1mm per day and has not been kept alive more than 6 months in culture (Brasier *et al*. 2003a).

Werres *et al*. (2001*a,b*) have reported detailed records of Phytophthoras on trees and shrubs in Germany, mainly from nurseries. Some of these extend previous European records (cf. Brasier 2000). They also report what may be the first isolation of the A1 sexual compatibility type of *P. cinnamomi* in Europe, from *Camellia*. Numerous other isolates could not be identified to a species on classical morphological grounds.

ITS-based phylogeny of Phytophthoras

Understanding the natural relationships of Phytophthoras, including the relationships of new *Phytophthora* taxa (Table 1) to known Phytophthoras, has been greatly enhanced by the advent of molecular phylogeny techniques. An example is the recent ITS phylogeny of Cooke *et al*. (2000). This study shows that most *Phytophthora* species comprise a tight natural cluster of about eight major clades. However, several species fall well outside this cluster and may represent new genera. The study also shows that the traditional six morphological groups of Waterhouse (1963) do not conform very closely to the evolutionary lineages in the

Table 1. New *Phytophthora* taxa from trees, shrubs or associated natural ecosystems in Europe

Taxon	ITS clade no[A]	ID by date	Host/Ecology/Distribution
Alder Phytophthoras ('*P. alni*')[B C]	C7a	FC, UK, 1990s	*Alnus* spp., swarm of species hybrids; Europe
P. taxon Pgchlamydo[B D]	C6	FC, UK, 1970s	*Prunus*, Douglas fir, Alder, wet site, riparian, nursery; UK, France, North America, Germany.
P. taxon Riversoil[B D]	C6	FC, UK, 1990s	Riverbank soils near alders; UK.
P. inundata sp. nov [E] (*P.* sp O-group)	C6	FC, UK, 1970s	Trees/shrubs (*Aesculus, Salix, Olea*), after flooding or in wet soils;UK, France, Spain Italy, South America.
P. quercina sp. nov[F]	C3	IFB, Munich, 1990s	Fine roots and soil of *Quercus* spp.; ubiquitous from UK and Sweden across Europe to Turkey.
P. uliginosa sp. nov[G]	C7a	IFB, Munich, 1990s	Wet oak forest soils, rare, central Europe.
P. psychrophila sp. nov[G]	C3	IFB, Munich, 1990s	Oak soils, rare, central Europe, South France.
P. europaea sp. nov[G]	C7a	INRA, Nancy / IFB, Munich, 1990s	Wet forest soils (*Quercus, Carpinus, Fraxinus*); localised, France, Germany, Austria, Sweden.
P. ramorum sp. nov[H]	C8a	BBA, Braunschweig, 1990s	*Rhododendron* and *Viburnum*; Germany, Netherlands, Poland; Sudden Oak Death *Phytophthora*, USA.
P. taxon Oaksoil[B D]	C6	INRA Nancy, 1990s	Oak soils ; rare, France.
P. taxon Forestsoil[BD]	C6	INRA Nancy, 1990s	Forest soils (*Quercus, Carpinus* etc.) ; rare, France.
P. pseudosyringae sp. nov[I]	C3	IFB Munich, 1990s	Oak soils, beech and alder bark; Europe, North America (=4 different species of *Phytophthora*?)
P. italica sp. nov[J]	ND	I.P.V. Palermo, 1990s	Myrtle, Italy

[A]Major ITS Clade no. of Cooke *et al.* (2000).
[B]Taxon not yet formally described in the literature.
[C]Brasier, ms in preparation.
[D]Brasier *et al* (2003a).
[E]Brasier et al (2003b).
[F]Jung *et al.* (1999).
[G]Jung *et al.* (2002).
[H]Werres *et al.* (2001c).
[I]Jung *et al.* (2003).
[J]Cacciola *et al.* (1996).

genus, and that the Peronosporas may actually be Phytophthoras.

The ITS gene tree of Cooke *et al.* (2000) was based upon 50 relatively well characterised taxa. Detailed studies of individual major clades are now in progress. These suggest much new information relevant to forest Phytophthoras is still to come. Thus a study of numerous additional isolates belonging to Clade 6 has now been completed. It has revealed seven previously undescribed taxa (Fig. 1). Several of these new taxa are listed in Table 1. Often they are represented by only one or a few isolates. Six of them are associated with forests or riparian ecosystems.

Much remains to be learnt about their origins, ecology, pathology and quarantine risk (Brasier *et al.* 2003a).

Mediterranean Europe: Evergreen oak mortality and *Phytophthora cinnamomi*

In the early 1990s *Phytophthora cinnamomi* was shown to be associated with a widespread mortality of cork oak, *Quercus suber* and holm oak, *Q. ilex* across Spain and Portugal. This work was summarised previously (Brasier 2000; Moreira, Ferraz & Clegg 2000). A decline hypothesis has been produced (Brasier, 1996). A NATO-supported research project on this problem, conducted mainly in Portugal, has recently ended
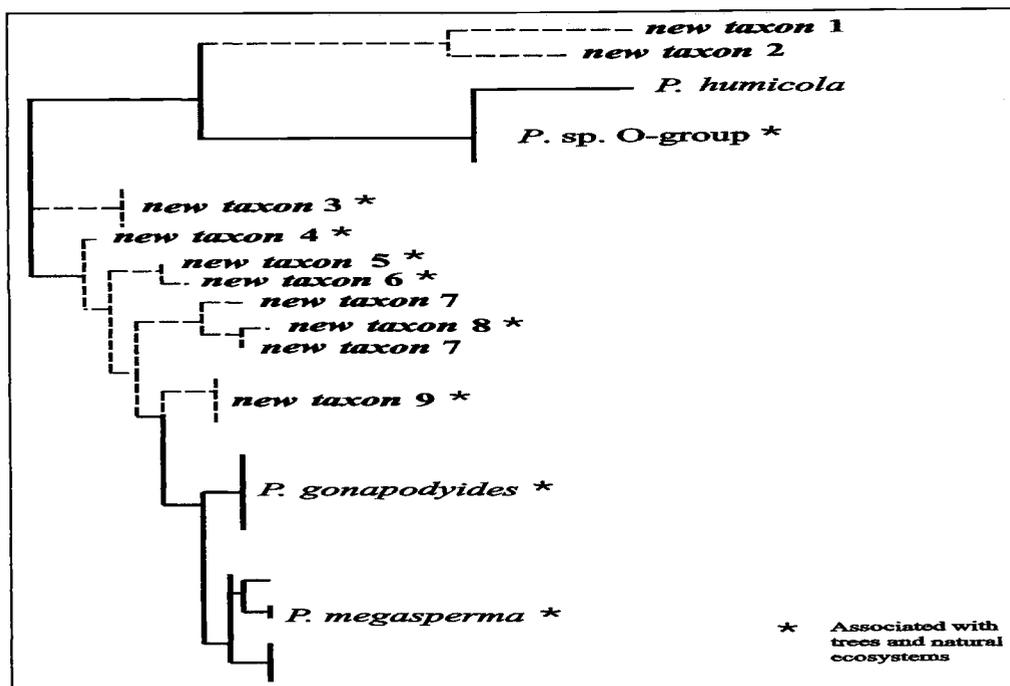
Fig. 1. Phylogram of *Phytophthora* taxa associated with major ITS Clade 6 of Cooke *et.al.* (2000). Solid lines: *Phytophthora* taxa included in the Cooke *et.al.* study. Dashed lines: 'new' phenotypically distinct taxa, most still undescribed. (Adapted from Brasier *et al.* 2003a).

(Ferraz and Cravador 2000). The project included a disease survey of 55 cork oak sites in Portugal. These showed infestation levels to be *c.* 12.5% of sites in the north of Portugal, 35.7-50% of sites in the centre and 74.1% of sites in the south (8 sites in Tras-os-Montes, 20 sites in Ribatejo and Alentejo and 27 sites in Algarve).

This distribution is believed to be consistent with conditions favourable to the pathogen and to disease development in these areas.

Another part of the NATO project concentrated on developing a PCR method for identification of *P. cinnamomi*, based on the cinnamomin elicitin gene. Four different cinnamomin genes were demonstrated (Coelho *et al.* 2000; Moreira *et al.* 2000). These have now been fully sequenced, and a phylogenetic tree showing all known *Phytophthora* elicitin genes has been produced (see Ferraz and Cravador 2000). In addition a recombinant, high yield β-cinnamomin gene has been developed to study the role of the cinnamomins in pathogenesis. Analysis of polypeptide profiles in infected and non-infected cork oak plants has also been carried out. This has revealed certain proteins that are expressed only in *P. cinnamomi*-infected roots. Likewise, specific cDNA fragments have been identified only in leaves of root-infected plants. It is hoped to isolate and characterise the proteins involved.

In Spain, a major expansion of foci of cork oak mortality, or 'sudden death' occurred in the south of the country after the exceptionally heavy winter rains of 1996/97 (E. Sanchez-Hernandez, pers. comm..). An EU-supported nation wide research programme on aspects of cork oak mortality, INTERREG II, was undertaken in 1998/99. In this programme, studies on isolation success of *P. cinnamomi* from soil around cork oak at 'sudden death' sites in south west Spain varied from 0-63%, mainly according to soil moisture levels (Sanchez-Hernandez *et al.* 2000). *P. cinnamomi* was consistently isolated, all the isolates exhibiting a high optimum temperature for growth of *c.* 30°C. Pathogenicity tests were carried out by incorporating *P. cinnamomi* inoculum into pots with *Q. suber* and *Q. ilex* seedlings. With six months conventional watering both *Q. suber* and *Q. ilex* suffered extensive fine root and lateral root loss compared with controls. With one month's continual saturation, *Q. suber* suffered greater root loss than did *Q. ilex*. Correspondingly severe foliar symptoms were observed, including defoliation of some *P. cinnamomi* infected plants.

6

An unidentified *Phytophthora* has been consistently isolated (along with *P. cinnamomi*) from cork oak 'sudden death' sites in Huelva, south west Spain. This *Phytophthora*, like *P. cinnamomi*, is highly pathogenic to roots of inoculated cork oak seedlings. It likewise has a high optimum temperature for growth (*c.* 30°C), produces chlamydospores, and has non-papillate sporangia. Its identity is under investigation (Sanchez-Hernandez *et al.* 2000).

In Italy *P. cinnamomi*, *P. cambivora*, *P. quercina* and eight other *Phytophthora* species were recovered from soil samples at 19 out of 30 oak forest areas, covering both the north and south of the country. The *Phytophthora* isolation frequency (35.2%) was correlated with soil pH. A significant association was found between declining oak trees and presence of *P. quercina* in northern Italy (Vettraino *et al.* 2001).

Central Europe: Deciduous oak decline (various Phytophthoras)

Studies in the mid- to late-1990s showed that a number of Phytophthoras were associated with the widespread and periodic decline of deciduous oaks, especially *Quercus robur* and *Q. petraea*, across much of Central Europe since the end of the 19th Century (summarised in Jung *et al.* 2000a, b; Brasier 2000). In Bavaria *P. quercina* sp.nov., *P. cambivora*, *P. citricola* (plus other *Phytophthora* species) were widespread on sites with sandy-loam to clay soils of mean pH (CaCl$_2$) ≥ 3.5. Significant correlations were obtained between crown transparency, fine root condition and the presence of *Phytophthora* spp., especially *P. quercina*. In soils of pH ≤ 3.9, no Phytophthoras were found and the correlations were mainly non-significant. Based on these observations, an EU funded project 'PATHOAK' involving 5 European countries was undertaken during 1998-2001 (Delatour 2001; see also Delatour *et al.* these Proceedings). The project had four main subtasks: to investigate water use efficiency of oaks, distribution of Phytophthoras in declining oak stands, pathogenicity of Phytophthoras to oaks, and the interaction of root pathogens with environmental parameters.

This EU project has produced a substantial range of information, much of which is summarised by Delatour *et al.* (these Proceedings). To illustrate some of the pathological findings, research data from the UK team will be discussed. A field survey was conducted in the UK (similar to that in the other contributing countries), involving 11 oak decline sites over 2 years. This showed that at least four species of *Phytophthora*: *P. cambivora*, *P. quercina*, *P. citricola*, and *P. gonapodyides* were present on 7 of the sites, but no Phytophthoras could be detected on the remaining sites, usually with more sandy/acid soils. These findings were similar to those of other EU partners (c.f. also Jung *et al.* 2000a, b). It was notable that at some sites, *P. cambivora* was isolated in the first year but not in the second; whereas *P. quercina* was isolated only in the second year. This suggests that there are considerable seasonal differences in the qualitative structure of *Phytophthora* populations in the soil; and therefore presumably also in the roots. To obtain a comprehensive picture, sampling would probably need to be carried out over a number of years.

Potted 1.8 m tall *Q. robur* were inoculated with representatives of these Phytophthoras by incorporating mycelium into the soil. The pots were then flood irrigated at 20° C. All the Phytophthoras caused significant mean fine root loss compared with non-inoculated controls (Fig. 2). The approximate order of aggressiveness was: *P. cinnamomi* (very aggressive control isolate) > *P. cambivora* > *P. quercina* and *P. gonapodyides* > *P. citricola* > *P. europaea* sp. nov. The percentage loss of fine roots was *c.* 62% for *P. cinnamomi*, 36-44% for *P. cambivora*, 38-57% for *P. gonapodyides*, 25-47% for *P. quercina*, 18-41% for *P. citricola* and 10% for *P. europaea*. There were also significant differences in pathogenicity between isolates of some of these species (C.M. Brasier and J. Rose, unpublished). The results are again similar to those obtained by other research groups in the Pathoak project (Delatour 2001; Jung *et al.* these Proceedings; Delatour *et al.* these Proceedings) and to earlier results (Jung 1998; Jung *et al.* 1999).

In order to remove a theoretical bias in favour of those Phytophthoras better adapted to 20° C, experiment was repeated at 14°C and 26°C. However, large differences were observed in the behaviour of the plant and soil systems at these two temperatures (Table 2). For example, there were substantial differences in top growth, dry weight increment, mycorrhizal activity and nitrogen utilisation. It was decided that the two experiments were not operating on the same ecological basis. Indeed, the observed differences suggest considerable caution is needed when comparing studies that
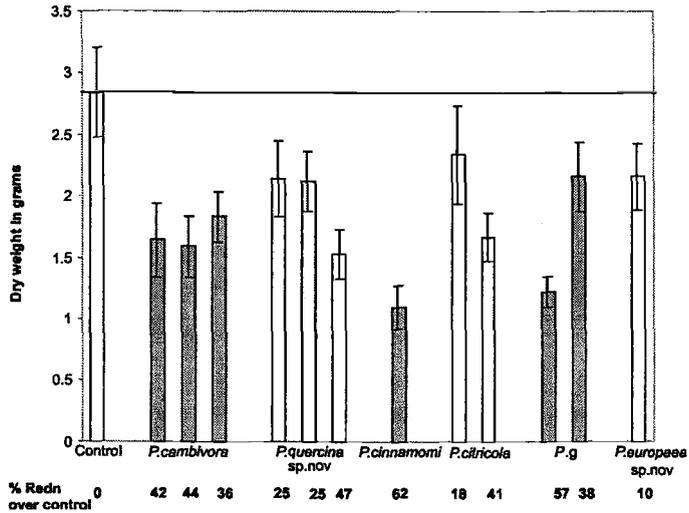
Fig. 2. Results of a pathogenicity test with *Phytophthora* isolates from UK oak sites. Phytophthoras were mixed with the soil of potted *Quercus robur* plants. Data shown are final mean dry weights of fine roots. Note the differences in pathogenicity between isolates within as well as between species e.g. *P. citricola* and *P. gonapodyides*. (C.M. Brasier and J. Rose, unpublished).

Table 2. Differences in plant and soil parameters of potted oak seedlings after twelve weeks incubation at 14° and 26° C[A]

	14°	26°
Root/shoot parameters		
Mean height gain (mm)	22.0 ± 14.01	191.00 ± 92.95
Mean diameter gain (mm)	1.0 ± 0.29	0.71± 0.24
Final mean top dry weight (g)	15.9 ± 1.31	14.0 ± 3.27
Final mean fine root dry wt (g)	1.96 ± 0.37	1.23 ± 0.29
Final mean lateral + tap root dry wt (g)	9.59 ± 1.19	3.30 ± 0.47
Foliar parameters		
Leaf yellowing	+++	-
Other leaf appearance	dull	15% marginal browning
Leaf loss	-	+
New leaf flushing	(+)	++
Aphids on leaves	+	-
Oak powdery mildew on leaves	-	+
Potting mixture parameters		
Trichoderma growth on surface	+++	+++
Fruiting of *Laccaria laccata*[B] at surface	++	-
Mycorrhiza visible on roots (7 trees)	4/7	0
Fungal gnats active in potting mixture	++	+++
Floodwater pH, second flooding[A]	6.3	5.0
Floodwater pH, third flooding[A]	5.6	4.5
Nitrogen concentration mg/l $^{-1}$ at third flooding[A] (2 pots only) : pot 1	5.57	63.28
pot 2	10.14	68.11

[A]Two year-old oak seedlings (already flushed), average 1.5 m height, were transferred into a vermiculite/peat mixture in 23cm dia pots for 3 months, beginning in June 1999. 20 seedlings/were maintained at each temperature, grown under lights in separate growth chambers at average 75% relative humidity. Pots were flooded 24 h on first day then again for 24h at one month and two months. Regular watering was carried out to maintain normal soil water capacity. [B]Mycorrhizal fungus (basidiomycota).

8

Quercus robur

224.20cm²

P.cambivora P.cinnamomi

% of largest lesion area

P.taxon pseudosyringae

P.citricola

P.europea sp.nov

P.uliginosa sp. nov

P.g

Control P.megasperma P taxon aldnurs P.quercina sp.nov P.sp O-group P. taxon Pgchlamydo

Phytophthora taxon

Fagus sylvatica

237.67cm²

P.cinnamomi

% of largest lesion area

P.cambivora P.citricola

P.sp 0-group

P.taxon pseudosyringae

P.europea sp.nov

P.uliginosa sp.nov

P.g

Control P.megasperma P.taxon aldnurs P.quercina sp.nov P.taxon Pgchlamydo

Phytophthora taxon

Fig. 3. Examples of wound inoculation tests on fresh cut logs of *Quercus robur* (above) and *Fagus sylvatica* (below). The mean lesion area of the most aggressive *Phytophthora* is shown in cm². All other isolates are shown as a percentage of this figure. Incubation was for 5 weeks at 20°C. (CM Brasier and SA Kirk unpubl.).

investigate interactions of root pathogens with trees, whether in pots or in the field

Host specificities of the oak Phytophthoras were investigated by wound inoculating fresh-cut logs of six different tree hosts (*c.* 25 x 110 cm logs; method of Brasier and Kirk 2001). Representative data are shown on Fig. 3. The results showed, for example, that (i) *P. quercina* did not attack the suberised bark of any host species tested. (ii) *P. cambivora* was an aggressive pathogen of *Q. robur* bark and was also active on *Fagus sylvatica*, *Castanea sativa*, *Pseudotsuga menziesii* and

Chamaecyparis lawsoniana, i.e. it exhibited, as expected, broad host range (cf. Brasier and Kirk 2001). (iii) *P. gonapodyides* was a moderate bark pathogen of most species including *Q. robur*, *Fagus* and *Castanea*. (iv) *P. pseudosyringae* (Table 1) was more aggressive to bark of *Q. robur* than to *Fagus*, whereas *P. inundata* (Table 1) did not attack *Q. robur* bark but was a moderate bark pathogen on Fagus (CM Brasier and J Rose unpublished). species including *Q. robur*, *Fagus* and *Castanea*. (iv) *P. pseudosyringae* (Table 1) was more aggressive to bark of *Q. robur* than to *Fagus*, whereas *P. inundata*

(Table 1) did not attack *Q. robur* bark but was a moderate bark pathogen on *Fagus* (C.M. Brasier and J. Rose, unpublished).

It is clear, therefore, that Phytophthoras associated with central European oak declines vary considerably in both their host and tissue-type specificities. The different Phytophthoras may be active during different seasons. At times they might compete with and at other times complement each other. Such possibilities need further investigation, as also does the issue of quantitative changes in their soil populations over time. *P. quercina* and *P. cambivora*, for example appear to have very different ecological strategies. It is postulated that *P. quercina* is a more ruderal species, tending to simultaneously attack large numbers of oak fine roots but then quickly replaced in these roots by other, often saprotrophic organisms. *P. cambivora* may be more stress tolerant; colonising fine roots of a wide range of hosts; but also progressing to, or directly invading, bark of larger suberised roots where it survives for a much longer period. *P. cambivora* might also concentrate its zoospore inoculum to a greater extent during its attack. This difference in ecology may also be reflected in breeding strategy. *P. quercina* is an inbreeding, homothallic species. *P. cambivora* is mainly outcrossing heterothallic. Large numbers of oospores of *P. quercina* may therefore return to the soil via the fine root detritus. These oospores may germinate readily when the next opportunity for infection of fine roots arises. Indeed, the latter is indicated by increased isolation frequencies of the pathogen when soil is air dried and pre-moistened before flooding (Jung *et al.* 1996). In *P. cambivora*, oospore formation may be much rarer, their main role being to generate the genetic variation needed to survive in a more heterogeneous environment, another to provide a long term resting stage.

Studies on oak decline Phytophthoras have recently been extended to Turkey, which has a highly diverse oak flora (Balci these Proceedings), and to Sweden and Serbia (T. Jung, L. Lundberg & K. Sonesson, and T. Jung and M. Glavendekic, unpublished data). A similar range of oak-associated Phytophthoras has been found in these countries together with yet more possible new taxa.

Role of Phytophthoras on chestnut

P. cinnamomi and *P. cambivora* have been associated with serious mortality and decline of chestnut (*Castanea sativa*) in southern Europe since the early part of the 20th Century. However, historical records suggest the appearance of the disease in Spain may date back to the early 1700s, perhaps reflecting the first importation of these pathogens into Europe. Since 1990, an increased intensity of disease has been observed, especially in Italy. Under the auspices of an EU funded project 'CASCADE' (1999-2006), sampling for Phytophthoras in soil is being carried out in chestnut stands in southern Europe. In Italy, these samples have revealed widespread presence of *P. cambivora* around the root zone, often accompanied by *P. citricola* and *P. cactorum*. *P. gonapodyides* is also commonly isolated from associated streams (Vettraino *et al.* 2001). Pathogenicity tests (1-year-old chestnut seedlings in pots infested with *Phytophthora* inoculum) showed that all four species are pathogenic, in an approximate order of severity *P. cambivora* > *P. citricola* > *P. gonapodyides* and *P. cactorum*. It is interesting to note, once again, that *P. gonapodyides* can cause significant damage (cf. Figs. 2 and 3 and Jung and Blaschke 1996; Jung *et al.* 1996). Although probably only a moderate pathogen, *P. gonapodyides* is a common forest inhabitant (Brasier, Hamm & Hansen 1993; Hansen and Delatour 1999). Its role in tree health may have been considerably underestimated and needs further investigation. *P. cinnamomi* was not isolated in the Italian survey, yet it is commonly isolated from diseased chestnut in southern France (C. Robin, personal communication). This difference may reflect the role of the nursery trade in spread of the, pathogen (see below).

Genetic status, pathogenicity and distribution of the hybrid alder Phytophthoras

New 'alder Phytophthoras' now spreading across Europe cause a serious collar rot of riparian Alnus (reviewed in Brasier 2000; Streito and Gibbs 2000; Gibbs et al. 2003.). These Phytophthoras are a swarm of 'still evolving' hybrids between *P. cambivora* and a *P. fragariae*-like *Phytophthora*. The main hybrid types have been termed the standard hybrid and the Dutch, German, UK and Swedish variants. They have different colony patterns, temperature -growth patterns chromosome numbers, gametangial morph-ology, levels of zygotic abortion and ITS and AFLP-types (Brasier *et al.* 1999; Brasier 2003). Since they are both behaviourally and genomically different many of the variants resemble separate cryptic species.
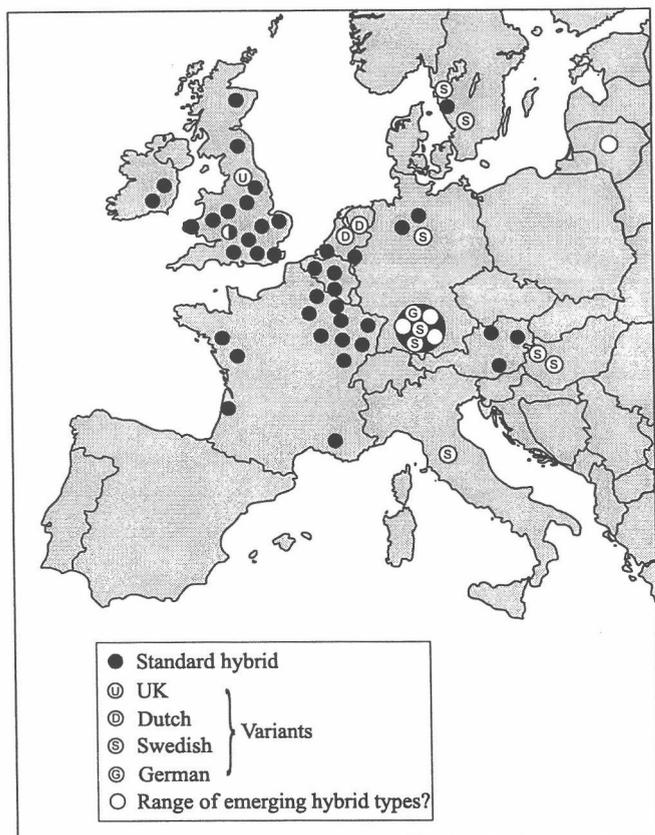
Fig. 4. Present known locations of alder *Phytophthora* hybrid types at sites across Europe. A highly simplified representation based on 280 samples, including 120 from Bavaria (large circle). Note the widespread distribution of the standard hybrid type and of the Swedish variant. Note also the 'hot-spot' of different hybrid types in southern Germany. (from Brasier 2003).

Distribution of different hybrid types

Since 1993 over 280 samples of the pathogen have been classified. Most fall into the above previously identified hybrid types. A few isolates have been identified as new major variants (previously unknown, unique hybrid types). The present known distribution of the different hybrid types is summarised in Fig. 4. The standard hybrid is fairly widely distributed. The Swedish variant is now known from Sweden, Germany, Hungary and Italy. This may represent the spread of this genotype by the international nursery trade. In Bavaria, southern Germany, from where over 120 isolates have been examined, several previously unknown variants have recently been found alongside the standard type and the German and Swedish hybrid types (C.M. Brasier, S.A. Kirk and T. Jung, unpublished). Bavaria may therefore represent either a hot spot of evolutionary activity; or an area into which many different alder *Phytophthora* genotypes have been introduced on infested plants. Previously unknown hybrid types are also present at one site in western Britain

(Hadley Brook, Worcestershire) and in Lithuania.

Pathogenicity and host range of different hybrid types

Twelve wound inoculation experiments, involving over 60 isolates, have been conducted on fresh-cut logs of *Alnus* and other trees species. These have shown that the standard hybrid is highly aggressive to *Alnus* bark. The Dutch variant is also highly aggressive; whereas the UK, German and Swedish variants tend to be less aggressive or only weakly aggressive. The behaviour of the variants is supported by observation of restricted lateral development of lesions caused by the Swedish and German variants in the field (Jung *et al.* The Proceedings). *P. cambivora* and a wide range of other *Phytophthora* species, including species from riparian ecosystems, were non-pathogenic or only weakly pathogenic to alder bark (Table 2) (Brasier and Kirk 2001).

The standard alder *Phytophthora* was non-pathogenic to bark of six other tree species;

whereas *P. cambivora*, *P. cinnamomi* and *P. citricola* were (as expected) moderately to strongly pathogenic. This indicates the standard alder *Phytophthora* has a high specificity to alder. In addition, behaviour of standard isolates indicated that critical thresholds of host resistance were operating in some tests, resulting in lesion suppression. In nature, this might result in recovery of affected trees. There was also evidence of strong seasonal influences on lesion development. Lesions on logs cut in July-October were generally much larger than those cut in November-March. No lesions occurred on logs cut in April (Brasier and Kirk 2001). This may reflect either the water content of the logs or ability of the host to mobilise carbohydrates for resistance.

Genetic stability and oospore viability in the hybrids

Standard hybrid isolates often change markedly in colony characteristics during storage. One standard isolate (P818) yielded a morphologically distinct sector having a unique ITS-type. Based on this observation, Delcan and Brasier (2001) have investigated the stability of asexual and sexual derivatives of the hybrids. One objective was to assess whether the natural variants could be obtained directly from the standard hybrid via genetic segregation. Thousands of zoospores and hyphal tip colonies of standard isolates were produced. These proved remarkably stable in phenotype. In contrast those of the UK and German variants were highly unstable. The UK variant in particular could not be 'stabilised' into a single morphological entity. When thousands of oospores of the different alder Phytophthoras were tested for germination, none could be germinated, although germination did occur in *P. cactorum* and *P. cambivora* controls. The lower chromosome number variants, such as the Swedish and German variants, did show higher levels of oospore vitality (by tetrazolium method) as opposed to germinability. Failure of the oospores to germinate was considered consistent with the frequent meiotic failure observed in the gametangia (see Brasier *et al.* 1999).

Although no evidence was obtained that variants are derived from the standard hybrid via genetic segregation, this could not be ruled out. The results also indicated that oospores were unlikely to play a significant role in dissemination. It was proposed that local dissemination may be mainly via zoospore spread; and long distance dispersal via spread of infested bark detritus or via infested planting stock (Delcan and Brasier 2001).

Further molecular characterisation of hybrid types

Recent isozyme studies have shown that the standard hybrids exhibit complex banding patterns characteristic of an allotetraploid. These studies also indicate that changes from heterozygosity to homozygosity are occurring in standard isolates, consistent with continuing evolution of their hybrid genome. The Dutch and German and UK variants shared a unique and characteristic isozyme pattern. The Swedish variant exhibited another unique pattern. The isozyme data also supported the view that *P. cambivora* is one of the parents of the hybrid, whereas *P. fragariae sensu stricto* is unlikely to be a direct parent (W. Man In't Veldt and C.M. Brasier, unpublished).

Occurrence of alder Phytophthora *inoculum in soil and river water*

In a study of soil around diseased alders along several rivers in southern Britain, with standard alder *Phytophthora* present, apple and alder leaf baits were used. Standard alder *Phytophthora* was readily recovered from necrotic bark lesions on trees along the riverbanks. Yet it was isolated only once from soil around the affected trees, whereas *P. gonapodyides* was frequently recovered from the same soil samples and *P. megasperma*, *P. citricola* and 'P. taxon Riversoil' were recovered occasionally (J. Delcan and C.M. Brasier unpublished; data in Brasier, 2002). Similarly in Bavaria, standard alder *Phytophthora* was readily isolated from bark of five common alders growing in a severely diseased plantation, whereas baiting of the rhizosphere soil only yielded *P. citricola* and *P. cactorum* (Jung *et al.* these Proceedings).

In a study of river water in northern France (standard alder *Phytophthora* present), rafts of live alder twigs were used as baits. The alder *Phytophthora* was rarely isolated from the lesions developing on the twig ends; whereas *P. gonapodyides* (or a *Phytophthora* similar to *P. gonapodyides*) was isolated frequently. Only during one sample period along one river was a higher incidence of alder *Phytophthora* recorded (J.C. Streito, unpublished; data in Brasier 2003). In another study in the UK, roots of five 1m alder saplings were immersed in an infested river

(with many diseased alders upstream) over two consecutive 6 month tests. Only one of the
These investigations indicate that alder *Phytophthora* inoculum is difficult to detect – by these baiting methods – in soil and river water. This suggests that inoculum is often at very low levels in these substrates. It may be concentrated in river water only when rare spikes of intensive zoospore release occur from infected root or collar tissue. The greatest amounts of inoculum may tend to be concentrated in infested bark detritus, which may become entangled with alder roots and stems during flooding

Surveys of the disease and disease management

National disease surveys have continued in EU member states, co-ordinated through an EU 'concerted action' project. An overall map of the survey sites in Europe showing the presence/absence of disease symptoms has been produced by J.C. Streito (Fig. 5).

A detailed *Phytophthora* isolation survey in Bavaria (see Jung *et al.* these Proceedings) indicates that 50% of riparian stands of *A. glutinosa* and *A. incana* along Bavarian rivers are infested; together with about 50% of non-flooded stands or plantations. On some rivers, >50% of

individual trees are now diseased. The source of inoculum can usually be traced back to infested stock, either planted upstream of a site or planted in a non-riparian sites that drains into a river. An investigative survey has therefore been carried out in Bavarian nurseries (see below).

Wound inoculation tests showed no useful resistance in seedlings of different European *A. glutinosa* provenances (Gibbs 2003). However, in Bavaria mature alders with healthy crowns and healed bark necroses have been observed in riparian stands which otherwise have a high incidence and a long history of the disease (T. Jung, unpublished). This suggested possible resistance among individuals. A selective resistance-screening programme has been started, based on cuttings from such survivors (Jung *et al.* these Proceedings). The feasibility of selecting for resistance of trees to introduced *Phytophthora* root pathogens is demonstrated by recent work on *Eucalyptus marginata* (Stukely *et al.* these Proceedings) and *Chamaecyparis lawsoniana* (Bower *et al.* 2000; Sniezko and Hansen 2000). Experiments are also in progress in the UK, France, Belgium and Germany on the response of disease levels to coppice management (Gibbs 2003; Jung *et al.* these Proceedings)
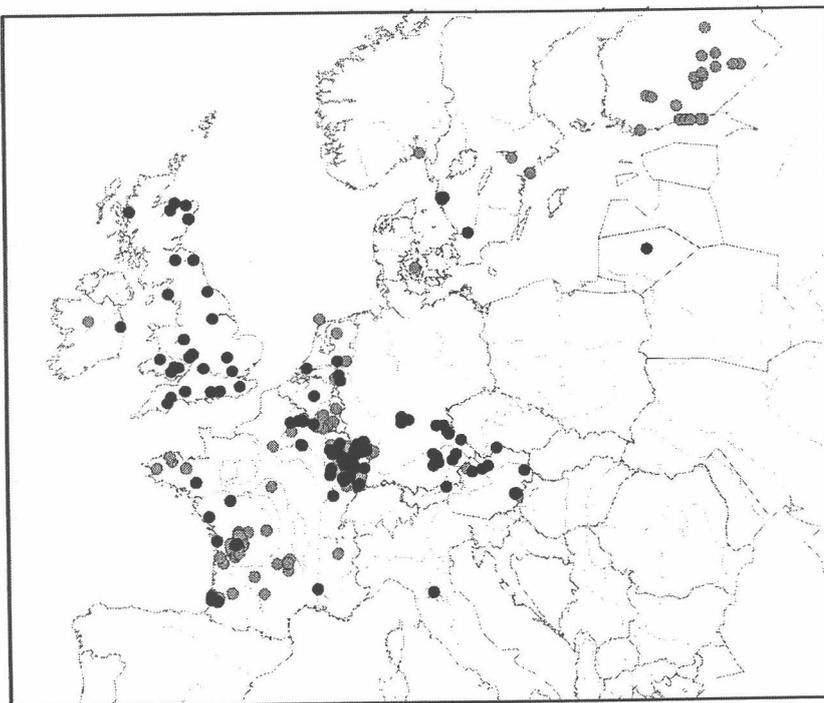
Fig. 5. Distribution of alder disease survey sites in Europe. Black circles, disease due to alder Phytophthoras present. Shaded circles, no disease recorded. Compiled by JC Streito (Nancy, France).

13

Spread via infested nursery stock

There is growing evidence that infested nursery stock plays a major role in the international and national spread of the alder Phytophthoras. In Bavaria, three out of the four commercial forest nurseries investigated have recently been shown to be infested. Moreover at one nursery the standard alder *Phytophthora* and four other *Phytophthora* species were isolated from soil around a single potted alder. Interestingly, alder *Phytophthora* was not detected in four nurseries belonging to the state forestry service (Jung *et al.* these Proceedings). Records of the disease at alder shelterbelt sites in the UK and in young plantations in Belgium, Netherlands, Germany and Italy - well away from riparian ecosystems – also indicate spread of the pathogen on infested planting stock. So too does the frequent occurrence of diseased riparian alder stands downstream, but not upstream, of affected plantations (Jung *et al.*, these Proceedings). The nursery issue is discussed further below.

Status of Californian 'Sudden Oak Death Phytophthora' *P. ramorum* in Europe

In the summer of 2000, a previously unknown *Phytophthora* was identified as the cause of a new aggressive disease of oaks and other trees and shrubs, the 'sudden oak death' (SOD), spreading in the San Francisco Bay area of California. Details are given elsewhere in this volume (Garbelotto *et al.;* Hansen; Frankel papers in these Proceedings). The new *Phytophthora* was closely related to *P. lateralis* on ITS sequence, but was distinctive in having abundant large brown chlamydospores and caducous sporangia. Because it attacked oaks and oak relatives, it was of considerable concern to European researchers as well to those in North America. Moreover, later in 2000 it was suggested that a *Phytophthora* with very similar morphological properties to the Californian SOD *Phytophthora* had been recorded in Germany in 1995. This *Phytophthora* caused a shoot blight of rhododendron and a stem canker of *Viburnum,*. It had subsequently also been recorded in the Netherlands. By early 2001 it was confirmed that the German/Dutch *Phytophthora* was almost certainly the same as that causing SOD in California. The rhododendron *Phytophthora* has now been formally named as *P. ramorum* sp. nov. by the German and Dutch groups (Werres *et al.*, 2001c).

This development raised the question whether *P. ramorum* posed a serious threat to European oaks, to other European Fagaceae such as chestnuts, or to other Ericaceae in addition to rhododendron. The extensive trade in rhododendrons and other potential hosts across Europe added some urgency to the issue. Pathogenicity tests have been initiated in Germany, the UK and the Netherlands to assess the level of risk. Preliminary experiments in the UK (wound inoculation of fresh cut logs, under licence in high security chambers) indicate that *P. ramorum* may be only a weak pathogen of *Quercus robur, Q. suber,* and *Castanea* bark compared with *P. cambivora* and *P. cinnamomi* controls. It may be a stronger pathogen of rhododendron and *Fagus* bark. However, in assessing such results, ecological conditions for infection and modes of entry of the pathogen need to be taken into account. Unlike *P. cinnamomi* or *P. cambivora, P. ramorum* is probably an aerially dispersed pathogen favoured in its spread by high atmospheric moisture (Garbelotto, these Proceedings). Build-up of inoculum can occur in the canopy. Still to be considered, therefore, is this pathogen's ability to infect leaves and shoots of *Quercus* spp. and other potential hosts in Europe. Also to be considered is its ability to directly enter the unwounded bark of potential hosts.

Role of nursery stock in *Phytophthora* evolution and spread

Over the past 200 years many highly damaging exotic Phytophthoras, such as *P. cambivora* and *P. cinnamomi,* have been introduced into European forests. This has most probably occurred through importation of infested planting stock. Recent developments suggest infested planting stock continues to play a major role in the evolution and spread of Phytophthoras. For example:

1. *P. lateralis* was recently discovered in a nursery in France (Hansen *et al.*, 1999). This pathogen threatens *Chamaecyparis lawsoniana* in its native range in the USA (Hansen 2000). It seems likely that it has arrived in Europe on imported, infested *Chamaecyparis* plants; or perhaps on another symptomless host.
2. Researchers in the UK, France and Germany recently found that oak seedlings obtained from local commercial nurseries for inoculation tests (EU research project,

14

PATHOAK) were already contaminated with Phytophthoras. The contaminating Phytophthoras included *P. cinnamomi*, *P. quercina* and *P. cambivora* (Delatour, 2001).

3. At one Bavarian nursery in 1999, five different *Phytophthora* species were isolated from a single potted alder seedling (Jung *et al.* these Proceedings).

4. Spread of the hybrid alder Phytophthoras to new sites appears to be associated with movement of infested planting stock (see above). The widespread distribution of the Swedish variant (Fig. 1) may well be an example.

5. The A1 sexual compatibility type of *P. cinnamomi*, previously absent from Europe, has recently been found on *Camellia* in Germany (see above). Most probably, it has been introduced on infested plants of *Camellia*. The latter is a host for the A1 of *P. cinnamomi* in North America (Zentmyer 1980).

6. *P. ramorum* (Californian SOD *Phytophthora*) was recently discovered in Germany and the Netherlands. On present evidence, it has probably spread on infested rhododendron and *Viburnum* nursery stock. It may have been introduced to Europe, or indeed to California, in this way from its geographic centre of origin.

Such incidents indicate the phenomenon remains widespread. There are also indications that infestation levels may be higher in intensive, large scale commercial nurseries; whereas organisations that raise their own planting stock from seed or cuttings, such as state forestry organisations, may be less affected (Jung *et al.* these Proceedings; A. Vannini, personal communication). The various processes and protocols involved suggest there is a significant risk to the health of European forests, as follows:

a. It appears that both Phytophthoras on existing European Plant Health schedules, such as *P. lateralis,* and previously undescribed *Phytophthora* species, such as *P. ramorum,* are entering Europe. The enormous environmental risk involved is evident. It is well documented that *Phytophthora* species have the potential to eliminate entire native tree populations (cf. the threat to *Chamaecyparis lawsoniana* in the USA; Hansen, this volume). They also have the potential to eliminate entire natural ecosystems (cf. *P. cinnamomi* and world heritage sites in western Australia; Shearer *et al.* these Proceedings

b. The occurrence of multiple *Phytophthora* species in the soil of a single potted nursery plant (see above) provides the potential for evolution and emergence of new *Phytophthora* pathogens, and new tree diseases, either via full interspecific hybridisation or horizontal gene transfer (see. Brasier 1995; 2001). Full hybridisation appears to have happened , for example, in the case of the new hybrid alder Phytophthoras (Brasier *et al.* 1999).

c. Introduction of a 'missing' mating type of a *Phytophthora* pathogen - such as the A1 of *P. cinnamomi* – also enhances the potential for evolution in aggressiveness, host range and other adaptive traits of *Phytophthora* pathogens. .

d. In the nursery environment Phytophthoras may thrive on roots of 'non-hosts' in a way that they could not manage to do in nature. This provides a pathway for insidious spread of Phytophthoras on visibly healthy 'non-hosts'. For example, although *P.quercina* is specific to oak, it was was recently baited from young alder seedlings grown in a nursery field that had harboured oak seedlings three years previously (Jung *et al.,* these Proceedings).

e. The nursery environment may also be selecting for more nursery-adapted genotypes (or hybrids) of *Phytophthora* pathogens. This could include selection for highly aggressive genotypes; or genotypes that remain latent in the nursery but become more damaging on mature outplanted material.

f. Widespread use of fungistatic (but not fungicidal) 'anti-*Phytophthora*' chemicals in nurseries may also be facilitating distribution of infected but visually symptomless plants.

g. Although molecular diagnostic tools are more likely to detect Phytophthoras in imported, visibly 'symptomless' nursery stock, these tools are not yet widely used by plant health inspectorates.

Concluding comments

Significant progress is being made in our understanding of *Phytophthora* pathogens in European forests. However, as indicated by this review, much of this progress is in response to an unprecedented increase in the appearance of new *Phytophthora* pathogens and new *Phytophthora* diseases across Europe. This in turn may reflect the increasing intensity of international trade in forest trees, shrubs and ornamentals. Climate change, improvements in the discrimination of *Phytophthora* taxa, and the dedication and insight of a few are also contributing to the broader picture. Nonetheless, if the perceived increase in *Phytophthora* activity is sustained, the future of more of our European trees and forests, like that of our presently threatened oaks and alders, seems far from secure.

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Phytophthora in the Americas -2001

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Abstract. A dramatic new disease dominates this update of the status of *Phytophthora* diseases and research in the Western Hemisphere. At the time our last meeting in Grants Pass, a disease called Sudden Oak Death was causing increasing concern in California around San Francisco Bay, but no causal agent had been identified. Not long after the meeting, an apparently undescribed species of *Phytophthora* was isolated from lethal stem cankers on coastal oaks, and activity (and concern) escalated. Now a major international research effort addressing origins, biology, and control, including quarantines, is underway. This has triggered *Phytophthora* surveys in forests in several parts of North America, leading to new host reports, and new species. On other fronts, active forest *Phytophthora* research continues in Argentina, looking for the cause of Mal des Cipres, in Mexico with *P. cinnamomi* and possibly other species on oaks, and in Oregon, focused on Port-Orford-cedar and *P. lateralis*. A new "Rangewide Assessment" of the status of POC and *P. lateralis* has been prepared. Breeding for resistance continues, with the containerised seed orchard organized by breeding zone to produce locally adapted seed, and the first seedlings expected to be available for operational forest planting in 2002.

Introduction

Two years ago in Grants Pass (Hansen 2000) at the conclusion of the first IUFRO Forest *Phytophthora* meeting, I worried a little that there wouldn't be anything really new in 2001 to report from the western hemisphere. We knew that big things were happening in Europe, and we had confidence that the Australians would continue their exciting work, but what was going to really change in the Americas? We needn't have worried. Not only are there new faces bringing new advances on old diseases, and a smattering of small new diseases, but Sudden Oak Death and *Phytophthora ramorum* has burst upon us, forcing an entirely new way of thinking about *Phytophthora* on our entrenched forest pathological minds. I also want to use this opportunity to review and update some of the old information presented in the first Proceedings - the stories of *P. cinnamomi* on chestnut and shortleaf pine in the SE United States and *P. lateralis* in the West are still relevant (Hansen and Sutton 2000).

Phytophthora was discovered in Europe, but that first epidemic came from the Americas. *P. infestans* was an inconspicuous indigenous *Phytophthora* on wild and native cultivated species of *Solanum* in Central America, but as potato late blight it set the standard for destruction by an exotic pathogen. Today sudden oak death, with its aerial pathology, is providing disquieting parallels to late blight. Apart from killing trees at an alarming rate, it has spawned new centers of forest *Phytophthora* research in California. We are reminded once again that the story of *Phytophthora* in forests of North and South America, as in other parts of the world, is largely one of devastating introduced pathogens. *Phytophthora lateralis*, in Oregon and California, exemplifies the dangers. The omnivorous, and seemingly ubiquitous *Phytophthora cinnamomi* is emblematic of any pathology meeting in Australia, but transformed the landscape of the south eastern United States about 200 years ago. It hasn't gone away. There is also an unfolding story of indigenous forest *Phytophthoras*. As we begin to understand bits and pieces of their pathology and their ecology, it is increasingly evident that they are important players as well.

Phytophthora ramorum

Phytophthora ramorum is the headline story. In this Proceedings, papers by Davidson, working with Rizzo and others, Garbelotto *et al.*, Frankel, and Svihra and colleagues contain the most complete account yet of this rapidly unfolding epidemic. Reports of unusual mortality in coast live oak, *Quercus agrifolia*, in coastal woodlands near San Francisco began in about 1993. At first oak bark beetles were assumed to be the cause of "Sudden Oak Death" (SOD), and then a variety of fungi, including *Hypoxylon* received attention. All proved to be

secondary. The trees were evidently dying from massive, girdling trunk cankers, often above seemingly healthy roots. It was only in the summer of 2000, as other possibilities were exhausted, that we began to suspect *Phytophthora* might be the cause. We collected bark samples on the hottest day on record for San Francisco, and Dave Rizzo from UC Davis successfully isolated a *Phytophthora*. It was a unique species, unlike anything reported before. Pathogenicity tests at Davis soon confirmed it as the cause of the disease, in both tanoak (*Lithocarpus densiflorus*) and coast live oak (Rizzo *et al.* 2002).

Coincidently, it was also in 1993 that a new disease of rhododendron and viburnum was first noted in landscape plantings and in nurseries in Germany and the Netherlands. An unusual "undescribed species of *Phytophthora*" was reported as the cause but little further attention was paid- it was just another foliar *Phytophthora* on rhododendron not causing any particular concern. Clive Brasier is never far from *Phytophthora* news. He saw the first isolates from California oaks and was impressed by their unique features. Not long after, he heard about the rhododendron isolates in Germany, and recognized that their unusual morphology (Werres *et al.* 2001) matched the unique species he had seen in California. The identity was confirmed by ITS sequence, and infected rhododendrons were soon found in California, beneath dead coast live oaks (Rizzo *et al.* 2002). The host range continues to expand, as *P. ramorum* is confirmed from a diverse array of unrelated forest trees and shrubs. Except for the oaks, symptoms are foliar necroses and shoot diebacks.

Curiously, the closest relative of *P. ramorum*, at least based on ITS sequence, is another exotic forest *Phytophthora* that is destructive in Oregon and northern California, *P. lateralis*. Although the two species are readily distinguished on pathogenicity, morphology, and cultural appearance, both form chlamydospores and caducous sporangia. The ITS sequence is so similar that a PCR probe designed to detect *P. lateralis* is also effective for detection of *P. ramorum* (Winton and Hansen 2001; Sutton pers. comm.).

This summer (August 2002), sudden oak death was found for the first time in Oregon, 300 km north of the known California infection areas. Nine areas of infestation, all within a 24 km² area, were located on forest lands near Brookings, in SW Oregon. Mortality centers ranged in size from 0.2 to 4.5 ha and included 5 to about 40 diseased trees. The pathogen was also isolated from necrotic lesions on leaves and stems of native *Rhododendron macrophyllum* and *Vaccinium ovatum* growing beneath diseased tanoaks on the Oregon sites. The disease was located and confirmed via the cooperative aerial survey flown by the USDA Forest Service and Oregon Department of Forestry (Goheen *et al.* 2002).

The wild hosts of SOD are not of great economic value, but they are often important members of the plant communities where they occur, and in California especially the coast live oak has great aesthetic value. Tanoak is a major component of mixed evergreen forests along the coast, growing with redwood and Douglas-fir. It sprouts back rapidly after wildfire or harvest. Several evergreen shrubs in the Ericaceae are susceptible, and *Vaccinium ovatum* and *Rhododendron macrophyllum* are killed in areas of high inoculum load. Ericaceous shrub communities dominate forest understories from northern California into British Columbia. Among the many important questions left to be answered is the potential of this pathogen to disrupt these plant communities beyond the range of tanoak. Can these foliar and dieback hosts sustain a damaging epidemic without the tree hosts? Will there be effective climatic and weather constraints on its spread?

In California the disease was widely established before the *Phytophthora* connection was demonstrated. There has been no attempt at direct control. In Oregon, by contrast, the infestations appear recent and small, and the decision was quickly reached to attempt eradication. All lands within 1 mile of the mortality centers are subject to Oregon quarantine, barring the transport of host materials. In the first phase of this operation all host plants within 15-30 m of symptomatic plants are being cut and burned. The total treated area is about 16 ha. Time will tell.

Other new Phytophthora species

Phytophthora ramorum is of course the big news, but there are other new diseases on trees as well. In this Proceedings, Tainter has described a new disease on plantation grown balsa (*Ochroma pyramidalis*) in Ecuador, caused

by *P. palmivora*. Oaks are well known suscepts to *P. cinnamomi*, but in Texas? Van Arsdel described it as cause of a yellowing disease of Texas oaks.

Also in this Proceedings is the first description of *P. cambivora* on chinquapin, *Castanopsis chrysolopis*. This is a small evergreen tree, in the Fagaceae, that grows in the Cascade Mountains of western North America. The disease was first noted about 2 years ago, in the southern Oregon Cascades. Dying trees have girdling basal bark lesions, extending upward from necrotic lesions on the main roots. The pathogen was readily isolated from active margins of bark lesions, and also from soil (by baiting) collected adjacent to dying trees. Interestingly, *P. cambivora* has also been baited from soil in other forest areas of Oregon, associated with healthy chinquapin, Douglas fir, and Oregon white oak.

Phytophthora lateralis

Phytophthora lateralis, the equally evil exotic twin of *P. ramorum*, kills Port–Orford-cedar (POC), *Chamaecyparis lawsoniana*, through much of its limited native range. POC is a unique forest tree, growing wild only in the geologically and climatically distinctive Klamath ecological province in SW Oregon and NW California. It has been planted widely as an ornamental, however, and it was in the ornamental nurseries, in 1923, that the disease first appeared (Zobel *et al.* 1985). There is only speculation about the origin of the pathogen, but it seems likely that unregulated international plant movements in the horticultural trade were somehow responsible for the introduction.

By the early 1950s *P. lateralis* was killing POC in the tree's native range along the southern Oregon coast. It spread quickly into the mountains, following road construction and timber harvest. POC regenerates prolifically in disturbed soil, and is especially abundant, and vulnerable, immediately adjacent to roads. Today the rate of disease increase has slowed dramatically, largely because most of the most vulnerable stands of cedar are already infected. A large, expensive, and multifaceted disease management and research effort has been launched by the Federal land management agencies. The goals are to halt the further spread of the pathogen, protect the remaining significant uninfected stands of POC, and to bring cedar back in the areas already infested. The strategies

include road closures, roadside sanitation, silviculture including targeted planting and spacing of POC, and genetic resistance (Hansen *et al.* 2000).

Recent work has focused on measuring the effectiveness of control operations, and disease resistance (Papers by Goheen, Sniezko, and colleagues in this Proceedings). Plant ecology is sometimes summarized as "the quantification of the obvious." Plant ecologists Erik Jules (Humboldt State University) and Matt Kauffman (U.C. Santa Clara) have taken a fresh and detailed look at the epidemiology of *P. lateralis*, using dendrochronology and modelling to plot the tree-by-tree spread of disease down streams from road crossings and other sources of introduction in several watersheds. The result puts a sharp point on things we have known in a general way, and can serve to refine several disease management guidelines. The current status of POC and the disease management effort is being summarized in a long-awaited "Rangewide Assessment of Port-Orford-cedar", to be published soon by the USDA Forest Service and the Bureau of Land Management in the United States.

Phytophthora cinnamomi

Phytophthora cinnamomi arrived in North America perhaps 200 or more years ago, unannounced, and spread silently but with lethal effect across the SE United States. Nothing is known of the early history, but by 1824 there were clear reports of sudden and unprecedented mortality of American chestnut and related *Castanea* species in forests and woodlands across the southern Appalachians. Nearly all of the trees in valleys and coves died within 2 or 3 years, with mortality extending upslope more slowly and trees surviving on dry ridgetops (Crandall *et al.* 1945). Chestnut was already largely gone from the southern Appalachian foothills before chestnut blight reached that region. It wasn't until 1932 that *Phytophthora* was associated with dying chestnut in the United States, and *P. cinnamomi* was convincingly shown to be the cause in 1945.

Littleleaf disease of shortleaf pine (*Pinus echinata*) first attracted attention in the 1930s (Hepting *et al.* 1945). Shortleaf pine grows broadly in the Piedmont and Coastal Plain of the SE United States especially on abandoned agricultural lands. Littleleaf disease was most destructive in stands older than 20 years on

severely eroded soils in this region (Tainter 1997), with a distribution that broadly overlapped the former southern range of chestnut. Above ground disease symptoms include chlorosis, stunted needles, and progressively reduced needle retention. Symptoms gradually intensify, with many trees dead within 2 to 16 years of first symptoms. Exhaustive searches for causal agents led in 1948 to the isolation of *P. cinnamomi* from symptomatic shortleaf pine trees (Campbell 1948), but because *P. cinnamomi* was seemingly everywhere across the south (Campbell 1949, 1951; Campbell *et al.* 1963), and because inoculations often did not result in symptoms, it took years more to confirm the etiology. The disease is explained today as the consequence of rootlet mortality (incited by *P. cinnamomi*) on stressed trees that are unable to efficiently replace those rootlets. Nutrient deficiency from loss of the fertile topsoils is exacerbated in older stands that are under maximum intertree competition for light and soil resources. *Phytophthora* also benefits on the eroded soils. Littleleaf sites characteristically have very poor internal soil drainage. Today littleleaf disease is still evident, but in fewer localities. In many stands susceptible trees have been replaced by tolerant ones, and soil structure and microbial activity have improved as a result of forest succession, to the detriment of *P. cinnamomi* (Zak 1961).

Phytophthora cinnamomi continues to attract attention in forestry in the SE United States. Littleleaf is still around (Tainter 1997), and other pines are damaged on poorly drained soils (Barnard *et al.* 1993). At this meeting, we will hear about damage to oaks from South Carolina to Texas. There is also continuing work on *Phytophthora* root rot of Frasier fir- a Christmas tree disease (Benson and Grand 2000, Frampton *et al.* in these Proceedings). The pathogen is apparently not present in native stands of *Abies fraseri*, found on organic soils above 1500m elevation, but it is present in transplant nurseries at lower elevations in the Christmas tree growing areas. One infected seedling per hectare may trigger an epidemic in downslope areas following heavy rains.

P. cinnamomi was presumably introduced to the Hawaiian Archipelago, perhaps with the first colonizing Pacific Islanders (Zentmyer 1988). Today it is implicated in Ohia decline, an episodic, locally devastating disease

of mature forest ohia (*Metrosideros colina*) (Kliejunas and Ko 1973). In one disease scenario, water drainage in the dense pahoehoe lava flows progressively deteriorates as organic matter and decomposing rock gradually plugs the cracks in the otherwise dense and uniform flow. Trees are progressively stressed by poor drainage, and mature trees, with their greater demands, are unable to replace rootlets killed by *P. cinnamomi*.

In the forests of the SE United States and in Hawaii, where *P. cinnamomi* has been present for hundreds of years, it is easy to forget that it is an exotic, invasive pathogen with the potential for devastation. The disease is now chronic; there are no longer advancing fronts of infestation marked by dead trees. The pathogen has reached its climatic limits. The dramatic ecological changes are history now, and new, disease tolerant plant communities have replaced what was lost. In many cases, we will never know what was lost. *P. cinnamomi* is not gone from these forests, however, and the Frasier fir Christmas tree story illustrates its potential to rise to new opportunities as they are created by human activity and perhaps changing climates. It is important to remember what *P. cinnamomi* has done to North American forests, and can do again.

The story is apparently replaying now in the state of Colima, Mexico (Tainter *et al.* 2000). *P. cinnamomi* is locally epidemic in an area of several hundred hectares around a village in that state, killing several native oak species and other susceptible vegetation in the surrounding communal woodlands. The mortality began in 1987. Spread appears to be primarily on the feet of cattle that graze freely. This may be another American ecological tragedy in the making.

Wild *Phytophthora*

Scattered information suggests that Phytophthoras are widespread but not often abundant in many temperate forest ecosytems, usually in the absence of dramatic disease (Hansen 2000; Wood pers. comm..). By contrast, there are very few reports of *Phytophthora* species from tropical forests. Certainly there are pathogenic species in the plantations of rubber, avocado, balsa (Tainter, this Proc.) and *Cinchona* (Crandall 1947). Zentmyer (1988) failed to isolate any *Phytophthora* from forest plants or soil, except in the immediate vicinity of

plantations. It must be presumed that this lack of reports is the result of very few surveys, however.

Phytophthora heveae is occasionally recovered from North American forest soils (Campbell and Gallegly 1965), but is best known as a pathogen of plantation rubber, and rhododendrons. In Central American rainforests, however, it is widespread and apparently ecologically significant. Davidson *et al.* (2000, and personal communication) demonstrated that it was an important cause of damping off of wild cashews *Anacardium excelsum* in a very diverse Panamanian tropical forest. Furthermore, it was most abundant, and caused the most damping off, where cashew seedlings were most abundant. Davidson hypothesized that *P. heveae* was exerting negative density dependent selection on cashew, and thus maintaining the diversity of the tropical forest.

P. gonapodyides is undoubtedly the most widespread species in temperate forests, in North America as well as Europe (Hansen and Delatour 1999). It seems to be ubiquitous in forest streams of the western United States, including very remote areas in Alaska and Oregon. It is commonly misidentified as *P. drechsleri, P. cryptogea,* or even *P. lateralis.* We recover it abundantly in all seasons from forest streams near Corvallis. We also recover it from soil in riparian hardwood stands in Oregon. *P. gonapodyides* has a reputation as a weak pathogen, and is capable of a saprophytic existence, but when circumstances are right, at least in artificial inoculation, it can induce significant disease. What is this abundant organism doing in the forest? Seemingly not much, but we have to wonder.

There are many more wild Phytophthoras; in our continuing work in Oregon, we have recovered 13 species from forest soils and streams. Only 3 of these, *P. ramorum, P. lateralis, P. cambivora,* are associated with recognized diseases in our forests. Most sampling to date has been in riparian hardwoods, with occasional forays into upland areas and conifer stands. Overall, we have recovered *Phytophthora* species from 29% of forest soil samples and 97% of water samples from forest streams. The latter are primarily *P. gonapodyides,* but the water list includes 8 other species. We regularly recover isolates that can not be readily assigned to any known species

(Hansen and Hamm 1988), but using ITS sequences we can at least associate them with near relatives.

We are left then with a very uneven picture of *Phytophthora* in the Americas. We can conclude that exotic pathogens are extremely dangerous in forest ecosystems and that *Phytophthora* species are widespread in forests, usually not causing recognizable disease. Most significantly, we must continue to acknowledge how little we know about forest *Phytophthoras.*

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Impact of *Phytophthora* in Australia and New Zealand
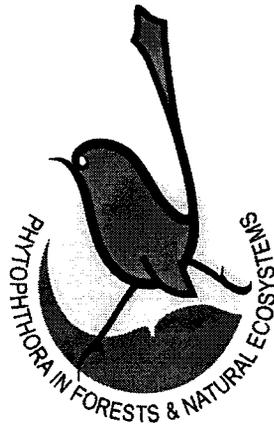
Foliar disease of *Eucalyptus* caused by *Phytophthora* spp.

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Abstract. Locally severe crown dieback of *Eucalyptus botryoides, E. fastigata* and *E. saligna* has been recorded in the central North Island – Bay of Plenty area of New Zealand since 1986. At least two different species of *Phytophthora* have been associated with twig and small branch lesions, leaf spots and petiole infection. Pathogenicity has been demonstrated in inoculation trials. Affected trees ranged from 4-12 years of age. Individual trees within a plantation can vary markedly in susceptibility, ranging from being unaffected to almost entire crown defoliation. Sporulation of the *Phytophthora* spp. has not been observed in the field. Although the *Phytophthora* spp. have been found in the soil beneath symptomatic trees the height of the trees precludes spore splash from the soil as a mode of fungal dispersal. Attempts to find evidence of the fungi on insects travelling up the trunks of the trees have not been successful. The fungi are probably undescribed species of *Phytophthora*.

Introduction

Locally severe crown dieback of *Eucalyptus* species, associated with foliage, shoot and twig infection by species of *Phytophthora* has been recorded in the Bay of Plenty bioregion (Crosby *et al.* 1976) of New Zealand since 1986. The disease was first recognised in a small stand of 19-year-old *Eucalyptus botryoides* in which trees developed marked foliage browning and leaf casting in early spring. Individual trees varied markedly in the degree of symptom expression, ranging from being unaffected to almost the entire crown wilting and then defoliating. During the spring and early summer symptoms on affected trees grew worse, but no healthy trees became infected. There was no further deterioration of the trees after November 1986, and by January 1987, all affected foliage had fallen. Apart from the sparseness of the crown of some trees there were no further symptoms until August 1987 (late winter) when the same pattern of foliage discolouration and leaf casting was repeated over the spring and summer.

Several fungi were routinely isolated, or recognised during microscopic examination of the affected tissues. These included species of genera such as *Botryosphaeria, Vermisporium* and *Phomopsis,* genera that contain some plant pathogenic species. A slow-growing, non-sporulating colony was also isolated but was initially paid little attention until it became apparent that these colonies were consistently isolated during the early stages of lesion development. The fungus was identified as a species of *Phytophthora*.

Subsequent to the first report of the disease in *E. botryoides* similar infection in stands of *E. fastigata* and *E. saligna* and in amenity plantings of *E. marcarthuri* and *E. sieberi* were recorded in the Bay of Plenty bioregion over the next 2 years. Severe defoliation of *E. delegatensis* was recorded some years later in stands in Southland bioregion, geographically at considerable distance from the Bay of Plenty.

As the severe defoliation was a hitherto unknown disease, pathogenicity tests were initiated with the fungi isolated from symptomatic material and, over a period of time, further studies into the disease were undertaken. Because occurrence of the disease is episodic, and because the non-indigenous host species were at the time neither economically important plantation species nor highly regarded amenity species, investigations into the ecology of the disease have been spasmodic and spanning nearly 15 years. This paper outlines some of the trials in which the ecology of the *Phytophthora* infection of the upper crown of *Eucalyptus* species has been investigated.

Symptoms

Initial symptoms of leaf spots on mature leaves are small light-green patches of irregular shape on the lamina. The tissue sinks and fades before becoming necrotic and lesions are surrounded by a deep-purple margin. Size of lesions is extremely variable and there may be a profuse scattering of very small spots. Lesions share a common appearance on different host species. Although leaf spotting is a common feature of the disorder, the lamina of many leaves fade and then become brown following an infection of the petiole. This frequently occurs in the absence of any leaf spots. Affected leaves are

readily abscised. Lesions on twigs may cause leaf death in the absence of dieback when the lesion surrounds a petiole. Seed capsules also become infected with the peduncle often infected before the capsule. Dieback follows lesion development on twigs and/or high levels of defoliation. The most consistent isolate from leaf petioles and from laminar lesions in early stages of development was the *Phytophthora* sp.

Materials and methods
Plant material
The origin of the seed from which plants were reared for inoculation experiments is unknown. Inoculations were carried out on 2-year-old *E. botryoides* and 4-year-old *E. saligna* planted in the extension area at Forest Research in Rotorua.

Two unimproved stands of *E. saligna* growing in New Zealand, AK 78/1 (Waipoua Forest) and R 77/50 (Rotoehu Forest) provided the seed for the stand of *E. saligna* where disease levels were monitored and where the soil sampling programme was carried out. The eucalypts were interplanted with *Pinus radiata*. The Australian origin of the two stands is unknown.

Inoculations
Experiment 1: Four twigs on each of 20, 2-year-old *E. botryoides* were wounded by lightly pricking with a cluster of 5 pins. Two wounded twigs per plant were inoculated, one with a mycelial plug and the other with an artificially infected 30 mm long piece of *E. botryoides* twig. The inoculum was placed on the wound, wrapped with damp cotton wool and fastened with parafilm. Mycelial plugs were taken from a 15-day-old culture grown on 3% malt extract agar (MEA). Woody inoculum was prepared by placing sterilised twigs on the surface of 3% MEA petri dishes on which the *Phytophthora* was growing and incubating at 18°C for 12 days. Controls consisted of attaching non-infected twigs and 3% MEA agar to the remaining wounded twigs on each plant. Plants were examined fortnightly and lesion development and dieback recorded. The final assessment was made ten weeks after inoculation. Presence of lesions, and dieback were recorded and lesion length measured.

Experiment 2: Small sterilised pieces of eucalypt leaves were placed on the surface of 3% MEA petri dishes on which the *Phytophthora* was growing and incubated at 18°C for 12 days to provide the inoculum. These colonised pieces of leaf tissue were placed in contact with leaf petioles and twigs and held in position with parafilm. Inoculated tissue was not wounded. Between four and six petioles, and between four and six twigs, on each of ten 4-year-old *E. saligna* were inoculated. Inoculations were made in late spring and inspections made at 3 week intervals for 15 weeks. Controls consisted of attaching non-infected leaf tissue to each of 10 leaf petioles and 10 twigs (one of each on each tree). Discolouration, lesion development and leaf casting were recorded and surrounding branches examined for any development of disease. After the final assessment material was gathered for isolations.

Seasonal disease development
Disease progress in a stand of 9-year-old *E. saligna* interplanted with *Pinus radiata*, in Rotoehu Forest in the Bay of Plenty, was monitored for 15 months. Symptoms were more severe in one end of the compartment where a count of the trees indicated that 40% had some signs of crown infection, ranging in intensity from foliage death on a single branch to death of all of the foliage. Two hundred trees, in an area of approximately 2 ha, were labelled in August 1987 and the first assessment of infection in these trees was made on the 1st September 1987. Trees were selected on the basis of reasonable visibility of at least half the crown. Assessments were carried out fortnightly for 3 months and then monthly until September the following year when the frequency of visits doubled again. Each tree was assigned a disease score according to the following scale.

Foliage Disease Score

1. crown healthy or trace infection
2. up to 20% foliage affected
3. 21 - 40% foliage affected
4. 41 - 60% foliage affected
5. 61 - 80% foliage affected
6. 81 - 99% foliage affected
7. all foliage dead

The term "trace infection" was applied to trees with less than 2% of the crown exhibiting browning leaves. In many such cases it was impossible to be certain, even with binoculars, of the cause of the symptoms and factors other than the disease under investigation may have been responsible. Leaf lesions that were not accompanied by foliage death were not clearly visible and so were not included in this evaluation of disease levels.

Dispersal of the fungus

Although sporangial and oogonial formation can be induced in the laboratory it has not been observed in the field. Sporangia are not deciduous and the means of dispersal of the fungus remains unknown. The *Phytophthora* sp. associated with the dieback has been found in the soil under affected trees and as insects are known to be capable of vectoring *Phytophthora* spp. from the soil into tree crowns (Evans 1971; Taylor 1977), this method of fungal dispersal was investigated during three separate seasons. Insect traps were employed on the ground, on tree trunks and in the canopy. Various types of trap were used (frass traps, pitfall traps and malaise traps on the ground, 3 different types of trunk trap and a malaise trap in the canopy) selected to catch as wide a range of species as possible. Traps were serviced and the contents collected at weekly intervals as crown symptoms developed and monthly otherwise. Because of the need to culture from the catch it was not possible to use preservatives. Some traps had an insecticide impregnated paper tissue in the catching container, while others were left bare despite the risk of losing some insects. The insecticide was a commercial mixture of tetramethrin, permethrin and piperonyl butoxide. Decay of the catch was a problem, especially with the longer servicing periods and in warmer weather.

The collected insects were categorised into broad groups and a subsample of each was placed onto a selective medium (PAR – containing pimaricin, ampicillin and rifampicin) to test for the presence of *Phytophthora* propagules. No specific determinations were made until or unless a positive result was obtained. Plates were incubated at 20°C and examined for colony type.

Soil samples

In order to determine whether the *Phytophthora* sp. was soil-borne, a bioassay method for isolations was first developed using artificially infested soil. Sections of *E. saligna* leaves with the petiole attached were found to be the most effective bait, and these were floated on top of 50 gm samples of either soil or litter flooded to a depth of 20 mm with distilled water. Flooded samples were incubated at 18°C. The *E. saligna* baits were removed after 7 days, rinsed in sterile water and plated onto PAR medium.

Experiment 1: Soil and litter was collected from beneath 8 infected trees in the *E. botryoides* stand where symptoms were first seen and from beneath 6 infected trees in Rotoehu Forest. Recently fallen leaves were discarded and litter in a stage of breakdown was collected from a 160 mm^2 area (outlined by a spade). The soil from the cleared area was dug from the surface to 100 mm deep and then mixed. Three replicates from each sample were bioassayed for *Phytophthora* sp. as outlined above.

Experiment 2: To determine whether soil colonisation follows deposition of infected leaves two *E. saligna* stands where the disease had been recorded were selected. At each site two 3 x 4m areas were cleared of all leaf litter. One area at each site was covered with a tarpaulin which was slightly raised in the centre. A 1 x 1m plot was marked in the centre of each cleared area. Replicate soil samples were collected from the centre plot each month for 14 months and bioassayed for the *Phytophthora* sp. To ensure that the *E. saligna* leaf material used as bait was free of any species of *Phytophthora*, 18 leaves were selected, cut into petiole plus lamina sections, surface sterilised in 10% hydrogren peroxide for 4 minutes and placed on PAR plates. Plates were incubated at 20°C for 10 days. No *Phytophthora* was isolated. Fallen leaves were removed from the cleared areas each month. Isolations were made from 15 freshly fallen (still green) leaves each sampling date per plot.

Results
Inoculations

Experiment 1: Twigs inoculated with *Phytophthora* developed dark lesions extending both distally and proximally from the inoculum point and many were subsequently girdled and died back (Table 1).

Inoculations were also carried out with isolates of *Botryosphaeria*, *Phomopsis*, *Vermisporium* and *Cytospora* derived from the diseased material. Symptoms varied with the fungal species inoculated. Symptoms of infection on the twigs inoculated with *Phomopsis* consisted of small shallow lesions that were seldom more than a few mm long. Some developed small cracks in the twigs. *Botryosphaeria* infections consisted of very small sunken spots on unwounded twigs and sunken lesions ranging from a few mm up to 50 mm in length associated with wounds. Only 4 lesions of the total inoculated with *Botryosphaeria* were greater than 20mm in length.
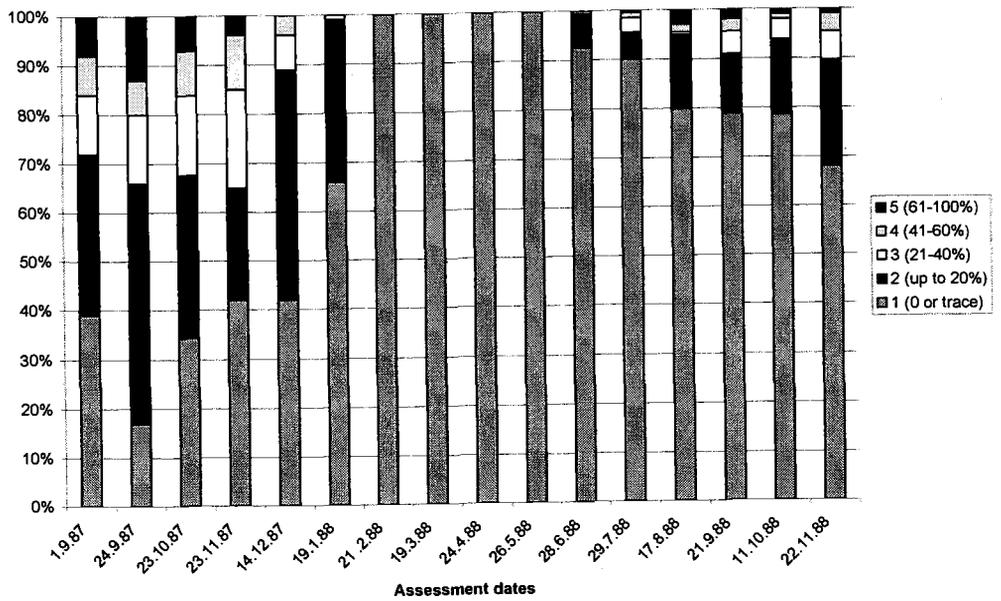
Fig. 1. Distribution of trees in each crown health category from September 1987-November 1988

Table 1: Percentage infection and dieback of *E. botryoides* twigs inoculated with the *Phytophthora* sp.

treatment	% infected	% dieback
wounded (mycelial inoculum)	100	50
not wounded (mycelial inoculum)	80	25
wounded (twig inoculum)	94	61
not wounded (twig inoculum)	100	33
wounded and unwounded (mycelial controls)	0	0
wounded and unwounded (twig controls)	0	0

Table 2: Lesion development on twigs and petioles of 10 *E. saligna* inoculated with the *Phytophthora* sp.

Tree No.	No. inoculations (twigs and petioles)	Percent developing lesions
1	10	30
2	10	60
3	10	90
4	8	38
5	10	60
6	10	60
7	8	13
8	8	50
9	10	50
10	10	20

Experiment 2: Results have been combined for the twigs and the petioles and are summarised in Table 2. No lesions developed on any of the control leaves or twigs.

Individual trees showed clear differences in susceptibility, with infection ranging from 13-90%. This paralleled field observations where defoliation of individual trees varied from 0–100%. Ninety percent of leaves inoculated at the petiole were cast without any infection spreading into the shoot/twig tissue. Development of infection was slow. Six weeks after inoculation 37% of all of the leaves were infected and this had risen to 57% at six weeks.

Seasonal disease development

The distribution of crown health categories at monthly intervals is shown in Fig. 1. Categories 5, 6 and 7 have been combined. Discolouration of the foliage continued to increase after assessments began in September 1987 until October in 1987, and from June until November in 1988, and then declined sharply. Assessments ceased in November 1988 but general observations of stand health were made monthly for the following 3 months. Affected foliage was almost entirely cast by the end of December in each year. Disease severity was markedly lower in the second year. Those individual trees that did not show symptoms (Category 1) during 1987 also remained symptomless in 1988. Nine percent of the trees showed no of infection throughout the entire period, with many others having only a trace infection. Severely affected individuals in the first year were also the worst affected in the second year. One was totally defoliated (Category 7) in 1987, and this tree lost 99% of its foliage (Category 6) in 1988. Those trees with a seasonal maximum score that fell between the two extremes were a little more variable from year to year, possibly because of the subjectivity of the scoring system. However the majority of trees scored as a 3 or 4 in the first season were ranked in the same order in the second season indicating that comparative individual tree susceptibility to the condition was quite consistent.

Although intensive monitoring of the trees was not continued yearly observations have been made of disease levels in this stand. From 1989 through 1991 the disease did not re-occur in this stand of *E. saligna*, or was at such a low level that it went undetected. Foliage and branch death was observed in 1992 and a similar pattern of winter/spring

build-up of symptoms followed by a cessation of symptoms during summer and autumn was documented during the following 3 years. No disease was recorded in 1996. The reason for the non-appearance of the disease in some years is not known.

Dispersal of the fungus

Many invertebrates other than insects were caught in the traps, and these were isolated from as they may have been potential vectors. On the ground, the pitfall traps collected mainly Collembola, Coleoptera, Arachnida and Mollusca, and the malaise traps Diptera, Hymenoptera and Arachnida. Frass funnels caught a variety of litter and debris, including frass, and mainly Coleoptera and Arachnida.

The trunk traps caught a wide range of organisms, Collembola being the most frequent, with Diptera, Coleoptera and Lepidoptera well represented, along with Arachnida and Mollusca. There was considerable movement of organisms up and down the trunks of the trees examined. Canopy traps yielded mainly Diptera, followed by Hymenoptera and Lepidoptera.

Significant numbers of specimens were caught; for example between July and November 1995, 4910 organisms were counted, and isolations were made from 3836. The numbers in trap collection jars in the other seasons were similar. Twenty percent of the insects and other invertebrates isolated in the July to November 1995 period carried one or more species of *Phytophthora* or *Pythium*, but the target species was isolated only once. The only positive result came from a large frass pellet which may have been the product of an organism that had eaten infected leaves. It was not possible to establish the identity of the organism responsible and no further cultures were obtained. The target *Phytophthora* sp. was however isolated from leaves collected each month from June–November in frass traps.

Soil samples

Experiment 1: The *Phytophthora* sp. was obtained only infrequently from either location. It was isolated only once, out of 24 attempts (8 samples, 3 replicates of each), from soil beneath *E. botryoides*, and twice from beneath *E. saligna* out of 18 attempts (6 samples, 3 replicates of each) at another location. The proportion of successful retrievals from leaf litter was slightly higher, 4

of 24 attempts from beneath *E. botryoides* and 5 of 18 attempts from beneath *E. saligna*.

Experiment 2: Recovery of *Phytophthora* sp. each month is given in Table 3. The *Phytophthora* was isolated more frequently from soil in the exposed plots than those that were covered. Results were not however strongly indicative that preventing infected leaves from contact with the ground would prevent soil infestation. The *Phytophthora* was seldom isolated from any source during the warmer months (December through April) but was recovered from fallen leaves on two occasions during this period and from soil from one of the uncovered plots.

Identity of the Phytophthora *sp.*

The *Phytophthora* sp. could not be satisfactorily placed in any of the 60 known species (Erwin and Ribeiro 1996) based on morphological and cultural characteristics. In recent developments in the taxonomic study of the genus Cooke *et al.* (2000) presented an ITS-based phylogenetic analysis of 50 described taxa comprised of *Phytophthora* and *Pythium* species and representatives from the Saprolegniales and Peronosporales, work based on the wider study of the rDNA ITS regions of over 234 *Phytophthora* isolates. Cooke *et al* (2000) found the genus *Phytophthora* to be paraphyletic with 47 of the 50 taxa examined falling into one cluster that was separable into 8 clades. The remaining 3 taxa (*P. macrochlamydospora P. richardiae*

and *P. insolita*), all originating in the Southern Hemisphere, were disposed into a further 2 clades and represented a distinct lineage. Examinations undertaken at Edinburgh University of the ITS region of isolates of the eucalypt foliar *Phytophthora* from New Zealand placed this fungus between clades 9 and 10 (David Cooke pers. com.). More detailed information on the identity will be the subject of a later publication. Cooke *et al.* (2000) suggested that the *Phytophthora* spp. falling into clades 9 and 10 may, on re-examination of morphogenetic and behavioural characteristics, be reassigned to one or more new genera.

Discussion

Severe foliage death, premature leaf casting and dieback were first recorded in 19-year-old *E. botryoides* and *E. saligna* in the 1980s. Although leaf spotting was a common feature of the disorder, the lamina of many leaves faded and turned brown evenly following an infection of the petiole and these leaves were readily abscissed. Dieback followed the formation of girdling lesions on twigs and/or high levels of defoliation. Fungi isolated from symptomatic material included species of *Botryosphaeria*, *Cytospora*, *Vermisporium*, *Phomopsis* and an undescribed species of *Phytophthora*. Apart from *Cytospora* sp. all of the fungi showed some pathogenic capability when *E. botryoides* were inoculated by applying mycelium to slits cut in the twigs. The most consistent isolate from leaf

Table 3: Isolation of *Phytophthora* sp. from soil and leaf litter in covered and uncovered plots.

| Sampling date | soil | | | | l eaf litter | | | |
	bare Site 1	plot Site 2	covered Site 1	plot Site 2	bare Site 1	plot Site 2	covered Site 1	plot Site 2
July 1999	+	+	+	+	+	-	+	-
August 1999	+	+	-	-	-	+	+	-
September 1999	+	-	+	-	+	+	+	+
October 1999	+	-	+	+	+	+	+	+
November 1999	+	-	-	-	-	+	+	+
December 1999	-	-	-	-	-	-	-	-
January 2000	-	-	-	-	-	-	-	+
February 2000	-	-	-	-	-	-	-	-
March 2000	-	+	-	-	-	-	-	-
April 2000	-	-	-	-	-	+	-	-
June 2000	-	-	-	-	-	-	-	-
July 2000	+	-	+	-	+	-	-	-
August 2000	-	-	-	-	+	+	+	-
September 2000	-	-	-	-	+	-	-	-

+ *Phytophthora* isolated

- not isolated

petioles and from laminar lesions in an early stage of development was the *Phytophthora* sp.

Although infection of eucalypt leaves, shoots and petioles by species of *Phytophthora* has been recorded on a number of occasions (Belisario 1990; 1993) these disorders have been generally confined to seedlings or young plants. Sprinkler irrigation systems have been considered to be responsible for the dissemination of infective propagules from the soil, the natural habitat of the *Phytophthora* spp., to the foliage. The isolation of a *Phytophthora* sp. from the leaves and twigs of trees up to 20 m in height raised questions about the method of dissemination. Unlike common aerially dispersed species of *Phytophthora* such as *P. infestans,* the agent causing potato blight, sporangia of the eucalypt *Phytophthora* are not deciduous. The means of dispersal remains unknown.

The *Phytophthora* sp. could be re-isolated from soil collected from beneath infected *Eucalyptus* spp. although only infrequently. The target *Phytophthora* was recovered only once although other species of *Phytophthora* and *Pythium* were often isolated in large numbers. Concurrently bioassays of soil beneath affected trees were undertaken and recovery percentages were consistently low. A programme where the soil was covered to prevent infected leaves coming into contact with the ground, and therefore preventing soil infestation from this source, did not provide conclusive evidence that the fungus occurs only transiently in soil following abscission of infected leaves.

In *E. saligna* and *E. botryoides* the overall pattern of appearance of the symptoms, commencing in the middle of the year and increasing in intensity of expression until October /November before declining in December, was consistent over the three seasons of 1986-89. Affected foliage was dropped throughout the period of symptom expression, but from December no new infection occurred and the continued production of new foliage restored the appearance of health to the stands. Subsequently occurrence of observable disease has been sporadic. There have been periods of 3 years when no foliage infection has been recorded. However during the period from June 1999 to September 2000, when soil and fallen leaves were examined monthly, and even though *Phytophthora* was isolated regularly there were no symptoms clearly visible in the crowns of the trees. Determining the presence of leaf lesions (and identifying lesion types), even with the aid of binoculars, when the base of the crown is 10 m or more from the ground, has proved difficult and the observations on disease levels refer to the type of infection that results in whole-leaf death. The disease has possibly been present, but in less manifest a form, in those years when no disease was recorded. The reason for the variation in disease levels from year-to-year is not known.

Individual trees in the *E. botryoides* stand, and in the four stands of *E. saligna* where the disease has been recorded, vary markedly in symptom expression. In contrast almost all trees affected in *E. delegatensis* stands in the southern part of New Zealand have suffered substantial dieback from which there has generally been no recovery. *Eucalyptus delegatensis* is an ash group eucalypt without the capacity for coppice growth and recovery following defoliation. The disease has not been studied in these stands and no experimental work with *E. delegatensis* has been undertaken. It remains unknown whether the *Phytophthora* infection is an important contributor to the decline of these stands.

The isolates examined by Cooke *et al.* (2000) were classifed, based on the ITS region of the genome, as a new species residing between clades 9 and 10 of their proposed phylogeny. Isolates of *Phytopthora* collected from eucalypt foliage since 1986 have varied in some cultural characteristics such as growth rate, colony morphology and production of oogonia and sporangia. The relationship of these isolates (deposited in NZFSCC at the Forest Reference Laboratory in Rotorua, New Zealand), whether there is more than one species involved or simply one highly variable species, will be addressed in a further paper.

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The distribution and impact of *Phytophthora cinnamomi* Rands in the south coast region of Western Australia

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Abstract. Climate, soils, topography and susceptible plant communities, in combination with the movement of infested soil by human activity, has resulted in *Phytophthora cinnamomi* becoming the most destructive plant pathogen in native plant communities in the south coast region of Western Australia. Centres of disease activity occur near Albany and in the Stirling Range and Cape le Grand National Parks. Incidence generally decreases in a north-easterly direction in association with drier climatic conditions. *P. cinnamomi* causes death of susceptible species, in particular members of the Proteaeceae, Epacridaceae, Papilionaceae and Myrtaceae, and results in changes in community structure and species composition. Two-thirds of the Stirling Range National Park, notable for its 1,530 plant taxa and high numbers of rare and endemic species, is infested. Sixteen of these species are threatened with extinction by *P. cinnamomi*, eight are critically endangered. The Montane Heath and Thicket Community of the eastern Stirling Range is also critically endangered. The Fitzgerald River National Park with 1748 plant taxa is largely disease-free although a sizeable infestation occurs in the core of the Park. Appropriate management of areas with high conservation values infested by *P. cinnamomi* as well as the protection of those areas currently disease-free, remains an ongoing challenge.

Introduction

One of the most significant threatening processes to the flora of the southwest is dieback disease caused by *Phytophthora cinnamomi* Rands (Oomycetes) (Wills 1993; Wills and Keighery 1994; Barrett and Gillen 1997). The distribution and severity of infestation in plant communities is influenced by temperature, soil type, nutrient status and water availability (Weste and Marks 1987, Shearer and Tippett 1989; Wilson *et al.* 1994). The greatest impact occurs where soils are infertile and drainage is poor, in particular in the *Eucalyptus marginata* forest understorey and in shrublands and heathlands associated with leached sands and laterite of the Northern and Southern Sand plains and the Swan Coastal Plain (Shearer 1994). The impact of this pathogen is especially devastating in the species-rich areas along the South Coast where there is some summer rainfall (Brown *et al.* 1998). Here, climatic conditions combine with soils, topography and susceptible plant communities to create ideal conditions for the sporulation and survival of the pathogen, and the dispersal of *P. cinnamomi* through root to root contact or in soil water flow. This paper will focus on the distribution and impact of *P. cinnamomi* in relation to climate, soils, plant communities and human activity in the South Coast region of Western Australia, in particular in that area between Albany (34.94°S 117.8° E) and Eucla (32.62°S 123.8° E). *P. cinnamomi* occurrence and impact data was complied from dieback survey and interpretation undertaken by Malcolm Grant

1986 - 2000 and from isolation databases from Vegetation Health Service, Department of Conservation and Land Management.

Study area

The South Coast region is generally referred to as that area of Western Australia along the southern fringe of the state with seaward draining river systems. The region is also referred to as the Southern Sand-plain. Although the South Coast experiences a Mediterranean climate characterised by mild wet winters and hot dry summers, there is considerable variation in climate across the region. Mean rainfall decreases northwards and eastwards from 939 mm per annum in Albany to 263 mm per annum in Eucla. Over 50% of the annual rainfall occurs during the winter months with episodic heavy summer rainfall. Summer rainfall events are generated from remnant cyclonic activity in the northwest of the state that eventually deteriorates into rain bearing depressions in the eastern south coast region. Mean summer maximum temperatures range from approximately 25°C at Albany to 30°C in the northeast, while mean winter minima range from 7°C at Albany to 4.5° C inland.

The geology of the region has been described and mapped on a scale of 1:250,000 on the Mount Barker-Albany (Muhling and Brakel 1985), Bremer Bay (Thom and Chin 1984), Lake Johnston (Gower and Bunting 1976) and Malcolm-Cape Arid Sheets (Lowry and Doepel 1974) and is comprised of three

distinct geological units, the Yilgarn Craton, the Albany Fraser Orogen and the Bremer and Eucla Sedimentary Basins. The south coast region lies on the southern margins of the Yilgarn craton and has experienced considerable surface weathering from previous sea level rises. As a result the topography is gently undulating with only the occasional incised river valley dissecting the landscape. Sub-surface soils are either derived from sedimentary sandstones, a feature of the early ocean inundation episodes, or heavy clays weathered from the granite substrate. Surface soils may be uniform in nature or duplex.

The vegetation of the South Coast region is confined largely to the South-West Botanical Province (Beard 1980). This province occupies the wetter southwest corner and south coastal fringe of Western Australia. The region is comprised of two major Biogeographic Regions (Thackeray and Creswell 1995), the Mallee and the Esperance Bioregions, and to a lesser extent the Warren and Jarrah Forest Bioregions in the west. Vegetation ranges from forests and woodlands to mallee, thickets and heathland plant communities. The South-West Botanical province is renowned for its high number of plant taxa and levels of endemism (Hopper 1979), in particular the heath and shrublands of the Northern and Southern Sandplains. Nodes of species richness are centered on the Mt Leseur area in the north and the Stirling Range

– Fitzgerald area in the Southern Sandplain (Hopkins *et al* 1983). The Proteaceae and Myrtaceae are the most dominant members of this 'kwongan' vegetation with the Epacridaceae and Papillionaceae also forming an important component. Of these families, the Proteaceae, Epacridaceae and Papillionaceae are highly susceptible to *P. cinnamomi* and to a lesser extent the Myrtaceae. Notably, 85% of Proteaceous species assessed in the Stirling Range National Park were susceptible (Wills 1993; Wills and Keighery 1994). The ecological impacts of *P. cinnamomi* infestation include loss of species richness and plant community structure and reduced biomass (Wills 1993; Wills and Keighery 1994). Heathlands and shrublands once rich with many species of flowering plants are converted to open low sedgelands, dominated by those species that have survived the effects of the root rotting pathogen. Changes in resource availability and habitat may also affect associated groups of fauna (Wilson et al. 1994; Nichols 1998).

Phytophthora cinnamomi in the South Coast region

Within the South Coast region, the area most susceptible to the impacts of *P. cinnamomi* is primarily from Albany inland to the Stirling Range, then eastwards to Esperance to a distance of approximately 60 km from the coast (Fig 1). From Esperance
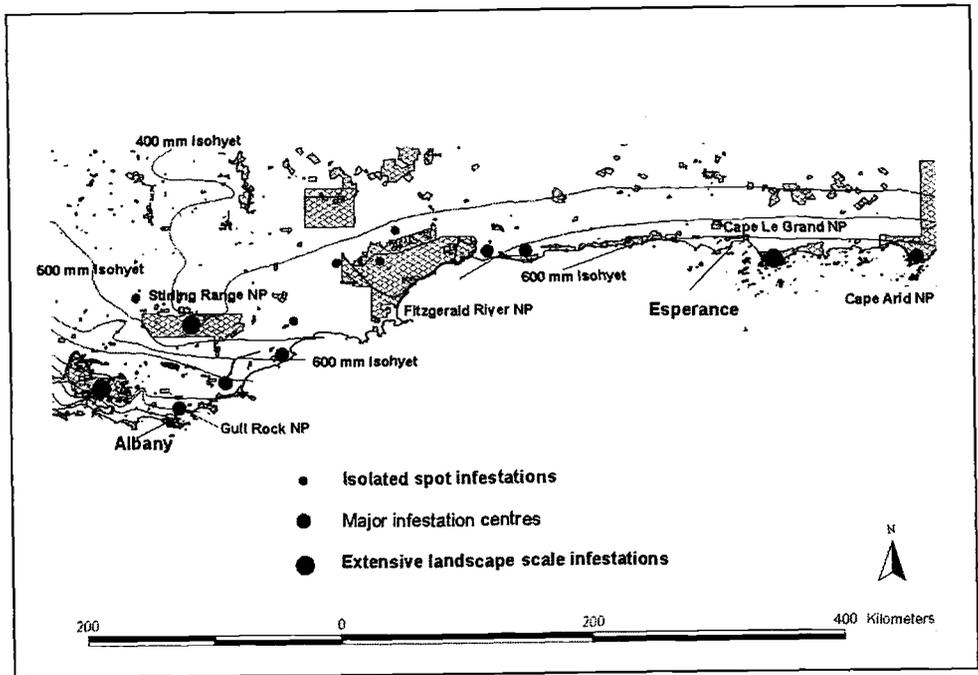
Fig. 1. Distribution of *Phytophthora cinnamomi* in the South Coast Region of Western Australia from isolation records.

eastwards to Israelite Bay the distance from the coast narrows to only 10 km. There have been no positive recoveries of *P. cinnamomi* east of Israelite Bay. In the South Coast Region, sites with homogenous deep sandy soils are most susceptible to the long-term impacts of *P. cinnamomi*. These sites are characterized by a dense, shallow root material layer in the upper soil horizon. The root mat layer is intertwined and as a result *P. cinnamomi* is capable of being transferred from susceptible host plant to another through root to root contact. This results in all susceptible host plants at the site being exposed to the passage of the *P. cinnamomi* infestation even though sub-surface drainage tends to be vertical. Poorly drained duplex soils have fragmented sub surface water flow that favors the production of spores and spread of the pathogen. Clay and laterite act as impeding layers causing sub-surface ponding necessary for the production of spores while the water tends to drain laterally spreading the pathogen further (Kinal *et al.* 993). Interrupted sub-surface water flow may allow some pockets of susceptible vegetation to temporarily escape infestation. However, within time frames of less than 10 years these pockets generally become infested.

Field interpretation for the presence of *P. cinnamomi* relies on locating recently dead and dying susceptible plant species. Sampling of root material and surrounding soil of recently dead individuals allows isolation of the pathogen and confirms presence of the pathogen. Key factors such as the presence of multiple species deaths, the pattern of development of plant deaths over time, proximity to a source of infestation and hospitable or inhospitable soil types are critical aspects of field interpretation. Several hundred kilometers of *P. cinnamomi* infestation boundaries occur across the south coast region contributing to a substantial loss of species rich plant communities each year.

Once introduced and established at a site susceptible to *P. cinnamomi*, infestations in lowland areas on deep sands typically progress in an outward spread pattern at rates ranging from 0.7 to 2.3 m per annum (Table 1) dependant upon the slope of the site, the nature of the subsurface soils and whether there is any additional surface water runoff. At a rate of spread of 1.5 m per annum, 1.5 ha of native vegetation is infested and modified for every 10 km of dieback fronts each year. This estimation is based on the spread of *P. cinnamomi* since 1965, a period that has undergone a 20% decline in total annual rainfall in the lower southwest. With 'normal' annual rainfall the anticipated rate of spread in the soil might be considerably greater. In wetter periods prior to 1965, it is likely that rate of spread *P. cinnamomi* was considerably higher. This may help explain the extent of *P. cinnamomi* occurrence across the south coast region, particularly those infestations in areas where there has been little vehicular activity and therefore less chance for the spread of infested soil.

Table 1. Annual rate of spread of *Phytophthora cinnamomi* in south coast *Banksia* shrublands on deep sands, Western Australia

Site	Latitude (S) Longitude (E)	Study Period	Length of front assessed (m)	Measurements per front	Mean annual rate of spread (m)
Gull Rock	35° 0' 10.1" 117° 58' 22.8"	1991-2001	40	4	1.5
Gull Rock	34° 59' 30.4" 117° 59' 24.8"	1991-2001	40	4	1.4
Gull Rock	35° 0' 50" 118° 2' 41"	1996-2001	100	20	1.7
Gull Rock	34° 57' 2.7" 118° 6' 32.2"	1991-2001	40	4	0.7
Gull Rock	35° 0' 47.3" 118° 2' 35.1"	1991-2001	40	4	0.7
Fitzgerald River National Park	33 54' 31" 119 29' 26"	1991-2001	80 100 100	9 4 4	2.3 2.1 2.03

Dieback mapping of *P. cinnamomi* infestations in conservation reserves across the south coast commenced in 1986. Mapping aimed to determine the extent of infestation in each reserve and the risk to known conservation values from further spread. Infestations on reserves in the Albany area such as the Stirling Range National Park, Two-Peoples Bay, Millbrook and Bakers Junction Nature Reserves were considerably greater than was anticipated. The establishment of early settlements for grazing, training of military personnel, wartime surveillance of coastal waters, mountain walking, 4WD activity and the construction of firebreaks have all contributed to the introduction and spread of *P. cinnamomi*. Of the more significant reserves, the Stirling Range, Cape le Grand, and Cape Arid National Parks and Two-Peoples Bay Nature Reserve were notable for the presence of extensive infestations.

Impact of *Phytophthora cinnamomi* on conservation reserves

The Stirling Range National Park (115,920 ha) is renowned for its 1,530 species of which more than 80 are endemic (Keighery 1993). The Park has unique climatic conditions when compared to the remainder of the south coast region. The mountain range of sedimentary sandstone results in considerable orographic rainfall, with not only a greater occurrence of rain events throughout the year but also significantly higher individual events (Courtney 1993). The unique heath and thicket plant communities of these elevated mountain tops, which may have been more widespread under wetter conditions in the past, reflect their mesic environment (Hopkins *et al.* 1993). The Park, surveyed for *P. cinnamomi* from 1986 to 1995, was determined to be extensively infested with approximately 60% of the Park affected and an additional 10% exposed to future uncontrolled spread of the pathogen. Only one mountain peak out of 17 with altitudes greater than 750 m elevation above sea level had escaped infestation. Orographic rain in combination with the presence of a species-rich plant community dominated by susceptible members of the Proteaceae, Papillionaceae, Myrtaceae and Epacridaceae growing on nutrient deficient shallow and duplex sandy soils provides ideal conditions for sporulation, survival, dispersal and infection. Once infested, the pathogen can spread at greatly increased rates of up to 250 metres per annum down slope from mountain summits.

Of the 24 threatened flora species that occur in the Park, 16 are susceptible to and threatened by *P. cinnamomi* with some or all populations infested, 14 of these are endemic to the Park and eight are currently classified as critically endangered. Of these eight taxa, seven have no *Phytophthora*-free populations. The Eastern Stirling Montane Heath and Thicket community, which occupies the mountain summits in the eastern end of the Park is classified as a critically endangered ecological community due to the impact of *P. cinnamomi* (Barrett and Gillen 1997). Phosphite application to those areas of the community that are still relatively intact is one of the few remaining management options in this widely infested plant community. In contrast, the western end of the Park, characterised by lower elevations, has the largest area of non-infested landscape. Management efforts have focused on minimising the introduction of the pathogen and the spread of existing infestations in this part of the Park. This area has been identified and nominated as a 'Special Conservation Zone' in the Management Plan for the National Park which enables all access to be strictly controlled and restricted to dry soil conditions and permits only key management and research activities to occur within the area.

Cape Le Grand National Park is a spectacular coastal conservation reserve located 40 km east of Esperance. More than 700 plant taxa have been recorded from the Park. This reserve is a narrow coastal feature constituting a remnant of native vegetation downslope from the agricultural farmlands. Cape Le Grand is the wettest point on the south coast east of Albany, receiving on average approximately 600 mm of rain per annum. This rainfall, combined with water table rise and increased run-off of surface water from the agricultural lands upslope, have enabled *P. cinnamomi* to flourish and spread rapidly. As a result Cape le Grand National Park is also extensively infested with *P. cinnamomi*. The only significant areas remaining free from infestation in this conservation reserve are the vegetation communities on the summits of its many granite hills. However, many of these have also become infested in the past due to the establishment of walking trails and the transport of infested soils. Two threatened flora species occur within the Park, of these, *Lambertia echinata* spp. *echinata* which is endemic to the area, is Critically Endangered due to the impact of *P. cinnamomi*. It is

currently known from less than 100 individuals in three populations, all of which are infested.

Cape Arid National Park is located on the coast 100 km east of Esperance but also extends a considerable distance inland. Mean annual rainfall at Cape Arid ranges from 500 mm per annum on the coast to 300 mm inland. While conditions are marginal for *P. cinnamomi*, surveys from 1985 to 1995 revealed the presence of extensive old infestations along the coastal sections of the Park. All of these infestations were located either around the base of the large granite rock features or in the creek-lines draining away from them. The early settlers established a settlement on the slopes of Mt Arid at a natural fresh water spring. The diary of one of the children who grew up at "Hill Springs" recorded that potted plant stock was brought in as a source of vegetables and fruit trees for their foods, thus introducing the pathogen. Although rainfall is less than optimal for *P. cinnamomi*, runoff from granite surfaces results in localised areas with higher soil moisture. This is obviously a key factor in the restricted occurrence of *P. cinnamomi* within Cape Arid National Park. Rainfall diminishes rapidly away from the coastal strip and while there have been suitable vectors for the spread of the pathogen inland, the conditions for survival and subsequent germination of *P. cinnamomi* have been unsuitable.

Two Peoples Bay Nature Reserve, a conservation reserve on the coast 30 kilometers east of Albany, is notable for the presence of the threatened Noisy Scrub Bird (*Atrichornis clamosus*) and Gilbert's potoroo (*Potorous gilbertii*) both rediscovered in relatively recent years after being considered to be extinct. The Two-Peoples Bay – Mt Manypeaks area is considered to be the most significant area for endangered birds in mainland Australia (Garnett 1992). The absence of fire and time since initial infestation has combined to create a situation where it is extremely difficult to interpret for the presence and absence of *P. cinnamomi*. On long-infested sites it is only through the presence of remnant trunks, root collar and fruit of species susceptible to *P. cinnamomi* that the presence of the pathogen can be determined once the original plant community has been dramatically altered. Surveys for *P. cinnamomi* the Mt Gardner area of the reserve determined that the area was extensively infested (Brittain 1989). *Hakea* and *Dryandra* dominated scrub and thickets on Mt Gardner have been replaced by a more open, sedge-dominated community.

Of four threatened vascular flora taxa recorded from the Reserve, two are threatened by *P. cinnamomi* and one, *Banksia verticillata* has become locally extinct due to infestation.

With 1748 taxa, of which 75 are endemic, the Fitzgerald River National Park is one of the richest flora conservation reserves in Western Australia (Chapman and Newby 1995). The park is also an International Biosphere Reserve recognised by IUNESCO. The Park contains some 20% of known plant species in Western Australia and 42% of known species in the South-West Botanical Province. Surveys for the presence of *P. cinnamomi* in the Fitzgerald River National Park have revealed only two infestations and current knowledge indicates that this is the least infested Park in the southwest of Australia. All of the factors that have led to high levels of infestation in other conservation reserves are present, namely the presence of suitable soils, sufficient rainfall (400 to 600 mm per annum), susceptible plant communities, previously uncontrolled access and poor quality vehicle track surfaces, and historical settlement and grazing practices. The Park's relatively warm climate is conducive to spore production particularly in association with episodic summer storms that can bring 100 mm to coastal and inland areas.

One major infestation is located within the central "Wilderness Zone" area of the National Park, on the Bell Track which was illegally constructed in 1971. The infestation is about 175 ha in area, and has the potential to spread to river catchments to the east and west (Smith and Grant unpublished). *P. cinnamomi* has resulted in gross structural changes in infested areas of *Banksia* shrubland. With more species of vertebrate fauna than any other conservation reserve in the southwest, the implications of future spread of this infestation and habitat change on associated fauna is considerable. The largest mainland population of the threatened fauna species *Parantechinus apicalis* (dibbler), occurs in the park. This small insectivorous dasyurid ocupies habitat characterised by mixed Proteaceous heath (Start 1998). Surveys in recent years within its former distribution in Arpenteur Nature Reserve east of Albany, a reserve extensively infested by *P. cinnamomi*, has failed to relocate the species (Baczocha and Start 1997). Of the 13 threatened flora species that occur in the Fitzgerald River National Park, none are currently impacted by *P. cinnamomi*. Only a proportion of these species are considered to be susceptible to the pathogen and at risk.

However, it can easily be envisaged that as in the Stirling Range National Park, once relatively abundant local endemics could rapidly become threatened with the spread of the pathogen.

Management of visitor vehicular access is an ongoing concern to avoid future introduction and or spread of the pathogen within the boundaries of the National Park. After significant rainfall events access to the reserve's gravel roads is restricted. At the Bell Track, phosphite has been applied to the infested area (Barrett 1999) and water diversion control measures undertaken to reduce additional water runoff from the track. Other management being considered include surface and sub-surface water control, the creation of a root free zone around the infestation and native animal exclusion from the infestation (Smith and Grant unpublished).

Conclusions

In summary the effect *P. cinnamomi* is having on the flora conservation values of the south coast region, in particular in areas such as the Stirling Range and Cape le Grand National Parks may be considered to be an ecological disaster of world significance. While the impact of major structural changes in the native vegetation upon invertebrate and vertebrate fauna suites in the south coast region has not been comprehensively studied, it is clear that there must be significant ecological consequences. Management of infested areas containing threatened plants and communities relies heavily on the use of phosphite to control the pathogen. Appropriate management of key non-infested areas such as the Fitzgerald River National Park is critical to prevent a similar catastrophe in this node of biological diversity.

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Phytophthora cinnamomi in New Zealand's indigenous forests

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Abstract: Phytophthora cinnamomi is not native to New Zealand, but perhaps it was introduced by Polynesians, as *Phytophthora*-like symptoms were observed in the early 1800's. The fungus is widespread in native forest soils. It can cause noticeable, localised damage during periods of weather suited to inoculum build-up (warm, wet winters), followed by periods of stress on the plants (unusually dry summers). However, the moderate New Zealand climate means that in native forests damage is rarely visible in above-ground parts of plants. Some roots are killed, but in New Zealand plants generally require only a proportion of their roots for normal growth. Despite major disease episodes being rare, *P. cinnamomi* could have subtle, longer term, effects on New Zealand's vegetation. Variation in plant susceptibility alone will cause changes to plant community structure over time. Seedlings of some major forest trees are highly susceptible. Although kauri (*Agathis australis*) forests appear to be re-establishing vigorously in many parts of northern New Zealand, at some sites seedlings survive only when protected against *Phytophthtora* by fungicides. In southern New Zealand, *Nothofagus* seedlings are susceptible to *P. cinnamomi* unless their roots are strongly mycorrhizal. This may prevent *Nothofagus* establishment in soils not already infected with ectomycorrhizal fungi.

Introduction

This paper reviews the impact that *Phytophthora cinnamomi* may have had, and may still be having, on the distribution and establishment of tree species in New Zealand's indigenous forests. *P. cinnamomi* was almost certainly introduced to New Zealand by humans. Given the human history of New Zealand, this introduction may have been at any time over the past 1000 years, the approximate date of the first permanent Polynesian settlement (Ogden *et al.* 1998). *P. cinnamomi* occurs on *Colocasia esculenta* in the Pacific (Dingley *et al.* 1981), one of the crops brought to New Zealand and cultivated by early Polynesian settlers (Mathews 1985). Although the first definite records of *P. cinnamomi* in New Zealand were made in the 1950s (Newhook 1960), *Phytophthora*-like symptoms on the highly susceptible *Meryta sinclairii*, were noted from the Hen and Chicken Islands in 1898 (Atkinson 1956; Beever 1984).

P. cinnamomi is now widely distributed in New Zealand's indigenous forests, being isolated from soils in apparently undisturbed forests in isolated situations (Newhook 1960; Hepting and Newhook 1962; Podger and Newhook 1971). However, the topic of *P. cinnamomi* in New Zealand native forests has been poorly investigated, being the subject of only 11 publications (Newhook 1960, 1970, 1978, 1989; Rawlings 1962; Robertson 1970; Podger and Newhook 1971; Hepting and Newhook 1962; Newhook and Podger 1972; Beever 1984; Horner 1984), many of these being review articles.

This lack of research interest reflects the fact that *P. cinnamomi* rarely causes conspicuous disease symptoms in New Zealand's native forests (Newhook and Podger 1972), and the perception is therefore that the fungus is a minor consideration in the health of these forests. Newhook (1989) noted that *Phytophthora* species invade chiefly unsuberised tips of feeder rootlets, and that rootlet death may be followed by rootlet replacement. It is the balance between these opposing factors that determines symptoms expressed in an infected plant, which may die suddenly, may die after a period of decline, or may show no external symptoms. The balance involves interactions between environmental factors such as soil moisture, temperature, nutrition, and the effect these have on growth of both the fungus and its host. Due to the moderate climate in New Zealand, trees can manage on a small fraction of their root system in most seasons (Newhook 1989). Disease associated with *Phytophthora* is noticeable only in seasons of unusual weather, that results in high stress on infected plants (Podger and Newhook 1971).

Two of New Zealand's major forest types will be discussed in relation to *P. cinnamomi* — kauri (dominated by *Agathis australis*) and beech (dominated by *Nothofagus* spp.). Information on their susceptibility to *P. cinnamomi*, and the effect that this may be having on the distribution and establishment of both kauri and beech, will be reviewed.

Kauri (*Agathis australis*)
Background

Kauri remains widely distributed over much of its natural range in the north of New Zealand, but

extensive mature forest is now restricted to the Waipoua Forest area. Most existing stands are less than 150 years old, the result of natural regeneration following the intensive logging and burning of these forests over the past 200 years. Kauri regeneration covers about 60-100 000 ha (Ecroyd 1982). Much of this regeneration is on reserve land, and the expectation is that these stands will lead ultimately to mature kauri forest. Kauri forests are amongst the most diverse for tree species in New Zealand (Ogden and Stewart 1995). Mature kauri trees have thin but widely spreading crowns, with individual trees widely spaced. This space enables a distinct sub-canopy of hardwood tree species to develop, such forests commonly having over 20 tree species per hectare (Ogden and Stewart 1995).

Natural regeneration of kauri typically occurs following disturbance, whether it be a gap formed after a tree falls, or a more catastrophic event such as fire (Ogden and Stewart 1995). Kauri seeds require high levels of light to germinate, and seedlings grow poorly under a heavy canopy. Following large scale disturbance, kauri preferentially establishes on ridges and northern slopes (Ogden and Stewart 1995). It grows better in the dry, warm conditions of such sites, and in addition, attributes of such sites make disturbance more likely.

Phytophthora cinnamomi is widely distributed in kauri forest, having been isolated from soil beneath both mature, apparently undisturbed stands of kauri in remote localities, as well as from beneath young, regenerating stands (Newhook 1960; Hepting and Newhook 1962; Podger and Newhook 1971). Field observations suggest that *P. cinnamomi* may be influencing forest establishment and succession through pre- and post-emergence damping off, as well as causing disease in stands of older trees (Podger and Newhook 1971).

Experiments investigating the effect of *P. cinnamomi* on kauri seedling establishment
Horner (1984) investigated experimentally the impact of *P. cinnamomi* on kauri seedlings. We summarise the methods and some of the results of that study. See Horner (1984) for full details.

Methods
Kauri seedlings 1 and 2 years old were planted in 2 vegetation types (scrub and forest), about 500 m apart at Cornwallis, to the west of Auckland City. Five areas were chosen, 3 in scrub and 2 in forest, and within each area 3

sites were selected according to position on the slope (top, middle, and bottom), to encompass a range of soil moisture conditions. The sites were subdivided into 4 even-sized plots. Four treatments were applied at each site, with and without fertilizer, and with and without Ridomil (metalaxyl) fungicide. Seven nursery-raised seedlings were planted in each plot.

The experiment was run for 13 months. Seedling growth (height increase, branch number increase, shoot dry weight, fibrous root dry weight, and total root dry weight) and mortality were recorded. All plants dying during the experiment were checked for infection by *P. cinnamomi*, and after 13 months all surviving plants from one of the scrub areas and from one of the forest areas were harvested and checked for infection by *P. cinnamomi*.

Results
Scrub vegetation areas were dominated by 3-6 m tall *Leptospermum scoparium*, and the forest vegetation by tall *Kunzea*-podocarp forest. Both vegetation types are likely to have had a similar history, being logged about 150 years ago, and cleared by burning about 80-100 years ago (Esler and Astridge 1974). Both have a similar soil type (Horner 1984), the differences in vegetation probably reflecting differences in exposure – higher exposure sites in this area tending to be dominated by *L. scoparium* rather than *Kunzea ericoides* (Esler and Astridge 1974) – and also later burning in the area with scrub (Horner 1984). The scrub vegetation was less dense, allowing more sunlight to reach ground level, resulting in higher soil temperatures at these sites. The forest sites were not as prone to waterlogging as the scrub sites, probably because of the greater protection provided by the dense canopy. The soils at all scrub sites became waterlogged after prolonged rain, but those at the top of the slope were prone to summer drought during extended dry periods, whereas the soil at the bottom sites never dried out over summer. *P. cinnamomi* was isolated from the soil at all 15 sites, following baiting with lupin radicles and pine needle bases.

The interaction between *P. cinnamomi* and the planted seedlings was distinct in the two vegetation types. In scrub vegetation there were significant differences in most growth measurements (height increase, dry weight of shoots, fibrous roots, and total roots) between the Ridomil-treated seedlings and those not treated, while there was no significant difference between the fertilizer treatments (Horner 1984).

Mortality was consistently lower in the Ridomil treatments (Fig.1). There were also

42

significant differences in mortality between sites; more seedlings at the top of slope dying than at the bottom (Fig. 1). *P. cinnamomi* was isolated rarely from the Ridomil treated seedlings, and although common in the control seedlings, the frequency at which it was isolated did not correlate with seedling mortality, the fungus being common right across the slope (Fig. 1).
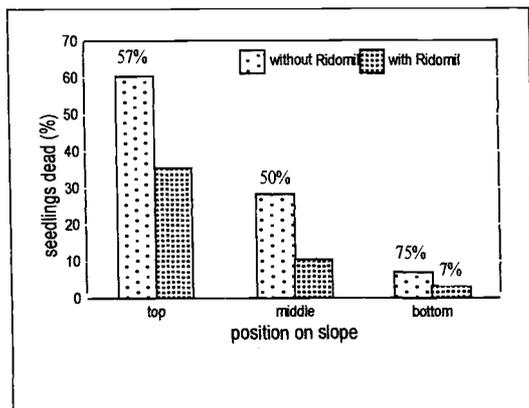
Fig. 1. Scrub vegetation areas, seedlings dying (%) over the 13 month period of the experiment, at top, middle, and bottom of slope, with or without treatment with Ridomil fungicide. The figures at the top of the bars are the % of all plants (both dead and alive after 13 months) from one of the replicates from which *P. cinnamomi* was isolated. (Position on Slope: df = 2, F = 17.326 P<0.001; Fungicide: df = 1, F = 6.536 P<0.05; Fungicide x Slope: df = 2, F = 1.083 NS).
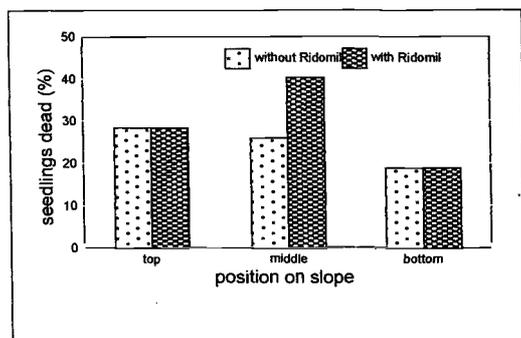
Fig. 2. Forest vegetation areas, seedlings dying (%) over the 13-month period of the experiment, at the top, middle, and bottom of the slope, with or without treatment with Ridomil fungicide. No significant differences between position on slope, or between fungicide treatments.

In the forest vegetation there was no significant difference in growth or mortality with Ridomil treatment, or with respect to position on the slope (Fig. 2). The only significant difference was with the fertilizer treatment, the fertilizer-treated plants being shorter. A confounding factor in the forest vegetation was damage to the seedlings by possum browsing. Much of the mortality at this site could be attributed to possum damage, and it is reasonable to expect that possums preferentially targeted the 'sweeter' fertilized plants.

Although *P. cinnamomi* was present in the soil beneath the forest vegetation, it was isolated from only 16% of the seedlings tested, all of these from plants untreated with Ridomil at the bottom of the slope.

Discussion

The higher mortality at the top of the slope in the scrub vegetation reflected the more harsh summer drought conditions at these sites. Although soils at both the top and bottom of the slope were poorly draining and easily waterlogged, those at the top became very dry during summer, whereas those at the bottom of the slope never completely dried out over summer. Although *P. cinnamomi* was more or less uniformly distributed across the site, and caused an overall depression in growth rate of the seedlings, the effect of the fungus on seedling mortality was strongly influenced by environmental stress. The plants were able to tolerate and survive infection except under conditions of high environmental stress, due to summer drought.

The low rates of infection of seedlings in forest vegetation suggest that under forest conditions, *P. cinnamomi* is unable to effectively infect or cause disease of kauri. One major difference in physical conditions between the sites was soil temperature, several degrees higher under the scrub vegetation (Horner 1984). Soil temperature is an important factor influencing *P. cinnamomi* activity (Newhook 1960). In addition, the soils in the forest sites rarely became saturated.

Consequences of the introduction of P. cinnamomi *to kauri forests*
1. Disruption to natural patterns of establishment and regeneration.
Ogden *et al.* (1987) considered kauri to have a 'cohort regeneration model', in which dense regeneration occurs in successional communities following large-scale disturbance, leading to self-thinning stands in which seedling recruitment is rare, this being more or less restricted to canopy gaps formed following local disturbance or the senescence of individual trees. The relatively recent introduction of *P. cinnamomi* to these forests may be disrupting natural patterns of regeneration at several levels. The moderating effect that New

Zealand's climate has on disease development by this fungus, where above-ground symptoms are expressed only at extreme sites or during extreme seasons of weather, means that such disruption may be subtle over short time scales.

The Horner (1984) experiments showed that the sites on which kauri seedlings preferentially establish, sites with high light levels, high temperatures, and potentially prone to drought, are those that also favour both infection by *P. cinnamomi* and the development of disease. Hence, large-scale disturbance in the presence of *P. cinnamomi* is likely to lead to a reduction in the sites on which kauri is able to re-establish, compared with that which may have been expected before the introduction of the fungus. Ogden *et al.* (1987) noted that only 5-15% of gaps within mature kauri forest are recolonised by kauri. Although these authors regarded this as a natural phenomenon, the question of whether this same pattern occurred before the introduction of *P. cinnamomi* is worth asking. If this low level of gap recolonisation is related to the introduction of *P. cinnamomi*, then the long-term viability of kauri forest without the revitalising episodes of large scale disturbance has been much reduced. The extreme age of the individual kauri trees within these forests means that even if *P. cinnamomi* was introduced 1000 years ago, its impact will only now be showing.

Newhook and Podger (1972) noted that the susceptibility of kauri may change as the trees mature, field observations suggesting that susceptibility increases as trees change from their juvenile to adult growth form, at about 100-150 years of age. Much of the regeneration initiated following the felling and clearing of kauri forests by European settlers in the 19th century, is now approaching the age where trees are taking on their adult growth form. There are several sites where trees of about this age are clearly diseased, with "little leaf"-like symptoms typical of *P. cinnamomi* infection (Podger and Newhook 1971). If this is an indication of a general increase in susceptibility with age, then there must be a risk that even where early stage regeneration appears healthy and widespread, the forests may collapse before maturity, truncating natural patterns of regeneration.

2. Decreased diversity within kauri communities.

New Zealand's native trees are known to vary in susceptibility to *P. cinnamomi* (Robertson 1970). The mixture of tree species in the high-diversity, mature kauri forests includes species of both low and high susceptibility (Podger and Newhook 1971). This alone means that the introduction of this fungus to a new site must impact on the overall diversity and structure, although New Zealand's cool, moist climate means that that impact may be subtle over the short term.

Nothofagus species
Background
Nothofagus forests are widespread in southern New Zealand, and have been gradually expanding their range from scattered refugia since the last glaciation (McGlone 1980, Wardle 1980). *Nothofagus* is obligately ectomycorrhizal, and Baylis (1980) postulated that the spread of *Nothofagus* is dependent on formation of the mycorrhizal association. Seedling establishment is generally successful only within a few metres of the margin of the forest, within the root zone of already established trees (Baylis 1980). This allows the newly germinated seedlings to become rapidly mycorrhizal through infection by fungal hyphae already within the soil.

Baylis (1980) noted an exception, where non-mycorrhizal seedlings of *Nothofagus* are able to establish on freshly exposed mineral soils. Similarly, Wardle (1980) noted the survival of non-mycorrhizal seedlings on gravel stream fans. Wardle considered that it is from such nuclei on recently formed soils that *Nothofagus* forest expands into the surrounding vegetation. Baylis (1980) hypothesised that the success of *Nothofagus* seedling establishment at such sites related to levels of available P, thought to be higher on these new soils, and estimated that available P levels of 15-30 ppm would be required for the growth of non-mycorrhizal *Nothofagus* seedlings.

In contrast to the Baylis' (1980) results, experiments we have carried out, planting *Nothofagus menziesii* and *N. solandri* seeds in soils from Tekapo grassland (grid reference NZMS 260 2293800E 5688900N) with available P of 3 ppm, have shown that these seeds germinate readily in soils with very low P levels (unpubl. data). Of 176 seedlings established in this soil, 90% survived for 14 months, and at 14 months 30% of the surviving plants were still non-mycorrhizal. The others had picked up mycorrhizal infections, presumably from air-borne spores from the surrounding environment, as the soils contain no ectomycorrhizal fungi. Less than 15% of the seedlings were mycorrhizal 6 months after planting. Although seedlings which developed mycorrhizae during the course of the experiment grew more rapidly than those that did not, survival rates were similar for both mycorrhizal and non-mycorrhizal plants. Our results suggests that P levels alone cannot explain

patterns of natural seedling establishment of *Nothofagus* in New Zealand.

Observations from restoration sites

Davis *et al.* (1997) tested vegetation restoration methods at an open-cast mining site at Giles Creek. The mine was established in mature *Nothofagus* forest on alluvial river terraces, the forest being progressively cleared along the advancing mine front. Amongst methods used to re-establish vegetative cover, was the planting of nursery-raised seedlings of tree and shrub species found in adjacent forest. Seedlings were planted in either overburden gravel, or in soils moved from recently cleared forest at the head of the mine overlayed onto overburden gravel. Most of the species that were planted survived well on both substrates for the 4.5 years of the experiment. Although *Nothofagus fusca* and *N. solandri* both survived reasonably well on the mine overburden, all those planted in the forest soil treatment had died after 4.5 years (Fig. 3). The mine overburden gravels had levels of available P of 11 ppm (Davis *et al.* 1997).

One of the major biological differences between the two planting substrates at Giles Creek was with respect to the presence of *Phytophthora cinnamomi*. The fungus was common in adjacent forest soils (unpubl. data), and thus will have been introduced to the forest soil treatments. In contrast, the mine overburden, comprising mineral soils from several metres beneath the ground, is likely to have been essentially sterile at the time the seedlings were planted. Root systems from 12 living seedlings planted on mine overburden were tested for the presence of *P. cinnamomi* 6 years after planting. Of the seedlings selected, 7 appeared to be unhealthy, 5 appeared to be healthy. *P. cinnamomi* was isolated from 5 of the unhealthy seedlings, but from none of those that appeared healthy (unpubl. data).

Rawlings (1962) reported the susceptibility of *Nothofagus* seedlings to *P. cinnamomi*, and one hypothesis to explain the Giles Creek observations is that *Nothofagus* seedling mortality was related to the presence of this fungus in treatments containing forest soil. One problem with this explanation is the large number of healthy *Nothofagus* seedlings growing in the surrounding forests as a result of natural regeneration. Why do these seedlings survive in soils with high levels of *P. cinnamomi*? We suspect an explanation for this is the interaction between *P. cinnamomi* and *Nothofagus* ectomycorrhizal fungi, the ectomycorrhizal roots being less susceptible to

infection by *P. cinnamomi*. Marx (1973) demonstrated
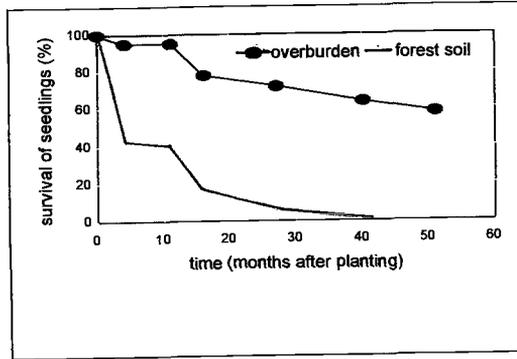
Fig. 3. *Nothofagus fusca* survival on mine overburden and forest soil substrates, Giles Creek coalmine. *Nothofagus solandri* shows a similar pattern, all plants in forest soil being dead after 50 months. Data adapted from Davis *et al.* (1997).

infection by *P. cinnamomi*. Marx (1973) demonstrated experimentally that ectomycorrhizal fungi protect roots of *Pinus* species against disease caused by *P. cinnamomi*. All natural seedlings examined had more heavily mycorrhizal root systems than the 5-year-old planted seedlings (Fig. 4). Examination of seedlings obtained from the
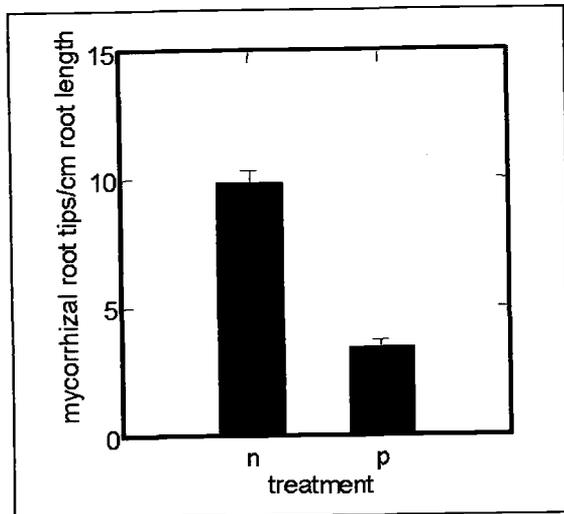
Fig. 4. Level of mycorrhizal infection of roots from naturally regenerating seedlings (n), and planted, nursery-raised seedlings (p), Giles Creek, October 1998. Number of mycorrhizal root tips per cm root length assessed using gridline intersect method (Brundrett *et al.* 1996). Bars are standard errors.

nursery that supplied the Giles Creek trial, and grown under the same conditions, suggested that the nursery-raised seedlings had no mycorrhizae at the time of planting.

Seedlings from a second restoration site, in graded alluvial gravels at the recently formed approaches to a bridge across Manson Creek in the Canterbury foothills (grid reference NZMS 260 2409600E 5785400N), were grown by the same nursery that grew the seedlings used at Giles Creek. At the time of planting, October 1998, these seedlings were about 200-700 mm high and appeared very healthy. The root systems of 5 plants examined were either non-mycorrhizal, or had <5% mycorrhizal roots. None were infected with *P. cinnamomi*. The plants had been raised in potting mix with high levels of fertilizer and treated with Trichopel to suppress damping-off fungi. After 16 months, about 15% of the seedlings planted at the Manson Bridge restoration site had died, but most of those still alive looked healthy. The root systems of 6 plants were examined, and although mycorrhizae were present in all of them, these were sparse and poorly developed. *P. cinnamomi* was not isolated from any of the 22 dead and 7 living plants examined (unpubl. data).

Discussion

Observations from the Giles Creek and Manson Creek restoration sites support the observations of Baylis (1980) and Wardle (1980) that *Nothofagus* can establish and grow without mycorrhizae in sites with new soils. Baylis and Wardle suggested that a heightened level of available P was likely to be the most important factor allowing the normally ectomycorrhizal species to establish at such sites. Experiments by Davis and Langer (1997) confirmed that low levels of P limit growth of non-mycorrhizal *Nothofagus* seedlings. However, the experiments we reported here on seedling establishment in Tekapo soils, and the successful establishment of seedlings on the Giles Creek mine overburden, show that although growth may be slow, seeds can germinate, and seedlings survive in low P soils. Observations from the Tekapo soil experiments and from both restoration sites suggest that such seedlings slowly accumulate mycorrhizae, presumably as a result of infection from the surrounding environment

The presence of *P. cinnamomi* may be the major factor limiting successful establishment of *Nothofagus* seedlings in soils lacking ectomycorrhizal inoculum. Observations from natural regeneration at Giles Creek showed that seedlings with strongly mycorrhizal root systems are not susceptible to *P. cinnamomi*, whereas the non-mycorrhizal, nursery-raised seedlings die rapidly after coming into contact with *P. cinnamomi*.

Consequences of the introduction of P. cinnamomi to Nothofagus forests

Explanations for current patterns of establishment and spread of *Nothofagus* in New Zealand are generally based on extrapolations from the past. The relatively recent introduction of *P. cinnamomi* to these systems is likely to have changed the rules governing such establishment and spread, so invalidating such extrapolations. The spread of *Nothofagus* forests may have been more rapid in New Zealand before the introduction of *P. cinnamomi.*

Nothofagus is still expanding its range in southern New Zealand, spreading out from glacial refugia from the last ice-age. Wardle (1980) suggested that to account for the post-glacial spread of *Nothofagus*, the average rate of expansion must have been at least 700 m per century. However, from observations at the margins of stands up to 400 years old, Wardle estimated actual rates of spread into adjacent closed forest of only about 6 m per century. Wardle observed that new stands develop most commonly on freshly exposed mineral soils, such as those associated with slips and alluvial gravel fans – and assumed that it was on these kinds of sites that *Nothofagus* has always expanded its range. He supposed that such sites would have been more common in the past, when landscape instability following glacial retreat would have provided extensive surfaces well suited to colonisation by *Nothofagus*.

Has the spread of *Nothofagus* into adjacent closed forests always been so slow? The sites with young, freshly disturbed soils and little or no organic content, provide protection from exposure to *P. cinnamomi.*In contrast, today's forest soils, likely to contain *P. cinnamomi*, will not provide that protection during the first few months of non-mycorrhizal growth. Thus, in a forest situation, the establishment of seedlings will be limited to those germinating within the root zone of already established plants, this allowing them to pick up mycorrhizae immediately after germination through direct contact with hyphae of mycorrhizal fungi already in the soil, and so providing protection from *P. cinnamomi.*

Allen (1988) noted that *Nothofagus menziesii* was expanding its range at many sites in the Catlins area, except those which were warm and fertile. *N. menziesii* is less able to compete on these sites than other forest trees in the region. Given that the impact of *P. cinnamomi* in New Zealand is temperature limited (Newhook 1960; Horner 1984), the possibility exists that this lowered competitive ability in part reflects an interaction with *P. cinnamomi.*

Conclusions

1. *Phytophthora cinnamomi* was almost certainly introduced to New Zealand by humans, some time within the last 1000 years.

2. The introduction of *P. cinnamomi* may have impacted on patterns of native forest establishment, regeneration, and spread.

3. The above-ground expression of symptoms in infected plants such as *Agathis australis*, is limited by New Zealand's climate.

4. Changing climate patterns associated with global climate change may alter the balance between *P. cinnamomi* and its hosts; the already widespread distribution of the fungus means its effect on native forests could be rapid and serious.

5. The introduction of *P. cinnamomi* to *Nothofagus* forests may have restricted natural patterns of regeneration, because of susceptibility of non-mycorrhizal seedlings to the fungus.

6. Predictions about the impact of climate change on the future distribution of New Zealand's native forests, based on extrapolations from their behaviour in the past, may have been invalidated by the addition of *P. cinnamomi* to these systems.

7. Most evidence about the introduction and impact of *P. cinnamomi* in New Zealand native forests is based on observation rather than experiment.

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The impact of *Phytophthora cinnamomi* on the flora and vegetation of New South Wales – a re-appraisal

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Abstract: Although *Phytophthora cinnamomi* is widely regarded as having a significant impact on native vegetation in many parts of southern Australia, the pathogen has been considered benign and possibly endemic in New South Wales. The evidence for the different behaviour in NSW has included that 1) *P. cinnamomi* is extremely widespread and easy to detect in soils, 2) generally susceptible genera such as *Banksia* are unaffected when it is present (suggesting a long host/pathogen interaction), 3) multiple plant deaths associated with *P. cinnamomi* are extremely rare and 4) *P. cinnamomi* has been recovered in remote areas (suggesting that it is endemic). Recent surveys of National Parks in eastern NSW have found that although *P. cinnamomi* is widespread it cannot be detected in some areas despite extensive soil sampling. Although most *Banksia* spp. seem to be relatively resistant to symptoms of infection, other taxa (especially some *Xanthorrhoea* spp.) are very susceptible. The loss of *Xanthorrhoea* cover may adversely affect threatened animals such as the Smoky Mouse and Southern Brown Bandicoot, which use the plants for cover and nesting. The NSW floral emblem, *Telopea speciosissima*, is also susceptible. *P. cinnamomi* may be widespread in NSW, however it is doubtful that it is endemic and it is certainly not always benign.

Introduction

Phytophthora cinnamomi is a serious pathogen of native vegetation in Victoria, Western Australia, Tasmania and South Australia causing changes in floristic composition and threatening rare species (Weste 1994). It has not been regarded as a significant threat to flora and vegetation in New South Wales (NSW) (Environment Australia 1999).

The Origin of P. cinnamomi *in NSW*

The belief that *P. cinnamomi* is not a significant threat to native vegetation in NSW is partly based on the concept that it is native in eastern Australia. The evidence for a long association between the pathogen and the NSW flora is that *P. cinnamomi* is widespread (Pratt and Heather 1973), is found in remote, apparently undisturbed areas (Pratt *et al.* 1973), and the flora shows considerable resistance to the disease. However logging, cattle grazing and gold prospecting may have introduced the pathogen to remote locations, and the apparent dominance of resistant species in drainage lines may be an effect of suppressive soils as is sometimes found in similar sites in Western Australia (McDougall 1997).

Recent genetic and molecular evidence does not support the view that *P. cinnamomi* is a native pathogen in NSW. Analysis of the mating populations of *P. cinnamomi* (A1 and A2) have shown that there are many more A2 than A1 populations (Old *et al.* 1984, 1988;

Dobrowolski *et al.* 2002). If the pathogen was native it would be expected that the two mating populations would be present in near equal numbers. Isozyme analysis indicates a low level of genetic diversity (Old *et al.* 1984, 1988; Linde *et al.* 1999) as does microsatellites (Dobrowolski *et al.* 1998, 2002), RAPD (Tommerup *et al.* 2000), and AFLP markers (Summerell unpub data). Additionally, both the A1 and the A2 isozyme and microsatellite types found in NSW are the only types so far isolated in Australia. There is no evidence of geneflow due to sexual reproduction, they have a global distribution and are quite distinct from the other A1s from Papua and New Guinea (Old *et al.* 1984, 1988; Dobrowloski *et al.* 2002). Low levels of genetic diversity are consistent with an introduced organism and are rarely seen in active, widespread indigenous organisms. Hence there is now substantial evidence that *P. cinnamomi* is introduced into all parts of Australia. The relevant question about *P. cinnamomi* in NSW is not whether it is native or introduced but whether it is having an adverse impact on natural systems that are regarded as having biodiversity, cultural or economic value. It is this question that we seek to address in the current paper.

Research on P. cinnamomi *in native vegetation in NSW*

Phytophthora cinnamomi was first associated with plant deaths in native vegetation in NSW in the late 1940s (Fraser 1956). Following the work that confirmed an association between the presence of *P.*

cinnamomi and decline of the commercially valuable timber species *Eucalyptus marginata* in Western Australia (Podger 1968) and dieback of hardwood forests in Victoria (Marks *et al.* 1972), much survey was done by the Forestry Commission of NSW to determine if the pathogen was a similar threat to forests in NSW. It was concluded that the pathogen was widespread in coastal forests and rarely caused disease symptoms (Gerrettson-Cornell 1986).

The spatial distribution of *P. cinnamomi* has been investigated in coastal forest in NSW by Arentz (1974) and Blowes (1980). *P. cinnamomi* was found to be widespread in *Corymbia maculata* forest, especially in gullies. Blowes (1980) found that the presence of *P. cinnamomi* in soil was closely associated with the occurrence of chlorotic plants of *Macrozamia communis*, although the pathogen could not be isolated from them. McCredie *et al.* (1985) assessed the comparative susceptibility in field trials of many *Banksia* species, including most species found in NSW which were ranked as of low susceptibility to *P. cinnamomi*. This contrasts with the majority of Western Australian *Banksia* species, which are of medium to high susceptibility.

Halsall (1982a) found that soil from an area on the Great Dividing Range, south-east of Canberra (Tallaganda State Forest) was suppressive to *P. cinnamomi*. The pathogen could apparently be present in this area without causing disease. Zoospore production and chlamydospore survival were found to be reduced as a result of soil micro-organisms and their interaction with inhibitory compounds present in the soil (Halsall 1982b).

P. cinnamomi has been isolated from soil at several sites in NSW (e.g. *Eucalyptus saligna* forest at Ourimbah, north of Sydney) where plants are dying but a clear association between its presence and disease has not been established (Gerrettson-Cornell 1986; Environment Australia 1999).

Reason for the current work
Following the discovery of *P. cinnamomi* in soil on the summit of Mt Imlay and the death of many plants there, the NSW National Parks and Wildlife Service (NPWS) decided to determine if *P. cinnamomi* is having an impact on flora and vegetation more widely in NSW. The study was limited in its scope and the resources allocated to it because, at the time the study commenced, the pathogen was not regarded as a significant threat.

Methods
Assessing impact
If *P. cinnamomi* were widespread and benign it would be commonly found in soil on sites without disease expression and on sites with plant deaths attributable to other factors such as insects, drought and other diseases. In the current study, *P. cinnamomi* was regarded as having an impact when it could be isolated from dying plants. The degree of impact was determined by assessing 1) evidence for change in vegetation on sites where *P. cinnamomi* could be isolated from dying plants, 2) extent of impact (i.e. whether it was rare or common in the landscape), 3) potential for concomitant damage (i.e. in plants and animals that are not immediately affected), and 4) potential for further damage in similar vegetation.

Locating possible sites with impact
Xanthorrhoea australis, an arborescent monocot, is regarded in Victoria as being highly susceptible to infection (eg. Weste 1981, Kennedy and Weste 1986; Aberton *et al.* 2002). Easily accessible sites in NSW where *X. australis* had been recorded in botanical surveys were searched. Sites with suspicious plant deaths, which were reported by NPWS staff or opportunistically observed, were also sampled.

Root / soil sampling
Roots were washed in tap water to remove adhering soil and were surface disinfested with 1% NaOCl in 10% ethanol. Roots were rinsed in distilled water and blotted dry. Small segments (5 mm) were dissected from the edges of discoloured lesions along the roots and plated on *Phytophthora* selective agar (PSA) consisting of carrot and potato puree and containing pimaricin, hymexazol and rifampicin modified from the recipe of Jeffers and Martin (1986). Plates were incubated in the dark at 25° C for 2-3 days and the resulting cultures were examined under the microscope. *Phytophthora cinnamomi* was identified according to the morphological characters outlined in Erwin and Ribeiro (1996).

Soil samples were collected by taking between 0.5 – 1.0 kg of soil from around the roots of plants at a depth of between 50 – 200 mm below the soil surface with a hand trowel. The trowel was decontaminated between each sample with 70% ethanol to prevent cross contamination. Two subsamples (about 50 g) from each soil sample were placed in polystyrene containers and flooded with 200

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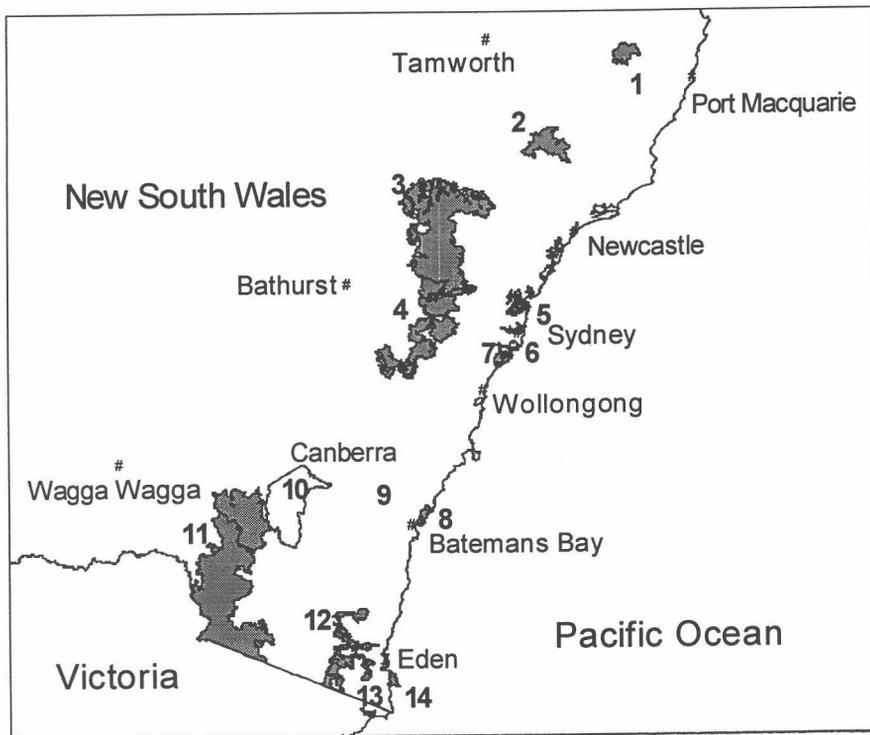
Fig. 1. Location of sites described in this paper.
Key: 1 Werrikimbe National Park; 2 Barrington Tops National Park; 3 Wollemi National Park; 4 Blue Mountains National Park; 5 Ku-ring-gai Chase National Park; 6 Sydney Harbour National Park; 7 Royal National Park; 8 Murramarang National Park; 9 Shoalhaven River; 10 Black Mountain (A.C.T.); 11 Kosciuszko National Park (Talbingo); 12 South East Forests National Park; 13 Mount Imlay National Park; 14 Ben Boyd National Park

mL of distilled water; 2 drops of Tween 20 were added to ensure even wetting of the soil. Four small PSA blocks (about 15 mm X 15 mm) were placed in the soil suspension, which was stirred to ensure contact of the soil suspension and the agar blocks. The baits were incubated at room temperature for two days except for a one hour incubation at 4°C to stimulate zoospore release. At the end of the incubation period the agar blocks were extracted, rinsed and blotted dry, plated onto PSA and incubated in the dark at 25°C for 2-3 days. *P. cinnamomi* was identified as outlined above.

All cultures of *P. cinnamomi* were purified by hyphal tip transfer onto potato carrot agar and were preserved by storage under sterile water in the culture collection of the Royal Botanic Gardens, Sydney.

Vegetation assessment
Permanent circular quadrats with an area of 50 m² were established in dieback areas in South East Forests National Park and Mount Imlay National Park. All species within quadrats were recorded and an estimate made of cover. Although it is too early to detect changes in the vegetation, the assessment

provides information on recruitment of susceptible species and differences between dieback-affected and healthy vegetation.

Results and Discussion
South Coast Infestations
Rainfall on the South Coast ranges from about 850 mm annually on the coast to 1200 mm on the coastal range and is distributed evenly through the year. Average maximum temperature is mostly greater than 20°C between October and April. Soils of the sites sampled range from sands on the coast to shallow, sandy loams on the ranges.

1) *Eucalyptus sieberi* dominated forest
Sites with *P. cinnamomi* in the soil and in dying plants were found in South East Forests National Park and Mt Imlay National Park (Sites 12 and 13, Fig. 1). Infestations were found in communities that are floristically similar to those in eastern Victoria, where disease was reported by Marks *et al.* (1975). Infestations were characterised by patches of dying and dead plants of *X. australis*,. Patches of dead *E. sieberi* and *E. obliqua* were rarely seen. Both species are regarded as highly susceptible (Podger and Batini 1971, Pratt and Heather 1973). *P. cinnamomi* was recovered

51

from the roots of dying plants of *Banksia spinulosa* var. *cunninghamii*, *Diplarrena moraea*, *Grevillea irrasa* subsp. *irrasa*, *Monotoca* sp. aff. *elliptica*, *Pultenaea benthamii*, *Tetratheca subaphylla* and *Xanthorrhoea australis*. Frequent deaths were also observed in the following species on dieback sites: *Acacia terminalis*, *Banksia serrata*, *Coopernookia barbata*, *Daviesia wyattiana*, *Epacris impressa*, *Oxylobium ilicifolium*, *Poranthera corymbosa*, *Pultenaea daphnoides*, *Stylidium graminifolium*, *Tetratheca pilosa*, *Xanthosia tridentata*.

A dieback boundary at a site in Mt Imlay National Park, which was marked in December 1999, moved between 0 and 4 m across and down the slope in the following year (based on new dead and dying plants of *X. australis*). Based on a comparison of 10, 50 m^2 quadrats placed on both sides of a distinct dieback boundary at Mt Imlay (marked by a line of dying *X. australis*), there was significantly less cover of *Acacia terminalis*, *Epacris microphylla*, *Tetratheca subaphylla* and *X. australis* on the dieback side of the boundary as determined by a *t*-test of arcsine transformed cover data. No seedlings of *X. australis* were found on the affected side of the dieback boundary at Mt Imlay. This contrasts with a dieback site at Mt Sugarloaf in South East Forests National Park, where seedlings were found to represent 67% of the total *X. australis* population, and the reported regeneration of *X. australis* on dieback sites in Victoria (Weste *et al*. 1999).

Changes in the structure and composition of ground flora have been found to affect populations of small mammals in other parts of Australia (Wilson *et al*. 1994; Laidlaw 1997). Two endangered mammals occur in the *E. sieberi* dominated forests in NSW: Smoky Mouse (*Pseudomys fumeus*) and Southern Brown Bandicoot (*Isoodon obesulus*). Several species of Epacridaceae and Fabaceae thought to be affected by *P. cinnamomi* are important components of the diet of the Smoky Mouse (Ford 1998). Southern Brown Bandicoots have been found to prefer *X. australis* for nesting in South Australia (Paull 1992) and so loss of *X. australis* in the *E. sieberi* dominated forests will potentially have an impact on this species.

The evidence indicates that the impact of *P. cinnamomi* is great in the *E. sieberi* dominated forests in south-eastern NSW. The potential future impact is also great. However, the area of uninfested vegetation (as defined by healthy populations of *X. australis*) is small. Most of the forest contains vegetation comprising

species commonly found in clearly infested areas. *P. cinnamomi* has been in the *E. sieberi* forests for many decades and most of the damage has probably already been done.

2) Coastal woodland and heathland *P. cinnamomi* was recorded at Green Cape in Ben Boyd National Park about 30 years ago (Pratt and Heather 1973) (Site 14, Fig. 1). We found several areas of dying and dead *Banksia serrata* in coastal woodland dominated by *Eucalyptus botryoides* and *E. baxteri* and an area of dying *Hakea* spp. (*H. dactyloides*, *H. decurrens* subsp. *platytaenia* and *H. ulicina*) in heathland. *P. cinnamomi* was commonly isolated from soil and from dying *Pultenaea daphnoides* plants. The apparent impact of *P. cinnamomi* is patchy in coastal vegetation. Stands of *B. serrata* can be found with (e.g. Bittangabee Bay) and without (e.g. North Ben Boyd) patchy deaths. *P. daphnoides* appears to persist regardless of *P. cinnamomi* infection as does *Dryandra sessilis* in the jarrah forest of Western Australia (McDougall 1997). Reductions in populations of *B. serrata* may be detrimental to nectar feeding animals such as Eastern Pygmy Possum, Squirrel Glider and Regent Honeyeater. A reduction in the abundance of *Xanthorrhoea australis* may affect the survival of Southern Brown Bandicoots in coastal heathland, although the vegetation of apparently affected heathland is dense and may provide habitat for Southern Brown Bandicoots regardless of the impact of *P. cinnamomi*.

3) *Corymbia maculata* forest Frequent deaths of *Macrozamia communis* in coastal forest dominated by *C. maculata* were noted in the 1970s (Colin Totterdell, ex-CSIRO pers. comm.) and sporadically since. *M. communis* is often the dominant species in the understorey and *P. cinnamomi* has proven very difficult to isolate from dying *M. communis* (Blowes 1980) and was not isolated in the current study. Blowes (1980), however, found that the distribution of *P. cinnamomi* was correlated with the presence of yellowing *M. communis*. Observations over the past three years at an infested site near Batemans Bay indicate considerable recruitment of *M. communis* in the vicinity of dying plants and that complete removal of the species is likely to be very slow.

Localised deaths of *C. maculata* have been observed in two areas. In one of these, in Murramarang National Park (Site 8, Fig. 1), there are numerous deaths of this species as well as *E. globoidea*, *E. botryoides*, *Pittosporum revolutum* and *Melaleuca*

hypericifolia. Almost all plants of *M. hypericifolia*, which was obviously once the dominant understorey species, have died at the site in the past three years. *P. cinnamomi* has not been found in the roots of any of the dying species although it was found in 8 out of 10 soil samples. Long-term monitoring is required in populations of dying *M. communis*. *C. maculata* is regarded as resistant to *P. cinnamomi* (Pratt and Heather 1973). *P. cinnamomi* may be contributing to the symptoms observed but is not necessarily the cause of them.

Southern Tableland Infestation

Average annual rainfall at Nimmitabel in the South Tablelands bioregion is about 700 mm, evenly distributed through the year, with an average maximum temperature mostly greater than 20°C between December and March. The site sampled is at an elevation of 950 m a.s.l and receives frequent frosts in winter. The soil is sandy loam.

A small area of dying plants was located on the edge of Nunnock Swamp (South East Forests National Park) to the east of Nimmitabel in regrowth forest dominated by *Eucalyptus dalrympleana* (Site 12, Fig. 1). The infestation was along a section of road that had been constructed to provide a crossing of the swamp. Deaths were observed in plants that had regenerated along the road and others at the edge of the swamp. No infestation was observed in the natural vegetation beyond the swamp. *P. cinnamomi* was isolated from dying plants of *Leptospermum juniperinum* and *Banksia marginata*. Numerous deaths occurred in *Grevillea lanigera*, *Stylidium graminifolium* and *Persoonia silvatica*.

Infestations of *P. cinnamomi* on the Southern Tablelands causing multiple plant deaths appear to be rare. Further survey is required, however, especially in vegetation containing *P. silvatica*.

Central Coast infestations

Rainfall in the Sydney coastal area, (Sites 5, 6 and 7, Fig. 1) is about 1100 mm annually and most abundant between January and June. The average maximum temperature is greater than 20°C between September and April. Soils of the sites sampled are sands, which are sometimes shallow over sandstone.

Royal National Park contains a range of plant communities and a very diverse flora. The vegetation is frequently burnt. One of the common species of heathland, *Xanthorrhoea resinifera*, has declined during the past 15 years (David Keith, NPWS, pers. comm.). In the current study, *P. cinnamomi* was isolated from dying *X. resinifera* and from *Sprengelia incarnata*, a species regarded as susceptible in Tasmania (Podger and Brown 1989). Other species recorded dying in this heathland community (but from which *P. cinnamomi* was not isolated) were *Grevillea oleoides*, which had abundant deaths, *Angophora hispida*, *Dilwynia floribunda*, *Hakea teretifolia*, and *Persoonia lanceolata*.

The consequences of the continued decline of *X. resinifera* are unknown but a considerable change in the vegetation is likely if this common species is lost. *P. cinnamomi* has previously been isolated from dying plants in Royal NP (Gerrettson-Cornell 1986). It does not appear to be present throughout the Park as it was absent in 100 soil samples from five areas containing *Telopea speciosssisima*, a species which is highly susceptible in cultivation (Walsh 2001; Summerell, unpubl. data). *P. cinnamomi* appears to be having a great impact in heath vegetation in this park. The impact was only detected through careful long-term monitoring of plant populations.

Forest dominated by *Angophora costata* and *Eucalyptus botryoides* occurs in remnants along the northern shore of Sydney Harbour. Crown dieback and tree death have been occurring in this vegetation for some years and have been variously attributed to polluted runoff and a nearby sewerage outfall. *Phytophthora cinnamomi* was recently isolated from areas of diseased *A. costata* at Middle Head, in soil and the roots of *A. costata* (Summerell unpublished data). An association between the dieback and *P. cinnamomi* is the subject of continued investigation, but it is notable that *P. cinnamomi* could not been found in an adjoining patch of healthy *A. costata*. There has been no regeneration of *A. costata* beneath the diseased canopy at Middle Head and there is considerable invasion of exotic shrubs and the native, fire-sensitive shrub *Pittosporum undulatum*. Since the seed reserve of *A. costata* is held in the canopy and seed is not thought to be long-lived (T. Auld, NPWS, pers. comm.), the loss of all mature trees in the diseased stand at Middle Head will probably be irreversible.

Other diseased sites on the Central Coast containing *P. cinnamomi* (eg. Ku-Ring-Gai Chase NP and Beecroft Peninsula) are currently under investigation. All display extensive but localised death of trees. Assuming that *P. cinnamomi* is the cause of disease at these sites and that at Middle Head,

its impact on the Central Coast is peculiar for NSW in that it effects mostly canopy species.

Northern Tablelands infestations

Rainfall is about 1500 mm annually, and most abundant between January and June. Average maximum temperature is greater than 20°C between November and March.

1) Snow Gum woodland Infestation by *P. cinnamomi* is dramatically impacting on subalpine woodland dominated by *Eucalyptus pauciflora* in Barrington Tops National Park (Site 2, Fig. 1). Deaths of the shrub *Oxylobium ellipticum*, which provides up to 100% cover in the understorey, were reported in the late 1990s. The species is regarded in Tasmania as being susceptible to *P. cinnamomi* (Podger and Brown 1989). *P. cinnamomi* has been isolated in soil samples (Mills 1999) and from dying plants of *O. ellipticum*, *Tasmannia purpurascens* and *Lycopodium deuterodensum* (McDougall and Summerell 2003). Given the rapid spread of the disease in the past four years, *O. ellipticum* seems destined for local extinction in this park. The fate of *T. purpurascens*, a species already regarded as threatened under Commonwealth legislation, is uncertain. In diseased areas, there has been a clear change in the understorey from vegetation dominated by dense shrubs (the remains of which are still evident) to vegetation dominated by grass (*Poa sieberiana*). Trials to assess the effectiveness of phosphite at controlling *P. cinnamomi* have commenced.

2) Communities containing *Xanthorrhoea glauca* subsp. *glauca*. Two areas of dying *X. glauca* subsp. *glauca* were located in Werrikimbe National Park, an area of coastal escarpment vegetation in northern NSW (Site 1, Fig. 1). *Phytophthora cinnamomi* was commonly located in soil beneath *X. glauca* plants and was found in the roots of *X. glauca Pultenaea pycnocephala* and *L. fraseri*. There were also numerous deaths of *Comesperma ericinum*, *Hakea dactyloides*, *Persoonia cornifolia* and *Petrophile canescens*. Due to low recruitment and high mortality, *X. glauca* will probably become extinct from one site within the next 50 years, despite being a co-dominant at present. Long-term monitoring at this site is important for evaluating impact and change.

The Wollemi Pine

The Wollemi Pine (*Wollemia nobilis*) was discovered in 1994. This tall tree is restricted to a few remote valleys in Wollemi National Park (Site 3, Fig. 1). Extensive soil sampling in Wollemi Pine sites failed to locate *P. cinnamomi*. Glasshouse trials have shown this species is highly susceptible (Bullock *et al.* 2000).

Conclusion

We can only speculate on the reasons for previous assessments that *P. cinnamomi* is a benign pathogen in NSW. The flora of NSW certainly appears to be more resistant to infection than that in Western Australia. The genus *Banksia*, for instance, although an excellent indicator of disease in Western Australia, is a poor indicator in NSW. Some *Xanthorrhoea* species do appear to be good indicators of disease in NSW but most vegetation does not contain *Xanthorrhoeas* or any other obvious indicator. The NSW landscape may indeed be largely a post-disease artifact. In addition, although some tree species in NSW are susceptible to *P. cinnamomi*, extensive death of overstorey species appears to be rare and localised. Much of the early focus on *P. cinnamomi*, both here and in other States, was on its effect on timber production. Much of the impact that we report in this paper is on understorey species. In the case of *Xanthorrhoea* death and decline in Royal National Park, the impact has only been detected through long-term monitoring. The evidence of dying understorey plants is often quickly lost as a result of fire. *X. australis* plants quickly degenerate after death (Duncan 1994). Impacts on flora and vegetation in NSW may have simply been overlooked because the impact on production timber species was not significant. The pattern of disease in NSW is also different to that commonly reported in early reports about disease in the jarrah forest of Western Australia, where there are clear disease fronts. Disease fronts were observed in the current study but they were rare. It was more common to find scattered deaths of susceptible species in an infested area. This pattern is not peculiar to NSW. Some infestations on the south coast of Western Australia are similarly characterised by diffuse disease fronts (Bryan Shearer, Department of Conservation and Land Management pers. comm.). Such infestations probably reflect the lower density of highly susceptible species.

Judging from our own surveys and those of other authors, *P. cinnamomi* is widespread in eastern NSW. However, there is clear evidence that *P. cinnamomi* is having an impact on flora and vegetation in several widely spaced parts of eastern NSW. Given that this study was only cursory, it is highly likely that there are many more areas where *P. cinnamomi* is

having an impact. There is also a high probability that *P. cinnamomi* will have further impact by directly or indirectly affecting threatened plants and animals that occur near infestations.

Although sampling of soil cannot prove the absence of *P. cinnamomi*, in the current study the pathogen could not be isolated from apparently diseased vegetation in Blue Mountains NP, Kosciuszko NP, Ku-ring-gai Chase NP, Sydney Harbour NP and Wollemi NP nor from healthy areas containing *Xanthorrhoea australis* in South East Forest NP. *P. cinnamomi* is not ubiquitous in eastern NSW.

Because *P. cinnamomi* is widespread in NSW and current and potential impacts are localised, there would appear to be no value in quarantine of large areas. Management should focus on hygiene and control trials using phosphite to protect threatened species and communities. *P. cinnamomi* cannot be assumed to be present throughout the landscape. In areas where hygiene is proposed and there is debate about the presence or absence of *P. cinnamomi*, a precautionary approach should be adopted and the pathogen assumed to be absent unless it can be proven to be present.

Acknowledgments

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How susceptible is the Flora of the South-western Australia to *Phytophthora cinnamomi*?

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Abstract. This study aims to compare variation in estimates of susceptibility to *Phytophthora cinnamomi* Rands between four databases and determine the proportion of the flora of the South-West Botanical Province of Western Australia that are susceptible to the pathogen. Estimates of the susceptibility of south-western flora to *P. cinnamomi* infection were obtained from databases for *Banksia* woodland of the Swan Coastal Plain, jarrah (*Eucalyptus marginata* Donn. ex Smith) forest, the Stirling Range National Park, and rare and threatened flora of Western Australia. For the woodland, forest and national park databases, hosts were naturally infected in uncontrolled diverse environments. In contrast, threatened flora were artificially inoculated in a shadehouse environment. Considerable variation within taxonomic units make occurrence within family and genus poor predictors of species susceptibility. Similar estimates of species susceptible to *P. cinnamomi* between the databases from the wide range of natural environments and an artificial environment, suggests that a realistic estimate of species susceptibility to *P. cinnamomi* infection in the south-west region has been obtained. The mean percentage of 40% susceptible and 14% highly susceptible equates to 2300 and 800 species of the 5710 species in the South-West Botanical Province susceptible and highly susceptible to *P. cinnamomi*, respectively. Undeniably *P. cinnamomi* in south-western Australia is an unparalleled example of an introduced pathogen with wide host range causing great irreversible damage to unique, diverse but mainly susceptible plant communities.

Introduction

The flora of south-western Australia is of international importance with regard to the high degree of richness and endemism and the high proportion of the flora at risk from threatening processes (Atkins 1998). Western Australia has about 8% of the world total of threatened, rare and poorly known flora, making the State one of the major centres for threatened flora in the world (Atkins 1998).

Phytophthora cinnamomi Rands infection is a major threatening process affecting the viability and genetic diversity of the flora of south-western Australia (Shearer 1994; Brown *et al.* 1998). Estimates of the susceptibility of south-western flora to *P. cinnamomi* infection have been obtained for the Stirling Range National Park (Wills 1993), jarrah (*Eucalyptus marginata* Donn. ex Smith) forest (Shearer and Dillon 1995), *Banksia* woodland of the Swan Coastal Plain (Shearer and Dillon 1996b), and rare and threatened flora of Western Australia (Shearer, Crane and Cochrane *unpublished*).

This study aims to determine the proportion of the flora of the South-West Botanical Province susceptible to the pathogen.

Materials and Methods

Specific details of the materials and methods for the *Banksia* woodland, jarrah forest, and Stirling Range National Park databases can be found in Shearer and Dillon (1996b and 1995) and Wills (1993), respectively. For the woodland, forest and national park databases, hosts were naturally infected in uncontrolled diverse environments. In contrast threatened flora were artificially inoculated in a shadehouse environment. Germinated seeds of threatened flora from Western Australia's Threatened Flora Seed Centre (Cochrane and Coates 1994) were grown potting mix of 1 part German Peat to 3 parts river-washed-sand in 15 cm-diameter pots and soil inoculated with *P. cinnamomi* isolates DP4 from the Northern Sandplains, DP51 from the Western edge of the wheatbelt and DP55 from the South Coast. Pots containing plants at least 6-months-old were inoculated in mid-summer and mortality was recorded thrice weekly for 5 months. Infection was confirmed by plating onto selective agar.

For *Banksia* woodland and jarrah forest databases, the assessment of susceptibility was based on the frequency of isolation of *P. cinnamomi* and plant mortality (Shearer and Dillon 1995, 1996b):

(1) no individuals of a species died in disease centres;

(2) individuals of a species died in one-third or less of the disease centres in which they occurred, and *P. cinnamomi* was not isolated from recently dead plants;

(3) individuals of a species died in one-third or less of the disease centres in which they occurred, and *P. cinnamomi* was isolated relatively frequently from recently dead plants;

(4) individuals of a species died in one-third or more of the disease centres in which they occurred, and *P. cinnamomi* was isolated relatively infrequently from recently dead plants; and

(5) individuals of a species died in one-third or more of the disease centres in which they occurred, and *P. cinnamomi* was isolated relatively frequently from recently dead plants;

Species in Groups 1 and 2 were considered to express resistance, those in Groups 3 and 4 variable susceptibility and those in Group 5 were most susceptible to infection (Shearer and Dillon 1995, 1996b).

For the Stirling Range National Park database the assessment of susceptibility was only based on the proportion of a plant population killed in a disease centre (Wills 1993):

(1) no apparent secondary symptoms of chlorosis, canopy dieback and/or death;

(2) < 20% killed at any location, and/or other secondary symptoms observed;

(3) 20-80% killed, varying with location; and

(4) > 80% killed.

Species in Group 1 were considered field resistant, those in Group 2 to express low susceptibility, those in Group 3 to express variable susceptibility and those in Group 4 to be highly susceptible (Wills 1993). For sake of comparison, the mortality rating of Wills (1993) was applied to the Threatened Flora database.

Differences between databases were analysed with Analysis of Variance (ANOVA) with database and susceptibility class independent variables and percentage of species in each susceptibility class the dependent variable. Percentage data was arcsine transformed.

Results
Variation Within and Between Families

The databases show considerable variation within and between families (Table 1). Taxa within Proteaceae are biased towards

Table 1. Percentage distribution of species among susceptibility group to *Phytophthora cinnamomi* infection for five families having the largest number of plant species in four databases

Database/ Susceptibility Group	Family Cyperaceae[A]	Epacridaceae	Mimosaceae	Myrtaceae	Papilionaceae	Proteaceae
Banksia Woodland						
1	80	50	50	24	40	21
2	20	0	17	41	10	29
3	0	25	17	12	10	7
4	0	0	17	12	20	7
5	0	25	0	12	20	36
Jarrah Forest						
1	40	0	50	20	33	0
2	60	14	30	40	8	33
3	0	43	10	20	0	0
4	0	14	10	20	42	40
5	0	28	0	0	17	27
Stirling Range National Park						
1	100	20	80	85	25	15
2	0	30	20	11	25	7
3	0	25	0	4	31	49
4	0	25	0	0	19	28
Threatened Flora						
1		0	87	22	15	19
2		0	7	14	8	9
3		40	0	31	23	32
4		60	7	28	54	40

[A]No Cyperaceae were tested for susceptibility to *P. cinnamomi* in Threatened Flora

Table 2. Number of species in each database and percentage of flora of south-western Australia susceptible and highly susceptible to *Phytophthora cinnamomi* infection derived from four databases

Database	Infection	Number of species	% Susceptible	% Highly susceptible
Banksia Woodland	Natural	95[A]	32	15
Jarrah Forest	Natural	105[A]	44	12
Stirling Range National Park	Natural	330	36	10
Threatened Flora	Artificial	142	49	21
Mean ($\pm$ standard error)			40 ± 4[B]	14 ± 2[C]

[A]Number of species occurring in $\geq$ 3 disease centres.
[B]The mean of 40 % equates to 2300 species of the 5710 species in the South West Botanical Province susceptible to *P. cinnamomi*.
[C]The mean of 14 % equates to 800 species of the 5710 species in the South West Botanical Province highly susceptible to *P. cinnamomi*.

susceptible groups in the Stirling Range National Park and Threatened Flora databases but bimodal and divided into resistant and susceptible groups for the *Banksia* woodland and jarrah forest databases (Table 1).

Epacridaceae and Papilionaceae taxa are biased towards susceptible groups in the Threatened Flora databases but divided into resistant and susceptible groups for the *Banksia* woodland, jarrah forest and Stirling Range National Park databases. Myrtaceae taxa are biased towards resistant groups in the *Banksia* woodland and Stirling Range National Park databases but divided into resistant and susceptible groups for the jarrah forest and Threatened Flora databases. Mimosaceae taxa tend to be resistant in all databases with a few taxa susceptible. All of the Cyperaceae were resistant.

Proportion of Flora Susceptible

Estimates from the four databases were relatively consistent with the proportion of species susceptible within the range 33-49% and those highly susceptible within the range 10-21% (Table 2). Estimates for the Threatened Flora data base were higher than those for the other three databases (Table 2), but differences between databases were not significant (P = 0.176). The mean percentage of 40% susceptible and 14% highly susceptible equates to 2300 and 800 species of the 5710 species in the South-West Botanical Province susceptible and highly susceptible to *P. cinnamomi*, respectively.

Discussion

Despite susceptibility being estimated by two different methods within different natural and artificial environments, the databases provide similar estimates of the percentage of species susceptible to *P. cinnamomi* in the South-West Botanical Province of Western Australia. The relative consistency between databases from a wide range of natural environments and an artificial environment, suggests that a realistic estimate of species susceptibility to *P. cinnamomi* infection in the south-west region has been obtained.

Estimates of susceptibility were highest for the Threatened Flora database. This would be expected as the taxa tested have been specifically selected for seed storage in Western Australia's Threatened Flora Seed Centre because of the likely threat of *P. cinnamomi* infection (Cochrane and Coates 1994), and therefore represent a biased sample.

Considerable variation within taxonomic units make occurrence within family a poor predictor of species susceptibility. Even though Proteaceae are considered in general to be a very susceptible family, variation is bimodal and divided into resistant and susceptible groups for the *Banksia* woodland and jarrah forest databases. More extensive databases of species susceptibility in plant communities threatened by *P. cinnamomi* infection must be a high research priority. A greater understanding of variation of host response within taxa may assist in understanding mechanisms of resistance and improvement of testing and assessment procedures.

Many highly susceptible species tend to occur frequently and be structurally dominant in the communities in which they occurr (Wills 1993; Shearer and Dillon 1995, 1996b). Thus infection by *P. cinnamomi* of the estimated 800 highly susceptible plant species in the South-West Botanical Province leads to an irreversible and conspicuous decline in

biomass with associated reduced floristic diversity and capacity of infested sites to support dependent biota (Wills 1993; Shearer and Dillon 1996a).

This paper quantifies the truly exceptional host range of *P. cinnamomi* in south-western Australia. The estimated 2,800 plant species susceptible to *P. cinnamomi* and the 810 highly susceptible plant species in the South-West Botanical Province make the region internationally unique with respect the number and diversity of taxa susceptible to infection. Undeniably *P. cinnamomi* in south-western Australia is an unparalleled example of an introduced pathogen with wide host range causing great irreversible damage to a range of unique, diverse but mainly susceptible plant communities.

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The Dieback cycle in Victorian forests

A 30 year study of the changes caused by *Phytophthora cinnamomi* in Victorian open forests, woodlands and heathlands, measured on defined quadrats

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Abstract Changes in both vegetation and in pathogen population and distribution were monitored periodically on defined infested quadrats and on similar pathogen-free quadrats on 13 sites representing major types of forest and woodland between 1970 and 2000. The susceptible eucalypts in the overstorey of infested sites showed severe dieback, loss of crown and deaths. All trees died on some sites, other presented dead leaders with epicormic growth on lower branches. Dieback of the understorey, followed by death occurred in 50-75% of the species in the healthy understorey, including the dominant *Xanthorrhoea australis*, thereby changing the community and the species composition. Species richness in infested quadrats declined, and percentage cover and percentage contribution to the community by susceptible species were almost eliminated. On steep slopes, the ground remained bare, but on other sites the susceptible flora was replaced by field resistant species of sedges and rushes, and by the partly resistant teatrees, *Leptospermum* spp., which formed a dense cover.

The pathogen was isolated from 100% of the root samples from infested quadrats from 1970 to 1984, but then gradually declined. In 2000, *P. cinnamomi* was rare on some sites and not isolated from four. Regeneration of 30 to 40 susceptible species, previously eliminated, was recorded from infested sites, and two thirds of these were growing on more than one quadrat. Copius regeneration of the previously dominant but highly susceptible *X. australis* occurred on four sites. Significant recovery and recruitment of the overstorey has not been observed. It is not yet clear whether the regeneration of the understorey is stable, or whether successive cycles of disease and recovery will occur.

Introduction

The disease cycle from invasion and destruction of a susceptible indigenous flora by *Phytophthora cinnamomi* Rands, to the decline of the pathogen and the regeneration of that same susceptible, indigenous flora was almost complete on some defined quadrats in each of the three Victorian forests studied. The major dieback threat is to open forests, woodlands and heathlands of Southern Australia. A study of the changes in the vegetation and in the pathogen populations and distribution over the past 30 years on defined quadrats in Victorian plant communities has revealed the plant communities and the species of each community which are particularly threatened by this pathogen.

The three regions which are assessed in detail are the Grampians in Western Victoria, Wilsons Promontory, the most Southern portion of mainland Australia, and the Brisbane Ranges, Southwest of Melbourne. Each of these regions has threatened plant communities in which many species are susceptible. The climate is characterised by a mean annual rainfall of approximately 600mm for the Grampians and the Brisbane Ranges, 1000mm at Wilsons Promontory which has sandy soils, and a temperature range conducive

to *P. cinnamomi* and zoospore production in Spring. The vegetation of these regions also experiences a period of water stress during the Summer-Autumn season which may be fatal for susceptible plants with infected roots

Disease caused by *P. cinnamomi* in Victoria was first recorded in 1970 for the Brisbane Ranges, in 1971 for Wilsons Promontory and in 1974 for the Grampians (Podger and Ashton 1970; Weste *et al.* 1973, unpublished report, Forests Commission of Victoria 1974).

Severe wildfire burnt the Brisbane Ranges in 1967, the Grampians in 1970 and Wilsons Promontory in 1962 and 1965-6. Fuel reduction burns have occurred since, but checks have shown that these have not changed the species composition of the quadrats.

This paper presents the evidence for the major phases of the disease cycle and for the factors controlling its progress. While the whole cycle is almost complete on some sites, it remains incomplete on sites with differing plant communities and in differing environments.

Methods

The thirteen sites studied are mapped (Fig. 1). Permanent defined quadrats were established in infested and in nearby, similar pathogen free vegetation in 1962 and 1970 in the Brisbane Ranges; in 1971 at Wilsons Promontory and in 1976 in the Grampians (Weste and Taylor 1971; Weste and Law 1973; Kennedy and Weste 1977). Quadrat size was determined from species:area curves for each site and therefore varied from 6 to 14m according to the plant diversity. Sites were selected to represent the different plant communities and were assessed biennially until 1986, then in 1995 and 2000 in the Grampians, 1996 and 2000 at Wilsons Promontory, and 1992, 1994, 1996 and 1998 in the Brisbane Ranges (Kennedy and Weste 1986, 1997; Weste 1981, 1986). At each assessment species composition, percentage cover and health of each plant were recorded for three replicate quadrats of the understorey. Samples containing roots and the accompanying soil were collected from living) from both infested and pathogen free quadrats at each assessment and were tested for the presence of the pathogen by lupin baiting (Weste 1981). Trees were measured separately on 30x30m quadrats. Species composition, percentage crown destruction, health of leading branches, and the presence of epicormic shoots were measured. Data were analysed statistically to compare infested with pathogen free quadrats. Plant species and nomenclature follow Walsh and Entwistle (1996 to 1999).

Results

The first phase of the disease usually occurred along a shallow depression in the topography. The pathogen was virulent, aggressive and infected all susceptible species contacted by the swimming spores along the gully. The boundary of disease was at first sharply defined with healthy vegetation on both sides of the gully. Epidemic disease was observed with chlorosis browning and death of all susceptible species, including the dominant *X. australis* which serves as a useful indicator of dieback disease. A mean of 50 to 75% of the understorey species were susceptible, but because the degree of susceptibility varied with the species, a mosaic of dead and dying plants was observed. Some species died completely and rapidly such as *X. australis* and *Isopogon ceratophyllous*, others died gradually, branch by branch. Plants growing on slightly elevated mounds of soil, for example around trees often escaped contact by the zoospores and survived. Species composition of the plant community changed, species richness and percentage cover were both reduced and bare ground increased by as much as 70%. The elimination of susceptible species from the infested quadrats has been documented (Kennedy and Weste 1977; Weste and Law 1973; Weste et al. 1973).

Approximately one year later symptoms appeared on the susceptible species of the overstorey. These included the dominant species of these forests and woodlands, *Eucalyptus obliqua*, messmate and the stringy barks, *E. baxteri*, *E. macrorhyncha*, and the peppermints, *E. radiata* and *E. dives*. All eucalypts on the Grampians sites, on both infested and pathogen free sites, showed crown loss from wildfire (1970) of 10 to 15%. This was the only damage observed on pathogen free sites and on resistant eucalypts such as *E. melliodora* and *E. dives*. On infested sites the leaves of susceptible eucalypts turned brown, and abscissed from the leaders, so that the crown density was reduced by 30 to 100%. Deaths of 45% of these species was observed. On some quadrats no living trees remained.

During this phase 100% of root samples taken from living susceptible plants with symptoms growing on infested quadrats yielded *P. cinnamomi* on baiting. The pathogen was isolated from all infested quadrats, but not from pathogen free quadrats.

The second phase began one to three years after infection with the colonisation of infested quadrats by field resistant species, such as the grasses, (except *Themeda triandra* which is susceptible). the sedges, the rushes and the rope rushes. *P. cinnamomi* infected the roots of these species, but new roots formed without other symptoms (Phillips and Weste 1984). *Leptospermum* spp. (*L. myrsinoides, L.continentis, L.scoparium*) were slightly susceptible, produced a dense growth on infested quadrats. Dieback of the terminal branches was observed, the plants did not flower, but survived. All these species grew as scattered components of pathogen free quadrats, and rapidly provided a dense understorey on infested quadrats consisting of a carpet of *Hypolaena fastigata* or a cover of *Gahnia radula* with clumps of *Lepidosperma semiteres* and other rushes and sedges. These are all wind pollinated and do not produce nectar, honey or nutritious pollen, and therefore do not attract birds or small mammals. On steep quadrats the bare ground was not colonised by field resistant plants, remained bare and subject to erosion. This phase lasted until 1985, about 15 years.
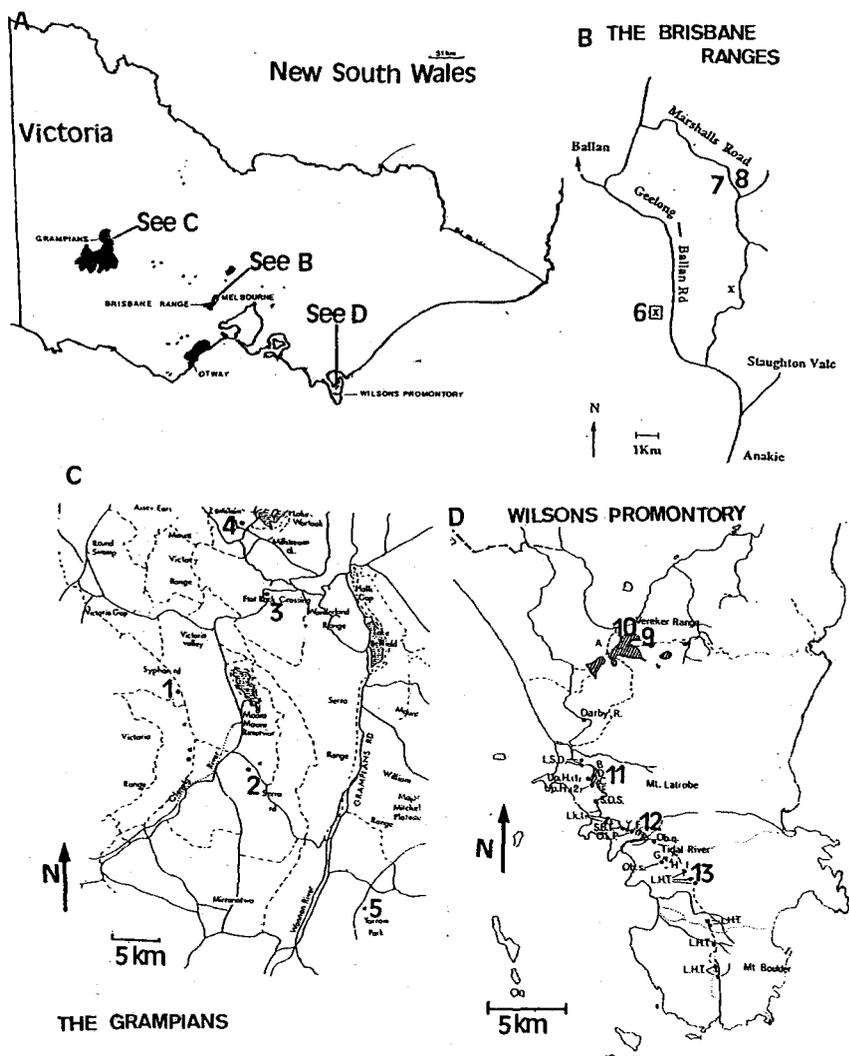
Fig.1. Location of permanent quadrats on sites 1-13 where population density and distribution of *Phytophthora cinnamomi*, flowering plant composition, percentage cover and disease status were monitored over a period of 20 or more years.

During the third phase of invasion, a decline in pathogen isolations from root samples was recorded, ranging from 0 to 97%. In this changed community, with few or no susceptible roots available, *P. cinnamomi* was rarely isolated. The number of infested quadrats from which *P. cinnamomi* was isolated also declined and ranged from 0 to 75% except on wet quadrats, such as those receiving drainage.

In the following or fourth phase the infested quadrats varied in reaction. In most quadrats, particularly those with low pathogen isolations, regeneration of susceptible species was recorded. Recruitment was mostly from seed, but also from underground stems or lignotubers. The first such regeneration was of moderately susceptible and subsequently of highly susceptible species which became infected and died. However in 1995 to 1996 that of susceptible species on infested quadrats included 27 from 11 different families from the Grampians, 15 from 7 families from the Brisbane Ranges and 19 from 9 families on Wilsons Promontory. These included seedlings of susceptible eucalypts (Weste and Kennedy 1997). The plants were young, small, and ranged from 0 to 4% of percentage cover. Most plants survived despite the sporadic recovery of *P. cinnamomi* from the same quadrat. In other infested quadrats the growth of the

resistant flora was so vigorous and dense that regeneration of small susceptible plants was scarce. The presence of large logs from fallen dead eucalypts also suppressed seedling development. Seedlings of the susceptible eucalypts regenerated and survived.

In 1998 abundance regeneration of susceptible plants, notably of the once dominant *X. australis* was recorded on some infested sites in the Brisbane Ranges, at Anglesea and at Wilsons Promontory. The regeneration was measured on three replicate infested quadrats of 5 x 5m for each of three sites in the Brisbane Ranges (Weste *et al.* 1999). Size, health and location of 77 living and 20 dead *X. australis* plants were recorded from three quadrats, and one third of these were less than 8 years old, that is the apex had appeared above ground since the previous assessment in 1990 (Gill and Ingwerson 1976). *P. cinnamomi* was isolated from three locations on each of these 5 x 5m quadrats and was active, infecting and killing the young *X. australis* plants nearby. Similar abundant regeneration of *X. australis* was observed on the Angelsea heathlands in an area where all plants of this species had previously been destroyed by *P. cinnamomi*. In 1998 abundant regeneration was also observed and photographed at two sites on Wilsons Promontory, on Five Mile Road at the intersection with Millers Landing Road and at Tidal Overlook. As a result of a control burn on the latter site the plants of *X. australis* all flowered (Gill and Ingwerson 1976). During the subsequent months these plants turned yellow and began to die. *P. cinnamomi* was isolated from the roots. Infection was apparently derived from repairs to the steps both at Tidal Overlook and on Mount Oberon. As a result of consultation with Dr. Cahill, M. Aberton from Deakin University and with the rangers of Wilsons Promontory National Park, these plants were sprayed with phosphonate in 1999. The spray provided protection which lasted 14 months, after which they were re-sprayed (Aberton personal communication).

Massive regeneration of *X. australis* was also recorded on two infested sites in the Grampians in May 2000. Mean counts of 73 and 68 plants were growing on one quadrat of 5 x 5m on sites at Yanam Park and on Syphon Road respectively.

The regeneration of *X. australis* was accompanied by regeneration of other susceptible and highly susceptible species such as *I. ceratophyllous*, *Leucopogon* spp. and *Dilwynia* spp. By May 2000, 74 of the 80 susceptible species recorded from pathogen free quadrats had recruited on the infested quadrats of the Grampians and 70% of these were growing on more than one quadrat, which probably increased their chance of surviving infection. Regeneration represented a recovery in both species richness and in percentage cover despite the sporadic occurrence of *P. cinnamomi* on the infested quadrats.

The overstorey also responded with vigorous epicormic growth and renewed crown vigour on surviving trees. Both saplings up to 8 years old and seedlings of one to two years old were recorded from most sites. However some of the saplings had 5 to 10% dieback.

The decline in pathogen population and distribution, reported in 1985, continued and by 2000 positive isolations from baiting tests were less than 1%, consequently no quantitative data can be presented. Pathogen distribution was also reduced. *P. cinnamomi* was not detected from two sites in each of the three study areas. In other words *P. cinnamomi* was no longer detectable at 6 of the 13 sites.

Discussion

The disease cycle is almost complete for some infested quadrats in the three areas of Victorian open forest, open woodland and heathland. The cycle began with infection 24 to 30 years ago and proceeded to the stage where regeneration was recorded for the same susceptible species that were initially destroyed by the pathogen. According to the data recorded for each infested quadrat in 2000 and in 2001 the original species composition and structure of each community, the species richness and the plant abundance were being restored. The habitat shelter, the nutritious pollen and the nectar attractive to local fauna will become available. The plant cycle was contiguous with the pathogen cycle. Populations and distribution of *P. cinnamomi* increased rapidly during the invasive, aggressive and epidemic phases of the cycle (Weste and Ruppin 1997), but declined during the second and subsequent phases.

However the situation at present is complex and varies with the site. On some infested quadrats the resistant flora remains dominant, and regenerating susceptible species have to compete for space, light, water and nutrients. On steeper sites such as Sena Road in the Grampians or on Mount Oberon and Tidal Overlook at Wilsons Promontory the top soil was eroded soon after the susceptible species were killed and 60 to 70% of the ground remained bare. On some devastated

sites seed for regeneration may not be available, as at Marshalls Road site in the Brisbane Ranges.

Factors operating within the cycle to reduce pathogen activity include low availability of susceptible roots as food base and sheltered habitat, microbial suppression and lack of free water at temperatures greater than 12°C for zoospore production, dispersal and infection. There is no evidence yet of selection for resistance among susceptible species or of a decline in pathogenicity of *P. cinnamomi*.

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Impact of *Phytophthora* in Europe
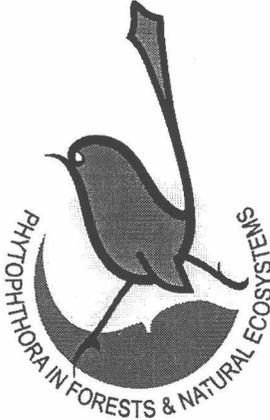
Occurrence of *Phytophthora* species in oak ecosystems in Turkey

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Abstract. Fifty-one healthy and declining natural oak forests in Turkey have been screened for *Phytophthora* species. Eight *Phytophthora* species and two unidentified species were isolated from the rhizosphere soil using the bating method with young oak leaves. The morphology, physiology and RAPD banding patterns of the isolates were compared with other *Phytophthora* species previously isolated from Austrian oak stands. rDNA ITS sequences of some isolates were similar to *P. citricola*, however morphological characteristics were highly variable and differed from the holotype. *P. quercina* was commonly isolated from slopes susceptible to drought and was more frequent from the European part of Turkey, although it occurred on some stands in the Asian part. *P. cinnamomi* was isolated only from one site in an open native oak-dominated forest. In declining stands, necroses of fine roots were commonly detected. Significant differences in the isolation frequency of various *Phytophthora* species were observed between healthy and declining trees. The possible role of *Phytophthora* species and oak decline is discussed and the results are compared with the other studies in Europe.

Introduction

There are early reports from the beginning of the 20th century on oak dieback in the former Soviet Union (Oleksyn and Przybyl 1987). Subsequently, the decline of oak is well documented elsewhere in the world (e.g. Donaubauer 1998 and references therein). Oak decline is a recurring problem which has become increasingly apparent in the least two or three decades and it is still a current problem world-wide wherever oaks occur (Delatour 1983; Donaubauer 1998). Recently, dieback of *Q. griffithii* of an unknown cause has been reported from Bhutan (D.B. Chhetri, per. Comm). Oak decline is a complex disease that is caused by many predisposing and triggering factors (Siwecki and Liese 1991; Luisi *et al.* 1993; Dreyer and Aussenac 1996).

Phytophthora species cause serious disease problems in forest ecosystems world-wide (Hansen and Sutton 1999). The isolation of *P. cinnamomi* from roots of *Q. suber* and *Q. ilex* (Brasier *et al.* 1993), the observation of the death of fine roots and necrotic lesions on roots of *Q. robur* and *Q. petraea* (Vincent 1991; Blaschke 1994) as well as the association of *Phytophthora* spp. with the decline of oak trees led many scientist to conclude that soilborne *Phytophthora* species could play an important role in oak decline in Europe and other parts of the world. So far *P. cinnamomi* has been isolated from various oak species in Spain, Portugal, France, Italy and recently in Mexico and most authors

suggested that this *Phytophthora* species is associated with the decline and mortality of oak trees (Brasier *et al.* 1993; Robin *et al.* 1998; Gallego *et al.* 1999; Bianco *et al.* 2000; Tainter *et al.* 2000). During comprehensive studies in oak forests in central Europe, ten other *Phytophthora* species including four new taxa were isolated, among which the newly described *P. quercina* was the most common species (Jung 1998; Jung *et al.* 2000). Likewise, in the course of a research project funded by the European Union (PATHOAK), eleven *Phytophthora* species were encountered in European oak forests (Delatour *et al.* these proceedings).

Apart from *P. cambivora* on sweet chestnut and *P. cactorum* on beech seedlings Phytophthoras have not as yet been detected in forest ecosystems in Turkey (Selik 1986). Six different *Phytophthora* species (*P. hibernalis*, *P. citrophthora*, *P. capsici*, *P. cactorum*, *P. drechsleri* and *P. infestans*) had been reported from agricultural crops so far (Biçici and Çınar 1990).

The present project aimed to examine the relationship between dieback symptoms of oak and the presence or absence of Phytophthoras in native deciduous oak ecosystems. A survey was conducted in order to elucidate the species composition and population structure of *Phytophthora* on various oak species in Turkey.
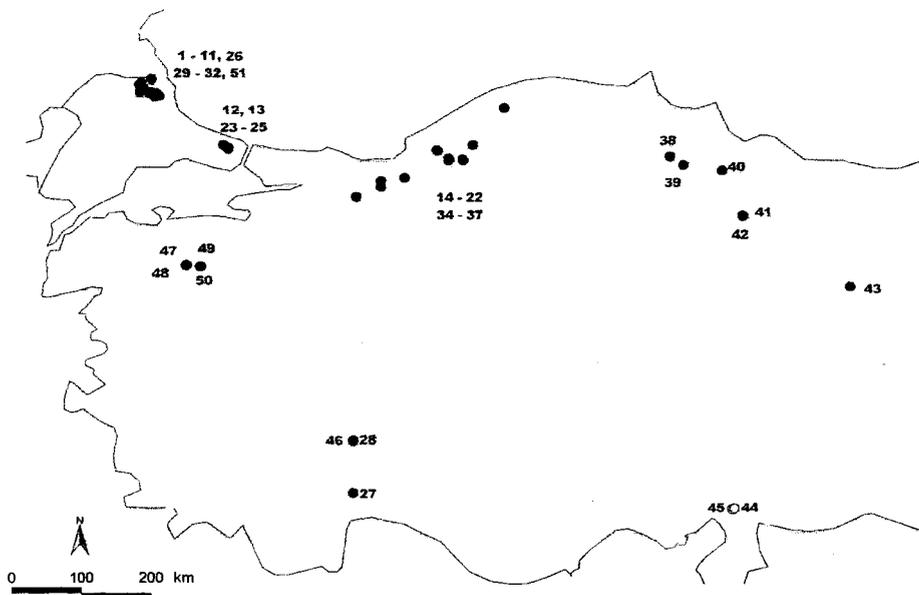
Fig. 1. Map of Turkey showing the location of the oak sites examined: **1** Demirköy/Karacadag (138), **2** Demirköy/ Karacadag (61), **3** Demirköy/Istihkam (64), **4** Demirköy/Papazkoru, **5** Demirköy/Bezirgan, **6** Demirköy/K.Bezirg., **7** Igneada/Macera (24), **8** Demirköy/Karacadag (99), **9** Demirköy/Pirençetepe (39), **10** Demirköy/Papazkoru (16), **11** Demirköy/Kadinkule, **12** Belgrad/Kuzey S., **13** Belgrad/Ayvat B., **14** Düzce/Hamamdagi, **15** Düzce/Muncurlu, **16** Kastam./Pınarbasi, **17** Yenice/Aksuagzı (45), **18** Karabük/Ulukaya, **19** Yenice/Çitdere (55/56), **20** Yenice/Kabakli (21), **21** Yenice/Bakirli (96), **22** Yenice/Sariot, **23** Belgrad/Topuzlu B, **24** Belgrad/Valide B. **25** Belgrad/IIMahmut B. **26** Demirköy/Bezirgan (128), **27** Antalya/Asar (614), **28** Egirdir/Yukarıgökdere **29** Demirköy/Sivriler (10), **30** Demirköy/Istihkam (36), **31** Demirköy/Karacadag (139), **32** Demirköy/Sarapnel-Manastır tepe **33** Devrek/Bulanikdere (81), **34** Dokurcun I (41), **35** Dokurcun II **36** Bolu/Sariçam, **37** Devrek/Bulanıkdere, **38** Vezirköprü/Tasören (282), **39** Havza **40** Amasya/Destek **41** Tokat/Yaylacık I **42** Tokat/Yaylacık II **43** Divrigi/Delidag **44** Dörtyol/Ufacık I, **45** Dörtyol/Ufacık II, **46** Egirdir/Kasnak, **47** Kepsut I/Catalarmut (15), **48** Kepsut II/Kansız köyü, **49** M.Kemal Pasa/Gürgendagi I (10), **50** M.Kemal Pasa/Gürgendagi II, **51** Demirköy/Isthkam   (Numbers in parentheses indicate the forest district number)

Materials and Methods

Study area and oak species in Turkey

The investigations were carried out during 1999 (Sept.-Nov.), 2000 (Aug.-Sept.) and 2001 (Apr.-May) at 51 oak stands at various parts of Turkey (Fig. 1). Most of the oak forests where studies were carried out, were located in productive forest. However, some stands such as the sites 12, 13, 19, 23, 24, 25, 28, 36 and 46 were located in nature protection areas and are therefore not subject to silvicultural practices. Stands 4, 9 and 10 were coppice forests. While tree age varied from 40 to 300 years, the majority of trees were about 80 years old. The sites were located in four different climate zones; the Mediterranean and submediterranean winter rain area, the warm humid zone and the mountain climate zone. The altitude ranged from 85 to 2030 m. a.s.l. and the annual rainfall differed between 400 mm and 1200 mm. Except for site 15 which was on a site with periodic flooding and a high ground water

level, almost all sites were located on steep slopes.

Oak trees cover 22.7 % (6.5 mil. ha) of the total forest area in Turkey (Mayer and Aksoy 1986). Among the eighteen oak species native to Turkey the following species were examined; *Q. petraea* subsp. *petraea*, *Q. petraea* subsp. *iberica*, *Q. robur* subsp. *robur*, *Q. frainetto*, *Q. pubescens*, *Q. cerris* var. *cerris*, *Q. cerris* var. *austriaca*, *Q. hartwissiana*, *Q. vulcanica*, *Q. ithaburensis* subsp. *macrolepis*, *Q. macranthera* subsp. *sypirensis*.

Sampling procedures and isolation of Phytophthora species

The investigation sites were chosen to be representative of the local forest community and the various site conditions. Between 2 and 16 trees (mean for all sites 5.7 trees) were selected and observed in each stand. Trees were selected to be representative at each site and had a scattered distribution within each

69

stand. Intensity of sampling varied between stands and some sites were two or three times more intensively investigated (sites 1, 11, 15, 17, 25 and 33) than others. Ideally, half of the sample trees belonged to healthy trees (group 1) and the other half of the trees showed symptoms of dieback (groups 2, 3 and 4). However, some stands were totally healthy and none of the trees showed any symptoms of dieback. In other areas only trees displaying dieback symptoms occurred. Soil and root samples were taken around the stem base of 291 oak trees. Soil samples were used for attempts to recover *Phytophthora* species. Four soil samples, 50 to 150 cm apart from the trunk and regularly spaced in four directions around the base of each tree were collected. After removing the organic matter one litre rhizosphere soil including fine roots were taken from each plot and put in a plastic bag. After careful mixture of the four soil samples from each tree, 300 ml of the soil mixture was flooded with deionized water in a plastic box. Baiting was performed at a temperature of 20°C with 2 or 3 days old leaflets collected from young *Q. robur*, *Q. petraea* and *Q. hartwissiana* plants. After 3 – 10 days incubation leaflets with brown discoloration were examined under a light microscope for the presence of sporangia of *Phytophthora*. If sporangia were observed leaflets were washed under tap water, cut in 1-3 mm pieces and placed on selective agar. Cultures of *Phytophthora* spp. growing out on selective media were transferred to V8 juice agar (100 ml/l vegetable juice from tomatoes, carrots, celery and beetroot [Biota AG, Trägerwilen, Switzerland], 3 g/l. CaCO$_3$ and 20 g/l agar) in order to get pure isolates. The selective medium was prepared by amending V8 juice agar media with 10 mg/l pimaricin, 200 mg/l ampicillin, 10 mg/l rifampicin, 25 mg/l PCNB, 50 mg/l nystatin and 50 mg/l hymexazol (Jung et al. 1996).

In case of negative isolation the method of Jeffers and Aldwinckle (1987) proved to be very useful. Soil subsamples with negative isolation results on baited leaves, were tested for a second time after air drying in the laboratory. All soils with negative isolation were re-examined within a period of six months. For soil samples infested with *Pythium undulatum* this method was repeated two or three times.

Crown status of declining trees was assessed according to Pollanschütz et al. (1985) as follows; Group 1, no symptoms of decline, crown transparency less than 10-15 %;

Group 2, weak tree, dieback of some tips of branches and twigs or decline in a small part of the crown, crown transparency 15 – 35 %; Group 3, apparent transparency in all parts of the crown, dieback of twigs and branches, yellowing and wilting of leaves, epicormic shoots often present, crown transparency 35 - 55 %; Group 4, highly damaged oak, considerable transparency of crown, many dead twigs and branches, yellowing of all of the leaves and many epicormic shoots detectable, crown transparency > 55 %.

Identification of Phytophthora *spp.*

Colony morphology and growth rate of representative *Phytophthora* isolates were studied on various artificial media. Plastic Petri-dishes (diameter 9 cm) containing PDA CMA, MEA, and V8 juice agar were inoculated with 5 mm discs cut from the margin of a growing culture and incubated for 7 days at 20 °C in the dark. On PDA and CMA, colony morphology and growth rate were generally recorded after 14 days. Slow growing cultures on various media were also inspected after 14 days. For determination of temperature-growth relationships, cultures were incubated from 5°C to 35°C at 2.5°C intervals on V8-juice agar in the dark. Growth rate was noted 5 days after the onset of linear growth. However, for the minimum and maximum temperature measurement was made after 10 days and for fast growing isolates after 3 days. Tests were repeated twice using three replicate plates per isolate. To examine the sporangia, a disc cut from the edge of a growing culture was placed in a Petri dish and flooded with non-sterile soil extract water (Erwin and Ribeiro 1996). After 24 to 72 hours incubation, 50 mature sporangia were randomly chosen to study sporangium morphology and dimensions. For measurements of oogonia, discs were cut from the surface of one-month old cultures grown in the dark. Of each isolate, 50 randomly chosen oogonia were measured using a light microscope at a magnification of 400x. Cultural and morphological characteristics were compared with authentic isolates kindly provided by T. Jung (Bavarian State Inst. of forestry (LWF) Munich, Germany) and obtained from CBS (Utrecht, The Netherlands). ITS sequencing of the isolates and comparisons with sequences of known *Phytophthora* spp. in Gene bank and other databases were done by D. Cooke (SCRI, Dundee, Scotland) in order to confirm the identifications derived from the morphological studies.

Statistical analysis

The relationship between the crown status of sample trees and presence/absence of *Phytophthora* species in the rhizosphere soil was tested using logistic regression analyses. Furthermore, contingency tables were used for analyse the relative risk that oaks develop severe above-ground symptoms by comparing of the healthy (group 1) and the entire declining (group 2, 3 and 4) trees. All statistical analyses were made using the programme package SPSS for Windows 8.0.

Results

Symptoms of decline observed in the Turkish oak forests

Compared to other sites in Turkey, trees from oak sites in Thrace showed dieback symptoms more frequently. In addition, higher levels of tree mortality and dieback were observed at the two upland forest stands in Tokat (41, 42), located in eastern Anatolia. On most sites dieback symptoms occurred on single trees, scattered within the stands or in small patches. An association of decline with valleys or high incidence of decline throughout large areas was not noted. A positive or negative rate of crown status was noted between the years at the sites which were

observed for three years. However, on many stands there was no change in crown status over the same period. Strip and bleeding cankers were rarely seen on inspected trees. Tarry exudations and tears on the bark were also seldom observed. Epicormic shoots were occasionally detected on branches or the trunk. Trees with decline symptoms in natural, undisturbed ecosystems showed dieback of some branches and parts of crown which were frequently covered with lichens or mosses that might indicate a slow decline process.

Isolation of Phytophthora *spp.*

At least eight *Phytophthora* species were isolated from soil samples around 117 out of 291 trees in 38 of the 51 sites (Table 1). The assemblage of species included *P. quercina, P. citricola, P. cinnamomi, P. gonapodyides, P. cryptogea, P. cambivora, Phytophthora* sp. 1 and *Phytophthora* sp. 2.

Three ecotypes of *P. citricola* were recognised. While type C occurred in the Asian part, the two other types (A and B) were found in the European part of Turkey. All types have morphologically similar oogonia which differ, however, in size. The semipapillate sporangia were variable in shape

Table 1. *Phytophthora* and *Pythium* species which were isolated from soil samples at various oak stands in Turkey.

Phytophthora species	% positive stands	No. of trees	Site number	*Quercus* species
P. quercina	57.0	89	1, 3, 4, 5, 7, 11, 14, 15, 17, 18, 21, 21, 24, 28, 29, 30, 31, 32, 33, 35, 39, 40, 41, 42, 44, 45, 46, 47, 50	*Q. petraea, Q. vulcanica, Q. cerris, Q .robur, Q. hartwissiana, Q. frainetto*
P. citricola A	3.9	2	1, 51	*Q. petraea*
P. citricola B	1.9	2	12	*Q. frainetto*
P. citricola C	1.9	4	38	*Q. cerris*
P. cinnamomi	1.9	4	25	*Q. petraea, Q. robur*
P. gonapodyides	1.9	1	16	*Q. petraea*
P. cryptogea	3.9	10	38	*Q. cerris, Q. petraea*
P. cambivora	1.9	-	47	*Fagus orientalis*
Phytophthora sp.1	3.9	9	11, 22	*Q. petraea*
Phytophthora sp. 2	1.9	2	12	*Q. petraea*
Phytophthora 'unknown'[1]	7.8	8	2, 8, 23, 45	*Q. petraea, Q. pubescens,*
Pythium undulatum	17.6	15	2, 6, 11, 15, 23, 41, 46, 48, 49	*Q. petraea, Q. cerris, Q. frainetto, Q. vulcanica, Q. robur*
negative	25.5	174	6, 9, 10, 13, 19, 26, 27, 34, 36, 37, 43, 48, 49	*Q. ithaburensis* subsp. *macroleis, Q. macranthera* subsp. *sypirensis*

[1]*Phytophthora* 'unknown' indicates that sporangia of *Phytophthora* were observed on leaflet baits but could not be obtained in pure culture. Negative= no *Phytophthora* spp. isolated from baits.

which was in general characteristic for *P. citricola* isolates. However, the cultures from Belgrade forest (site 12) showed the greatest variation in shape of sporangia, whereas the other isolates mainly had ovoid-elongated and ellipsoid sporangia. Other differences refer to the growth rate and colony morphology. The type A of *P. citricola* showed an optimum temperature at 27.5°C with a growth rate of 8.0 mm/day and had the maximum temperature for growth at 30°C with a radial growth rate of 7.5 mm/day. The type B isolates showed an optimum growth at 25°C and had a higher optimum growth rate (9.43 mm/day) compared to the type A cultures. The maximum temperature for growth was similar with type A, but a markedly lower radial growth rate was recorded (2.65 mm/day). The two types could be easily distinguished from each other by their colony morphologies on potato-dextrose agar (PDA). While type A isolates produced a stellate colony, type B showed a floral colony morphology which varied on V8 juice agar. The two types were clearly divided into two separate groups by their ITS sequences.

Type C isolates showed similar growth-temperature relationships compared to type A with a lower growth rate (5.93 mm/day) at the maximum temperature for growth (30°C). Slightly floral unique colony morphologies were observed on PDA medium, whereas a radiate colony type with a distinct ring was occasionally produced on V8A media after 7 days incubation. This colony morphology on V8 juice agar was rarely observed of type B isolates as well. Sporangium formation on solid media (MEA and V8A) is another distinct feature that differentiates type C from the other *P. citricola* strains from Turkey.

Based on the unique combination of vegetative, gametangial and physiological characters the *Phytophthora* sp. 1 is considered as a new species (Balci *et al.* in prep.). However, it shares identical ITS DNA sequences with *P. citricola* (D.L. Cooke, pers. comm.). This species was recovered from *Q. petraea* at two different sites in Thrace (stand 11) and in Yenice (stand 22) (Fig. 1). *Phytophthora* sp. 2 showed a close affinity to *P. citricola* as well but is distinguished from it by its colony morphology and growth–temperature relationships. The sporangia of *Phytophthora* sp. 2 are markedly ovoid-ellipsoid and occasionally possess a short stalk (caducous), a character which is not

common for *P. citricola* isolates (Erwin and Ribeiro 1996).

P. cambivora (mating type A2) was recovered only once from soil samples and from up to seven meters high strip-lesions of a beech tree (*Fagus orientalis*) in Kepsut (stand 47). Interestingly, *P. cambivora* could not be recovered from oak trees in the same forest. Although *P. cinnamomi* (mating type A2) was isolated from Belgrade forest in Istanbul, an association of this species with decline of oak in this forest which consisted of *Q. petraea* and *Q. robur* trees (>100 years) was not apparent. It is possible that *P. cinnamomi* was introduced to this site from a big nursery close to the forest. Other attempts to recover *P. cinnamomi* in this area did not result in positive isolation. Furthermore, associated tree species *Carpinus betulus* and *Fagus orientalis*. as well as understorey plants, which could be considered as indicators of the presence of *P. cinnamomi* did not show any symptoms of decline. *P. cryptogea* was recovered twice from *Q. cerris* and *Q. petraea* trees at moist-wet sites with clayey soil substrates in Vezirköprü (stand 38) and in Muncurlu (stand 15).

P. quercina was generally the most common species and was recovered from soil samples of 29 out of 51 sites (Table 1). It occurred in every main forest ecological zone and in each of the four climate zones considered in the survey. Notwithstanding that all stands in Anatolia were located on steep slopes and are generally characterised by long dry periods during the growing season, *P. quercina* occurred frequently. This is exemplified by the sites in Tokat (42, 43) which have an annual rainfall less than 400 mm per year and shallow sandy soil layers. These sites were among those with the highest isolation frequencies of *P. quercina*. Because of its slow growth, *P. quercina* was not easily recovered at the first isolation attempts. All soil samples with negative isolation results were again tested a second or third time after storing them in dry conditions in the laboratory. These repeated isolation attempts often resulted in positive isolation of *P. quercina* at sites where first isolations were not successful. All isolated *P. quercina* cultures showed the characteristic morphological and cultural features as indicated in the description of the species by Jung *et al.* (1999a). However the optimum temperature of 22.5°C was lower than that of isolates from central Europe. With an average of 4.66 mm/day (range 4.47 to 5.61 mm/day) the growth rate of Turkish isolates at the

optimum temperature was faster than that of the ex-holotype isolate (optimum 25 °C, growth rate 3.7 mm/day).

The field collections to isolate *Phytophthora* resulted in the lowest isolation rate in late summer 2000 (data not shown), when all soil samples were dry after an unusually long hot and dry season. From these soil samples *P. quercina* and the new species, *Phytophthora* sp. 1, were commonly recovered after repeated flooding, although the soil had been dry at the time of collection. During the other two years of field collections soil samples were often moist or even wet and thus many *Pythium* species occurred constantly in the course of the isolation routines. Among them, *Py. undulatum* and *Py. annandrum* were the most frequently isolated species.

On infested sites, there was a significant relationship between the crown status and the occurrence of *Phytophthora* species in the rhizosphere (logistic regression: odds ratio = 0.594, P= 0.005). Oaks with *Phytophthora* in their rhizosphere were 1.6 times more likely to exhibit decline symptoms than oaks without *Phytophthora* (relative risk= 1.620, 95% CI: 1.184 - 2.216). As shown in Fig. 2, *Phytophthora* spp. were less frequently isolated from healthy trees (group 1, 19 out of 88 trees) than from trees with crown status 2 (44 out of 100 trees). Phytophthoras were most common on trees with deteriorated crown transparency (groups 3 and 4; 54 out of 103 trees).

Discussion

The present study confirms that a diverse assemblage of *Phytophthora* species occurs in the rhizosphere of oak ecosystems in Turkey. A similar spectrum of Phytophthoras was encountered in oak ecosystems in various part of Europe (Jung 1998, Delatour *et al.*, these proceedings). The suspected new species, *Phytophthora* sp. 1 and *Phytophthora* sp. 2, as well as *P. citricola* type C were only detected in Turkey, but not in the European studies. In contrast, we did not recover *P. syringae*, *P. cactorum*, *P. megasperma* or *P. europaea*, which were common in European oak forests. The differences in the assemblages of *Phytophthora* spp. encountered in Turkey and in the European studies are probably due to differences in site (topography), climatic and biogeographic factors between Turkey and Europe. It was somewhat surprising that *P. cinnamomi* was only detected at one site in Turkey (site 25, Belgrade forest). *P. cinnamomi* was reported to be involved in the
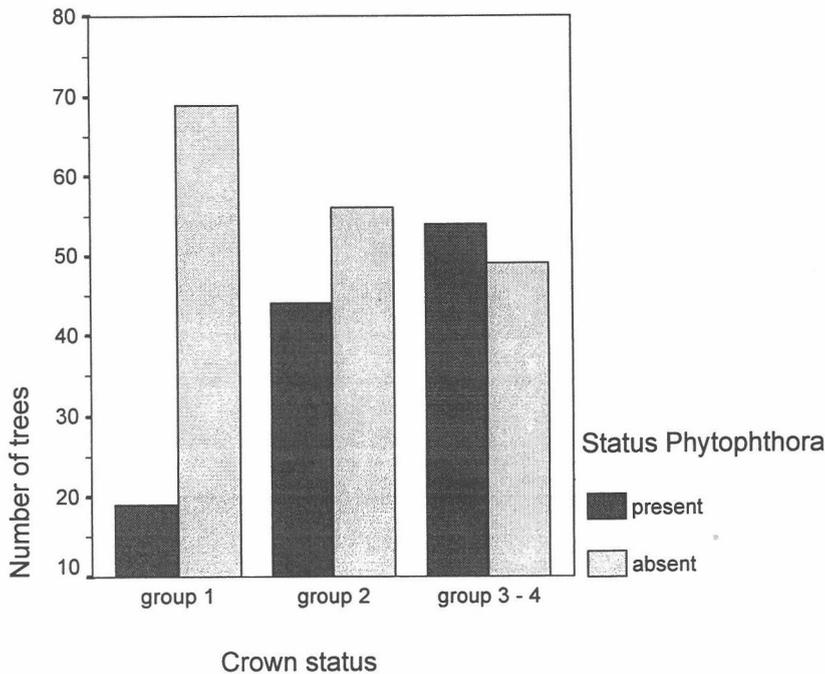
Fig. 2. Occurrence of *Phytophthora* spp. related to crown status.
Group 1: crown transparency < 15 %, group 2: crown transparency = 15 - 35 %, group 3 and 4: crown transparency > 35 %.

decline of *Q. suber* and *Q. ilex* in Spain and Portugal (Brasier *et al.* 1993) and we thus expected to encounter it more frequently, especially in parts of Turkey with a Mediterranean climate. The isolation results clearly show that *P. cinnamomi* does not appear to play a role in the decline of oak in Turkey.

It is noteworthy that *P. citricola* (type C), a species which is easily recognisable, was only once recovered in the Asian part of Turkey (site 38), while the other forms of *P. citricola* (types A and B) were only detected in the European part of the country. Types A and B of *P. citricola* were also recovered in oak forests in Austria (Balci *et al.* in prep.). Based on isozyme analyses, Oudemans *et al.* (1994) showed that *P. citricola* can be divided into five groups and some of these groups show host specificity. Isolates of *P. citricola* from Turkey could be divided at least into three types which support the view that this widespread species is separated into genetically and morphologically distinct types which form a species or subspecies complex. We therefore conclude that further investigations are needed to clarify the taxonomy of *P. citricola*. *P. cambivora* was commonly encountered in oak forests in Germany (Jung *et al.* 1996, 2000). In our study *P. cambivora* was only once recovered from *F. orientalis* in a mixed beech-oak forest (site 47, Kepsut), but not from oak trees in the same forest or from any other oak sites in Turkey (Table 1). This is consistent with results of Hansen and Delatour (1999) who did not isolate *P. cambivora* in oak forests in north-eastern France. However, in Italy Vettraino *et al.* (2000) encountered *P. cambivora* very frequently from chestnut but only once from an oak tree. Likewise, we rarely detected *P. cambivora* in a survey of Phytophthoras occurring in oak forests in Austria, notwithstanding similar sampling and isolation procedures were applied in the studies in Turkey and Austria (Balci *et al.* in prep.). *P. cambivora* is mainly known from chestnut and beech trees in Europe (Erwin and Ribeiro 1996) and probably shows host specificity or is only locally distributed. *P. gonapodyides* was only once isolated from a wet site with puddles which confirms that this species is common on sites with higher water level or in streams and where water accumulates in forest after heavy rain (Hansen and Delatour 1999).

P. quercina was the most common species both in the Asian part of Turkey and in Thrace. It was also the most frequently isolated

Phytophthora species in the European studies (Jung *et al.* 2000, Delatour this volume). Three new hosts (*Q. vulcanica*, *Q. hartwissiana* and *Q. frainetto*) of *P. quercina* were detected in the course of our studies in Turkey. Its common occurrence even on the endemic species *Q. vulcanica* which shows a local distribution in a mountain climate zone at 1600 m a.s.l. is particularly noteworthy. The widespread occurrence of *P. quercina* in oak ecosystems in Turkey and even on oak species with locally restricted distribution range clearly confirms a strong association of *P. quercina* with oak and suggests that this species is naturally distributed in oak forests of Europe and parts of Asia. It seems extremely unlikely that *P. quercina* was introduced into oak ecosystems in Turkey, because planting of oak seedlings is not a common silvicultural practice in Turkey. Furthermore, the high frequency of *P. quercina* in natural oak ecosystems that have not been influenced by human activities as well as in unmanaged sites support the view that *P. quercina* is a native pathogen of oak. In addition, *P. quercina* proved to be host-specific to the genus *Quercus* in pathogenicity tests (Jung *et al.*, 1999b).

P. quercina showed considerable adaptations to site conditions, since it was detected on steep upland areas with dry climate, on wet sites as well as in different forest ecological zones with very variable soil conditions (data not shown). In contrast, other species were detected only on moist or wet sites. Furthermore, the repeated isolation attempts one or two years after sampling and initial isolation from soil samples that had been dried for some weeks yielded only positive isolation of *P. quercina* and *Phytophthora* sp. 1. This indicates that this species might be well adapted to dry conditions and could survive for a long time in the soil at unfavourable environmental circumstances. The isolation rate of *Phytophthora* species, especially of *P. quercina*, was high during late dry summer and autumn. This indicates that Phytophthoras were probably more competitive during this dry season because of less activity of microbial antagonists such as bacteria and reduced activity of other organisms (Malajcuk 1983, Shearer and Tippett 1989). This became apparent when soil samples taken from wet sites or from soil profiles with a high portion of organic material were air dried several times in order to obtain pure cultures of *Phytophthora* spp. Due to the high activity of other organisms, initial isolation attempts of

Phytophthoras were often unsuccessful from soil samples of such sites.

The decline of oak in Turkey could be characterised as a progressive dieback. The high isolation frequency of Phytophthoras, especially *P. quercina* from trees with progressing symptoms of dieback suggests that Phytophthoras could possibly be involved in oak decline in Turkey. Jung *et al.* (2000) proposed that a *Phytophthora*-mediated oak decline occurs in central Europe at stands that are characterised by specific site factors (pH and soil type) which favour the development of the disease. The host-pathogen interactions are probably influenced by many predisposing and triggering factors, as previously discussed in the diverse literature on oak decline in Europe (e. g. Delatour 1983; Oleksyn and Przybly 1987; Hartmann *et al.* 1989; Brasier 1993; Luisi *et al.* 1993; Dreyer and Aussenac 1996; Jung *et al.* 2000). Phytophthoras could be a selective evolutionary factor in natural ecosystems (Delatour *et al.* these proceedings) or under some circumstances be the main cause of oak decline, interacting with many other factors, as reported from Spain and Portugal (Brasier *et al.* 1993). Nevertheless, Phytophthoras could express their pathogenic potential more effectively in combination with other physical factors (e.g. lasting drought, flooding) (Shearer and Tippett 1989; Hansen and Sutton 1999; Delatour *et al.* these proceedings). This was recently shown in a drought stress experiment in which damage of roots caused by *Phytophthora* spp. was significantly more severe on seedlings affected by drought (Jung *et al.* this volume). At the sites in Tokat which showed the most severe symptoms of dieback and the highest isolation rates of *P. quercina* drought might have predisposed trees to infection by *Phytophthora* and might have contributed to prolonged disease succession. These sites were the driest among those included in the survey. Notwithstanding, at the sites in Dörtyol (sites 44, 45) a possible influence of silvicultural treatment on soil biology and stand susceptibility is suspected. While *P. quercina* was recovered from half of the oak trees at site 44 (untended stand) it was isolated from all trees at the other stand (site 45) which was thinned three years ago.

As already reported by Hansen and Delatour (1999) and Jung *et al.* (2000) *P. quercina* was isolated from the soil of oak stands that usually do not favour the development of Phytophthoras. Jung *et al.* (2000) emphasise that *P. quercina* is the least demanding species among the Phytophthoras on oak with regard to site conditions. Our isolation results indicate that *Phytophthora* sp.1 is another species which occurs in natural oak ecosystems and is adapted to thrive well in a wide range of sites, including dry sites which are usually not appropriate for most *Phytophthora* species. Therefore, natural ecosystems and undisturbed forest areas should be examined for the presence of Phytophthoras, even if symptoms of disease on trees or on the understorey vegetation are not apparent. Such studies could greatly improve our understanding of the behaviour of Phytophthoras in natural ecosystems. Phytophthoras in natural and undisturbed ecosystems could be considered as a biotic factor contributing to negative selection of genotypes displaying high susceptibility to the pathogen. Other than viewed as serious pathogens, *Phytophthora* species may have contributed to genetic diversity in natural forest ecosystems (Hansen 1999). As the influence of human activities increases, forests may become more susceptible to infection by *Phytophthora* spp. and decline of trees may become apparent. The *P. quercina* – oak pathogen – host system may be a model to study and compare the interactions between Phytophthoras and their host trees in natural and managed and other human-influenced forests.

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Phytophthoras and Oaks in Europe

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Abstract   An approach to the study of decline processes in oak was developed, integrating long-term ecological dynamics and short-term ecophysiological processes. Extensive forest surveys showed that many *Phytophthora* species, *P. quercina* (41% of 58 positive sites); *P. citricola* (38%); *P. cinnamomi* (17%); *P gonapodyides* (16%); *P. cambivora* (16%); *P .europea* (14%); *P. megasperma* (10%), *P. cactorum* (7%); *P. psychrophylla* (2%); and *P cryptogea* (2%) were detected in the soil of European oak forests. *Phytophthora* spp. were not detected at half the sites. These sites were usually located on the most acid soils where, nevertheless, decline could be severe. In soil infestation experiments on 2 year-old oak seedlings, root reduction by *P. cinnamomi* and *P. cambivora* was up to approximately 50-60% within 3-4 months; compared with *P. quercina* (up to 50%). Damage by other species was generally lower, but occasionally high. Dramatic impairment in water relations of seedlings only occurred when roots were very severely reduced. In central Europe where decline is more prevalent, *P. quercina* could be involved in some atypical symptoms observed. In oak forest, Phytophthoras may act as an important selective pressure parameter. Specific conditions are required for a significant disease impact.

Introduction

Oak forest in Europe

Europe extends from the Arctic to the Mediterranean Sea, and from the Atlantic Ocean to the Urals. High mountains such as the Alps and the Carpathians are also present. Thus the European continent has a variety of climatic conditions and consequently a variety of forest types. From the north to the south, three principal forest ecosystems can be identified. In the northern regions, the boreal forest consisting primarily of conifers, covers a wide area in Scandinavia and Russia. In the middle regions there are large forests of deciduous trees where oaks (*Quercus robur* and *Q. petraea*) and beech (*Fagus silvatica*) are prevalent. At the southern limits of Europe there is the Mediterranean forest where holm oak (*Q. hex*) and cork oak (*Q. suber*) are prevalent. Broadleaved trees account for about 50 % with the exception of the boreal area (less than 10%). In addition to the four oak

species mentioned above (Fig. 1), there are about 10 other oak species, including *Q. pubescens* (west, central and south), *Q. cerris* (Italy and Balkans), *Q. frainetto* (Iberian, south Italy, Balkans). Another important Fagaceae species is sweet chestnut (*Castanea sativa*) which grows in the Mediterraneans and west France.

Management of oak forest

Like other European forests, oak forest are regularly managed by foresters, and have been for a long time. This is especially so for the pedunculate and sessile oaks which produce very high quality timbers at 150-200 years old (1 cubic meter = 600 EURO = 500 $; 100,000 $ per hectare). Other oak species are less intensively managed but they are very important for the landscape and environment. Cork oak is specifically cultivated for cork production.

Oak diseases

Not considering insect defoliations which in some years can be very damaging, oaks my suffer greatly from oak mildew. The fungus (*Microsphaera alphitoides*) invaded Europe at the beginning of the 20 century. Root rot fungi may also impair oaks in specific situations as it is for *Collybia fusipes* whereas in comparison Armillarias do not seem to be important. A number of stem rots can also decrease the wood value (*Fistulina hepatica, Laetiporus sulfureus*, etc). In the Mediterranean, oaks may suffer much from *Biscognauxia mediterranea*.

Oak decline

Decline of mature oaks was described a long time ago in Europe (Delatour 1983 ; Fig. 2). It occurred repeatedly during different periods, and in different forests. It was a big concern to foresters in regions where many oaks in a forest declined and died within a few years. Another situation is when oaks exhibit chronic symptoms of decline with a few trees dying. The latter situation is presently quite frequent in Europe in mature oak forest, at least in some places, as it can be seen through the European monitoring program started in 1986 (Eichhorn and Pan 2000).

'Oak decline' is often a disputed concept. Indeed, many very different detrimental factors are usually present in the declining forests. The symptoms of decline are not helpful in identifying the cause because they are very general and non-specific. They basically consist of progressive dieback of twigs and branches. Associated or additional

symptoms can also be present and include occurrence of epicormic shoots, leaf clusters, reduced size and possible yellowing of leaves.

In the eighties a severe oak decline that occurred on mature trees in the high forest of Tronçais (center of France) was analyzed. Pedunculate oak died back, but not sessile oak, even when they occurred in a mixed stand. Decline and mortality developed most severely on the poorest and acidic soils, and on waterlogged soils. In 1980, dendro-chronological analyses showed that the severe drought that occurred in 1976 had reduced much of the radial growth of both oak species. Several situations were differentiated for radial growth in the trees still present. Sessile oak recovered in growth earlier than pedunculate oak. Other pedunculates did not recover in growth, some having very poor but even increments, and some with increasingly poor increments. Pedunculates with all these types of reduced growth also displayed crown symptoms. while any sessile oaks present showed slight symptoms. Pathologists showed that on non-waterlogged soils, oaks for which growth did not recover suffered from severe root rot by *Collybia fusipes*. Overall Levy *et al.* (1994s in Tronçais, was induced by a drought with the help of *Collybia* in some places. This was the result of it being planted on unsuitable sites and maintained artificially by humans for more than 100 years (Becker and Levy 1982). The 'Tronçais scenario' was considered quite general and valid for many forests, at least in France where pedunculate oaks are frequently grown beyond their ecological range. It also illustrated that different factors could participate in decline of oaks. Since this time the ecological and ecophysiological status of oaks, and *Collybia fusipes*, has been studied more intensively in France.

Phytophthoras — background.

The only damaging *Phytophthora* disease that has been present in European forests for a long time is the ink disease caused by *Phytophthora cinnamomi* or *P. cambivora* (Grente 1961). It has killed many chestnut trees since its introduction in the Mediterranean. A similar disease on red oak (*Quercus rubra*) also caused by *P. cinnamomi* was described in south west France (Barriety *et al.* 1951). It causes a very damaging stem bark canker that grows upwards to several meters height. No decline or death of trees is associated with this disease. Other oak species can be also infected. There has been a considerable amount of research on *P.*
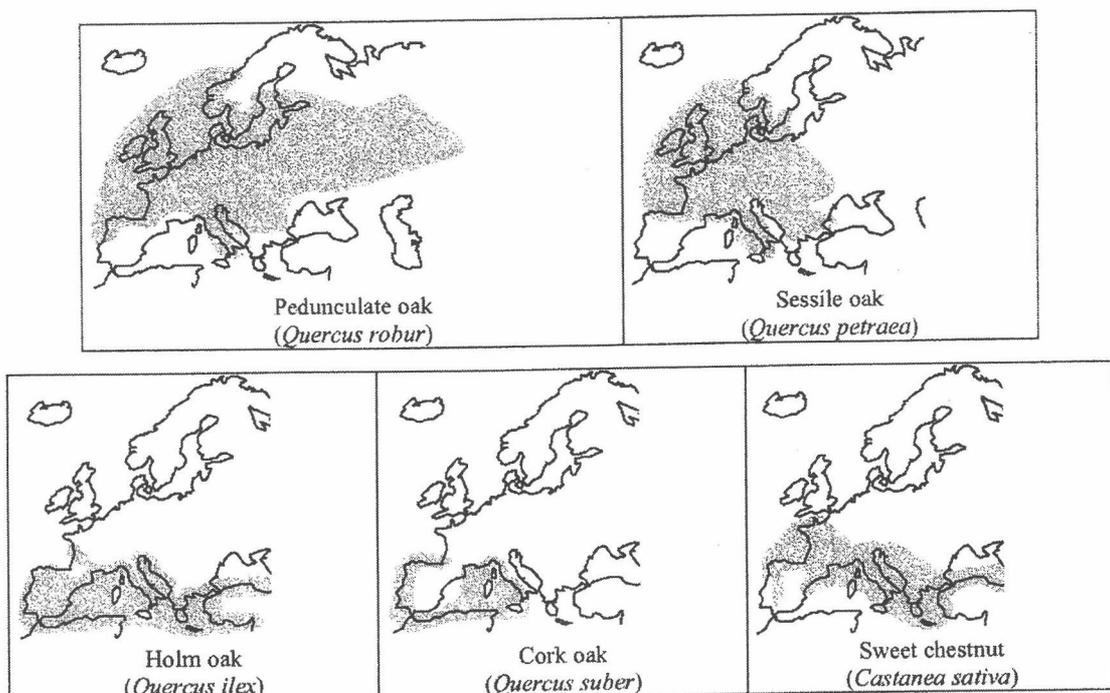
Fig. 1. Distribution of four oak species and of chestnut in Europe (from to Becker *et al*. 1982)
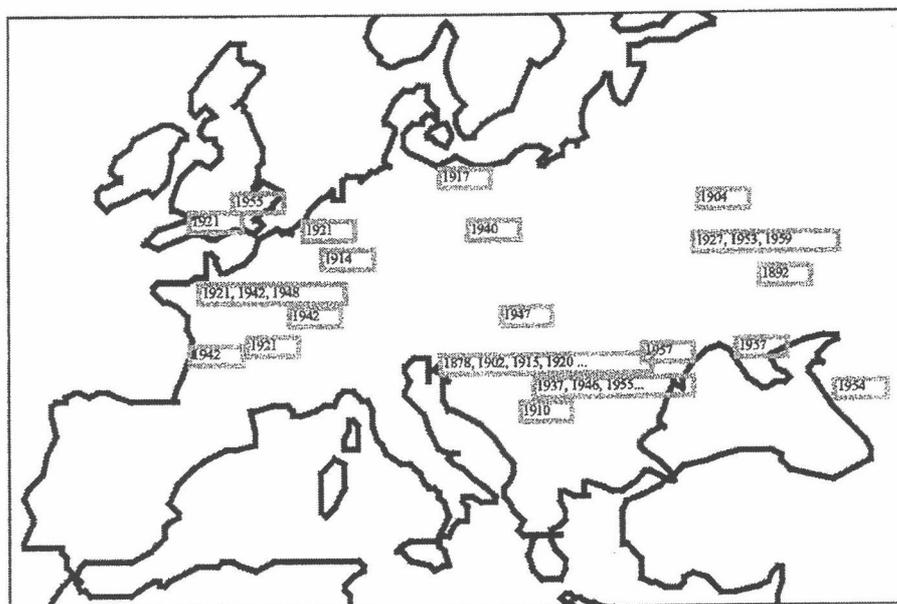
Fig. 2. Occurrence of decline of Pedunculate and Sessile oaks throughout Europe until the 1960's (from Delatour 1983).

cinnamomi in France in the last decade (Robin *et al.* 2001).

Two more recent events have further stimulated European research on the relation between Phytophthoras and decline of oaks.

Phytophthora cinnamomi in Iberia: Cork oak decline has been known in Portugal for many years. However, an unusual intensive decline and mortality occurred between 1980-90 in Portugal and Spain, in association with a series of unusually dry years. Brasier (1992) showed that *P. cinnamomi* was associated with that decline. A European research project

('Phyode') studied this question between 1995 and 1997.

Phytophthoras in central Europe: In Germany, Blaschke (1994) hypothesized the involvement of *Phytophthora* spp. in oak root damage, based on histopathological evidence. Subsequent work (Jung *et al.* 1996) showed that a number of Phytophthoras, including the new *species P. quercina* (Jung *et al.* 1999) present in the soil of oak forest was associated with decline of mature trees. A European research project ('Pathoak') was launched in 1998. It was aimed at increasing understanding of the role of Phytophthoras in oak decline in France, south UK, Germany and Italy. The main results obtained are presented here and elsewhere in these proceedings.

Are Phytophthoras present in the soil of oak forests?

Numerous sites were investigated throughout Europe (i) 27 sites before 1996 (Jung *et al.* 1996); (ii) 107 sites between 1998 and 2001 ('Pathoak'). Detection of Phytophthoras was attempted by baiting soil samples flooded with water with tender young leaves of pedunculate oak. Phytophthoras were detected in the soil at about half the sites investigated (Table 1). Eleven species were detected (including 3 new species *P. europea, P. psychrophila* and *P. uliginosa* [Jung *et al.* 2001]), and some isolates were 'unidentified'. *P. citricola* and *P. quercina* were the most frequent species (Table 2). Several species of *Phytophthora* were detected at individual sites, or below individual trees, in some cases abundantly (Hansen and Delatour 1999). When sampled repeatedly, some sites were positive or negative, or resulted in different species of *Phytophthora* on different occasions. This suggested fluctuation in the *Phytophthora* species over time.

Phytophthoras were detected across a wide range of ecological situations. However, in the most acidic soils, Phytophthoras were absent or infrequent (Fig. 3) and below a pH (H_2O) value of about 4 no *Phytophthora* was isolated. As a rule, Phytophthoras were widespread across a large geographical area and no clear limits can be stated at present. The only exception is *P. cinnamomi* which is typically located in the south and west of Europe (Fig. 4). It is worth to noting that no stem bark symptoms were observed in the forests investigated except where there was an association with *P. cinnamomi,* or occasionally *P. citricola.*

Pathogenicity of Phytophthoras

Damage to fine roots was assessed in several soil infestation experiments performed in different laboratories. *Quercus robur* seedlings (2 years old) were common to all the experiments; substrate was basically a peat-sand mixture. Inoculum was added to the substrate as millet seeds infected in the laboratory. One to 6 floodings were applied depending on the experiments which lasted 3 or 4 months. Assessment of root damage was done visually or by measuring different root parameters (dry weight, number and length of roots, number of tips, and combination of that parameters ; as detailed in Jung *et al.* [2000a, b]).

No plants died and the seedlings generally grew quite well whatever the situation of the roots. As compared to the controls, roots were usually reduced in the seedlings in the presence of the *Phytophthora* species tested. Results were quite variable

Table 1. Frequency of oak forest sites with *Phytophthora* present in the soil.

Reference Country	Number of sites		Positive sites (%)
	total	*Phytophthora* present	
Jung *et al.* 1996			
Germany	18	17	94
Switzerland	1	1	-
Slovenia	1	1	-
Hungary	3	3	-
Italy	4	4	-
Pathoak 2001			
France	60	30	50
Great Britain	17	19	53
Italy	30	19	63
Total	134	84	63

Table 2. *Phytophthora* species detected in the soil of oak forests

Phytophthora species	Country (No. of positive sites)								
	Germany (17)[a]	Switzerland (1)[a]	Slovenia (1)[a]	Hungary (3)[a]	Italy (4)[a]	France (30)[b]	Britain (9)[b]	Italy (19)[b]	Total (%)
citricola	13	1		2	1	11	3	13	41(49)
quercina	5			1	3	13	5	6	33(39)
gonapodyides	3					6	4	2	15(18)
cambivora	1					3	2	4	10(12)
cinnamomi						5		5	10(12)
europaea	1					8			9(11)
cactorum	2		1					4	8(10)
syringae						6		2	8(10)
megasperma						5		1	6(7)
psychrophila	1					1			2(2)
cryptogea								1	1(1)

[a]Jung et al. 1996, [b]Pathoak 2001.
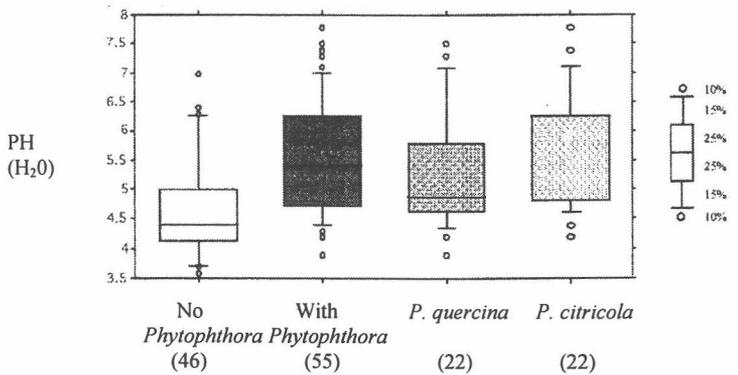
Fig. 3. Distribution of forest sites with or without Phytopthoras according to the soil pH (number of sites in parenthesis)

between the experiments but the *Phytophthora* species were widely different in pathogenicity as summarized in Fig. 5 for *Quercus robur*. As expected, *P. cambivora* and *P. cinnamomi* induced the highest root reduction. Similar root reductions were also produced by *P. quercina* and *P. citri*cola. The other *Phylophthora* species were less or only occasionally damaging. In addition to the fine root reduction, bark lesions were observed on thicker roots and taproots associated with *P. cinnamomi* and, occasionally with *P. quercina*.

Other experiments using stem bark inoculation of seedlings or excised logs, showed that *P. cambivora* and *P. cinnamomi* were the most aggressive species to bark, *P. citricola* was moderately aggressive, and *P. quercina* not aggressive to bark (Brasier and Kirk 2001; Pathoak 2001).

No major difference in susceptibility was detected between the oak species tested, including *Quercus rubra,* with the exception of

Q. ilex which was the most susceptible to the aggressive Phytophthoras. This result agrees with the earlier observations that *Q. ilex* is more susceptible to *P. cinnamomi* than *Q. suber* (Tuset et al. 1996 ; Robin et al. 1998). However, it is important to note that the susceptibility of oaks to *P. cinnamomi* was much lower than that of *Castanea sativa* (Robin et al. 1998).

Are Phytophthoras associated with crown symptoms and root damage of oaks in forests?

Three types of relationships were considered-

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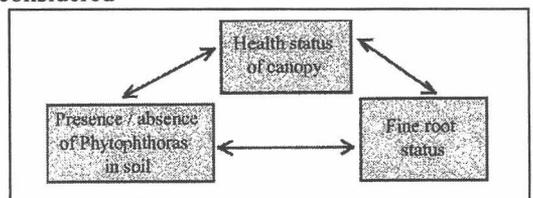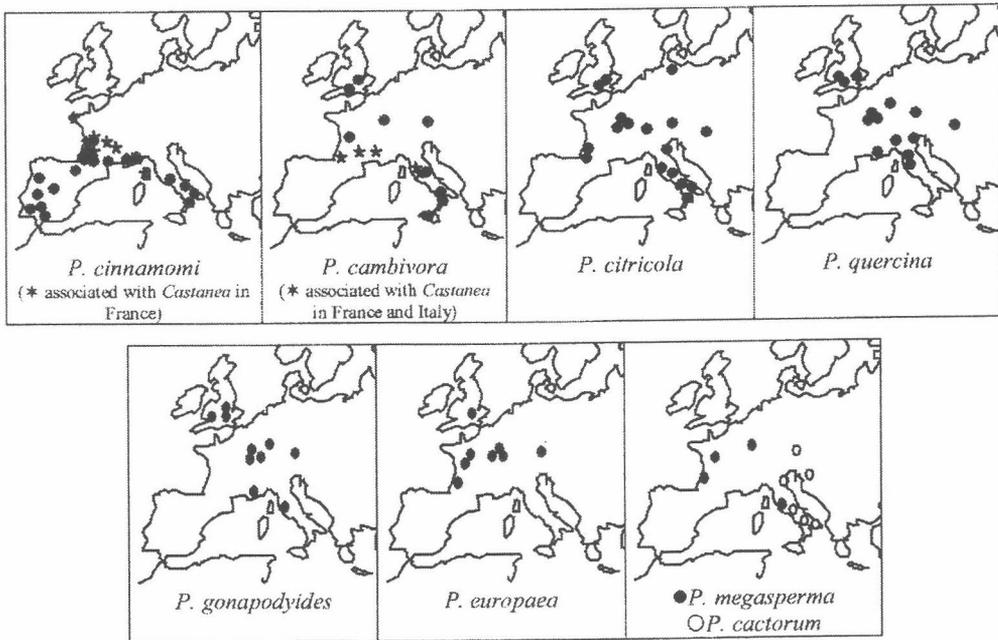
Fig. 4. Distributions of Phytophthoras in oak forests in Europe. (Compiled from: Brasier 1992; Phyode 2nd report 1995; Jung *et al*. 1996 Robin etal. 1998; Levy 2000; Vettraino *et al*. 2000; Pathoak final report 2001; Morel *et al*. 2001)
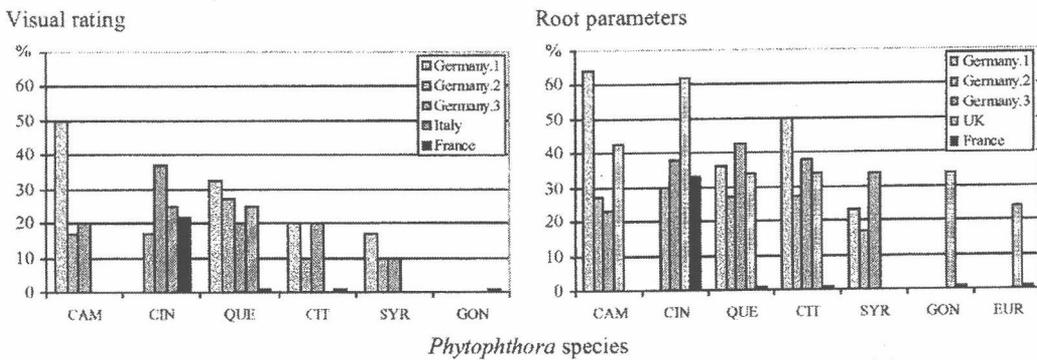
Fig. 5. Root loss in *Quercus robur* seedlings in the presence of Phytophthoras in soil (% of the control; mean values estimated from several experiments assessed in different ways. (Compiled by Pathoak 2001). CAM *Phytophthora cambivora*; CIN *P. cinnamomi*; QUE *P. quercina*; CIT *P. citricola*; SYR *P. syringae*; GON *P. gonapodyides* EUR *P. europaea*

Health status of canopy was assessed visually as the percentage of defoliation, according to the European system (Anon. 1997). Trees rated below 25% defoliation were rated as healthy, and those with more than 25% defoliation as declining. Fine root status was assesses on the roots present in soil samples collected around each tree at a distance of 40-150cm from the stem base, to a depth of about 20cm. Only roots more than 5mm in diameter were included. Fine roots were quantified according to Jung *et al*. (2000a,b)

Relation between health of canopy and presence of Phytophthoras in soil

Considering all the *Phytophthora* species together no general relationship was found between their presence in the soil and the health of the canopy. In Bavaria however

In Germany (Bavaria) (Jung et al., 2000ab) 124 oaks in 19 stands	In other countries (Pathoak, 2001)
	France : 433 oaks in 60 stands UK : 157 oaks in 17 stands Italy : 141 oaks in 30 stands
	No relation was found

6a- all Phytophthoras

6b- *Phytophthora quercina*
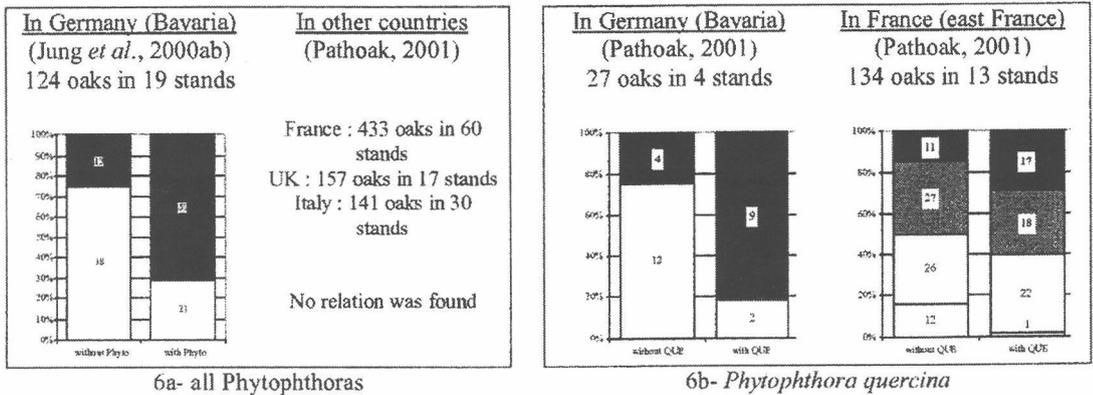
Fig. 6. Distribution of healthy (❸) and declining (v) oaks according to the presence of Phytophthoras in the soil. In each pair of bars the left hand side is without the pathogen(s) and the right hand side in the presence of the pathogen.
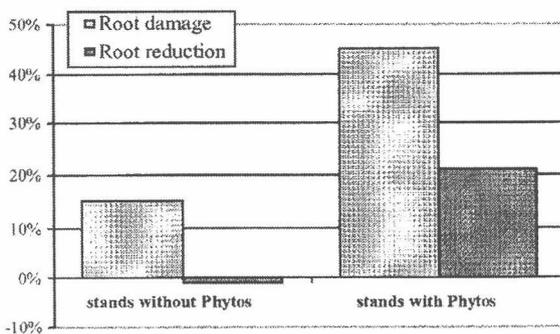
Fig. 7. Fine root status in declining oaks in Bavaria, Germany, in stands infested or not infested with Phytophthoras. (Compiled from Jung *et al.* 2000a b. Relative values as compared to healthy trees (16 stands with phytophthoras and 19 stands without; 47 healthy and 46 declining oaks, and 59 healthy and 65 declining oaks respectively; 'root damage' was assessed visually; 'root reduction' is the average value of the 4 root parameters measured by the authors).

Phytophthoras were detected significantly more often below declining oaks than healthy oaks (Fig. 6a). In stands in Bavaria where *P quercina* was present, a significant relationship was found and there was a similar trend in east France (Fig. 6b). Apart from this, in France no relationship was found for *P. citricola* (121 oaks in 11 stands), *P. europaea* (106 oaks in 8 stands) and *P. syringae* (112 oaks in 6 stands).

Fine root status of declining oaks.

In stands where Phytophthoras were present in Germany (Bavaria), fine root stratus of declining oaks was consistently poorer than that of healthy oaks (Jung *et al.* 2000a,b; Fig. 7). Results were similar in the stands investigated in France and Italy, but with erratic differences between declining and healthy trees in some stands (Pathoak 2001). In stands without Phytophthoras present in the soil (only investigated in Germany), declining and healthy oaks were not significantly different for fine root status (Fig. 7).

Fine root status of oaks with or without Phytophthoras

In stands with Phytophthoras present, fine root status was also poorer in oaks with Phythophthoras than in those without, in Germany (Jung *et al.* 2000ab) and in Italy (Pathoak, 2001). In France, however, no consistent differene was detected when considering the situation of pedunculate oaks with *P. quercina, P. syringae* or *P. cinnamomi*, (Pathoak, 2001). In general it was clear that presence of Phytophthoras in the soil of a stand, or below individual trees, was often associated with a reduced amount of roots.

How damaging is root reduction to oak health?

This question was examined experimentally on seedlings (3 years old) in pots with *P. cinnamomi* added to the substrate.

Table 3. Effect of root infection by *Phytophthora cinnamomi* on oak and chestnut saplings

	Sweet chestnut	Holm oak	Pedunculate and red oak	Hybrid chestnut
Root loss (% biomass)	100	64	34-36	~0
Predawn leaf water potential		reduced	not affected	
Leaf nutrient content		Decreased N and P	not affected	
Biomass partitioning		Increased leaf mass ratio	not affected	
Mortality	Up to 100%	None at 70% root loss	none	none

Several, parameters were monitored for one year after infection (Table 3). In addition to oak species, sweet chestnut and hybrid chestnut (*Castanea crenata X C. sativa*) were included in the experiment as highly susceptible and resistant species, respectively.

Results showed that pedunculate and red oaks did not suffer significantly from root infection, as compared to the highly susceptible holm oak in which several plant parameters were impaired, even if there were no seedling deaths (Table 3).

Conclusion

The general concept of "Oak decline" does not correspond to a unique phenomenon. Indeed, in some stands decline consists of a quick death of many trees within a few years. In others, decline is chronic and develops over many years or decades ; death of the defective

For a pathologist, it is important to be aware that pathogens are only one of the factors that may impair the oak ecosystem. An example of a root pathogen involved in the long lasting process of oak decline, in interaction with site conditions, is with the root rot fungus *Collybia fusipes*. In France, work of Marçais and colleagues showed that the pathogen is widespread in oak ecosystems where it may be responsible for the severe reduction of root systems. It has been shown that severity of infection and the subsequent impact on tree health, including death, strongly depends on the ecological site conditions, and Collybia-oak-decline occurs mainly on *Q. robur* (Marcais *et al.* 2000 ; Piou *et al.* 2001; Camy and Marçais 2001)

Another example of such an interaction is with the littleleaf disease of

trees is only occasional. In a number of such cases of "oak decline", caterpillar defoliation or unusually severe droughts were clearly a major cause. Especially in the case of drought, decline severity was also strongly modulated by other factors, such as soil conditions, and was not consistent across oak species.

Not presented in the present paper, recent results obtained by carbon balance simulation showed that tree dynamics, then death, are paralleled by decreases in stored and remobilised carbon. Declining trees suffered a large imbalance in carbon, and the hypothesis put forward is that tree death occurs once stored carbon decreases below the cost of new early wood and leaf construction during spring. In addition, the water use efficiency was lower in *Q. robur* than in *Q. petraea*, showing their different ecological behaviour (Pathoak 2001).

shortleaf pine (*Pinus echinata*) in the southern United States (Hansen 2000). The disease developed in a context of nutrient deficiency on eroded soils with poor internal drainage where stressed trees were unable to efficiently replace rootlets killed by *P. cinnamomi*.

Reviewing the evidence on Phytophthoras on oaks in Europe- what is now their status with regard to the oak decline story? As several species of *Phytophthora* are widespread in many oak forests in Europe, whether declining trees are present or absent, it is difficult to conclude that *Phytophthora* is a general threat to all forests. However, the situation of some particular species must be examined more specifically.

The introduced species *P. cinnamomi* and *P. cambivora* were detected in oak forests and their pathogenicity to oak roots and bark

confirmed. Interestingly, *P. cinnaniomi* is distributed only in the parts of Europe where frost does not limit its development. There are many arguments for its involvement for the recent decline and death of the Mediterranean oak species in the Iberian countries and some parts of France. *P. cambivora* is present across a large area. No evidence of decline of oaks was obtained in association with *P. cambivora* but this species is most often associated with chestnut deaths in Italy (Vettraino *et al.* 2000).

Among all the Phytophthoras, *P. citricola* and *P. quercina* are the species most frequently detected in a large part of Europe. Both are pathogenic to fine roots of oaks, but *P. quercina* is not pathogenic to stem bark. There is evidence of an association in oak forests between the presence of *P. quercina*, or of *Phytophthora* in general, and a deterioration of fine roots and the tree canopy. This type of association, and the wide distribution of *P. quercina* focuses attention on that species. However evidence obtained from forest conditions cannot be interpreted directly as cause-effect relationships and many questions remain.

The amount of root damage an oak may suffer before showing a significant impact on health is not known. Seedlings may bear considerable root loss without major disturbance of their development. Root loss in mature oaks must be examined differently as effects are cumulative over many years. However we poorly understand the fine root compartment in oak forests, together with its associated soil microbes, *Phytophthora* and other fungi including mycorrhizal species. It is particularly important to understand how parasites contribute to the fine root turnover and to determine the level at which turnover becomes detrimental for an oak in a given environment. Information on the deep root system of oaks is also lacking. In the case of *P. cinnamomi* on *Eucalyptus* it was shown that it was important to consider damage to the vertical roots especially in relation to the tree's reaction to drought conditions (Shea *et al.* 1983). *P. cinnamomi* and *P. cambivora* are able to cause root bark damage. Thus those species could be potentially more harmful to trees than species such as *P. quercina* that are able only to destroy fine roots. Finally we do not know the relative importance of chronic destruction of fine roots and increase of turnover which concerns food management, compared with destruction of part of the root system concerned with transportation of nutrients and water.

For Phytophthoras to threaten the health of oaks drastically, involvement of other factors is probably required. Water relations were investigated on oak seedlings inoculated with *P. cinnamomi*. Despite root infection resulting in effects resembling water stress, limitations of water loss in leaves allowed infected trees to survive root losses, even in susceptible host species. In a forest however, because of their reduced capacity to explore the soil and extract water, infected adult trees would be more vulnerable to drought. Such a *Phytophthora* impact was probably illustrated by the cork oak and holm oak decline and mortality that occurred in Iberia in conjunction with the severe droughts of the 80's (Brasier 2000).

Climate change and anthropogenic nitrogen in put are of concern (Jung et al. 2000a). When nitrogen input into forest soils in Europe increases it is difficult to forecast the balance between positive and negative effects, and experiments suggest that the different *Phytophthora* species could react differently. Large differences can also exist between sites and parts of Europe. Nitrogen emission varies from more than 100 kg/ha in south Germany to less than 40 kg/ha in most parts of France (EMEP 1998). For climate change, forecasting is similarly difficult. The only documented effect is for *P. cinnamomi*. With rising temperature there is the potential for enlargement of its range to the north of Europe (Brasier 1996).

Whether they are introduced or endemic, Phytophthoras in the soil of oak forests may bring a selective pressure on oak ecosystems, especially in the reduction of the amount of fine roots and contribute to a long lasting process of health decline of oaks. However it is difficult to draw a general picture for Europe where oak forests are very diverse in many respects: -the oak species present, ecological situations, history of the stands, silviculture etc. In many oak stands trees are quite old, -more than 150 or 200 years, and derived from coppice with standard stands transformed gradually in high forest, and no oak forest can be considered as genuine natural forest in Europe.

In the case of the *Phytophthora* species involved in fine root reduction, but harmless to main roots or stem bark, we can conclude that they may contribute to the oak decline process in certain circumstances. Accordingly, foresters cannot consider that oaks are resistant to everything. They will have

to grow and manage the different oak species more cleverly than before taking in account the respective ecological requirements and the local site potentialities. That way, oak forests in Europe will be in a better condition to challenge the coming environmental change, and many instances of decline of oaks may be avoided.

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Effect of environmental constraints on *Phytophthora* – mediated oak decline in Central Europe

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Abstract. *P. quercina, P. citricola, P. cambivora* and 3 other *Phytophthora* species *in vitro* produced significantly higher numbers of sporangia with increasing nitrate concentrations in soil leachate with nitrate concentrations of 2, 25, 50 and 100 ppm. The effects of different nitrate concentrations (0, 50 and 100 ppm) in the flooding water and drought stress on root damage of *Quercus robur* saplings by *P. cinnamomi, P. quercina, P. cambivora, P. citricola* and *P. syringae* aff. were studied in two separate soil infestation tests. In both experiments non-inoculated control plants showed almost no root damages, while *P. cinnamomi* and *P. quercina* were the most aggressive pathogens. Root condition of oak was significantly more influenced by *Phytophthora* infection than by drought stress alone. All *Phytophthora* species were able to survive and produce root damage under repeated drought stress. Root damage caused by *P. cinnamomi* and *P. quercina* tended to be more severe when interacting with drought. Increasing nitrate concentrations stimulated root growth of non-inoculated control plants, and increased the difference in fine root length and number of root tips between non-inoculated oak seedlings and those growing in *Phytophthora*-infested soil. The potential implications of these results for the etiology of the disease in the field is discussed.

Introduction

Since the beginning of the twentieth century oak decline has been a serious and frequently recurring disease of European oak forests (Delatour 1983; Oleksyn and Przybyl 1987; Hartmann *et al.* 1989). Being an episodic phenomenon of local or regional importance in the past, oak decline in its current phase has been progressing since the beginning of the 1980's, and is widespread in Europe (Delatour 1983; Hartmann *et al.* 1989; Siwecki and Liese 1991; Luisi *et al.* 1993). Above-ground symptoms include dieback of branches and parts of the crown, formation of epicormic shoots, high transparency of the crown, yellowing and wilting of leaves and tarry exudates from the bark (Siwecki and Liese 1991; Luisi *et al.* 1993), all symptoms indicative of water stress and poor nutrition.

Several studies have observed deterioration of oak fine roots in Central and Western Europe (Näveke and Meyer 1990; Vincent 1991; Eichhorn 1992; Blaschke 1994; Jung 1998; Thomas and Hartmann 1998). Isolations from fine roots and rhizosphere soil samples revealed the widespread occurrence of several *Phytophthora* species including *P. cactorum, P. citricola, P. cambivora, P. gonapodyides*, and the new species *P. quercina* in 29 out of 33 stands investigated in 6 European countries (Jung *et al.*1996; Jung

1998). In Northern France, Galoux and Dutrecq (1990) isolated a *Phytophthora* species from fine roots of declining oaks, and Hansen and Delatour (1999) demonstrated a diverse *Phytophthora* population including *P. quercina* in oak forest soils. In Bavaria, a survey was made on the occurrence of soil-borne *Phytophthora* species and the fine root status of 217 healthy and declining mature trees of *Quercus robur* and *Q. petraea* in 35 oak stands on a range of geologically diverse sites (Jung *et al.* 2000a,b). The results showed that depending on the site conditions at least two different complex diseases are referred to under the name 'oak decline'. *P. quercina, P. cambivora* and *P. citricola* were common on sites with a mean soil-pH (CaCl$_2$) $\geq$ 3.5 and sandy-loamy to clayey soil texture and seven other *Phytophthora* species were infrequently isolated from rhizosphere soil. In these stands highly significant correlations existed between crown transparency, fine root condition and the presence of *Phytophthora* spp., especially *P. quercina*. In contrast, in stands with sandy to sandy-loamy soils and a mean soil-pH $\leq$ 3.9, *Phytophthora* spp. were not found, and correlations between crown transparency and various root parameters were either less significant or not significant. Jung *et al.* (2000*b*) concluded that *Phytophthora* species are strongly involved in oak decline on sandy-

loamy to clayey sites with a mean soil-pH ($CaCl_2$) $\geq$ 3.5, and defined this widespread decline type as '*Phytophthora*-mediated oak decline'

In several soil infestation tests *P. quercina* and *P. cambivora* were the most aggressive species to root systems of young *Q. robur* plants (Jung 1998; Jung *et al.* 1996 & 1999*a*). In another test with various Phytophthoras and a range of broadleaf tree species, *P. quercina* proved to be host specific to the genus *Quercus* (Jung *et al.* 1999*b*).

The long duration and the wide geographical range of the current episode of oak decline suggests that large-scale and long-term environmental changes in Central and Western Europe during the last decades might have imbalanced the host-parasite relationship between oaks and the naturally occurring soil-borne *Phytophthora* species. One major factor certainly is an excess of anthropogenic nitrogen input into forest soils which are now becoming over-saturated with nitrogen (Nihlgard 1985; Kreutzer 1991; Mohr 1994; Thomas and Kiehne 1995). Another potential threat is the continuous increase in mean winter temperatures of 0.03K per year combined with a significant seasonal shift of 20-30% of the summer precipitation into the cold season in Central Europe and parts of Western and Northern Europe since the 1960's (Rapp and Schönwiese 1995; Schönwiese *et al.* 1994). Environmental constraints can increase the susceptibility of a host to root pathogens, and thus indirectly promote pathogen infection. However, they can also favour a pathogen directly. There are examples for both nitrogen fertilization as well as prolonged drought periods enhancing the severity of *Phytophthora* diseases (Schmitthenner and Canaday 1983; Shearer and Tippett 1989; Brasier 1993 & 1996; Erwin and Ribeiro 1996).

The objectives of this experimental study are focussed on the effect of (i) different concentrations of nitrate on the formation of sporangia, and the effects of (ii) different concentrations of nitrate, and (iii) drought stress on root growth and root damage produced by various soil-borne *Phytophthora* species in a defined pathosystem with *Q. robur* saplings.

Methods
Effect of nitrate on sporangial production

For this test 100 g nonsterile luvo-vertic cambisol soil of a natural oak stand in Bavaria was flooded with 1 L demineralised water, and incubated at 19°C in the dark. After 24 hours the soil suspension was filtered, and amended with KNO_3 resulting in a concentration series of 2 (natural concentration), 25, 50 and 100 ppm nitrate. The nutrient contents and the pH of the test solutions are given in Table 1.

Two isolates of *P. quercina* and one isolate each of *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. gonapodyides* and *P. syringae* aff. were used. All isolates except *P. cinnamomi* (*Castanea sativa*, Switzerland) and *P. syringae* (*Fagus sylvatica*, Bavaria) were isolated from oaks in Bavaria. Although *P. cinnamomi* was never recovered from Central European oak forests it was included in this test because of its known ability to occur in acidic soils (Shearer and Tippett 1989). For each isolate two discs (10 mm diam.), cut from the edge of a 5-7 day-old culture growing on malt-extract agar (MEA) at 20°C in the dark, were placed in a 5 cm Petri dish and flooded with the test solutions just over their surface. The petridishes were incubated at 19°C and natural daylight, and the test solutions were replaced after 24 and 48 hours. Measurements of the pH of the discarded test solutions revealed that within 24 hours changes of the adjusted pH values were always less than 0.1. After 72 hours the numbers of sporangia per field in the light microscope at 80 x magnification (= 6.2 mm^2) were counted. For each isolate 10 fields (5 per agar disc) were chosen at random. The significance of differences between different nitrate concentrations was tested using the parametric Student's t-test.

Effects of drought stress and different nitrate concentrations on root damage by Phytophthora *spp.*
Fungal isolates and soil infestation method

Surface sterilised acorns of *Q. robur* were sown into JUMBO rootrainers ® (1 acorn per rootrainer, cell depth 25 cm, volume 1000 mL; Ronaash Ltd., Roxburghshire, Scotland) filled with sterilized potting medium (mixture of vermiculite – sand – peat 1/1/1 v/v/v) . When the seedlings were 2 months old, a vermiculite-millet seed- V8-Juice broth - inoculum of *P. quercina* (2 isolates), and 1 isolate each of *P. cinnamomi*, *P. cambivora*, *P. citricola* and *P. syringae* was

Table 1. Mean contents (ppm) of selected minerals and mean pH of soil leachates used for the sporangia test

NO$_3^-$ conc.	pH (CaCl$_2$)	NO$_3^-$	Cl$^-$	SO$_4^-$	Ca^{2+}	Mg^{2+}	K$^+$	Na$^+$	Al^{3+}	Fe^{3+}	Mn^{4+}	Cu^{2+}
2 ppm	5.1	2.0	1.1	1.7	6.7	0.3	0.7	1.1	0.1	0.1	0.2	0.02
25 ppm	5.1	24.8	1.2	1.8	4.0	0.3	15.7	0.9	0.2	0.1	0.3	0.02
50 ppm	5.1	49.5	1.1	1.8	4.0	0.3	33.9	0.8	0.2	0.1	0.2	0.02
100 ppm	5.1	98.6	0.9	1.6	6.7	0.3	61.5	0.7	0.1	0.1	0.2	0.02

prepared and incubated at 20°C in the dark (Jung *et al.* 1996, 1999*a)*. After 4-5 weeks, the inoculum was carefully mixed, rinsed in running demineralised water to remove excess nutrients, and without manual disturbance of the root system 30 mL of inoculum were added directly sidewise into each JUMBO rootrainer ®. Controls received rinsed uninfested vermiculite-millet seed- V8-juice mixture at the same rate. In both experiments 10 rootrainers per isolate and treatment each containing 1 oak seedling were placed in plastic tubs (40 x 30 x 20 cm) with drainage holes to allow alternating episodes of flooding and drainage. At the end of both experiments reisolations were made from necrotic root tissues, direct plating and baiting soil samples with *Q. robur* leaflets (Jung *et al.* 1996, 1999*a*, 2000*b*).

Experimental design of the nitrogen experiment

Plants were incubated in a growth chamber for 3 months with a temperature regime of 20°C during the day and 12°C at night, then 2 months at a constant temperature of 10°C, followed by 4 months at 20°C / 12°C. Tubs containing the 10 rootrainers per isolate and treatment were flooded once per month for 3 days with demineralised water amended with 0, 50 and 100 ppm nitrate (applied as KNO$_3$). Two weeks after the flooding each oak was watered with 100 mL demineralised water. After each flooding episode mineral elements in the flooding water released from each tub were analysed.

Experimental design of the drought stress experiment

The experiment was conducted over 6 months in a greenhouse at 18°C and 65% relative humidity. The tubs containing the 10 rootrainers per isolate and treatment were flooded once per month for 3 days followed by drainage. While drought stressed seedlings were not watered between the flooding episodes, seedlings growing under non-limited water supply received 100 mL demineralized water 2 weeks

after flooding. The soil moisture was measured with a TDR probe (TRIME-FM, version P2, IMKO Mikromodultechnik GmbH, Ettlingen, Germany) once per week after the first two flooding episodes, and from the third flooding on in monthly intervals one day before the next flooding episode.

Evaluation and statistical analysis

The percentage of root damage was estimated visually after spreading the roots of each oak seedling uniformly on trays (30 x 50 cm) etched with gridlines (2 x 2 cm squares). In addition, the final evaluation of the fine root system (parameters determined: mean total root length and mean total number of root tips per seedling, - referred to as 'root length' and 'number of root tips') was made using the image analysis software WINRhizo, Version 3.9 (Regent Instruments Inc., Quebec, Canada). Experimental data were statistically analysed by one-way ANOVA using Prism 3 (GraphPad, San Diego, CA).

Results
Effect of nitrate on sporangial production

The production of sporangia was stimulated *in vitro* by different concentrations of nitrate added to a nonsterile soil leachate of an oak stand which naturally contained negligible amounts of nitrate (2 ppm), and therefore was used as control. All *Phytophthora* species, and in particular *P. quercina*, *P. citricola* and *P. syringae*, produced significantly higher numbers of viable sporangia with increasing concentrations of nitrate (Fig. 1, Table 2). No stimulation was found with ammonium irrespective of the concentration (Jung *et al.*, unpublished).

Effect of nitrate on root damage of Q. robur seedlings caused by Phytophthora spp. ...

With all nitrate concentrations tested the control plants showed almost no root damages after periodic flooding (Table 3). Root growth of
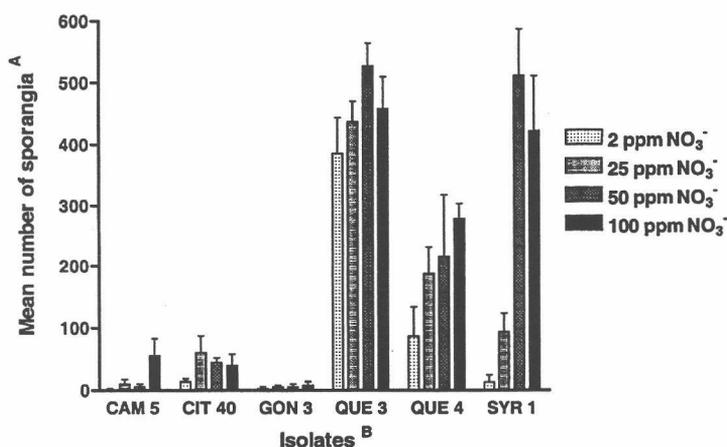
Fig. 1. Influence of different nitrate concentrations on the formation of sporangia of various soil-borne *Phytophthora* species
[A]mean number of sporangia per field in the microscope (=6.2mm^2) formed after 72 h at 20°C in non-sterile soil leachate (pH 5.1). [B]CAM = *P. cambivora*, CIT = *P. citricola*, GON = *P. gonapodyides*, QUE = *P. quercina,* SYR = *P. syringae* aff.

Table 2. Significance[A] of differences between the numbers of sporangia formed by *Phytophthora* spp. at different nitrate concentrations

Isolates[B]	Nitrate concentrations (ppm)					
	2 / 25	2 / 50	2 / 100	25 / 50	25 / 100	50 / 100
CAM 5	**	**	***	ns	**	***
CIN 4	**	***	ns	ns	*	**
CIT 40	***	***	**	ns	ns	ns
GON 3	ns	ns	*	ns	ns	ns
QUE 3	*	***	*	***	ns	*
QUE 4	**	**	***	ns	***	ns
SYR 1	***	***	***	***	***	*

[A] Student's t-Test; ns = not significant, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.
[B]CAM = *P. cambivora*, QUE = *P. quercina*, CIN = *P. cinnamomi*, CIT = *P. citricola*, GON = *P. gonapodyides*, SYR = *P. syringae* aff.

control plants was consistently stimulated by increasing nitrate concentrations in the flooding water as reflected by the increase of root length and number of root tips (Table 3).

In contrast to the control plants, no such stimulation was found in oak seedlings growing in soil infested with *P. quercina*, *P. citricola* and *P. syringae*. However, in oaks growing in soil infested with *P. cinnamomi* and *P. cambivora*, respectively, the condition of the fine root system was improved by increasing nitrate

concentrations even though it remained worse than in the particular control plants.

Amongst all isolates tested both isolates of *P. quercina* (mean root damage ranging from 26 to 38%) together with the *P. cinnamomi* isolate P382 (21 - 30%) were most aggressive to *Q. robur* seedlings. All nitrate concentrations resulted in significant differences in root damage between *P. quercina* and the control, but the parameters root length and number of root tips

Table 3. Effect of different nitrate concentrations in the flooding water on root condition of 1-year-old *Quercus robur* plants growing in *Phytophthora* - infested soil

Nitrate concentration	Isolates[A]						
	C	QUE 96	QUE 67	CIN P382	CAM 1	CIT 58	SYR 6
			Root damage (%)[B]				
0 ppm	5.0	32.5	26.0	30.0	25.5	16.5	19.8
	(2.4)	(20.6)	(15.3)	(12.5)	(14.0)	(15.7)	(16.4)
		**	*	**	*	ns	ns
50 ppm	6.3	37.8	30.0	21.0	13.3	14.0	14.8
	(4.0)	(19.0)	(20.2)	(17.7)	(10.5)	(13.4)	(15.7)
		**	**	ns	ns	ns	ns
100 ppm	6.3	36.5	35.0	28.3	17.8	21.0	16.0
	(4.0)	(18.1)	(23.7)	(15.7)	(15.2)	(12.4)	(15.3)
		**	**	*	ns	ns	ns
			Total root length (cm)[B]				
0 ppm	1542	1270	1193	891.3	1123	1512	1443
	(315.8)	(335.1)	(327.8)	(128.7)	(358.7)	(408.8)	(331.1
		ns	ns	**	*	ns	) ns
50 ppm	1737	1165	1398	1444	1333	1515	1553
	(314.8)	(309.5)	(380.4)	(222.8)	(317.8)	(506.2)	(401.1
		**	ns	ns	ns	ns	) ns
100 ppm	1785	1195	1253	1177	1399	1253	1414
	(428.5)	(335.2)	(382.4)	(308.3)	(387.9)	(347.1)	(380.5
		**	*	**	ns	*	) ns
			Number of root tips[B]				
0 ppm	2866	2380	2251	1468	2107	2620	2654
	(494.6)	(642.1)	(640.2)	(183.5)	(722.7)	(458.1)	(507.9
		ns	ns	**	*	ns	) ns
50 ppm	3076	2214	2532	2624	2476	2558	2805
	(499.0)	(518.1)	(687.5)	(427.6)	(449.1)	(610.5)	(699.3
		**	ns	ns	ns	ns	) ns
100 ppm	3166	2361	2176	2315	2537	2072	2547
	(727.7)	(500.9)	(713.1)	(726.4)	(453.3)	(613.9)	(643.7
		*	**	*	ns	**	) ns

[A]C = control, QUE = *P. quercina*, CAM = *P. cambivora*, CIN = *P. cinnamomi*, CIT = *P. citricola*, SYR = *P. syringae* aff.
[B]Mean, (standard deviation), significance of differences to the control calculated with One-Way ANOVA combined with a Dunnett's post test.

were only significantly different in the 50 and 100 ppm NO_3^- treatment (isolate QUE 96), and the 100 ppm NO_3^- treatment (isolate QUE 67), respectively.

When no nitrate was added to the flooding water, *P. cinnamomi* (P 382) a similar proportion of the root system as the *P. quercina* isolate QUE 96. However, root length and number of root tips were markedly lower than in QUE 96, indicating infections in an earlier stage of the test and, as a consequence, an advanced root rot process. Isolate P 382 produced significant damages also with 100 ppm, but not with 50 ppm NO_3^-.

The *P. cambivora* isolate CAM 1 produced significant root damages in the 0 ppm NO_3^- treatment, and was only slightly aggressive with 50 and 100 ppm NO_3^-, respectively. In contrast to isolate CAM 1, the *P. citricola* isolate CIT 58 caused a significant decrease in root length and number of root tips only in the 100 ppm NO_3^- treatment as compared to the control. *P. syringae* aff. (SYR 6) was only slightly pathogenic with all nitrate concentrations.

Effect of drought stress and infection on root growth
The volumetric soil water content decreased in both treatments from 20% 3 days after

93

flooding to 16% 14 days after flooding (Fig. 2). At this time each oak seedling growing under non-limited water supply received 100 mL demineralised water, while seedlings subjected to drought stress were not watered for 1 month until the next flooding. Consequently, 4 weeks after each flooding the mean water content was significantly lower in the drought stress treatment (7%) than in the non-limited water supply treatment (14%) (Fig. 2).

As expected, root growth of the control plants was slightly reduced by drought stress. However, all root parameters were significantly more influenced by *Phytophthora* infection (especially by *P. quercina* and *P. cinnamomi*) than by drought stress alone (Table 4).
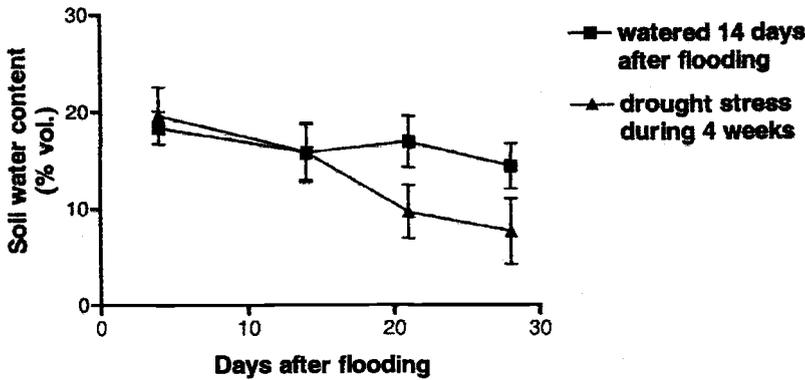
Fig. 2. Development of mean soil water content under different water regimes

Table 4. Effect of different water regimes on root condition of 1-year-old *Quercus robur* plants growing in *Phytophthora*-infested soil

Water regime	Isolates[A] C	QUE 96	QUE 67	CIN P382	CAM 1	CIT 58	SYR 6
Root damage (%)[B]							
water supply not limited	6.3 (4.0) *	29.0 (9.4) *	27.3 (15.5) *	42.8 (26.1) **	25.3 (12.8) *	24.3 (13.4) ns	15.8 (13.5) ns
drought stress	5.0 (1.4) ns	22.3 (14.7) ns	39.3 (18.3) **	58.8 (21.3) **	25.3 (12.0) *	25.0 (19.4) *	21.3 (13.5) ns
Total root length (cm)[B]							
water supply not limited	1956 (688.4) **	1056 (292.7) **	1116 (315.2) **	1190 (528.2) **	1447 (461.2) ns	1210 (380.7) **	1338 (399.1) *
drought stress	1646 (650.2) ns	1400 (416.1) ns	1092 (377.9) ns	890.5 (141.9) *	1089 (387.9) ns	1100 (432.4) ns	1464 (769.4) ns
Number of root tips[B]							
water supply not limited	3375 (1109) **	1995 (539.5) **	1886 (551.9) **	2137 (1094) *	2760 (911.3) ns	2169 (738.0) *	2092 (1038) **
drought stress	2654 (953.3) ns	2728 (832.6) *	1675 (567.2) *	1570 (228.1) *	2084 (610.1) ns	1854 (562.8) ns	2553 (1260) ns

[A]C = control, QUE = *P. quercina*, CAM = *P. cambivora*, CIN = *P. cinnamomi*, CIT = *P. citricola*, SYR *P. syringa* aff.
[B]Mean, (standard deviation), significance of differences to the control calculated with One-Way ANOVA combined with a Dunnett's post test.

P. cinnamomi was the most aggressive species to
Q. robur followed by P. quercina, P. cambivora
and P. citricola. The drought stress experiment
demonstrated different infection strategies of P.
quercina and P. cinnamomi. While P. quercina
proved to be mainly a fine root pathogen, P.
cinnamomi was damaging both fine roots and
suberized cortical tissues, sometimes girdling the
taproots. All Phytophthora species tested were
able to survive under repeated drought stress,
and caused root damage during reflooding.
Drought stress did not significantly increase root
damage by any of the Phytophthora species
tested. However, root damage attributed to the
infection by P. quercina (QUE 67) and P.
cinnamomi (P 382) tended to be more severe
under drought stress.

Discussion
In the last decade several studies have
demonstrated that a diverse population of soil-
borne Phytophthora species were associated with
declining oaks on a wide range of soil types in
Central and Western Europe (Galoux and
Dutrecq 1990; Jung 1998; Jung et al. 1996,
1999a,b, 2000a,b; Hansen and Delatour 1999;
Vettraino et al. 2001; Delatour et al. 2002). In
general, the effects of nitrogen on Phytophthora
diseases vary with different host-pathogen
combinations and with the nitrogen form applied
(Huber and Watson 1974; Schmitthenner and
Canaday 1983). For instance the severity of
crown rot of apple trees caused by P. cactorum
(Utkhede and Smith 1995), root rot of citrus trees
caused by P. parasitica (Klotz et al.1958) and
little leaf disease of pines in New Zealand caused
by P. cinnamomi (Newhook and Podger 1972)
was increased by nitrogen fertilization especially
if the nitrogen/phosphate ratio becomes
imbalanced. However, there are also many
examples of nitrogen decreasing disease severity
(Schmitthenner and Canaday, 1983).

Sporangia production of P. quercina, P.
citricola, P. cambivora and 3 other Phytophthora
species in non-sterile soil leachate with nitrate
concentrations ranging from 2 to 100 ppm was
significantly stimulated by increasing nitrate.
This might at least partly be due to the observed
nitrate-based stimulation of bacteria and
protozoae in the soil leachate. In contrast, effects
of ammonium nitrogen on sporangia formation
were inconsistent and less pronounced (data not
shown). Furthermore, it is known that the uptake
of nitrate by fine roots increases the rhizosphere
pH, thus indirectly stimulating the formation of
sporangia of most Phytophthora species (Jung et
al. 2000a,b), whereas the uptake of ammonium
has the opposite effect. The primary nitrogen
form in the soils of 19 Phytophthora infested oak
stands in Bavaria was nitrate with a mean
concentration of about 36 ppm compared to
about 2 ppm ammonium, indicating favourable
conditions for sporangia formation in the field
(Jung et al. 2000a,b).

Increasing nitrate concentrations in the
flooding water stimulated root growth of non-
inoculated oak seedlings, and improved all root
parameters of oaks growing in soil infested with
P. cinnamomi and P. cambivora. This
corresponds with Schmitthenner and Canaday
(1983) who report a recovery of pines in the
USA suffering from little leaf disease caused by
P. cinnamomi following nitrogen fertilization. In
general, increasing nitrate concentrations during
flooding did not significantly increase the root
damage caused by any Phytophthora species
tested. However, the differences between the
oaks growing in soil infested with P. quercina or
P. citricola and the control plants in the non-
infested soil as well as the significance of these
differences were increasing with increasing
nitrate concentrations. This was most probably
due to the observed nitrate-induced stimulation
of root growth in the control plants, and a
destruction of a high proportion of the
regenerated fine root system by P. quercina and
P. citricola in the infested soils.

The visual estimation of the percentage of
root damage was a better assessment of the
actual root status than the morphometric
parameters root length and number of root tips.
The reasons were most probably the relative
short duration (9 months) of the experiment and
the lack of saprophytic soil organisms at least at
the beginning of the experiment. Therefore, a
high proportion of infected and necrotic fine and
small woody roots were still attached to the
healthy root system, and included in the
morphometric measurements.

Being well aware that additional
experiments with more isolates per Phytophthora
species are required, and that the significance of

results from *in vitro* experiments on tree seedlings is generally rather limited with respect to mature trees growing in the field, the results of our nitrogen experiments could be interpreted as follows:

Increasing nitrogen input into forest soils (Nihlgard 1985; Kreutzer 1991; Thomas and Kiehne 1995) leads to the observed decrease in mycorrhizal frequency associated with lower diversity of ectomycorrhizal morphotypes (Zare-Maivan 1983; Meyer 1987; Blaschke 1994; Van Driessche and Piérart 1995; Kovacs *et al.* 2000). On sites with low populations or absence of soil-borne *Phytophthora* pathogens, a reduced efficiency of root systems in absorption of water and minerals can be compensated by a nitrate induced stimulation of root growth. In soils infested with *P. quercina* or *P. citricola* the enhanced nitrogen input stimulates sporangia formation of the pathogens, and increases the susceptibility of newly formed fine roots to zoospore attacks due to the lack of protection by ectomycorrhizae, which are known as an effective mechanical and biochemical barrier against infection by *Phytophthora* species (Zak 1964). As a consequence, the ratio between rootlet death and replacement becomes imbalanced, and the necessary compensation of the reduced water absorption capacity by enhanced fine root growth is prevented. Furthermore, recent ecophysiological measurements in the field indicate partial stomatal closure, reduced CO_2 assimilation rate and lowered water use efficiency in symptomatic oaks (Heyne 2002). Over the decades the process of steady inoculum build-up and a progressive destruction of the fine root system leads to a weakening of the oaks, thus predisposing them to drought stress (Leininger 1998) and attacks by secondary parasites e.g. *Armillaria mellea* s.l. and/or *Agrilus biguttatus* (Jung *et al.* 2000*b*; Hartmann and Blank 1992, 1998).

Since the 1960's a significant climate change has occurred in most parts of Europe. The frequent occurrence of mild-humid periods during wintertime and springtime of the last decades indicated by a rise in mean winter temperature of 0.03K per year and a seasonal shift of precipitation from summer into winter (Schönwiese *et al.* 1994; Rapp and Schönwiese 1995) might have favoured the infection of non-mycorrhizal roots by zoospores during the cool season thus triggering an increasing population of *Phytophthora* and a progressive destruction of fine root systems from year to year. This is confirmed by the fact, that the *Phytophthora* species occurring in oak stands are able to form sporangia and to release zoospores in SEW at temperatures between 2 to 8°C (Jung 1998).

Furthermore, the summer climate in Central Europe shows a tendency to more frequently occurring droughts and heavy rain (Schönwiese *et al.* 1994; Rapp & Schönwiese 1995). During prolonged drought periods in summertime oaks are suffering from their degenerated fine root systems thus showing aboveground symptoms of water stress and malnutrition. During periods of heavy rain the oaks are not sufficiently able to regenerate their fine root systems because high proportions of freshly formed fine roots are destroyed in the wet soils by exploding populations of soil-borne Phytophthoras. Such a pattern of interaction between climatic perturbations and *Phytophthora* fine root damage was demonstrated by Brasier (1993 & 1996) for Iberian oak decline caused by *P. cinnamomi*, and is supported by the results of our drought stress experiment. All *Phytophthora* species tested were able to survive repeated drought stress, and produced significant fine root damages when soils were reflooded. *P. cinnamomi* and one isolate of *P. quercina* isolated from a site with highly fluctuating water tables and very dry conditions during summertime caused even more root damage with drought stress between the floodings than under non-limited water supply, whereas another *P. quercina* isolate isolated from a permanently moist site produced less root damage under drought stress. This might indicate adaptations to site conditions in the *P. quercina* population.

Recapitulating, the results of our experiments support the view that the two major longterm and largescale anthropogenic constraints, nitrogen input into forest soils and increase of frequency and duration of summer droughts combined with a tendency to heavy rain, might have favoured the build-up of *Phytophthora* inoculum, thus imbalancing the ratio between rootlet death caused by *Phytophthora* infection and rootlet replacement, weakening the oaks and predisposing them to droughts and attacks by secondary parasites and pathogens.

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98

New findings in *Phytophthora* root rot of walnut in Italy

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Abstract. Root rot caused by *Phytophthora* species has recently been recorded in several plantations of *Juglans regia* in Northern and Central Italy. Isolation trials from soil and infected roots confirmed the presence of *Phytophthora* species. Symptoms of the disease are very similar to those of Ink disease of Chestnut. Morphological and molecular identification indicated the presence of five species. Amongst them *P. cinnamomi*, whose presence in Italy has probably been confused with *P. cambivora* in the past; *P. cambivora*, *P. cactorum*, *P. citricola* and *P. cryptogea*. This is the first record of the latter two species on walnut in Italy. Pathogenicity tests carried out on 2-year old seedlings through soil infestation, confirmed *P. cinnamomi* as the most aggressive species on *J. reglia*.

Introduction

English (Persian) walnut (*Juglans regia* L.) is widely utilized in Italy for reforestation activities in pure and mixed plantations of broad-leaved species. Increasing decline of this tree has been recently reported from several areas. Symptoms resemble those typical of root and collar rot, however decline and sudden death is not uncommon.

In Europe *P. cinnamomi* Rands (1922) has been frequently reported as the main cause of walnut collar and root rot especially in France (Gravatt 1953; Grente and Averseng 1966; Prunet and Herman 1995). The presence of this species on walnut in Italy still remains a matter of discussion. In fact collar and root rot of walnut was first described in Italy by Curzi (1933) and then Petri (1937) and attributed to *P. cambivora* (Petri) Buisman (1927). Such difference with the European situation is not surprising since another tree species, sweet chestnut, is mainly affected by *P. cinnamomi* in France, Spain and Portugal and by *P. cambivora* in Italy (Vettraino *et al.* 2001a).

The aim of the present work was to investigate the *Phytophthora* complex associated with walnut decline and death in Italy.

Methods

Samplings

Nine declining walnut orchards and a nursery in Northern and Central Italy were investigated. Samples were collected in each site from collar, roots, and soil at the base of symptomatic trees in order to assess the presence of *Phytophthora* spp. After collection, tissue samples were sterilized in ethanol and plated in PARBhy agar (per liter: pimaricin, 10 mg; ampicillin (sodium salt), 250 mg; rifampicin, 10 mg; hymexazol, 50 mg; benomyl, 15 mg; malt extract, 15 g; agar, 20 g).

Soil samples (200 ml) were moistened and incubated at 20°C for 3 days and flooded with 500 ml of distilled water. Five fresh-picked leaves of *Rhododendron* spp. were placed directly on the water surface and incubated at 20°C until the development of spots or leaf discoloration, but not longer than one week. The leaves were then blotted on filter paper, cut in small pieces and placed on PARBhy agar (Robin 1991). *Phytophthora* isolates were maintained on carrot agar (CA) (Brasier 1969) at 20° in darkness and sub-cultured at 4-week intervals.

Species identification

Isolates were identified by comparing colony growth patterns and morphological features of sporangia, oogonia, antheridia, chlamydospores and hyphal swellings with known isolates and with species descriptions reported in literature (e.g. Stamps *et al.* 1990; Erwin & Ribeiro 1996). Colony morphology was described on 10-day-old cultures grown on carrot agar (CA), potato dextrose agar (PDA), malt agar (MA), and V8 in 90-mm-diameter Petri dishes at 20°C in darkness. Identification of the isolates was confirmed by comparing the total soluble proteins patterns according to Laemmli (1970) and the RFLP patterns of their ribosomal DNA (rDNA) with those of known isolates of *Phytophthora* spp. according to the methodology of Vettraino *et al.* (2001a). Sporangia were produced by placing a disk of mycelium from a 7-day-old culture grown on V8 in soil extract prepared according to Chee and Newhook (1965). Morphology was assessed by light microscopy and length and breadth of 100 sporangia were measured for each isolate.

Pathogenicity tests

Two field isolates of each *Phytophthora* species identified were used for pathogenicity tests. Persian (English) bare root 2-year-old seedlings were planted in 5L pots containing steam-pasteurized potting mix (50% peat, 25% sand, 25% ground pumice). The seedlings were randomly divided in 11 block of 10 individuals each. Plants in each block were inoculated with one isolate of

Table 1. *Phytophthora* species isolated from different sources in walnut stands

Species	Locality	Site	Damage	Source
P. cactorum	Roma	nursery	sudden death	collar
	Rovigo	orchard	decline	collar
P. cambivora	L'Aquila	orchard	decline	collar and root
	Benevento	orchard	decline	collar and root
P. cinnamomi	Alessandria	orchard	sudden death and decline	soil, collar and root
	Treviso	orchard	sudden death and decline	soil, collar and root
	Cuneo	orchard	sudden death and decline	collar and root
	Venezia	orchard	sudden death and decline	collar and root
P. citricola	Benevento	orchard	decline	root
	Napoli	orchard	decline	root
P. cryptogea	Alessandria	orchard	sudden death and decline	soil
	Treviso	orchard	sudden death and decline	Soil
	Viterbo	orchard	sudden death and decline	soil

Locality

1 Treviso

2 Venezia

3 Rovigo

4 Cuneo

5 Alessandria

6 Viterbo

7 Roma

8 Aquila

9 Napoli

10 Benevento

● *P. cinnamomi*

○ *P. cambivora*

⊛ *P. citricola*

● *P. cactorum*

○ *P. cryptogea*
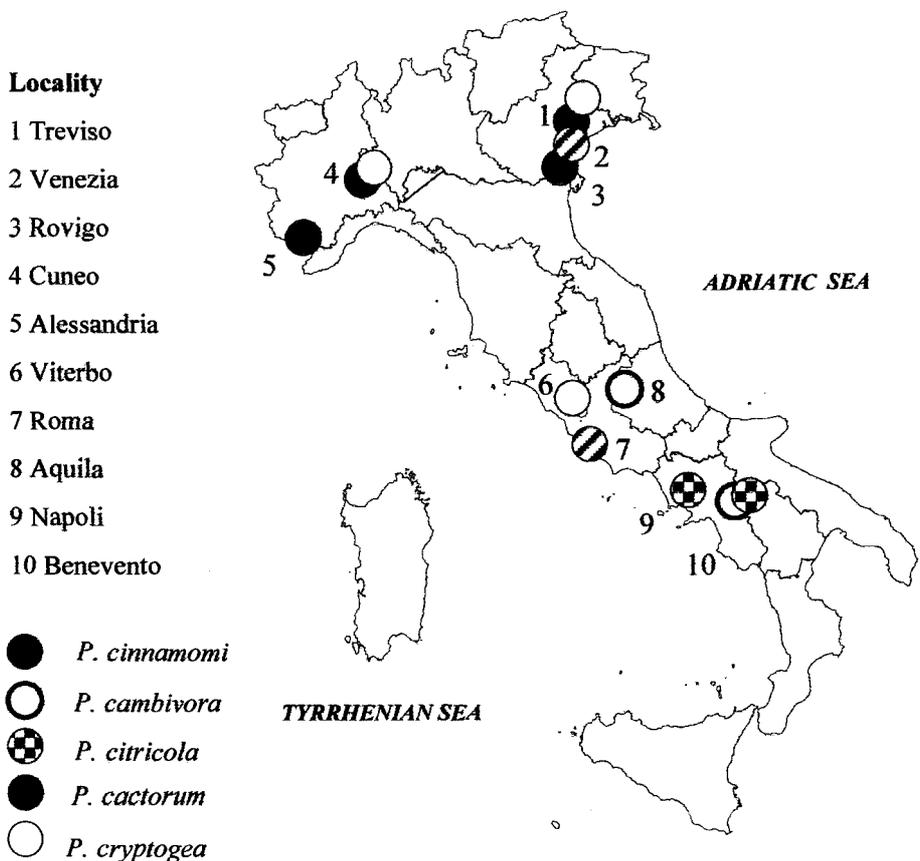
Fig. 1. Recovery of *Phytophthora* spp. from declining walnut trees in Italy.

100

Phytophthora spp. *Phytophthora* inoculum was prepared according to Vettraino *et al.* (2001a). *Phytophthora* sporulation in the soil was started by flooding for 24 h. One uninoculated block was left as control.

Results

Survey and isolation activities.

Five species of *Phytophthora* were identified: *P. cinnamomi*, *P. cambivora*, *P. cactorum* (Leb. and Cohn) Schröeter (1886), *P. citricola* Sawada (1927) and *P. cryptogea* Pethybridge and Lafferty (1919) (Fig. 1). More than one species was sometimes recovered from the same sample.

P. cinnamomi was associated with sudden death events and recovered from collar, roots and soil; *P. cambivora* was associated with declining trees and isolated from collar and roots; *P. citricola* was recovered in orchards from roots of declining trees; *P. cactorum* was the only species recorded in nursery on wilted walnut seedlings, however it was also isolated from collar of declining trees in an orchard; *P. cryptogea* was recovered only from soil in heavily declining stands (Table 1).

Pathogenicity tests.

After 30 days from inoculation mortality was recorded only for the plants inoculated with *P. cinnamomi* with a mortality of 80%.

Discussion

The results of this study confirmed the presence of *P. cinnamomi* as the causal agent of root and collar rot of walnut in Italy. However this species was present only in orchards in Northern Italy from where *P. cryptogea* in soil and *P. cactorum* from collar necroses were also recorded. *P. cambivora* is present as agent of collar rot on walnut in Central and Southern Italy confirming its better adaptation to a warmer climate. *Phytophthora citricola*, another species widespread in hardwood forest in Italy (Vettraino *et al.* 2001a,b), was also isolated from root necroses. Among the five species isolated, *P. cinnamomi* was confirmed the most aggressive to walnut, being the only species able to cause death at high rate 2-year old plants within one month.

In conclusion a *Phytophthora* complex is associated with walnut collar and root rot in Italy. The recovery of more than one species from the same stand and same sample could suggest a synergic activity on the host. The later aspect needs more studies in order to highlight the role of different *Phytophthora* species in disease development.

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101

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Variability in resistance to *Phytophthora cambivora* of *Castanea sativa* populations and selected cultivars in Italy and Spain

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Abstract. The behaviour of four wild populations of *Castanea sativa* from Sicily (Southern Italy), Pedmont (Northern Italy), A Coruña (Northern Spain), Malaga (Southern Spain) to *Phytophthora cambivora* was tested on 4-month old half-sib progenies through soil infestation. A number of qualitative and quantitative parameters were assessed including growth in height, number of leaves, dry weight, length of the stem and root necroses, crown condition and mortality. Results showed a wide variability in response to the pathogen challenge. Some progenies showed a resistance to *P. cambivora* similar to that of the resistant hybrid Marsol used as a control. An additional experiment was carried out through stem inoculation on 87 cultivars of *Castanea* spp., including French hybrids. In this case a relatively wide variability in susceptibility was assessed among the Italian cultivars, some of which showed comparable behaviour to the French hybrids.

Introduction

Studies aiming to select chestnut genotypes resistant to ink disease have focused mainly on hybridisation programs between *Castanea sativa* L. and the Asiatic species *C. crenata* Sieb. et Zucc. and *C. mollissima* Bl. Interspecific hybridisation programs were carried out in the last century, especially by INRA, in order to select hybrids tolerant to *Phytophthora cinnamomi* Rands for use mainly as rootstock for high quality fruit cultivars. Among them the most utilised are 'Marsol' (CA07), 'Maraval' (CA74), 'Ferosacre' (CA90), 'Marigoule' (CA15) and 'Marlhac' (CA118) (Salesses *et al.* 1993). These hybrids are *C. crenata* x *C. sativa* or the reciprocal. However genetic incompatibility of hybrid rootstocks with clones of *C. sativa* has limited utilisation of such resistant clones outside France (Craddock *et al.* 1999).

In the last 15 years more hybridisation programs have been conducted in France, Spain and Portugal to select hybrids with tolerance to *Phytophthora* and good vigour and nut quality (Gomes-Pereira *et al.* 1993; Fernández-López *et al.* 2001). Although inter-specific hybrids are the most tolerant to *Phytophthora*, wide variability has also been found in progenies from intra-specific crosses (Guedes-Lafargue and Salesses 1999).

A large screening of half-sib progenies of *C. sativa* from different European countries, including France, Italy, Spain, Greece and United Kingdom, is on going under the EC project CASCADE (EVK2-CT1999-00065) in order to assess the variability in tolerance to *Phytophthora cambivora* (Petri) Buisman of wild and domesticated populations of chestnut in Europe. A further study is ongoing, sponsored by the Calabria Region, and aims to test the resistance to ink disease of selected cultivars of *C. sativa.*

The present study reports the preliminary results of resistance tests to *P. cambivora* carried out on *C. sativa* provenance from Italy and Spain, and on cultivars selected from a chestnut germplasm collection in Southern Italy.

Materials and Methods

Study sites

For the provenances tests, two chestnut sites localized at latitudinal extremes both in Italy and Spain were chosen. In each site up to three chestnut typologies were investigated: coppice, orchard and naturalized forest. In each typology 8 trees were selected along a transect crossing the site. In October 2000, 40 nuts were collected from each tree and considered open-pollinated progenies. The sites in Italy were chosen in Pedmont (northern site) and in Sicily (southern site). The sites in Spain were chosen in Coruña (northern site) and Gaucin (southern site).

Resistance tests

For resistance tests on cultivars, material was obtained from a collection of chestnut germplasm in Calabria that is managed by the Regional Agency for Development and Services in Agriculture of the Calabria Region (ARSSA).

Resistance tests on half-sib progenies and on selected cultivars were performed respectively by soil infestation and inoculation of excised sprouts using a method described by

Browne and Mircetich (1986), with some modifications (Vettraino *et al.* 2001). For the soil infestation tests, *C. sativa* seedlings were obtained from surface sterilised seeds planted in 600 ml pots containing steam-pasteurised potting mix (50% peat, 25% sand, 25% ground pumice). Seedlings were grown in a greenhouse until their lower stems were well lignified and their average height was 20-30 cm. *Phytophthora* inoculum was prepared by growing *P. cambivora* isolate P15FC2 for 4-6 weeks at 20°C on sterilised millet seeds thoroughly moistened with V8 broth (V8 juice 200 ml; $CaCO_3$, 3 g; distilled H_2O, 800 ml).

The inoculum was repeatedly rinsed with sterile water to remove unassimilated nutrients and then added to the potting mixture at the rate of 10 mL inoculum/600 mL potting mixture. *Phytophthora* sporulation in the soil was initiated by flooding for 24 h.

Each progeny consisted of 20 seedlings. Plants were watered to field capacity every second day. Two months after the inoculation, 50 g samples of soil from each of 6 randomly chosen pots were flooded with water and baited separately with 15 rhododendron leaf disks 1 cm in diameter.

For sprout inoculation tests, five, one-year-old dormant sprouts, 2 cm in diameter and 1m in length, were collected from each tree, placed in test tubes with sterile H_2O and maintained at 20°C with a 12h photoperiod. Shoots were inoculated as the buds started to open. A cork borer was used to remove a 3 mm bark disk from the excised shoot. The bark disk was replaced by a 3mm plug of a 10-day-old culture of isolate P15FC2 of *P. cambivora* grown on PDA. Three inoculations for each sprout were performed. Controls (one for each sprout) were represented by inoculation with a PDA plug. After inoculation, shoots were incubated in a ventilated chamber for one week at 20°C and 100% relative humidity. After incubation the length of the bark necrosis was measured on each shoot.

Statistical analysis of data was performed with the software InStat 3 (GraphPad, San Diego, CA, USA).

Results

Resistance tests on progenies

Percentage of mortality of provenances was significantly higher than that of the control French hybrids (Fig. 1). No significant differences in mortality have been found among the four geographic provenances. However a wide variability in mortality at any given site was found among provenience classified by typology (Fig. 2). Over all sites there was no trend based on typology of parents. Mortality was significantly different from the French hybrids in only four provenances out of the ten tested (Fig. 2).

Resistance tests on cultivars

Results of the resistance test carried out through shoot inoculation on 75 different cultivars including Euro Japanese hybrids, are shown in Fig. 3. There was a high level of variability in the response to the pathogen. Most of the variability was in the cultivars from Southern Italy and in particular those from the Calabria region (Fig. 3).

Discussion

The results confirmed the existence of high variability in chestnut populations in regard to resistance/tolerance to *P. cambivora*. Considering the strict condition under which these experiments were carried out, it can be concluded that a large part of this variability has a genetic basis. Generally the lower resistance of *C. sativa* individuals to ink disease compared to the French hybrids was confirmed, although some *C. sativa* provenances showed a low percentage of mortality. However mortality can be considered just one of the measurable variables to estimate resistance at progeny level. Activities within the CASCADE project have considered many additional variables estimating resistance whose values for each seedling and progeny are still under statistical elaboration. It is impossible at the moment to speculate on the origin of resistance. The analysed progenies are half-sibs as chestnut is an out-crossed species. Consequently each seedling has to be considered a unique genotype.

Orchards in Pedmont and Gaucin were characterised by significantly lower per-centages of mortality compared with other regions. These two sites deserve more detailed investigations. Data shown for selected cultivars, highlight the variability among cultivars in their response to pathogen challenge. It has to be considered that extent of necrosis on lignified shoots estimates just one aspect of the resistance to ink disease.
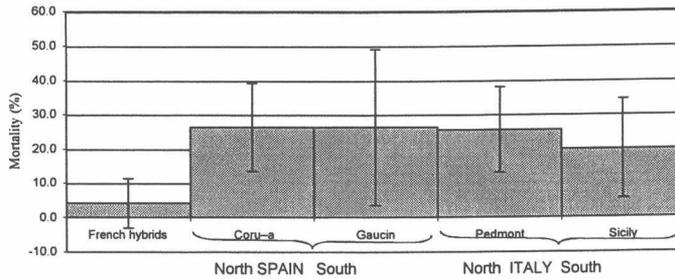
Fig. 1. Percentage of mortality of inoculated seedlings from different geographic regions. Vertical bars correspond to standard deviation.
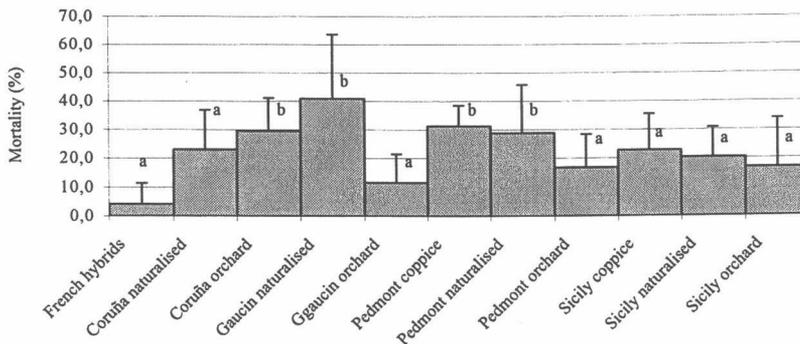
Fig. 2. Percentage of mortality of inoculated seedlings grouped at geographic origin and typology level. Vertical bars correspond to SD. Different letters indicates significant differences from the French hybrids at P<0.05.

These results are a first contribution to the studies on the potential of resistance existing in European natural and domesticated populations of *Castanea sativa*.

Acknowlededements

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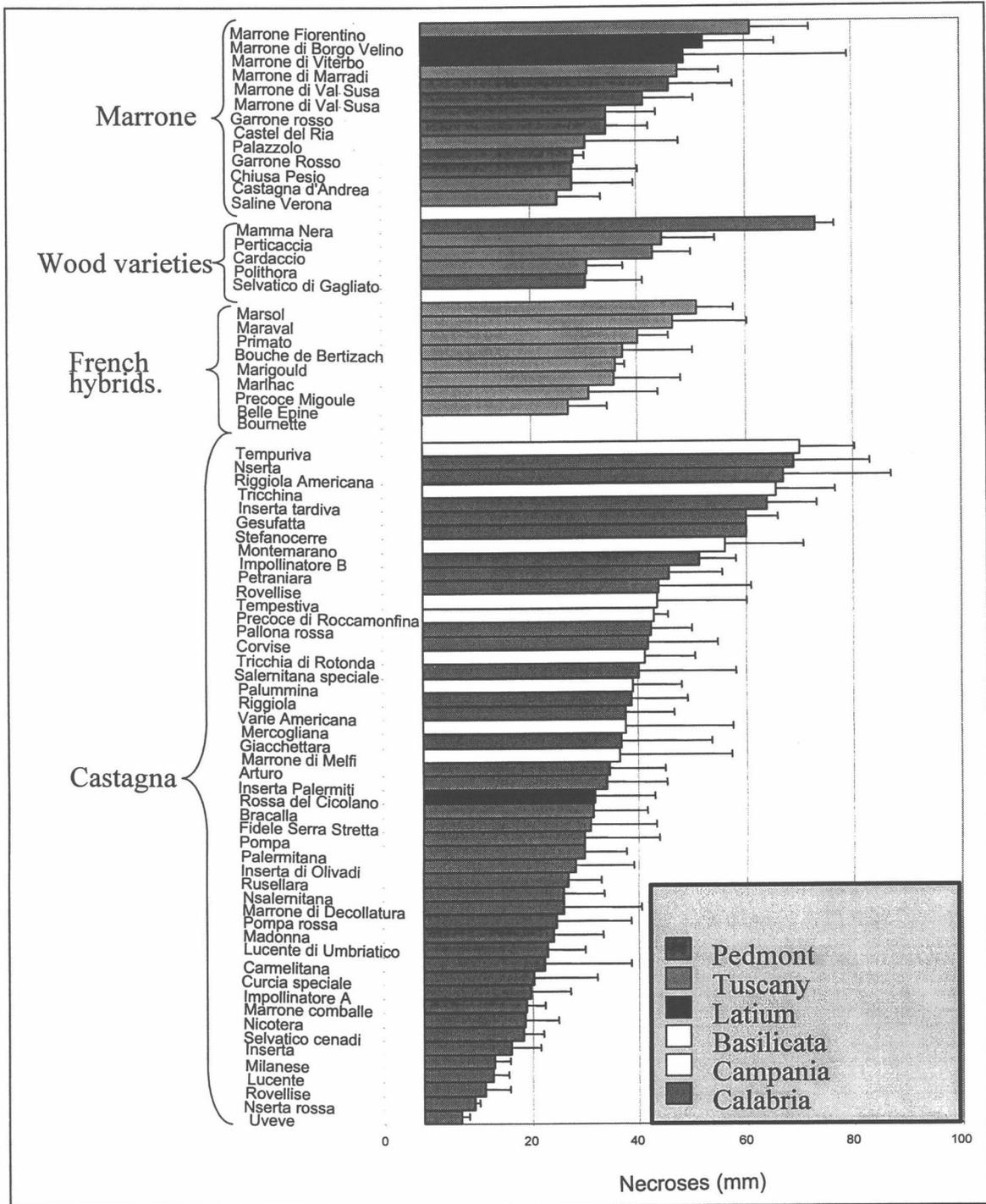
Fig. 3. Length of necrosis measured on shoots from 75 cultivars of chestnut inoculated with *Phytophthora cambivora* isolate P15FC2. Bars correspond to standard deviation.

Impact of *Phytophthora* in the Americas
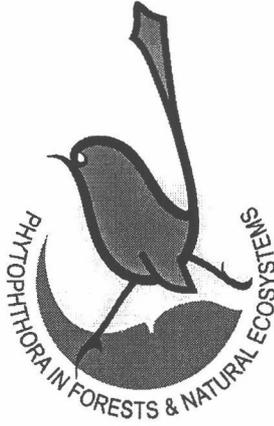
Evidence for aerial transmission of *Phytophthora ramorum* among *Quercus* and *Lithocarpus* in California woodlands

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Abstract. The newly discovered *Phytophthora ramorum* canker disease of oak (Sudden Oak Death Syndrome) threatens millions of acres of California woodlands where *Quercus agrifolia* (coast live oak), *Lithocarpus densiflorus* (tanoak), or *Quercus kelloggii* (black oak) are dominant species. An important step in controlling this disease involves understanding how it is spread. We provide evidence for an aerial pathway of transmission for *P. ramorum*. The presence of diseased oaks at all elevations on hillsides and the above-ground nature of the disease indicate an aerial component to the movement of spores. Although viable spores have yet to be found on infected oak tissue, foliar hosts may serve as sources of inoculum that are produced and aerially dispersed in rain. In the laboratory, abundant sporangia form on moistened leaves of infected *Umbellularia californica* (bay) and *Rhododendron* spp. within 72 hours. These sporangia are highly caducous and easily disperse in water. Chlamydospores were also observed on the surface of moistened *U. californica* and *Rhododendron* leaves. Consistent with these results, *P. ramorum* has been recovered from rainwater collected from woodlands with infected oak and bay trees. *P. ramorum* has also been found in soil, litter, and streamwater. Laboratory experiments suggest that spores of *P. ramorum* that land on oak bark could survive in moist conditions for at least one month. In addition, *P. ramorum* can survive in living moistened *U. californica* leaves throughout the summer months. In a field inoculation trial, spores did not need a wound to infect oak trunks. This mode of transmission, including spore production on foliar hosts, aerial transport in rain, survival in moist conditions such as rain-moistened trunks, and infection without wounds, may help explain the rapid spread of *P. ramorum* within a given geographical site.

Introduction

The newly discovered *Phytophthora ramorum* canker disease of oak (Sudden Oak Death Syndrome) threatens millions of acres of California woodlands where *Quercus agrifolia* (coast live oak), *Lithocarpus densiflorus* (tanoak), or *Quercus kelloggii* (black oak) are dominant species (McPherson *et al.* 2000, Garbelotto *et al.* 2001). An important step in controlling this disease involves understanding how it is spread, both on a small scale between oak trees in an infected area, and over a larger scale from infected to uninfected woodlands.

On all known hosts to date, *P. ramorum* infects aerial parts of the plant. Infection on *Quercus* and *L. densiflorus* occurs on the main trunk and branches. Cankers have been noted as high as 20 m on the main trunk of *L. densiflorus*. On other hosts such as *Rhododendron*, *Arbutus menzesii* (madrone), *Manzanita*, and *Vaccinium ovatum* (huckleberry) *P. ramorum* infects stems and leaves. On *Umbellularia californica* (bay), *P. ramorum* is only found in the leaves. If infection is initiated on the aerial parts of plants, it follows that aerial movement of inoculum must occur. In addition, the observation of infected oak trees at all elevations on hillsides, not just valleys or stream courses, further suggests aerial spread of this pathogen. To explain this spatial pattern of disease, we need to understand the transmission biology of *P. ramorum*, consisting of spore production, movement of spores, and infection of a new individual. Knowledge of the necessary conditions for each of these steps may help us establish barriers to the spread of infection.

Identification of sources of spore production for *P. ramorum* in California oak woodlands is complicated by the presence of many host plant species in the forest. Currently, the host list includes 10 non-oak plant species in five plant families (Rizzo *et al.* unpublished

data). These other hosts may serve as sources of inoculum and act as infectious links among oak trees.

Production, movement, and survival of spores is likely to be highly affected by seasonal climatic changes in temperature and moisture in central coastal California. The current range of *P. ramorum* occupies areas with a Mediterranean climate influenced by maritime weather. Rainfall occurs during the cool winter months from December to April. During the summer, although morning fog is generally present, drought and high temperature conditions prevail in a significant portion of the range. Because production and survival of spores usually require very high humidity (Duniway 1983), abundance of *P. ramorum* spores may be highly seasonal and coincide with the winter rains. Rain splash has been shown to move *Phytophthora* spores of other species over several meters (Ristaino and Gumpertz 2000), making this a potentially effective means of local transport, especially for spores requiring moisture for production and survival. However, *Phytophthora* spores also may survive adverse summer conditions as dormant resting spores residing in host tissue.

Once spores are produced and transported to oak trunks, suitable conditions are needed for survival of spores and infection of new host individuals. *Phytophthora* may infect through pruning wounds or naturally occurring wounds (Bostock and Doster 1985). However, in many cases, *Phytophthora* species do not need wounds to infect host plants.

In this paper, we provide data on a possible aerial pathway of infection for *P. ramorum* on oaks to explain the observed patterns of disease. We report on the types of spores produced by *P. ramorum* on tissue of several important hosts under laboratory conditions. We then monitor the abundance of spores in rainwater, soil, litter, and stream water in forest sites throughout the year. We test for the survival capabilities of spores under dry and moist conditions in the laboratory as a first attempt to understand survival potential on oak bark. We also test for survival of *P. ramorum* throughout the summer in living leaves of a wide-spread foliar host, *U. californica*. Finally, we perform a non-wounding inoculation on *L. densiflorus* and *Q. agrifolia* to investigate the potential of these to infect trees under natural conditions.

Methods
Production of spores

Production of spores was monitored on pieces of naturally infected host tissue collected from forest and nursery sites within the 300 km host range. Eight excised *Q. agrifolia* cankers, 9 infected *U. californica* leaves, and 15 infected nursery *Rhododendron* leaves were placed in moist chambers at 18 – 23° C for 72 hours and monitored for spore production. In addition, the bleeding sap was collected when possible from 13 *Q. agrifolia* at weekly intervals from 22 April to 16 July, 2001, and plated on PARP medium to test for the presence of viable pathogens.

Movement of spores

Recovery of spores was attempted from rainwater, soil, litter, and stream water in a time series spanning the winter rains and the drying summer months.

Rainwater: Raintraps were used to collect rainwater in a coast live oak woodland at Fairfield Osborn Preserve in Sonoma Co. Both *Q. agrifolia* and *U. californica* trees were infected with *P. ramorum* at this site. Raintraps consisted of a 165 cm x 75 cm vinyl sheet stretched over a pvc frame and folded into a funnel with a 4 liter collecting jar at the bottom. Traps were set up on 2 February, 2001 and used to collect rainfall though winter, and for one unusually late rain on 27-28 June. Two traps were placed 0.5 m in front of cankers on each of 7 oak trees, for a total of 14 traps. Previous isolations confirmed that the cankers were caused by *P. ramorum* infection. Two "distant" traps were placed at a distance of 5 m from all infected oak trees. Four additional "distant" traps were installed on March 15, 2001. Rain water was collected at approximately 2 day intervals during a storm event. Rainwater was stored overnight at 4-5° C to allow spores to settle. One liter of water was suctioned off of the bottom of containers and filtered through a Millipore 3 μm cellulose esterase filter to capture all spore types. The filter was then cut into strips and placed filtrate side down on selective medium plates. After 7 days strips were removed, and colonies of *P. ramorum* were counted.

Soil: Beginning in March, 2001, soil and litter were collected on a monthly basis from around the base of 15 diseased oak trees at the Fairfield Osborn Preserve to test for the presence of *P. ramorum*. Previous isolations confirmed

that all trees were infected by *P. ramorum*. At the base of each tree on the infected side, soil was collected in zip-lock bags from three separate spots and pooled to equal 500 g. Litter was collected at three spots to fill 1/4 the volume of a gallon zip-lock bag. A green d'anjou pear was pressed into the soil or litter in each sample so that 1/3 of the pear was immersed, and diH20 was added to the sample until 1/2 of the pear was immersed in water. Samples were allowed to remain for 6 days at 18 – 23 C. Pears were then removed, washed, and monitored for signs of *Phytophthora* lesions. Tissue from likely lesions was plated on PARP selective medium to verify *P. ramorum* presence.

Stream water: Water was collected from Bean Creek, Santa Cruz Co., on a bi-monthly basis from April through June, 2001 in a forested area with infected *Q. agrifolia* and *L. densiflorus* trees. Sampling ended in June when the stream dried up. For each sample, 8 liters of stream water were stored in plastic bins at 18 – 23 C. Two d'anjou pears were added to each bin so that pears were half immersed in stream water. After 5 days, pears were removed and *P. ramorum* infection was assessed as for soil baiting (see above).

Survival of spores
Laboratory: To assess survival of spores under various moisture regimes, suspensions of zoospores and chlamydospores of *P. ramorum* were each added to water, moist filter papers, or dry filter papers (Fisherbrand P4 4.25 cm) and monitored for viability for one month. The suspensions for both types of spores consisted of a pool of spores from fifteen isolates. Approximately 200 spores in 100 µl of suspension were added to either (1) 100 µl of diH20; (2) a filter moistened to saturation with 100 µl diH20; or (3) a dry filter. The moistened filters were kept in closed screw-cap tubes. An additional 150 µl dH20 was added to the moist filter at two week intervals to maintain saturation. The dry filters were allowed to dry completely at room temperature (23° C, 30 % rh, 30 minutes) and placed in a crisper. All treatments were stored at 15 ° C. Five replicates of each treatment were plated on selective medium at 0, 3, 7, 15, and 30 days for chlamydospores, and 0, 7, 15, and 30 days for zoospores. Colonies were counted at 36 and 72 hours to assess viable spores.

Living U. californica leaves: To test for survival of *P. ramorum* inoculum in attached leaves of an important foliar host during the hot, dry summer, three infected leaves from each of 14 *U. californica* trees at the Marin Municipal Water District, Marin Co. were collected for isolation of *P. ramorum*. *P. ramorum* was isolated on pimaricin-ampicillin-rifampicin-PCNB agar (PARP). Collections began in July and occurred monthly. The number of positive leaves from each tree was recorded.

Non-wounding inoculation of Q. agrifolia *and* L. densiflorus

To test for the ability of spores to infect *Q. agrifolia* and *L. densiflorus* without an apparent wound in the bark, a non-wounding inoculation with spore suspensions of *P. ramorum* was carried out on the Marin Municipal Water District on 5 April 2000. For *Q. agrifolia*, 5 trees each were inoculated with spore suspensions from either isolate O-13 (from *L. densiflorus*) or O-16 (from *Q. agrifolia*), and 5 controls were inoculated with agar suspensions. For *L. densiflorus*, 4 trees each were inoculated with spore suspensions from either of the two isolates and there were 4 controls. The *L. densiflorus* trees were located in a coast redwood forest. The *Q. agrifolia* trees were located in a closed canopy, mixed-evergreen forest. Each of the inoculated trees received a sporangia suspension on one side and a chlamydospore suspension on the other side. Sporangia suspensions were made by placing 5 V8 agar mycelial plugs in 5 ml soil extract water at 18 – 23 ° C for 24 hours to induce sporangia production. (For methods, see Rizzo *et al.* 2002) The sporangia solutions averaged 180 spores / ml. Each chlamydospore solution was formed by blending a 6 cm diameter mycelial disk from isolates grown on CMA in a 100 x 15 mm petri dish for 6 weeks. Abundant chlamydospores were noted on the cultures before blending in 100 ml diH$_2$O. The chlamydospore solutions averaged 173 spores / ml. For the inoculation on each tree, the trunk was first wet with tap water. Treatments (O-13, O-16, control) were assigned at random to trees and 5 ml of sporangia suspension (or mock) and 100ml of chlamydospore suspension (or mock) were then applied to opposite sides of the tree in a 15 cm^2 patch. The inoculated area was then wrapped in plastic and sealed with duct tape at the top and bottom to maintain moisture. Gaps in the taped end were sealed with wet cotton. Plastic was removed after a week. Trees were checked on a

110

monthly basis for bleeding sap and canker formation.

Results

Production of spores

Spore production was observed on tissue of some host species. Sporangia were present on the surface of 3 of 9 infected *U. californica* leaves and all 15 *Rhododendron* leaves within 72 hours. Chlamydospores were also observed on the surface of one bay leaf. No spores were observed on the surface of oak bark cankers. Sporangia were observed microscopically in the bleeding sap of one oak tree. However, none of the 81 plating attempts of bleeding sap from the 13 trees resulted in colony formation.

Movement of spores

Raintraps: Rainwater contained viable spores of *P. ramorum* (Fig. 1). At some point during the sampling period, *P. ramorum* was recovered from rain captured at all 7 of the *Q. agrifolia* trees. Propagule counts were low, but they were present in rain water at each of the collection periods except for the brief summer storm in late June (13mm rainfall). Spore counts were variable from tree to tree and in positive samples ranged from 0.25-7 spores per liter. Of the six traps placed 5 m away from infected oak trees two were positive for *P. ramorum* during the sampling period.

Soil Baiting: Soil and litter also contained viable propagules of *P. ramorum.* During March, 3 of 15 soil samples and 1 of 15 litter samples tested positive. During April, no soil samples tested positive, and only 1 of 15 litter samples tested positive. During May, no litter samples tested positve, and only 1 of 15 soil samples tested positive. The positive May sample was from the same tree as the positive April sample. In June and July, all samples were negative.

Stream Baiting: The April sample from Bean Creek was positive for *P. ramorum.* Samples from May and June were all negative.

Survival of spores

Laboratory: A portion of both the zoospores and chlamydospores survived in the water and moist filter treatments (Fig. 2). Both types of spores were killed by the drying process at 30% rh at room temperature in the 'dry filter' treatment (data not shown). Chlamydospores survived better than zoospores in both the water and moist filter treatments. Zoospore survival averaged less than 20% at the end of the 30 day period while chlamydospore survival at 30 days in water and on moist filters still averaged 75% and 41% of starting values, respectively.

Living U. californica *leaves:* Inoculum of *P. ramorum* survived 4 months of hot, drying conditions in *U. californica* leaves. Inoculum levels did not decrease with time. The number of positive leaves for *P. ramorum* were 15, 17, and 20 out of 42 for July, August, and September, respectively.

Non-wounding inoculation of Q. agrifolia and L. densiflorus

Five weeks post inoculation, one *Q. agrifolia* and one *L. densiflorus* exhibited bleeding symptoms. Five months after the inoculation, two *Q. agrifolia* and two *L. densiflorus* showed bleeding symptoms. Other *L. densiflorus* may also be positive, yet not initially show bleeding, as determined by an earlier wound inoculation experiment (Rizzo *et al.* 2002). On all four infected trees, bleeding symptoms appeared on the side of the tree inoculated with sporangia. Three of the positive inoculations were caused by O-16 (from *Q. agrifolia*) and one by O-13 (from *L. densiflorus*

Discussion

Rapid production of spores on foliar hosts, such as *U. californica* and *Rhododendron*, may be the key to the spread of *P. ramorum* within a locality, and perhaps between different geographic locations. *P. ramorum* produced sporangia on moistened leaves of both *U. californica* and *Rhododendron*, the two foliar hosts tested. Chlamydospores were also noted on infected *U. californica* leaves. In addition, we have observed chlamydospores on infected *Rhododendron* leaves in moist chambers for other experiments.
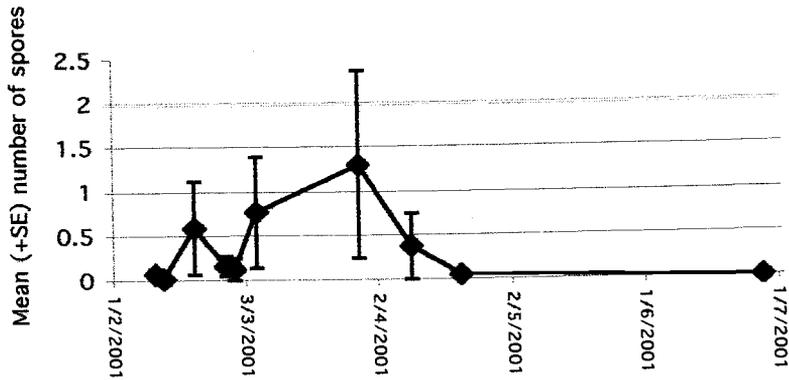
Fig. 1. Mean number of *P. ramorum* spores per liter of rainwater (± standard error) for storms occurring from February through June, 2001 at the Fairfield Osborn Preserve, Sonoma Co. Means were based on the average from two collecting traps at each of seven *Q. agrifolia* trees.
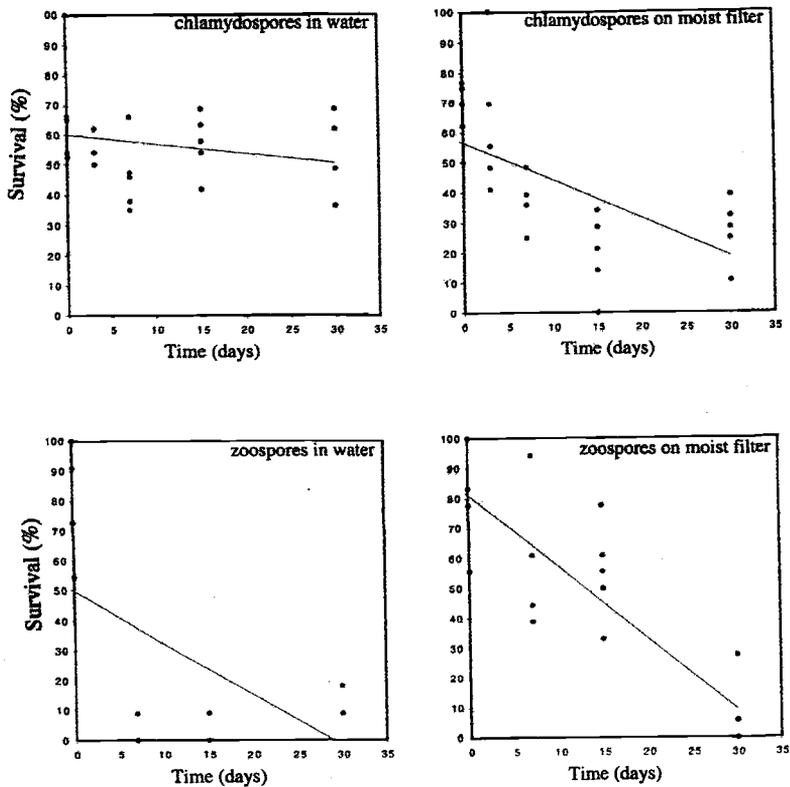
Fig. 2. Survival over time of *P. ramorum* chlamydospores and zoospores in water and on moist filter paper at 15 C. Each datum represents the spore count of one of five treatment replicates plated to agar at a given time point. Spore count data is scaled as a percent of the highest spore count at time (0) to facilitate comparison among graphs.

112

While rhododendron species are not major components of most California forests, *U. californica* is a dominant species in many coast live oak forest types. Consistent with laboratory results on spore production, evidence from the field suggests that inoculum from *U. californica* leaves may be very important in vectoring *P. ramorum* to oaks. Swiecki (2001) found a significant association between infected oaks and the presence of *U. californica* trees. Rainwater traps in this study placed 5 m from infected *Q. agrifolia* contained viable spores that may have come from overstory infected *U. californica* trees. Furthermore, we often observe an increase in *Phytophthora*-like leaf spots on *U. californica* growing within oak infection centers. To further investigate the importance of *U. californica* as an inoculum source, studies are underway to determine temperature requirements for sporangia production on *U. californica* leaves, the viability of *P. ramorum* in leaves attached to trees or fallen in litter, and the distance spores can travel from *U. californica* leaves.

The negative results of spore production on oak bark cankers or in bleeding sap require further investigation. Rapid contamination of oak cankers by fungal species may have prevented sporulation of *P. ramorum*. In addition, the seasonal state of the bark may not have been conducive to spore production at the time of the test (Brasier and Kirk 2001). However, successful amplification of *P. ramorum* DNA from bleeding sap (Garbelotto, unpublished data), suggests the presence of propagules and underscores the need for additional isolation attempts from sap.

Viable spores of *P. ramorum* were shown to be carried in rainwater, soil, litter and stream water. The abundance of viable propagules in these media exhibited a distinct seasonality, peaking in March and early April of 2001, a low rainfall year. Production of sporangia by other *Phytophthora* species depends on high moisture levels (Duniway 1983). Hence, the presence of moisture from rain on infected plant tissue is likely necessary for production of spores that eventually fall down to soil, litter or stream water. Generally increasing levels of inoculum in rainwater in early April, and the lack of spores in the isolated 2-day June rain, may suggest that a time of prolonged moisture is needed for inoculum buildup. Warming temperatures in early April during the period of consistent rainfall may also

have contributed to the peak in spore production. Failure to recover *P. ramorum* from soil and litter in the summer months may indicate that seasonal drying is sufficient to reduce viability of spores in these substrates.

Survival of *P. ramorum* spores also depended on moisture levels. In laboratory tests, both chlamydospores and zoospores placed in suspension on filter paper were killed by drying for one-half hour at 30% relative humidity. However, with moist conditions, zoospores and chlamydospores of *P. ramorum* can survive for at least a month, and this study suggests that chlamydospores probably survive much longer. Thus spores transported to the trunks of oak trees may survive for significant periods of time during the wet winter months. Because moisture loss appears to be one way to kill spores of *P. ramorum*, we are initiating studies to determine survival times for spores under a range of humidity levels. Pairing laboratory data on moisture requirements for spore survival with climate data from forests may help us predict how long spores are present in litter and soil after rains cease, and hence, when closure of areas to the public or logging may be warranted.

Survival of *P. ramorum* in leaves of *U. californica* trees throughout the hot, dry summer may allow relatively high levels of inoculum to persist in forests even though soil, litter, or ephemeral streams may no longer harbor viable spores. The presence of inoculum may allow for rapid spread of *P. ramorum* from many leaf surfaces once winter rains begin, and lead to an exponential spread of infection among the numerous host plants.

P. ramorum does not need wounds to infect *Q. agrifolia* and *L. densiflorus* trees. If trunks stay wet during winter months with rains occurring every few days, spore survival times of up to 30 days in moist micro-habitats such as grooves in bark or beds of moss would allow for a long window of opportunity to initiate infection. Although some *L. densiflorus* in this experiment may be infected yet fail to show bleeding symptoms, it appears that not all trees became infected in this trial even though inoculum levels were quite high. This raises the hope that some type of resistance is present at the onset of infection.

Given the ability of *P. ramorum* to produce spores on foliar hosts which can then be

carried in wind-blown rain, survive on oak trunks in moist conditions, and infect oak trees without a wound, it is understandable how *P. ramorum* could readily spread among oaks within a given location. It is harder to explain long-distance jumps between known sites with oak disease such as the 300 km gap between Mendocino, California and Brookings, Oregon. Aerial dispersal of spores in wind without rain can move spores up to a kilometer (Ristaino and Gumpertz 2000). However, only two of the 60 species of *Phytophthora* are known to have this kind of dispersal (Duniway 1983). Although anthropomorphic spread of infection cannot be ruled out, it remains a primary research priority to investigate forested corridors between oak disease sites for the presence of infected foliar hosts serving as infection pathways.

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Rethinking *Phytophthora*

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Abstract. Our conceptions of the genus *Phytophthora*, its systematics, ecology, and pathology, are in flux. Recent work, much of it reported in the Proceedings of the 1st international meeting of this IUFRO Working Group (Hansen and Sutton 2000), challenges old conceptions, suggests new paths of inquiry, and offers hope to those dedicated to disease management in forests and wildlands around the world. Indeed, this group itself has triggered some of the important advances, by fostering communication between previously isolated research groups, and contributing to a sense of activism in *Phytophthora* research and forest protection. In this review I want to highlight some of the key changes in our understanding, including new diseases and new and evolving knowledge and commitment, as well as outline some of the new research and disease management challenges as I see them.

New diseases

Two newly described and dangerous forest tree diseases with aerial epidemiology challenge our preconceptions about the capabilities of *Phytophthora*. Sudden oak death (SOD), caused by the newly described P. *ramorum* (Werres *et al.* 2001), is killing *Lithocarpus, Quercus, Rhododendron,* and *Arbutus* species in areas of California and Oregon, and causing foliar blight and dieback on numerous other woody hosts in affected areas (Goheen *et al.* 2002; Rizzo *et al.* 2002). This *Phytophthora* kills oaks with girdling stem cankers, but the main avenue of spread seems to be rain splash, with important inoculum (deciduous sporangia) produced on infected foliage and twigs high in the crowns of trees and shrubs. The pathogen has been isolated from soil and streams, but it is not yet clear whether these sources are important in the disease cycle. Root infection is not evident, and stem cankers seldom extend to the roots.

SOD has already caused dramatic damage in the San Francisco Bay area of California, and its broad and seemingly unpredictable host range makes it a threat to many other forest ecosystems as well. The emergence of this disease in a heavily populated and generally affluent area has created both problems and opportunities for disease management, and revealed weaknesses in the pathology infrastructure in the United States. Tremendous political pressure for a "cure" has been generated as trees died in the back yards of rich and influential people. As a result, unprecedented amounts of money have been allocated to research and disease management. But forest pathology research programs in the

Forest Service and at the University of California at Berkeley had atrophied, so there was no chance for a quick response. Dave Rizzo, U.C. Davis, and Matteo Garbelloto, U.C. Berkeley Extension, have risen to the challenge, but Forest Service research is still invisible.

Despite the obvious danger posed by this new pathogen, the California and Federal regulatory systems have proven incapable of timely, useful response. There is still no Federal quarantine. Calls for quick action were met by demands for more information about the pathogen. It seems that only well known organisms can be regulated through quarantine.

Direct efforts at disease control in infested areas of California are difficult because of lack of knowledge to support control actions, shortage of research personnel to develop the disease control strategies, and the headstart the pathogen had before the true cause was diagnosed. Affected lands are largely privately owned, or managed by local agencies. So far, timber industry lands and National or State Forests are unaffected, with the consequence that there is no clear administrative responsibility for action. The State and Private branch of the Forest Service has lead the charge in gathering and disseminating information, raising financial resources, and in survey and monitoring, but has attempted no direct control. A SOD Taskforce, comprised of more than 70 organizations and hundreds of individuals, keeps the pot stirred, but is too cumbersome for effective disease management.

The recent detection of new SOD infections in a confined area of SW Oregon

forests presented a fresh opportunity for disease management. Oregon led in establishing a state quarantine to try and prevent transport of the pathogen, and in establishing an effective survey and detection effort. The Oregon infections appear to not be associated with human activity. Because the infections appear to be new, and affected areas are small, and because we are not encumbered by the California SOD Taskforce, Oregon has mounted an eradication effort. Only time will tell the success, but given the evident danger of P. *ramorum*, and the short window of opportunity to have any chance of eradication, we chose to act.

At this meeting, another new, foliar *Phytophthora* was reported. Margaret Dick described the disease on eucalyptus in New Zealand. The species is still undescribed, but ITS sequence analysis places it with *P. macrochlamydospora* in a rather poorly understood clade of species, linked most obviously by their geographic ties to Australia and New Zealand. Here, the epidemiology is still unclear since the sporangia are not deciduous, but the damage is real, and because of the importance of eucalyptus in many countries, the disease must be considered very dangerous. It will be interesting to see if the southern hemisphere response is any more effective than the frustrating regulatory efforts against SOD.

New knowledge

Significant new understandings in the areas of *Phytophthora* systematics, biogeography and ecology, and pathogenesis have been achieved in recent years. Perhaps most fundamental, and with the most diverse ramifications, is the new molecular phylogeny of the genus based on ITS DNA sequence, presented by Cooke and colleagues (2000). For the first time we have an objective presentation of the evolutionary relationships within the genus. The authors grouped the species into 10 clades, or clusters of closely related species, based on sequence similarity. This architecture is already serving as the basis for new, and testable, hypotheses about evolutionary and speciation processes, ecology, and pathogenesis. For example, the new *P. ramorum*, with deciduous sporangia, is very close in ITS sequence to *P. lateralis*, but appears to be misplaced among species with non-caducous, non-papillate sporangia and soil-based ecologies. Does this indicate a unique speciation process?

The ITS data base has already proved invaluable in isolate identification. It is clear that there are many undescribed species already in hand, and surely more to be found in nature. As new species are recognized, especially within old morpho-species such as the *P. megasperma* complex now seen to contain at least 6 distinct species (Hansen and Maxwell 1991; Cooke *et al.* 2000), it is evident that we need new species concepts to guide our taxonomy. It is also evident that we need to improve our understanding of speciation processes to support the new taxonomy. How do we explain the very close relationships between homothallic *P. megasperma ss*, sterile *P. gonapodyides*, and morphologically similar heterothallic isolates? Is the polyploidy evident within *P. megasperma ss* an important clue?

Our concept of the genus must change as well. *Peronospora* is in reality a *Phytophthora*, at least by ITS sequence. *Halophytophthora* seems to be a legitimate segregate, and other groups, perhaps including *P. macrochlamydospora* and the new species on eucalyptus leaves in New Zealand, should be segregated as well. The line between *Pythium* and *Phytophthora* needs to be reexamined (Cooke *et al.* 2000).

At a very practical level, the new phylogeny and sequence data base greatly facilitate the design and testing of new molecular diagnostic tools. Unique ITS sequences can be directly detected, and specificity of primers can be rationally tested against other species of most similar sequence. Diagnostics based on other parts of the genome, or other molecules, can also be tested for specificity more efficiently, with this new knowledge of which species are most likely to be similar.

It has only recently become evident that *Phytophthora* species are widespread, diverse, and locally abundant in soils and waters of reasonably undisturbed forests. This was first highlighted in Germany, in work by Jung and colleagues (1996), and has been confirmed and expanded through the efforts of the E.U. PathOak project coordinated by Claude Delatour (Hansen and Delatour 1999; Robin *et al.*, this Proceedings). A similar situation evidently holds in North America (Sutton and Hansen, this Proceedings). New species are being described (Jung *et al.* 1999; Hansen *et al.* 2002), and there certainly are more to follow. With a few

116

dramatic exceptions, these "forest Phyto-phthoras" are not associated with any recognized disease of surrounding vegetation.

Some species are clearly of exotic origin, but most give every appearance of being indigenous in forest soils. What are they doing there? At least the most widespread and most abundant species, *P. gonapodyides*, is capable of a saprophytic lifestyle in streams, colonizing leaves as they fall after insect attack or normal autumn defoliation. Is this truly a "genus of plant pathogens," as so often stated, or does it include a diverse array of life styles? Some well known agricultural pathogens, such as *P. cactorum*, *P. megasperma*, and even *P. cinnamomi*, are also being found in forest soils, seemingly not causing disease of mature plants at least. Some are seemingly ephemeral damping off pathogens of newly emerged seedlings (*P. cactorum, P. hevea* in some topical forests; J. Davidson *et al.* 2000). Probably some represent scattered and short lived introductions by birds or other animals, but it seems likely that many if not most are living as fine root pathogens of forest plants. Populations, and damage, are perhaps kept low by coevolved tolerance and resistance of the associated plants, generally well drained soils, a numerous and diverse competing soil microflora, and variable, generally unfavorable, soil environments.

Developing trends
It is exciting to me to come to these meetings and learn that *Chamaecyparis lawsoniana* is not the only host tree being successfully bred for resistance to a dangerous *Phytophthora* species. We learn of developing programs with European alder and chestnut, but also the advanced and successful programs for resistance to *P. cinnamomi* in jarrah eucalyptus and radiata pine. Increasingly, resistance is real, useful, and available. The challenge is now not so much to find and develop resistant trees, but to intelligently propagate and deploy them in forest and plantation settings. Under what circumstances is it appropriate to use clonal material or major resistance genes, and when is the chance of altered pathogen virulence too great? This in turn demands new knowledge about pathogen variability and population structure, dispersal mechanisms and clonal lineages.

How can deployment of resistant trees be integrated with an overall forest management operation, especially in situations where diverse ecosystem attributes are valued over timber, as in Oregon with Port-Orford-cedar (Hansen *et al.* 2000), or where the pathogen attacks many species in addition to the dominate tree, as in the jarrah woodlands of Western Australia? And how can we keep managers from seeing resistance as a panacea and excuse to drop other disease management efforts such as hygiene and road closures.

The important role of infested nursery stock in the epidemiology of *Phytophthora* diseases is increasingly recognized, and concerted actions are being organized. Nurseries seem to have played an important role in both the generation and the dissemination of the alder *Phytophthora* in Europe. Even with pathogens that are already widely dispersed, such as *P. cinnamomi*, nursery infection can lead to more damaging disease and local epidemics in previously uninfested microsites.

Demanding *Phytophthora*-free planting stock of the nursery growers is the obvious solution. Quality control, however, is a challenge, especially with the widespread use in nurseries of fungistatic compounds, such as metalaxyl and phosphonate and its allies, that limit infections and keep plants appearing healthy, but don't eliminate the pathogen. If fungistatic compounds are not allowed, it will force nursery managers to adopt rigorous programs of hygiene, soil water and organic matter management, and inspection. It is entirely possible to grow healthy planting stock without reliance on *Phytophthora* suppressing chemicals, and this would be a major step forward. It must be understood, however, that *Phytophthora* species may still be present without causing dramatic losses, just as they are present in many well-drained forest soils without causing visible disease. The only assurance of *Phytophthora*-free planting stock is strictly regulated nursery production in soil-free, containerized production systems.

New emphasis is also being placed on a number of other areas that can be loosely grouped as "pathogen ecology." The complex interactions between *Phytophthora* species and climatic fluctuations are increasingly recognized as important to understanding disease in the forest, but they remain very poorly understood. Just why is it that so many *Phytophthora* diseases occur in plant communities that at least

117

on average are very dry? The Mediterranean cork oak woodlands, and the Jarrah forest of Western Australia are dramatic examples. Is the flare-up of disease in the year or two following unusually heavy rains predictable enough to be used in disease forecasting, and perhaps trigger forest protection measures?

New knowledge and communication opportunities foster active disease management efforts. The challenge of halting, even reversing *Phytophthora* diseases in forests and wildlands is daunting, but all over the world people keep trying. The importance of *Phytophthora* diseases in forests is evident in the large cooperative efforts that are underway to combat them. The multifaceted, multi-partnered *Phytophthora* dieback research and disease management program here in Western Australia, involving the productive collaboration of ALCOA, CALM, CSIRO, Murdoch, and local groups, has been a model, providing inspiration, as well as new research, to the rest of us. The practicality and efficacy of phosphonate in forests and wildlands was demonstrated here. It provides a valuable new research and disease management tool that will be used on several disease systems around the world.

In Europe several multinational research and information projects focused on *Phytophthora* are sponsored by the European Union. The PATHOAK project looking at *Phytophthora* and oak decline presented its results at this meeting. Sudden Oak Death has engendered a huge consortium of organizations in California, while in neighboring Oregon, a much smaller group emphasizes speed and agility over mass and political clout. In each case, pathologists from different organizations are coming together to get the job done.

One product of these efforts is strategic planning for future research and disease management. In Australia, *Phytophthora cinnamomi* was addressed in a national assessment, spurred by its national recognition as amongst the "top 5" threats to the environment. California has produced a "risk matrix" summarizing information relevant to quarantine decisions. In Oregon, a new "Range Wide Assessment" of the status of Port-Orford-cedar and *P. lateralis* will soon be available, including risk assessment and disease management options. It isn't all talk and paper. Operational, on the ground (and in the air), disease

management programs are at work in Australia with *P. cinnamomi*, in Oregon with *P. ramorum*, in Oregon and California with *P. lateralis*, and in Bavaria with the alder *Phytophthora*.

The special challenge of exotic pathogens

Prof. Hal Mooney, Stanford University, titled a recent talk: "Fight Them on the Beaches, or Let the New World Order Begin." He was referring to the worldwide ecological crisis perpetrated by invasive organisms of all kinds. Blocking the initial establishment of an exotic pest is indeed the one best chance to stop its damage. This is widely recognized, and an elaborate (and cumbersome) international system of quarantine and regulatory authority is in place. The weaknesses and outright failures of the system are all too obvious, and successes are difficult to document. In the current political and economic climate of globalization, the task only gets more difficult. Nevertheless, we must support and strive to improve the system, to protect our forests. At the same time, however, we must support and improve the national survey and monitoring programs that give us a chance to respond to exotic pests early enough to have a chance of eradication. And there must be the will to act, when given the chance.

It is a bold step to attempt an eradication. You must act on incomplete knowledge. You can't afford to wait for all of the answers, because the pathogen won't wait. You must act on faith, because most often a rational assessment of the odds for success, or a vote among your colleagues, will suggest you will fail. Fear of failure is often misplaced, however. "Eradication" sounds too much like extinction. Eradication need not be forever; it doesn't need to be a "once and final" success to make it worth while. In western North America, we "eradicate" gypsy moth from our forests again and again, seemingly annually. The program is not a failure just because new introductions continue-- the repeated eradications mean that 20 or so years on and gypsy moth is still not established in western forests. That is a colossal achievement. And in those 20 years, a generation of westerners has grown up able to enjoy a healthy forest -invaluable, even if the record is broken next year. But entomologists have not only bought time. They have used that time and now there are new, promising biocontrol agents that may make a difference in the future.

118

Phytophthora cinnamomi has presented a daunting challenge to generations of pathologists. There was no chance for eradication-- it had been spread worldwide before most people new such organisms existed. It has destroyed ecosystems. But it is not everywhere! Here at this meeting, we have stood, literally straddling the dieback front. It advances, seemingly relentlessly, but at a finite speed, when left to its own devices. The sense of an "unstoppable epidemic" comes largely from human-abetted spread. The skilled efforts of the dedicated corps of "*Phytophthora* interpreters" have created opportunities for control by delimiting infestations, monitoring spread, and highlighting failures in control efforts, so they can be remedied.

There has been in the past a major effort to limit the human vectoring of *P. cinnamomi* in western Australia. That effort may have waned recently, but it is, I think, because of those past efforts that there are still uninfested forests and wildlands to protect. And in the intervening years, a new tool, phosphonate, has been developed. Phosphonate won't eradicate *Phytophthora*, at least not quickly, but it does protect plants from infection and death, and it does stop advance of the pathogen. The 20 years saved by old fashioned road closuand they have bought the chance for a new tool to give new energy to the campaign.

It is very true that continuing political and public support is necessary for control programs that depend on human cooperation. But with few exceptions, pathologists are lousy politicians. And politicians and the public are fickle -their attention wanders, or is yanked away by events that transcend even *Phytophthora*. A long-term strategy for disease management must look for systems and processes that will stay effective through times of neglect.

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Potential pathogenicity of four species of *Phytophthora* to twelve oak species

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Abstract   Four species of *Phytophthora* were recovered from soil or roots around declining urban oak trees in the states of South Carolina, Florida, Texas, USA and Colima, Mexico: *P. cinnamomi, P. citricola, P. citrophthora,* and *P. megasperma.* Relative pathogenicity of individual isolates was determined by inoculating stems of potted seedlings of 12 species of *Quercus*, measuring lesion lengths, and then calculating lesion expansion rates. After 40 days, it was apparent that oaks were differentially susceptible to *Phytophthora* spp. Lesion expansion rates varied among species of *Phytophthora* on any one oak species and among oak species inoculated with any one species of *Phytophthora.* The range of lesion expansion rates per day were: 0.5 mm on *Q. ellipsoidalis* to 11.0 mm on *Q. muehlenbergii* for *P. citrophthora*; 1.1 mm on *Q. phellos* to 9.8 mm on *Q cerris* for *P. cinnamomi*; 0.1 mm on *Q. phellos* to 3.0 mm on *Q. prinus* for *P. citricola*; and 0.0 mm on *Q. cerris* to 1.1 mm on *Q. borealis* for *P. megasperma*, which was the least virulent species in these tests. These four species of *Phytophthora* have potential to attack a variety of oak trees in urban landscapes.

Introduction

Nursery, landscaping, and urban forestry businesses have become a multi-billion dollar industry in the United States Trees are purchased and planted in a variety of sources and places, some of which may contain infested plant material and/or soil. Because of the increased movement of potentially contaminated plants and/or soil, many pathogens, especially *Phytophthora* species, have the potential to devastate native plants where the pathogens or susceptible hosts have been introduced. Thus it is increasingly important to understand the role of pathogens in tree decline.

Phytophthora spp. are among the most serious soilborne pathogens found in both forest ecosystems and urban landscapes and are becoming increasingly recognized as important pathogens of oaks. *P. cinnamomi* Rands has proven to be a serious root-destroying pathogen to over 1,000 plant species worldwide (Erwin and Ribeiro 1996), especially in regions where soils have a high clay content or are saturated periodically. These environmental conditions favor the development of *Phytophthora* spp. and may compromise roots of *Quercus* spp. The fungi invade and colonize stressed feeder and taproots, causing a decline and premature mortality of trees of all ages. The objective of this study was to determine the pathogenicity of four species of *Phytophthora*, which had been recovered from soil and roots around declining mature oak trees, to species of *Quercus* that are commonly used in landscapes.

Materials and Methods
Seedlings of Quercus spp.

Acorns of twelve oak species commonly planted in the United States were obtained from commercial sources: sawtooth oak (*Quercus acutissima* Carruthers), white oak (*Q. alba* L.), jack oak (*Q. ellipsoidallis* E. J. Hill), bur oak (*Q. macrocarpa* Michx.), chinquapin oak (*Q. muehlenbergii* Engelm.), northern red oak (*Q. rubra* L.), willow oak (*Q. phellos* L.), chestnut oak (*Q. prinus* L.), European turkey oak (*Q. cerris* L.), and English oak (*Q. robur* L. *fastigata*). Acorns of Mexican red oak (*Q. peduncularis* Bello 509) and Mexican willow oak (*Q. salicifolia* Bello 1562) were obtained from personnel of SEMARNAP in Mexico City.

Acorns were stratified in moist sphagnum peat at 5-10°C for several months and then planted in a peat-based soilless mix. In February 1998, after seeds had germinated, seedlings were transplanted into the soilless mix in 10-cm diameter pots. Seedlings were grown in a greenhouse with natural light at a temperature range of 15-30°C. Seedlings were watered and fertilized as needed until June 2000. At the time of inoculation, the seedlings were 25-75 cm tall and 4-7 mm in diameter. The number

of seedlings within each species that were available for inoculation was different.

Isolates of Phytophthora *spp.*

Eight isolates of four species of *Phytophthora* were used in this study—*P. cinnamomi, P. citricola, P. citrophthora,* and *P. megasperma.* All but one isolate of *P. cinnamomi* were recovered from soil around declining oak trees in diverse geographical locations. Isolates of *P. cinnamomi* were: MX-12 from Colima, Mexico, which previously was shown to be pathogenic to three species of Mexican oaks (Tainter *et al.* 2000); FL-97 3059 recovered from roots beneath a declining oak tree in Florida, USA provided by Ernie Ash, Florida Forestry Commission; AF-027 from field soil in an ornamental crop nursery in South Carolina, USA; and three isolates from Texas, USA; *P. citricola* (TX-15 and TX-16), *P. citrophthora* (TX-3), and *P. megasperma* (TX-1 and TX-RP). Isolates were maintained on acidified potato-dextrose agar (aPDA) medium. Agar plugs 5 mm in diameter from 10-day-old cultures were used for inoculation.

Inoculation of oak stems

Stem inoculations were conducted over a period of several days in June 2000. A total of 15 seedlings of each oak species were selected for stem inoculations. Individual seedlings were used as replicates. For each oak species, there were three replications of each fungal isolate used and three controls. For *Q. muehlenbergii* there were two controls and two replications for each fungal isolate. Due to the limited availability of some seedlings of oak species, not all isolates were tested on every oak species.

A sterile razor blade was used to make a downward-slanting wound through the bark and just into the xylem 15 cm above the soil line on each seedling. The bark flap was hinged outward, an agar plug from the selected isolate was placed in the wound, and the wound was covered with Parafilm and aluminum foil to prevent desiccation. A control consisted of inoculation with a sterile agar plug. After inoculation, the seedlings were maintained in the greenhouse and watered as needed. Seedlings were observed daily and symptom development was recorded.

A seedling was harvested when all of its leaves had wilted, which was between 20-42 days after inoculation. The length of necrosis in phloem tissues was measured as an indication of pathogenicity. To measure lesions, the Parafilm and foil were removed and the outer bark was carefully shaved with a sterile razor blade from the inoculated area. The length of discoloration in the phloem and xylem was recorded. To attempt to re-isolate causal agents, tissue was removed from the upper and lower edges of each lesion and plated on pimaricin- chloramphenicol-hymexazol (PCH) (Shew and Benson 1982) or PARPH/CMA selective media (Jeffers and Martin 1986). Isolation plates were incubated at room temperature (21-24°C) for seven days and then observed for presence of *Phytophthora* spp. Seedlings that developed no foliage symptoms or stem lesions were maintained in the greenhouse. After 42 days, they were harvested and examined for lesion development as described above.

Soil infestation

For comparison, 35 seedlings each of *Q. peduncularis* and *Q. salicifolia* were grown in infested soil. All isolates of *Phytophthora* spp. were used except for the Florida isolate of *P. cinnamomi.* Inoculum was grown in V8-Juice vermiculite medium (Tuite 1969) for 28 days at room temperature.

To infest the soil, 5 to 7 1-cm-diameter holes were evenly distributed across the surface of the medium in each pot. A 1-cm^3 aliquot of inoculum from a 28-day-old culture was placed in each hole. The holes were made to a depth of approximately half the pot height to maximize soil and root contact. Five seedlings were used for each isolate for each of the two oak species. The seedlings were grown in the greenhouse and were harvested after 3 months.

Statistical Analysis

For the stem inoculation, lesion length data for each oak species were analyzed using one-way analysis of variance (ANOVA) to determine if significant differences (*P*=0.05) among isolates occurred. If there was a significant treatment effect, all isolate means were compared to the mean of the non-inoculated control treatment using Dunnett's test (*P*=0.05). Data were analyzed in Minitab statistical software (version 12.0). Lesion length data for each of the two oak species planted in infested soil were analyzed using ANOVA and Duncan's multiple range test (SAS 1989).

Results

Inoculation of oak stems with different isolates

All isolates produced a measurable lesion compared to the controls (Table 1) Therefore, each isolate exhibited some degree of pathogenicity within most of the oak species tested. Upon harvesting the seedlings, it was apparent that oaks were differentially susceptible to *Phytophthora* spp. and lesion lengths varied among oak species inoculated with different isolates of *Phytophthora*.

All oak species, compared to the controls, were susceptible to *P. cinnamomi* except for *Q. acutissima* (*P*=0.075) and *Q. muehlenbergii* (*P*=0.664). There was no significant difference in lesion lengths between these two oak species inoculated with species of *Phytophthora*. Both *P. citrophthora* and *P. citricola* produced significant lesion lengths on 4/12 species of oaks. *Q. phellos* and *Q. rubra* were susceptible to all four species of *Phytophthora* and produced significant stem lesions compared to the controls (P<0.001).

After 40 days, it was apparent that oaks were differentially susceptible to *Phytophthora* spp. Lesion expansion rates varied among species of *Phytophthora* on any one oak species and among oak species inoculated with any one species of *Phytophthora*. The range of lesion expansion rates per day were: 0.5 mm on *Q. ellipsoidalis* to 11.0 mm on *Q. muehlenbergii* for *P. citrophthora*; 1.1 mm on *Q. phellos* to 9.8 mm on *Q. cerris* for *P. cinnamomi*; 0.1 mm on *Q. phellos* to 3.0 mm on *Q. prinus* for *P. citricola*; and 0.0 mm on *Q. cerris* to 1.1 mm on *Q. borealis* for *P. megasperma*, which was the least virulent species in these tests.

Soil infestation with different isolates

The extent of root lesions on the two Mexican oak species following the soil infestation is shown in Table 2. *Q. peduncularis* was more susceptible, with 18/34 (53%) of the seedlings showing lesion formation, compared to *Q. salicifolia*, with only 3/34 (9%). Each fungal isolate was associated with at least one, or more, lesions on taproots. *P. cinnamomi*, isolated in South Carolina, was associated with root lesions of up to 32mm on *Q. peduncularis* and was

Table 1. Mean stem lesion lengths (mm) exhibited by oak species inoculated with isolates of *Phytophthora* species.

Phytophthora sp	Isolate	*Q. acutissima*	*Q. alba*	*Q. cerris*	*Q. ellipsoidalis*	*Q. macrocarpa*	*Q. muehlenbergii*	*Q. phellos*	*Q. peduncularis*	*Q. prinus*	*Q. salicifolia*	*Q. robur fastigata*	*Q. rubra*
P. cinnamomi	AF-027	152				29*			139*			154*	
	FL-97	87				34*		42*				126*	
	MX-12	69	41*	251*	74*	58*	176	44*	171*	160*	139*	194*	196*
P. citricola	TX-15	16		25	16			27*	78			40	48*
	TX-16	31	26	33	25	29*	58	5	44	77*	35	43	34
P. citrophthora	TX-3	34	46*		17	21	220	22*	41	104*	34	48	47*
P. megasperma	TX-1	10				13		20*	0		67	18	37
	TX-RP	7	28*	0	20	15	14	16	20	36	10	10	43*
Control		0	0	0	0	0	0	0	0	0	0	0	0
ANOVA: *P* value		0.075	0.004	0.004	0.004	<0.001	0.664	<0.001	<0.001	0.001	0.009	<0.001	<0.001

Oak Species[a]

[a]Means within a column with a significant ANOVA *P* value (≤0.05) were compared to the control treatment by Duncan's Test (*P*=0.05); means significantly different from the controls are followed by an asterisk (*).

Table 2. Root lesion length (mm) in two Mexican oak species planted in *Phytophthora*-infested soil.

Phytophthora Species	Isolate	Oak Species[a]	
		Q. peduncularis	*Q. salicifolia*
P. cinnamomi	AF-027	35*	15
	MX-12	32*	3
P. citricola	TX-15	7	0
	TX-16	4	0
P. citrophthora	TX-3	2	0
P. megasperma	TX-1	8	0
	TX-RP	2	0
ANOVA *P* value		0.0177	0.063

[a]Means within a column with a significant ANOVA *P* value (≤ 0.05) were compared by Duncan's Multiple Range Test ($P=0.05$); means significantly different from each other are followed by an asterisk (*)

significantly different ($P=0.018$) from the remaining isolates of *Phytophthora* spp. excluding the Mexican isolate of *P. cinnamomi* with a *P*-value of 0.063. Lesion lengths were both more abundant and longer on *Q. peduncularis* than on *Q. salicifolia*. 90% of the seedlings of *Q. peduncularis* exhibited root lesions produced by the two isolates of *P. cinnamomi*, whereas only 3 of 10 seedlings of *Q. salicifolia* produced visible lesions. Each of the remaining isolates of *Phytophthora* spp. produced at least one measurable root lesion on *Q. peduncularis*.

Discussion

Ten out of the twelve oak species were susceptible to *P. cinnamomi*, primarily the Mexican isolate. *P. citricola* and *P. citrophthora* were pathogenic to four of the ten of the oak species inoculated. Of all the oak species inoculated, only *Q. phellos* and *Q. rubra* were susceptible to *P. megasperma*.

The apparent difference in susceptibility of the two Mexican oaks to *P. cinnamomi* to root infection reflects readily visible differences in susceptibility seen in the field (Tainter *et al.* 2000). The incidence of root lesions associated with the other *Phytophthora* species tested, although low, suggests a similar differential susceptibility. This preliminary study suggests that these four species of *Phytophthora* have the potential to attack a variety of oak trees used in landscapes.

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Phytophthora palmivora, the cause of red foot in balsa (*Ochroma pyramidalis*).

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Abstract. Red foot, a lethal canker disease of plantation-grown balsa (*Ochroma pyramidalis*) in Ecuador, is described. Red foot is first visible as a triangular-shaped sunken area at the base of the tree. The most striking symptom is exudation of bright reddish watery sap that runs down the stem and dries to produce a rusty powdery residue. Removal of the bark revealed a watery gray lesion in the phloem that quickly changed to dark red-brown. *Phytophthora palmivora* was consistently isolated from margins of active cankers. Artificial inoculations of healthy balsa trees produced cankers in 90 days identical to those on naturally infected balsa trees. *P. palmivora* was re-isolated from these cankers, thus fulfilling Koch's Postulates and identifying a new host for this pathogen. There was no apparent effect of site quality on canker initiation or subsequent development. The fungicide fosetyl-AL (Alliette ®) reduced the width of phloem lesions by about 50% but had no effect on lesion length.

Introduction

Balsa, *Ochroma* spp., is the lightest of commercial timbers and because of this attribute, the wood has value for a very unique mixture of products (Record and Hess 1943). In order to produce a product of uniform quality and assure its availability, much of the balsa entering the market is now grown in carefully managed plantations. On well-drained sites with deep, rich soils, balsa may attain commercial harvest age at from 4 to 6.5 years with a height of 18-25 m and a trunk diameter of 25-40 cm. Until age 6 years or so, balsa develops a shallow, spreading root system.

In Ecuador, balsa plantations are established on former agricultural lands. As a result, plantations have experienced a variety of pest problems, some of which have not been recognized in natural forests where balsa is a relatively minor component. The condition known as Apata roja®, or red foot, may appear at any time during a rotation (Fig. 1A). Red foot is first visible as a triangular-shaped sunken area at the base of the tree. A striking feature is exudation of a bright reddish watery sap that runs down the stem, accumulates on the lower stem, and dries to produce a rusty powder-like residue. On small trees (ca. 20 cm diameter at 1.4 m above ground) the externally visible canker and bleeding area may extend upward only 0.3 m or so. On larger trees (ca. 70 cm or larger) the canker may extend upward more than 2 m. The canker appears to originate from the lower stem area between two major roots. The canker extends vertically up the tree stem but also develops at a slower rate circumferentially around the root crown, eventually girdling the cambium, and this causes death of the tree.

Although not devastating to net wood volume production of the rotation, red foot requires constant attention throughout the rotation to maintain optimum stem density and maximum wood production. Occurrence and incidence of red foot is predictable only to the extent that it is more prevalent in low-lying areas where water accumulates during the summer rainy season. It may also occur where the soil has a hard pan at a depth of 30cm or so.

Inspection of cankered trees during the past two decades suggested that *Phytophthora* might be involved. In October/November, 2000 two of us (WDK and FHT) examined red foot cankers in balsa plantations which ranged in age from 6 months to 4 years. Chips were removed from the leading edge of phloem lesions of several cankers and plated into tubes containing slants of pimaricin-chloramphenicol-hymexazol (PCH) selective medium (Shew and Benson 1982). In addition, phloem and xylem tissues from the cankers were collected and razor blade sections were prepared. These were examined at the field station with a light microscope at 200X magnification. Discolored vessel elements, adjacent companion cells, and phloem cells contained large coenocytic hyphae and structures which resembled chlamydospores.

Methods

Each of 36 isolation tubes yielded *Phytophthora palmivora* (Erwin and Ribeiro 1996). One of the isolates was arbitrarily selected for subsequent field inoculations and maintained on modified PARPH-V8 medium (Ferguson and Jeffers, 1999). To prepare for field inoculations, the isolate was transferred to acidified potato-dextrose-agar medium.

Four sites were selected on land owned by Plantaciones de Balsa, Guayaquil, Ecuador. Two sites were of site quality among the best for growth of balsa. Two other sites were of much lower productivity. In December, 2000 on each site eight trees were wounded and inoculated with *P. palmivora*. Inoculations were conducted at a height of 1.4 m on the stem for ease of inoculation and subsequent observations and to minimize contamination from possible undetected natural infections. An additional 8 trees on each site were wounded and inoculated only with a sterile agar plug. Inoculations were as previously described (Tainter *et al.* 2000). The duct tape wrappings were removed after 2 weeks in order to not restrict diameter growth of the trees. At the time of inoculation, 4 of the inoculated trees at each of the sites were arbitrarily selected for treatment with fosetyl-AL (Alliette®) fungicide at a recommended maximum dosage of 1 lb. fungicide/1 gal. water. This treatment was performed by spraying to runoff the lower 2 m of stem.

At the time of inoculation, several trees afflicted with natural infections of red foot were inspected and tissue samples collected from various portions of the canker. In addition, the morphology of the cankers was examined in detail and internal symptoms recorded.

The tissue samples were fixed in non-chilled 3.5% glutaraldehyde/cacodylate fixing buffer as previously described (Tainter *et al.* 1999). Sections 8-10 μm thick were affixed to glass microscope slides and viewed either unstained or following staining with either 0.05% aqueous toluidine blue-O or 2% ferric chloride in 95% ethanol to identify polyphenolics, or with iodine (0.5%) in 5% aqueous potassium iodide to identify starch, as previously described (Tainter *et al.* 1999). Sections were viewed at 100X and 200X magnification and representative photomicrographs taken.

In March 11 - 16, 2001 each inoculated tree was inspected and the height above and below the inoculation point, and total width, of phloem lesions were measured. From each tree a single set of three chips was removed from the upper lesion edge and plated into a tube of PARPH-V8 medium. Date were analyzed using Duncan's Multiple Range Test (SAS Institute Inc. 1989).

Results

Inoculations

In every tree, inoculation with *P. palmivora* produced a bleeding canker with phloem lesions identical with those observed in natural infections. Some phloem lesion statistics are shown in Table 1. Mean phloem lesion length above the point of inoculation was 37.4 cm, significantly greater (P = 0.05) than the mean length of 23.8 cm below the point of inoculation. Total lesion length of 61.7 cm on

Table 1. Canker growth in balsa trees 90 days after inoculation with *Phytophthora palmivora*

				Canker Dimensions (cm)			
Treatment	Site	No.	DBH (cm)	Above/Below	Total	Width	% Stem Girdled
N[u] + A[v]	Good	16/16[y]	20.8a**	37.6a*/24.1a	61.7a*	20.8a*	32.4a**
N + A	Poor	13/12	11.5b	37.2a/23.5a	58.1a	23.6a	65.0b
All N=s		13			62.1a	30.9a	
All A=s		15			58.4a	13.5b	

[u] Trees inoculated with *P. palmivora*.

[v] Trees inoculated with *P. palmivora* followed by a basal spray of fosetyl-AL (Alliette ®).

[y] Sixteen trees within each treatment were inoculated, but some succumbed before end of experiment.

* Means within a column followed by the same lowercase letter are not different at P = 0.05.

** Means within a column followed by the same lowercase letter are not different at P = 0.01.

125

the good sites was not different from total lesion length of 58.7 cm on the poor sites (P = 0.05). Mean diameter of the trees at 1.4 m was 20.8 cm on the good sites and 11.5 cm on the poor sites, significantly greater at P = 0.01. The proportion of the stem circumference girdled by *P. palmivora* was 32.4 % on the good sites compared with 65.5 % on the poor sites, this difference being highly significant. While there was no difference in lesion width on trees growing on the two qualities of sites, these results suggest that the smaller trees on the poorer sites are probably girdled quicker because of their smaller diameter.

Each of the inoculated trees treated with fungicide at the time of inoculation also developed a bleeding canker similar to those observed in natural infections and the phloem lesions were similar to those in natural infections although they appeared somewhat lighter in color. For the trees treated with fungicide, mean lesion length of 58.4 cm on treated trees was not significantly less than the mean lesion length of 62.1 cm on untreated trees (P = 0.05). However, there was a highly significant difference (P = 0.01) in lesion width between treated (= 13.5 cm) and untreated (=30.9 cm) trees.

P. palmivora was re-isolated from 74 % of the *P. palmivora*-only inoculated trees and from 81 % of the *P. palmivora*-inoculated-fosetyl-AL (Alliette ®)-treated trees, thus successfully completing the fourth, and final, step in the confirmation of Koch's Postulates.

Internal symptomatology
Removal of bark on the canker face revealed a triangular-shaped mass of affected inner phloem tissues (Fig. 1B). Three distinct zones were visible. Zone 1 appeared to be the youngest part of the canker, consisting of light brown patches delimited by a dark brown zone line. These patches were initially small, separate, and distinct at the advancing edge of the canker but increased in area as the zone line delimiting each patch migrated. As the patches increased in area, they coalesced to form a contiguous patchwork which was designated as zone 2. The patches in zones 1 and 2 were initially a watery gray color just after the bark was removed but this oxidized within a few seconds to a light brown color. The patches in zone 2 continued to be delimited by a dark zone line but it was red to maroon in color. Zone 3, or what is interpreted as the oldest part of the

canker, appeared as a similar series of irregularly shaped dark brown patches each of which was delimited by a narrow, dark maroon border, with the only differences being a darker color and visible disintegration of the tissues.

Histology
The exact mode of entrance into the balsa tree by *P. palmivora* was not determined, but after penetration of living tissues is achieved, fungal colonization was concentrated in a layer of inner phloem cells (mostly sieve elements) of 10-20 cells thick just exterior to the cambium (Figs. 2A, 2B, 2C) and almost all of the fungal colonization was upward and almost exclusively intercellular. There was very little invasion of rays and no growth within the alternating bands of fibers. From these hyphal clusters, individual hyphae then ramified upwards within the inner phloem to initiate the satellite infection patches and, thus, continually formed new zone 1's. Each of the satellite patches then appeared to have subsequently expanded in area, somewhat more so in a vertical direction, and then ceased expansion as it met its neighboring patches.

The presence of *P. palmivora* was associated with the deposition of large quantities of polyphenolic materials in axial and ray parenchyma cells, and in any other living cells within the inner phloem that had not yet differentiated. Although scattered starch granules were encountered within phloem cells, the histochemical stains revealed that most of the discoloration forming the red to brown color of the visible phloem lesion was due to the deposition of polyphenolics. The polyphenolics appeared either as small-, to medium-sized droplets which partially filled each cell, or as a single droplet which almost completely filled each phloem cell.

As the hyphae of *P. palmivora* colonized the inner phloem, the phloem cells were partially displaced and crushed (Figs. 2A, B). Phloem cell contents also became disorganized and were less visible. Localized areas within the phloem became desiccated, appeared shrunken, and small cavities formed which were filled with a reddish watery liquid. Presumably this material then leaks out and runs down the bark exterior to produce one of the most visible external symptoms of red foot. Tissues within zone 3 of the canker had lost their structural integrity and the bark split and cracked

126
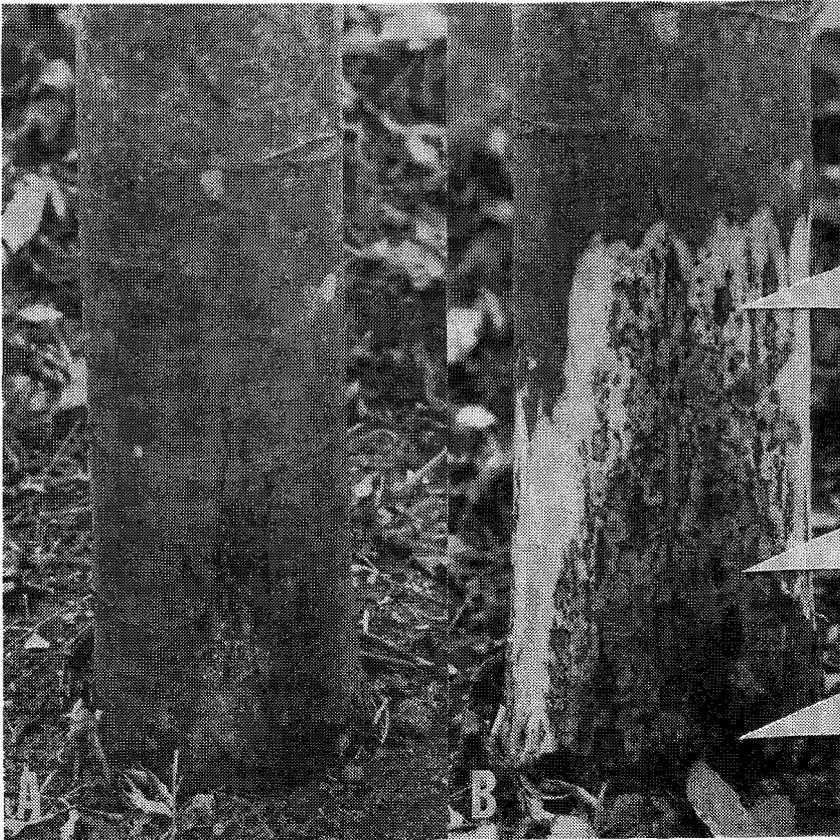
Fig. 1. Red foot canker on eighteen-month-old balsa tree. A. External view; B. With outer bark removed to show the three visible internal zones.

open. At this advanced stage of development it was difficult to isolate *P. palmivora*.

There was little histological evidence that *P. palmivora* invaded the xylem to any great extent, although large, coenocytic hyphae were sometimes visible in vessel openings. As fungal colonization proceeded within the phloem to form zone 1, ray parenchyma and axial parenchyma within the xylem several cm beyond the visible margin of that zone had also begun to form polyphenolics (Fig. 2D). Also, walls of adjacent vessel elements were a brownish color and their companion cells were filled with polyphenolics. In the earliest visible stages of zone 1 in the phloem, the cambium was a uniform brown in color with discolored rays extending inward several mm. As one moved from zone 1 into zone 2, the brown discoloration within the xylem extended deeper and the area nearest the cambium was a darker brown as most of the axial and ray parenchyma cells and some

of the axial tracheids became filled with polyphenolics (Figs. 2E, 2F).

Discussion

P. palmivora is a notorious tropical pathogen, attacking at least 158 plant species (Erwin and Ribeiro 1991). Because most of the present balsa plantation lands were formerly used to grow some of these hosts, there should be no mystery as to the source of inoculum.

The reddish color of the exposed canker face, presumably due to the oxidation of large quantities of polyphenolics within the phloem cells, is similar to a response observed in three species of Mexican oaks infected with *Phytophthora cinnamomi* (Tainter *et al.* 1999). In the Mexican oaks, the possible toxicity of polyphenolics may force the invading pathogen to colonize host tissues in an advancing front with a uniform leading edge. In balsa, the polyphenolics may force *P. palmivora* to invade the healthy phloem in an advancing front consist-

127
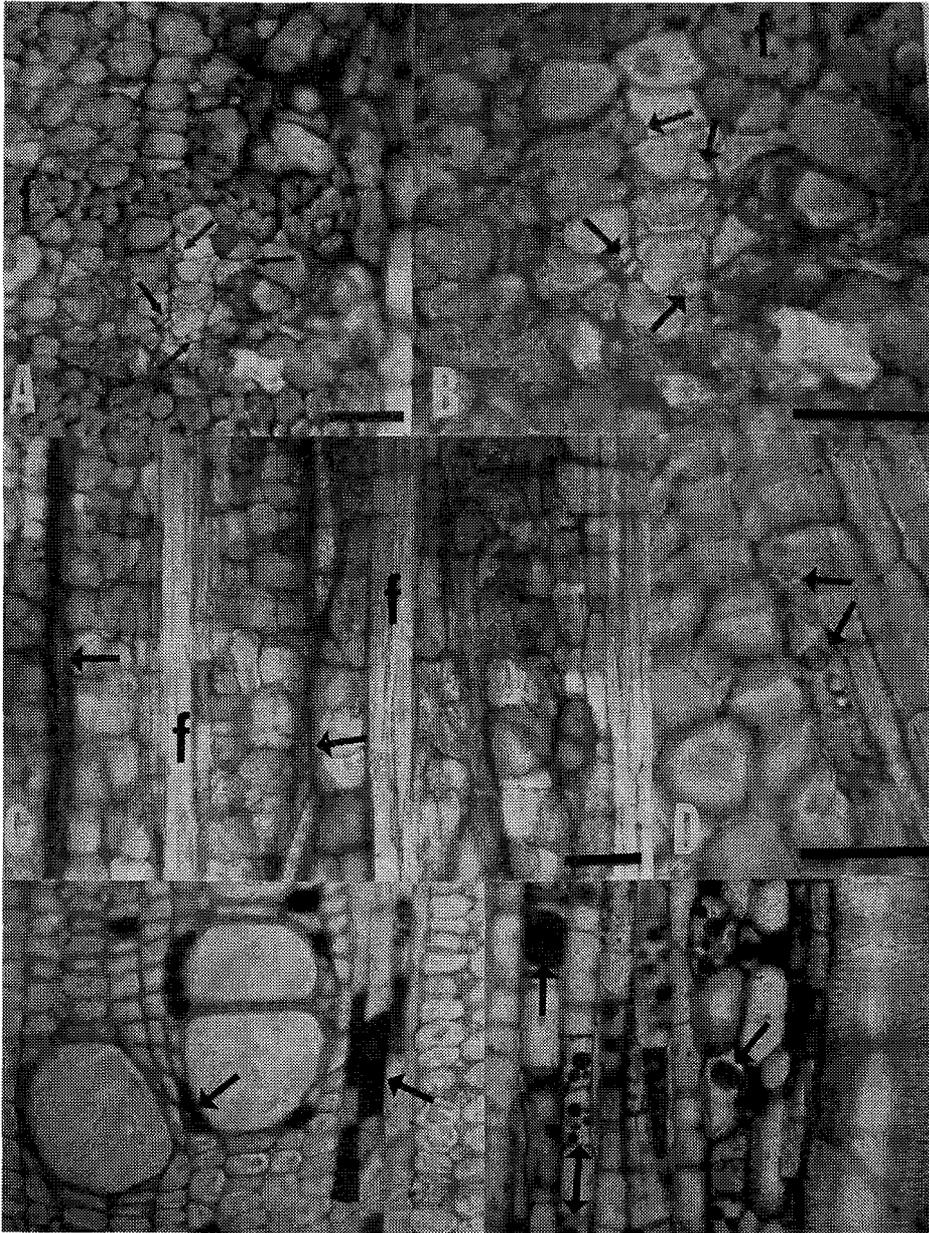
Fig. 2. Inner phloem in zone 1 of red foot canker. A. Transverse section, showing bands of fibers (f) and intercellular fungal hyphae (arrows) among sieve elements and axial parenchyma; B. Magnified view of portion of A, showing fibers (f) and fungal hyphae (arrows); C. Radial view through same area, showing same features as in A and B. Secondary xylem associated with phloem lesion of red foot canker. D. Transverse section 2 cm above visible xylem discoloration, showing limited presence of polyphenolics (arrows) in ray parenchyma; E, transverse section, and F, tangential section, showing polyphenolics (arrows) in ray and axial parenchyma adjacent to zones 1 and 2 of phloem lesion. Horizontal bar=60 μm.

ing of a series of circular patches, with each patch representing a local invasion of tissues and a host response to it. There is little evidence that the invading pathogen is slowed very much by the polyphenolics, because it then colonizes adjacent healthy tissues where it initiates similar reactions.

Except for high-valued balsa trees, such as those in seed orchards or those nearing rotation age, chemical control of red foot using fosetyl-AL (Alliette ®) is probably not justified. However, the low degree of chemical control in the present study may have resulted from the inability of the fungicide to readily pass the layers of fibers in the bark. Further research could clarify whether fungicidal application is possible.

A degree of practical control is already achieved by avoiding low-lying areas where water is liable to stand for several weeks. On sites with a hard pan, some experimental effort is being done to mechanically break the hardpan to improve internal drainage. Sanitation during the entire rotation is vigorously implemented and diseased trees are thinned out as soon as red foot is detected. The felled trees are left lying on the ground. What effect this has on maintaining or increasing inoculum levels in the soil is not known.

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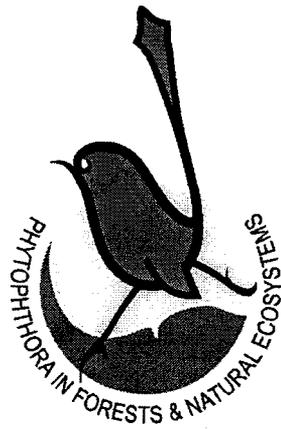
Monitoring of aerial phosphite applications for the control of *Phytophthora cinnamomi* in the Albany District

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Abstract. Operational phosphite applications in autumn 1997 were monitored at the Bell Track in the Fitzgerald River National Park and on Bluff Knoll in the critically endangered Eastern Stirling Range Montane community. The Montane community, which is extensively infested by *Phytophthora cinnomomi*, is notable for 11 threatened plant taxa, four of which are critically endangered. Aerial phosphite application at 24 kg a.i. ha^{-1} resulted in significantly higher percentage survival of *Phytophthora*-susceptible species in sprayed compared with non-sprayed quadrats on a dieback front at the Bell Track for up to two years post-spray. Similar observations were made at Bluff Knoll after phosphite application to uniformly infested vegetation. *Sphenotoma* sp. Stirling Range sampled from Bluff Knoll had considerably higher phosphite concentrations at two weeks and five months post-spray than *Lambertia inermis* sampled from the Bell Track. Phytotoxicity symptoms in terms of foliar necrosis and defoliation post-spray were generally mild although selected species showed greater sensitivity. Growth abnormalities were observed in a small percentage of species assessed at the Bell Track site. Concerns regarding potential phytotoxic effects of phosphite on plant health must be balanced by the threat posed to the survival of critically endangered species and plant communities by *Phytophthora cinnamomi*.

Introduction

The soil-borne pathogen *Phytophthora cinnamomi* Rands is a major threat to Australia's native flora (Shearer and Tippett 1989; Wills and Keighery 1994; Barrett and Gillen 1997). Application of the fungicide phosphite, by stem injection or foliar spray has been shown to control *P. cinnamomi* in a number of native species (Shearer and Fairman 1991; 1997a,b; Komorek *et al.* 1997; Aberton *et al.* 1999; Pilbeam *et al.* 2000). Operational aerial phosphite applications have now been conducted in natural plant communities to protect threatened flora and communities (Gillen and Grant 1997; Barrett 1999). Aerial phosphite application at 24 kg a.i./ha has achieved disease control for up to two years (Komorek *et al.* 1997), however, this may vary in different plant communities and in vegetation of different ages. Faster growing young plants may lose their resistance to *P. cinnamomi* after phosphite application at 24 kg/ha within three years (Komorek and Shearer 1998). *In planta* phosphite concentrations may also vary between species and decline at different rates (Barrett 2001). While phosphite has been found to increase the survival of selected susceptible native species along a dieback front, the ability of phosphite to enhance plant survival in infested areas in the long-term is unclear.

Although phosphite is considered to have low toxicity to plants (Guest and Grant 1991), foliar phytotoxicity has been reported in selected horticultural and ornamental species (Walker 1989; Anderson and Guest 1990; de Boer and Greenhalgh 1990; Wicks and Hall 1990; Seymour *et al.* 1994) and native species (Komorek *et al.* 1997; Pilbeam *et al.* 2000; Barrett 2001). Aerial phosphite application rates must ensure a balance between achieving adequate phosphite concentrations for disease control while avoiding phytotoxicity symptoms in the range of species present.

This paper investigates i) the effectiveness of phosphite in controlling the spread of the *P. cinnamomi* to non-infested vegetation in the Bell Track area of the Fitzgerald River National Park, ii) the prevention of further species decline in infested areas on the summit of Bluff Knoll in the Stirling Range National Park, iii) *in planta* phosphite concentrations in selected species post-spray and subsequent decline in phosphite levels and iv) and symptoms of phosphite phytotoxicity in the plant communities sprayed.

Methods

The two study sites occur within the south coast region of Western Australia which experiences a Mediterranean climate characterised by mild wet winters and hot dry summers. The Bell Track site (S 33°54'31", E 119° 29'26") is in the Fitzgerald River National Park, a UNESCO Biosphere Reserve located between Albany and Esperance. The area is significant for an extensive *P. cinnamomi* infestation (175 ha) in the centre of a reserve which is otherwise relatively free of

P. cinnamomi and noted for the richness of its flora. The vegetation is thicket and scrub over heath on deep sand, last burnt in 1986.

The Bluff Knoll site (S 34 '22' 32", E 118 °15'17") is located on the summit area of Bluff Knoll (15 ha), the highest peak in the Stirling Range National Park and is within the critically endangered Eastern Stirling Range Montane community which is extensively infested by *P. cinnamomi* (Barrett and Gillen 1997). The community is notable for 11 threatened plant taxa; nine of which are endemic to the area and four of which are currently ranked critically endangered. Vegetation is dwarf scrub on skeletal sandy clay loam regenerating after a fire in 1991. The Bluff Knoll site experiences considerable orographic precipitation.

Six 5 x 5 m plots were established at the Bell Track in non-infested vegetation on a dieback front within the spray target area and another six control plots in an area which was excluded from phosphite application. On Bluff Knoll, six 5 x 1 m monitoring quadrats were established within the target area, another six control quadrats were covered with clear plastic during phosphite application. Target areas were aerially sprayed using a Cessna Agwagon 1 88B, equipped with a Micronair Rotary Atomiser spray system that resulted in a droplet size of between 300-500 μm with coverage of approximately 60 droplets/cm² The spray formulation was applied from an altitude of 10 m above the plant canopy in 15 m wide swathes, at a rate of 30 L/ha using 40 % phosphite (Foli-R-fos 400, UIM Agrochemicals Pty Ltd.). One application of 40% product applied at a rate of 30 L/ha resulted in an output of 12 kg/ha. The initial aerial application was followed up by a second application six weeks later to achieve a final output of 24 kg/ha. Synertrol Oil, a vegetable oil concentrate, was added as a surfactant at a rate of 2% of total volume.

In May 1997, at two weeks post-spray, numbers of selected *Phytophthora* susceptible species were counted in monitoring quadrats. These were *Banksia baxteri* R. Br. and *Lambertia inermis* R. Br. at the Bell Track site and *Sphenotoma* sp. Stirling (P.G Wilson 4235), *Andersonia echinocephala* (Stschegl) Druce and *A. axilliflora* (Stschegl) Druce on Bluff Knoll. Due to the difficulty of reliably distinguishing *Andersonia axilliflora* seedlings from *A. echinocephala* seedlings, data from these two species were combined. Recruitment of seedlings in these three species from the

family Epacridaceae occurred over the study period on Bluff Knoll and the original counts were amended to reflect this. At two weeks after the second spray in May 1997 and at five months post- spray in October 1997, shoots of *L. inermis* (Bell Track), and *Sphenotoma* sp. (Bluff Knoll) were sampled for phosphite analysis. *Sphenotoma* sp. was also sampled at three years post-spray from Bluff Knoll. Samples were washed, oven dried at 37°C, ground and weighed to 0.5 g ± 0.002 g. Phosphite content was analysed by the Western Australian State Chemistry Centre by gas chromatography using a P-sensitive column (D.B-Wax) and a phosphorous-specific flame photometric detector (Hewlett Packard). The limit of phosphite detection was 1 μg/g dry wt material.

At two weeks post-spray all species within monitoring plots were assessed for signs of phytotoxicity taking into consideration any foliar necrosis that was present pre-spray. Control plots were assessed for changes in plant health at the same time. Phytotoxicity was rated using a phytotoxicity rating system where 0 = zero necrotic foliage, 1 = 1 - 20% of foliage affected (mild), 2 = 21 - 40% of foliage affected (moderate), 3 = 41 - 60% of foliage affected (moderately severe), 4 = 61 - 80% of foliage affected, 5 = 81 - 100% of foliage affected. A further three assessments were conducted in October 1997, March 1998 with the final assessment in November 1998 at 18 months post-spray. By the second assessment defoliation, growth abnormalities and chlorosis were evident in some species and these symptoms were incorporated into the rating system. Phytotoxicity was also scored in additional monitoring quadrats at the Bell Track site, in mallee-heath vegetation on gravel-loam-sand.

Percentage survival of *Phytophthora*-susceptible species were analysed by means of Analysis of Variance (ANOVA) with spray treatment as the independent variable and percentage survival as the dependent variable. Data were transformed by arcsin square root (x/100) and tested for meeting the assumptions of ANOVA prior to analysis. Homogeneity of variances was assessed using Bartlett's univariate test, scatter plots were used to assess correlation of means and variances, and the distribution of the dependent variable was assessed by the Chi-square test of normality and histogram of the distribution. Data which failed to meet the assumptions for ANOVA were analysed using a Kruskal-Wallis test.

Data for each species were analysed separately for each assessment period.

Results

Mean percentage survival of *B. baxteri* from Bell Track was significantly (P < 0.05) higher in sprayed, compared with non-sprayed quadrats at 12, 18 and 24 months post spray (Fig 1a). For *L. inermis*, mean percentage survival was significantly (P < 0.05) at 12 months post-spray (Fig. 1b). While percentage survival continued higher in sprayed quadrats at 18 and 24 months post-spray, the difference between sprayed and control quadrats was not significant (P > 0.05), although at the final assessment percentage survival was higher (78.9%) in *L. inermis* compared with *B. baxteri* (68.5%).

Mean percentage survival of *Sphenotoma* sp. and *Andersonia* ssp. in sprayed quadrats on Bluff Knoll was not significantly (P > 0.05) higher in sprayed compared with non-sprayed quadrats at 12 months post-spray (Fig 2a,b). There were considerable numbers of plant deaths in sprayed as well as non-sprayed quadrats. However, mean percentage survival for both species was significantly (P <0.01) higher in sprayed compared with control quadrats at 18, 24 and 36 months post-spray. From 12 to 36 months post-spray, mean percentage survival increased slightly in sprayed quadrats for *Andersonia* spp. while for *Sphenotoma* sp. there was a slight increase at 18 and 24 months post-spray.

In planta phosphite concentrations in shoots sampled at two weeks post-spray were more than six times higher in *Sphenotoma* sp. Stirling Range from Bluff Knoll Site than in *Lambertia inermis* from the Bell Track (Table 1). At five months post-spray, levels had dropped to almost 25% of initial levels in *L. inermis* while those in *Sphenotoma* sp. were over 60% of initial concentrations. Low levels (3.9 µg/g) persisted in *Sphenotoma* sp. at three years post-spray.

Phytotoxicity was expressed initially as foliar necrosis which ranged from tip and marginal necrosis to full leaf necrosis depending upon the severity of the symptoms. Phytotoxicity was observed in 88 (60%) of the 146 species assessed however, ratings at any of the four assessments were mild (ratings ≤ 1) in 69 of these. No symptoms were observed 58 (40%) of species assessed. Moderate to moderately severe symptoms (1 to ≤3) were observed in 19 (13%) species which were from the families Myrtaceae (7), Epacridaceae (5), Proteaceae (5), Papillionaceae (1) and Mimosaceae (1) and these were predominantly from the Bell Track site. By the final assessment symptoms persisted in 20 (13.6%) of species and these were mild in all but one species. Growth abnormalities were apparent in 15 (10%) of the 146 species assessed. These were most common in the Proteaceae (8) followed by the Myrtaceae (5), Epacridaceae (1) and Dilleniaceae (1). The most common growth abnormality was 'little leaf' with or without rosetted foliage. Little leaf was accompanied in certain species by spindly, elongate shoot growth. Chlorosis of foliage, in particu (6.8%) of species in the Proteaceae (3), Myrtaceae (4), Epacridaceae (2), Dilleniaceae
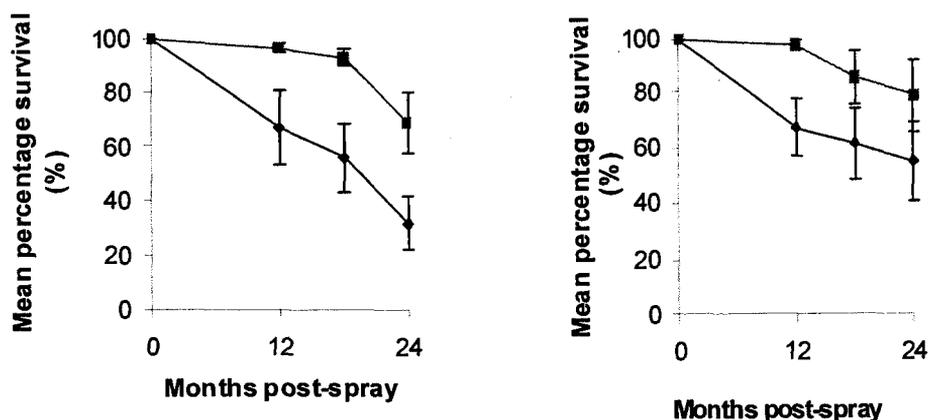

Fig. 1. Mean percentage survival of a) *Banksia baxteri* and b) *Lambertia inermis* plants in monitoring quadrats on a dieback front, Bell track, from May 1997 to April 1999 after phosphite application at ♦ 0 kg/ha, and ■ 24 kg/ha. Values are the means of data from six quadrats ± standard error.
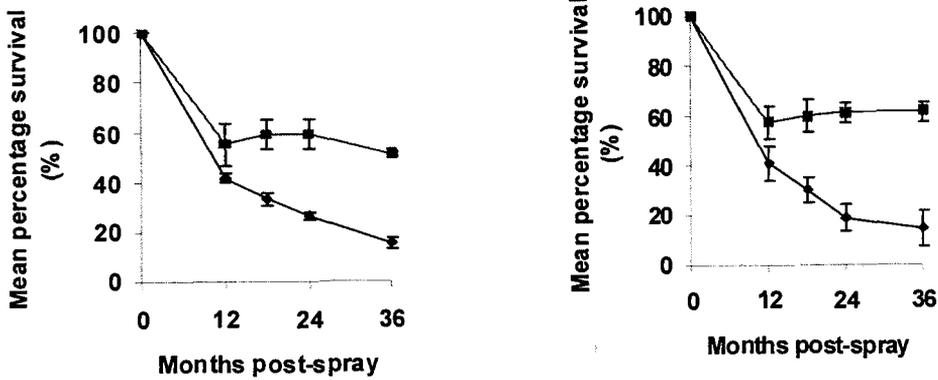
Fig. 2. Mean percentage survival of a) *Sphenotoma* sp. Stirling and b) *Andersonia* plants in monitoring quadrats within a dieback infestation, Bluff Knoll, from May 1997 to April 2000 after phosphite application at ♦ 0 kg/ha, and ■ 24 kg/ha. Values are the means of data from six quadrats ± standard error.

Table 1. Mean *in planta* phosphite levels in *Lambertia inermis* and *Sphenotoma* sp. Stirling two weeks after phosphite application at 24 kg/ha and five months later, and 36 months later for *Sphenotoma* sp. Data are the means of 24 *L. inermis* and 20 *Sphenotoma* sp. samples ± standard errors.

Species	Phosphite concentration (µg/g)		
	May 1997	Oct 1997	April 2000
Sphenotoma sp. (Stirling)	242.9 ± 31.3	154.0 ± 25.2	3.9 ± 0.9
Lambertia Inermis	38.2± 4.6	10.5± 1.7	-

(1) and Papilionaceae (1). Chlorosis also occurred in combination with growth abnormalities. Growth abnormalities and chlorosis were most frequent in species from the Bell Track site particularly in those growing on deep sands. An abundance of new growth was apparent in *B. baxteri* in sprayed compared with non-sprayed plants which was mainly normal in appearance. However, some individuals had small leaves with spindly new shoots. On Bluff Knoll, only one species (*Calothamnus crassus* (Benth.) Hawkeswood) showed some stunting of new growth while three demonstrated chlorotic foliage. By the final assessment at 18 months post-spray, these three species had recovered with the exception of *Kunzea montana* (Diels) Domin. which continued to show chlorosis.

Discussion

At the Bell Track site, there was minimal decline in numbers of plants of *B. baxteri* and *L. inermis* sprayed with phosphite at 24 kg/ha for up to 12 months post- spray. While mean percentage survival continued significantly higher in *B. baxteri* for up to two years post-spray, the reduction in percentage survival from 18 months to two years post-spray, suggested that individuals were becoming more susceptible to the pathogen and re-application of phosphite was required at this point to maintain disease control. This is supported by anecdotal observations of plant deaths in sprayed quadrats at three years post-spray (M. Grant pers. comm.). In the case of *L. inermis*, reduced disease control was apparent even earlier at 18 months post-spray. At the Bluff Knoll site percentage survival rates were significantly higher in *Sphenotoma* and *Andersonia* in sprayed compared with the control quadrats from 12 months to three years post-spray. While there was still a considerable decline in numbers in the sprayed plots in this dieback-infested site, most of this decline took place in the initial year post-spray and many of these individuals were likely to have been infested at the time of spraying. Phosphite application post-inoculation with *P. cinnamomi* is considerably less effective than application pre-inoculation (Davis 1989; Marks and Smith 1992). It is also possible that some of the plant deaths observed in

Sphenotoma may have been related to naturally thinning of dense seedling populations. While percentage survival rates of the Bluff Knoll species were lower at each assessment post-spray than for those at Bell Track, there was little decrease in percentage survival in sprayed plants from 12 to 24 months post-spray in contrast to the Bell Track species. The higher initial phosphite concentrations achieved in *Sphenotoma* and presumably in *Andersonia* spp. from the Epacridaceae compared with the Proteaceous species *L. inermis* may have led to longer disease control. Differences in phosphite tissue concentrations may be related to species specific factors such as leaf characteristics (Barrett 2001), although the more open canopy cover on Bluff Knoll may also have influenced spray retention and uptake. In addition, the Bluff Knoll vegetation, although burnt more recently in 1991, was very slow growing due to extreme environmental conditions encountered at this site. Therefore, there may have been less dilution of phosphite concentrations with growth compared with individuals of *L. inermis* and *B. baxteri* which were still growing strongly at 11 years post-fire. Fast growing plants may lose their resistance to *P. cinnamomi* after phosphite application at 24 kg/ha within three years (Komorek and Shearer 1998). There is likely to have been considerable dilution of *in planta* phosphite concentrations in these larger shrub species which is supported by the results of phosphite analysis at five months post- spray. As phosphite levels in *L. inermis* had dropped almost four-fold by five months post spray it is likely that phosphite concentrations were negligible by the last assessment at 24 months post-spray. In contrast low levels of phosphite were recorded in *Sphenotoma* sp. Stirling at three years post-spray.

At the application rate of 24 kg/ha, 40% of species assessed showed no symptoms of phytotoxicity and the majority of the remainder species had mild symptoms. However, more severe phytotoxicity in a small percentage of species suggests the need for caution where the sensitivity of target species to phosphite is unknown, particularly in the case of rare species. Further research is required to clarify the causes of the growth abnormalities and chlorosis observed and the relationship between soil type and the incidence of these symptoms. As well as vegetative impacts, effects on reproduction must be considered. Reduced flowering (Pilbeam *et al.* 2000) and fruiting Barrett (2001) has been recorded in selected native species after phosphite application. Reduced flowering in spring was observed in the two species from the Myrtaceae (*C. lechenaultii* and *B. preissiana*) after autumn application at 24 kg/ha at Bell Track (Barrett 1999). Tip defoliation may reduce bud set and flowering or phosphite may exert a hormonal or nutritional influence (Barrett 2001). This should be taken into consideration when selecting application rates, particularly where there are concerns about the seed banks of target species.

In conclusion, the results of these operational sprays in terms of species survival rates suggest that phosphite proved effective in reducing the spread of *P. cinnamomi* to non-infested vegetation for up to two years post spray in the Bell Track shrubland community. Phosphite was also effective in increasing plant survival rates in *Phytophthora*-susceptible species in dieback-infested vegetation on Bluff Knoll. If sufficient juvenile plants can be kept alive until the onset of flowering, fruiting and seed set, phosphite may be an effective tool in maintaining and rehabilitating this critically endangered plant community. Selection of phosphite application rates needs to consider the phosphite concentrations necessary to prevent plant death in target species as well as potential phytotoxicity in phosphite-sensitive target and non-target species.

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Control of *Phytophthora cinnamomi* by the fungicide phosphite in relation to *in planta* phosphite concentrations and phytotoxicity in native plant species in Western Australia

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Abstract. Low volume aerial phosphite applications has been used in recent years at rates of up to 24 kg ha^{-1} to protect native plant species and communities threatened by *Phytophthora cinnamomi* while the recommended rate for spray to run-off phosphite application is 5 g L^{-1}. Phosphite uptake and *in planta* phosphite concentrations in native plant species may vary considerably between species and with application rate as may the effectiveness of disease control and the duration of this control. Phytotoxicity symptoms post-spray include foliar necrosis, defoliation, growth abnormalities and chlorosis, reduced root growth and reproductive effects and these may also vary considerably between species. Phytotoxicity symptoms increase with increasing application rate but are generally mild at recommended rates. However, a percentage of species in the plant communities assessed show greater sensitivity to phosphite. Aerial phosphite application rates for native plant communities aims to maximise *in planta* phosphite concentrations in *Phytophthora*-susceptible species for disease control while minimising phytotoxicity symptoms in the species present.

Introduction

The soil-borne plant pathogen *Phytophthora cinnamomi* Rands is recognised as a key threatening process to Australia's biodiversity (Commonwealth Environmental Protection and Biodiversity Conservation Act 1999). Its impact is particularly devastating in the southwest of Western Australia (Wills and Keighery 1994). The fungicide phosphite has been used regularly in horticulture and agriculture for the control of plant pathogens from the genus *Phytophthora*. Research in recent years has shown that application of the fungicide phosphite, by stem injection or foliar spray, can control *Phytophthora cinnamomi* Rands in a number of native species (Shearer and Fairman 1991; 1997a; 1997b; Komorek *et al.* 1997; Ali and Guest 1998; Aberton *et al.* 1999; Tynan *et al.* 2001, Wilkinson *et al.* 2001). The term phosphite refers to the salts of phosphonic acid (H$_3$PO$_3$). Phosphite is a systemic fungicide translocated both in the phloem and xylem (Ouimette and Coffey 1990). In the phloem it is translocated through the plant along typical source-sink pathways (Ouimette and Coffey 1990).

Phosphite exhibits a complex mode of action, acting directly on the pathogen and indirectly in stimulating host defence responses to inhibit pathogen growth (Guest and Bompeix 1990; Guest and Grant 1991). Increasing phosphite concentrations have been generally found to correlate with better disease control (El Hamalawi and Menge 1995) however the presence or absence of phosphite in stem tissue may not strictly correlate with inhibition of *P. cinnamomi* growth (Hardy *et al.* 2001a). The ability of phosphite to control *P. cinnamomi* in native plant species may vary considerably between species, season of application and community (Hardy *et al.* 2001a). Plant tissue phosphite levels and control of *P. cinnamomi* decline over time and this rate of decline varies between species. Phosphite application aims to achieve optimal *in planta* phosphite concentrations for the control of *P. cinnamomi* while minimising phytotoxicity symptoms in target plant species. Although phosphite is considered to have low phytotoxicity (Guest and Grant 1991), symptoms including foliar necrosis have been recorded in a number of native species following its application (Komorek *et al.* 1997; Aberton *et al.* 1999; Ali and Guest 1998; Pilbeam *et al.* 2000; Barrett 2001, Hardy *et al.* 2001b;). In evaluating fungicides it is important to balance phytotoxic effects with the degree of control provided. Current low volume aerial application rates in Western Australia range from 12 to 24 kg ha^{-1} with disease control anticipated for approximately two years post-spray at these rates. The recommended rate for high volume

138

spray to run-off applications and for trunk injection is 5 g L^{-1}. This review investigates factors, which may influence the selection of appropriate phosphite application rates for use in native plant communities.

Phosphite concentrations and control of *Phytophthora cinnamomi*

Phosphite may act directly on *P. cinnamomi* to inhibit growth or alternatively indirectly to enhance host defence responses. The concentration of phosphite in the plant may determine the relative importance of each mode of action (Afek and Sztejnberg 1989). At low levels phosphite may increase the host defence mechanisms but at higher levels act in a fungistatic mode. Mode of action may also be related to whether target plants have well developed or poor dynamic defence systems (Smillie *et al.* 1989) Control of *P. cinnamomi* in native plant species has been achieved with foliar phosphite concentrations of 4.24 and 17.7 µg g^{-1} dry weight. These concentrations reduced colonisation by *P. cinnamomi* of inoculated *Adenanthos barbiger* and *Daviesia flexuosa* stems, respectively, following high volume application at 2 g L^{-1} (Pilbeam *et al.* 2000).

Control of *P. cinnamomi* in plant species in native plant communities following low volume aerial phosphite application has been achieved with *in planta* levels in the order of 5-50 µg g^{-1} dry weight. However, the duration of control varied from one year at low levels to two years at higher concentrations (Komorek *et al.* 1997). Thus the longevity of disease control depends upon the initial phosphite concentrations achieved in plants post-spray. Faster growing young plants may lose their resistance to *P. cinnamomi* more quickly after phosphite application, presumably due to dilution of the chemical with increased plant biomass (Komorek and Shearer 1998). Low volume phosphite application at 12 kg ha^{-1} to *Banksia telmatia* A S George seedlings resulted in leaf concentrations of 115.4 µg g^{-1} two weeks post spray, one year later this had decreased to less than 7 µg g^{-1} and then to a undetectable level at two years (Komorek *et al.* 1997).

In planta phosphite concentrations after low volume or spray to run-off applications may vary considerably between species sprayed at a fixed rate. Phosphite analysis of shoot samples from five shrubland species (*Adenanthos cuneatus* Labill, *Banksia coccinea* R.Br,

Jacksonia spinosa (Labill.) R.Br, *Lysinema ciliatum* R.Br., and *Melaleuca thymoides* Labill.) five weeks after low volume phosphite application at rates of 36, 72 and 144 kg ha^{-1} showed a significant (P < 0.01) difference in *in planta* phosphite concentrations between species (Barrett 2001). *J. spinosa* had phosphite concentrations some 20 times higher than those of *A. cuneatus* at these rates. Similar differences in species uptake have been recorded in other native plant communities after high volume applications at recommended rates (Hardy *et al.* 2001b). For example the jarrah forest species *Leucopogon verticillatus* R.Br. had concentrations of 26 µg g^{-1} compared with 299 µg g^{-1} in *Hibbertia furfuracea* (DC) Benth after application at 5 g L^{-1} (Tynan *et al.* 2001). Concentrations in *Daviesia decurrens* Meissner were generally 10 times higher than those of *Adenanthos barbigerus* Lindley sprayed at 2, 5 and 20 g L^{-1} (Pilbeam *et al.* 2000). Comparatively high *in planta* phosphite concentrations and low phytotoxicity have been recorded in plants grown in glasshouse conditions compared with those of the same species growing in native vegetation (Hardy *et al.* 2001a). Stem phosphite levels in glasshouse grown *Banksia grandis* Willd. were 750 and 380 times greater than field-grown plants treated with 5 or 10 g L^{-1}, respectively (Wilkinson *et al.* 2001).

Colonisation by *P. cinnamomi* generally decreases with increasing phosphite application rate (Smith 1994; Wilkinson 1997; Jackson *et al.* 2000, Pilbeam *et al.* 2000; Barrett 2001). However, there is not always a clear relationship between application rate and disease control. In *A. barbigerus*, control of *P. cinnamomi* colonisation was similar when sprayed at 5 and 20 g L^{-1} despite a 11-fold difference in *in planta* phosphite concentration while fungal colonisation decreased significantly as the application rate increased from 2 to 5 g L^{-1} despite small differences in phosphite concentrations (Pilbeam *et al.* 2000). Control of *P. cinnamomi* in five native species, stem-inoculated and sprayed with 5 and 10 g L^{-1} in a glasshouse study did not consistently correlate with phosphite concentrations in stems (Wilkinson *et al.* 2001). Extremely phytotoxic concentrations may also decrease the effectiveness of phosphite. For example, Shearer (pers. comm.) observed an increase in disease severity and plant mortalities in *B. coccinea* after trunk injection with 100 g L^{-1} phosphite.

Similarly, colonisation of roots of *Xanthorrhoea preissii* Endl. by *P. cinnamomi* sprayed at 20 g L^{-1} was more extensive than in plants sprayed with 5 g L^{-1} while foliage showed extensive foliar necrosis (Pilbeam *et al.* 2000). It is possible that excessive phytotoxicity may inhibit phosphite translocation in the phloem (Groussal *et al.* 1986).

The duration of control of lesion growth in five native species in a glasshouse trial sprayed at 5 and 10 g L^{-1} ranged from 6 to 18 months indicating that a single plant species cannot be used to determine the time for reapplication of phosphite (Wilkinson *et al.* 2001). Foliar application at 5, 10 and 20 g L^{-1} to species growing in two native plant communities that were stem-inoculated post-spray, reduced colonisation for between 5 and 24 months depending on species and rate (Hardy *et al.* 2001*a*). Loss of control was associated with a marked decline in phosphite concentration between 6 to12 months post-spray. After high volume phosphite application of 10 g L^{-1} in autumn to *B. grandis* growing in jarrah forest, *in planta* phosphite was not detectable after 12 months although *P. cinnamomi* was controlled up to 24 months (Tynan *et al.* 2001). However, as the limit of phosphite detection was less than 50 μg g^{-1}, low levels of phosphite may still have persisted. At 12 months post-spray, phosphite was detected in inoculated stems of *Leucopogon verticillatus* growing in the same plant community but *P. cinnamomi* growth was no longer contained (Tynan *et al.* 2001). After phosphite application to *B. grandis* in spring at 10 and 20 g L^{-1}, phosphite was detected for up to 24 months post-spray but *P. cinnamomi* was only contained at 20 g L^{-1}. In contrast, trunk injection at 50, 100 and 200 g L^{-1} to naturally growing wound inoculated plants of *B. grandis* and *E. marginata* resulted in disease control for up to four years (Shearer and Fairman 1997*b*). Phosphite applied at 6 g L^{-1} controlled *P. cinnamomi* for at least two years in *Xanthorrhoea australis* R.Br. growing in infested vegetation. Percentage survival of *Sphenotoma* sp. Stirling (P G Wilson 4235) growing in infested vegetation following aerial application at 24 kg ha^{-1} remained higher than in non-sprayed plants for up to three years post-spray (Barrett unpublished).

Phosphite concentrations and phytotoxicity symptoms in vegetative growth

Phytotoxicity symptoms may also vary considerably between species and application rate (Barrett 2001). In a study of 207 species sprayed at rates ranging from 24 to 144 kg ha^{-1}, plant families sensitive to phosphite included the Epacridaceae, Myrtaceae, Anarthriaceae, Proteaceae and Papilionaceae. However, trends within families and genera were not consistent (Barrett 2001). At the operational rate of 24 kg ha^{-1}, 92% of species showed either no symptoms or only mild symptoms over a 15-month study period. In a study of 18 plant species in the Northern Sandplains of Western Australia most species were unaffected by an application of 5 g L^{-1} but applications of 10 and 20 g L^{-1} caused damage to as much as 50 to 70% of foliage (Hardy *et al.* 2001*a*). Application of 5 g L^{-1} generally caused less than 25% damage to the canopy in most species in *E. marginata* forest (Tynan *et al.* 2001). Necrosis of leaves was observed in 9 of 36 native plants in Victoria sprayed at 6 g L^{-1} but in none sprayed at 2 g L^{-1} (Aberton *et al.* 1999). High volume foliar application of phosphite at 5 g L^{-1} caused burning of leaf tips in *Xanthorrhoea minor* R.Br. and *X. australis* (Ali and Guest 1998).

Recovery from foliar necrosis and defoliation also varies with application rate and species. At 7 months and 2 years after application, there was very little evidence of gross phytotoxicity symptoms remaining in Northern Sandplain species sprayed at 10 and 20 g L^{-1}. Following aerial application at rates above 24 kg ha^{-1}, *Nuytsia floribunda* rapidly shed and replaced foliage within a few months whereas other species had not fully replaced necrotic foliage by 15 months (Barrett 2001). Leaf regeneration in defoliated stems of *Banksia marginata* Cav. sprayed at 6 g L^{-1} did not occur for up to two years post-spray (Aberton *et al.* 1999). Phosphite stimulated new growth in individuals of the resprouter species *A. barbigerus*, *D. decurrens* and *X. preissii* with high phytotoxicity ratings particularly at application rates of 5 and 20 g L^{-1} (Pilbeam *et al.* 2000).

Plant deaths were recorded in *Astroloma xerophyllum* (DC) Sond and *Trymalium ledifolium* Fenzl sprayed at 5 and 10 g L^{-1} (Hardy *et al* 2001*a*). Phosphite applied at 5 and 10 g L^{-1} also killed individuals of the annuals *Pterocheata paniculata* F. Muell. Ex. Benth., *Podotheca gnaphalioides* R. A Graham and *Hyalosperma cotula* Benth with up to 60% and

90%, respectively of *P. gnaphalioides* plants dying (Fairbanks *et al.* 2001). The incidence of plant deaths in species from four plant communities sprayed at 24, 36 and 48 kg ha^{-1} ranged from 0 to 10% (Barrett 2001).

Growth abnormalities, in particular rosetting of foliage, a reduction in leaf size, chlorosis and stunted or spindly growth were recorded after low-volume phosphite application at rates ranging from 24 to 144 kg ha^{-1} (Barrett 2001). Of 207 species assessed, 32 % showed growth abnormalities and 36 % chlorosis. At the lowest rate applied of 24 kg ha^{-1}, the incidence of growth abnormalities ranged from 2 to 11 % in four plant communities. Growth abnormalities were more apparent in members of the plant family the Proteaceae while there was a trend towards a greater incidence of growth abnormalities in communities growing on low nutrient deep sandy soils (Barrett 2001). This may be due to an inverse relationship between plant nutritional inorganic phosphate levels and phosphite uptake (Carswell *et al.* 1996). Further research may reveal whether soil or plant nutrient levels influence phosphite uptake and metabolism.

Phytotoxicity symptoms general show a linear relationship with application rate. *In planta* phosphite concentrations in nine species sprayed at 36, 72 and 144 kg ha^{-1} were significantly correlated with phytotoxicity symptoms (Barrett 2001). Even at the extremely phytotoxic rate of 144 kg ha^{-1} (six times the recommended rate) certain species such as *A. cuneatus* showed minimal symptoms and relatively low phosphite concentrations while *J. spinosa* showed necrosis of more than 80% of canopy cover. Similarly, phosphite concentrations in the jarrah forest species, *A. barbiger* and *D. decurrens* showed a positive correlation with phytotoxicity symptoms after high volume application at 2, 5 and 20 g L^{-1} (Pilbeam *et al.* 2000). Mild phytotoxicity was evident in *A. barbigerus* and *D. decurrens* with foliar dry weight phosphite levels in the order of 10 µg g^{-1} however more severe symptoms occurred with concentrations in the order of 100 µg g^{-1} or higher (Pilbeam *et al.* 2000). Komorek *et al.* (1997) reported severe phytotoxic symptoms in *Lambertia multiflora* Lindl. following low volume phosphite application at 18 and 36 kg ha^{-1} that resulted in phosphite levels in plant tissues several weeks post-spray ranging from a few hundred to several thousand µg g^{-1}.

Mild phytotoxicity symptoms occurred with *in planta* phosphite concentrations in the order of 100 µg g^{-1} in *D. tenuifolia*, *A. cuneatus* and *M. spathulata* sprayed at 36 L ha^{-1}, the severity of symptoms increased above this concentration (Barrett 2001). In *B. coccinea*, concentrations of up to 200 µg g^{-1} resulted in mild symptoms in the majority of individuals (Barrett 2001). In contrast, shoots of *Corymbia calophylla* Lindley grown in a glasshouse showed only mild phytotoxicity (0 to 20% necrosis of the canopy) despite *in planta* shoot phosphite concentrations of 731 µg g^{-1} in plants sprayed at the 'phytotoxic' rate of 96 kg ha^{-1} (Barrett 2001). High phosphite concentrations of 1534 µg g^{-1} were recorded in fine root material although shoots showed minimal foliar necrosis in glasshouse grown *C. calophylla* after low volume application of 24 kg ha^{-1} (Barrett 2001). This data suggests that water deficit and osmotic stress may affect phosphite uptake in native vegetation and exacerbate foliar necrosis. Phosphite concentrations in necrotic foliage of *Eucalyptus redunca* were approximately four times higher than that of healthy foliage (Barrett 2001). Excessive foliar necrosis in target species is likely to result in direct loss of phosphite through defoliation or phosphite retention in necrotic foliage. In either case, a reduced quantity of phosphite may be available for translocation to root tissues.

Investigation of the relationship between phosphite phytotoxicity and selected plant characteristics showed that plant height, leaf shape, leaf hairs, the distribution and position of stomata relative to leaf surface and the presence of oil glands influenced phytotoxicity ratings (Barrett 2001). Growth form, leaf size, leaf orientation, fire response and the position of veins relative to the leaf surface were not related to phytotoxicity symptoms. This suggests that phosphite uptake may be influenced by species specific macroscopic and microscopic plant characteristics and this may explain some of the variation in *in planta* phosphite concentrations recorded in native species. Ultra-microscopic characteristics such as wax composition may also influence pesticide retention and uptake, as may cuticle thickness or a combination of all these factors.

Discussion

This review demonstrates the wide variation in initial phosphite uptake, phosphite decline post-spray, control of *P. cinnamomi* and

the duration of this control which may occur between species and plant communities. Phytotoxicity symptoms may similarly vary between species. While there is not always a clear relationship between *in planta* phosphite concentrations and disease control, in general control increases with increasing application rate. *Phytophthora*-susceptible target species such *Dryandra* and *Banksia* may have relatively low phosphite uptake compared with other members of the same plant community. To achieve optimal *in planta* phosphite concentrations for control of *P. cinnamomi* with aerial or high volume phosphite application in such species, plant health may be compromised in other non-target species. More frequent phosphite application at lower rates may optimise *in planta* phosphite concentrations while minimising phytotoxicity. However cost of application increases with more frequent application while potential phytotoxic effects on plant reproduction (Barrett 2001; Fairbanks 2001) may be exacerbated by, for example, annual application. Phosphite applied at current recommended rates may result in mild symptoms in the majority of species however some may show more severe symptoms. A test application with a hand-held sprayer prior to application is recommended to assess the range in sensitivity of the species present to phosphite and to assist in the selection of an appropriate rate. Timing of application should consider soil moisture levels and temperature to avoid undue osmotic stress.

Short-term phytotoxicity in non-target species may be acceptable as long as there are no long-term consequences for plant health and reproductive ability. Current aerial applications on the south coast of Western Australia target plant communities containing declared rare flora, which are 'critically endangered' or 'endangered' as a direct result of *P. cinnamomi* (Barrett pers. comm.). Management options are limited at the majority of these sites as most or all populations of these taxa are currently infested by the pathogen. Aerial phosphite application may maintain viable populations or allow time for other management actions to be undertaken such as the collection of seed or other germplasm. While enhancing plant survival is the primary consideration, phytotoxicity must not compromise disease control, cause plant death, or reduce the seed store. Monitoring of target species is recommended to ensure that seed banks and the maintenance of viable populations are not compromised. Further

research is required to determine the impact of repeated spraying in terms of phytotoxicity, the long-term efficacy of phosphite in enhancing plant survival and to develop optimal application methodologies in terms of plant phenology and environmental conditions. Research into its mode of action may hopefully lead to other more effective treatment options in the future. In conclusion, phosphite application remains the only method currently available to control *P. cinnamomi* in native plant communities, in particular those that are uniformly infested and contain threatened species. It can continue to be valuable tool in the overall management of *P. cinnamomi* provided it is used with caution.

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Variation exhibited by isolates of *Phytophthora megasperma* causing seedling and tree decline in south-west Australian coastal National Parks

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Abstract. Phytophthora megasperma is an active plant pathogen in National Parks directly to the north of Perth, and along the south coast in the Fitzgerald River National Park. Where active, *P. megasperma* has high impact on species contributing to habitat structure, e.g. *Banksia attenuata* in the northern sandplain and *B. speciosa* on the south coast. Eighty three isolates of *P. megasperma* retrieved from diseased native plants exhibiting foliar dieback symptoms were assessed for morphological and isozymic variation. Isozyme analysis of *P. megasperma* isolates from s-w Australia, three from South Australia, and six isolates from overseas representing the six putative taxa within the *P. megasperma* complex were undertaken. Genetic distances were determined according to Rogers (1972), and unweighted pair groupings with arithmetic averaging (UPGMA) phenograms were constructed using the computer program BIOSYS-1 (Swofford and Selanger 1981).

Introduction

Phytophthora megasperma is an active, primary, non-specific plant pathogen causing seedling root-rot and tree decline in the coastal national parks of s-w Australia. This disease poses a threat to the conservation status of various geographically restricted plant taxa. Within the Proteaceae family, *Banksia* and *Dryandra* were the two genera from which the pathogen was most often retrieved. This introduced soilborne pathogen, changes community structure following infestation because virtually all species susceptible to *P. megasperma* are woody perennials contributing to the plant community structure.

The plant pathogen *Phytophthora megasperma* has earned a reputation as a very variable and diverse plant pathogen (Hansen and Hamm 1983). Stamps *et al.* (1990) revised the well-used key of Waterhouse (1963). Two subspecies of *P. megasperma* were described on the basis of oogonium size and cardinal temperatures for colony growth. The accumulation of knowledge of non-morphological characters and host specificity of *P. megasperma* has seen a revision of the taxonomy of the pathogen. Biochemical and molecular techniques have provided another tool to augment traditional morphometric approaches to discriminate sub-groups within the taxa. Together, these techniques have been used to discriminate three new species: *P. sojae, P. medicaginis* and *P. trifolii* (Hansen and Maxwell 1991). On the basis of numerical analysis of mitochondrial DNA restriction fragment length polymorphisms, a further six distinct "molecular groups" have been identified (Forster and Coffey 1993).

Our study aimed to quantify some of the variability in form and function of the isolates retrieved from s-w Australia, the six protein groups identified by Hansen *et al.* (1986) and some isolates from South Australia.

P. megasperma impacts throughout WA

To the north of Perth, intermittent infections of *P. megasperma* extend over a range of 160 km in the Moora District. Commencing in the Moore River National Park (NP), the infections extend through areas adjacent to Badgingarra NP and Nambung NP to Eneabba (CALM 1990). *P. megasperma* has also been identified as an active primary, non-specific plant pathogen in the Fitzgerald River National Park (FRNP) particularly around East Mount Barren, lying to the east of Hopetoun (CALM 1991). *P. megasperma* has also been recovered from the Esperance area and east to Cape Arid NP .

Visual symptoms of disease impacts

The diagnostic disease symptoms commence with a chlorosis in the lower leaves. The plants lose vigour and the leaves die. This has been observed in seedlings and adult trees. In the majority of cases, the highest disease impacts were associated with sites that were either water gaining, water-logged and/or associated with impeded drainage.

Plant community simplification as a result of infestation

At two diseased sites in Cape Arid NP, (i.e. Thomas Fisheries Gravel Pit and Ranger's Residence at Thomas River), species/area data were collected in both diseased and contiguous healthy plots of vegetation. New species were

recorded in quadrats increasing in area, i.e. 0.25, 1.0, 4.0, 16.0, and 64 square meters.

In all instances, the cumulative number of species in diseased plots was less than the healthy counterparts. The most susceptible plant species belonged to the Proteaceae and Myrtaceae, as found by Wills and Keighery in their 1994 survey of the Stirling Range NP. This pathogen apparently acts to reduce the biodiversity of the floristic assemblage by eliminating the dominant plant taxa. With the loss of plant species, there is also a decline in vegetative cover and an increase in bare ground. This concomitant change in vegetation structure has been associated with degradation of wildlife habitats and food availability (Wilson *et al.* 1994).

Pathological variation

A gravel pit infested with *P. megasperma* 9.5 km along the Point Ann Road (FRNP) was the site for the *in situ* field inoculation. *Banksia baxteri* plants were selected from along the edge of one of the northern pits. Six isolates of *P. megasperma* were screened in this trial (all isolates being retrieved from within the FRNP). The experiment was a Randomised Complete Block design, with individual stems of *B. baxteri* receiving a single isolate of *P. megasperma*. Control stems (within each block) received an uncolonised agar plug. After three and six weeks, stems were retrieved from the field and lesion extension assessed and the fungus re-isolated from the lesions developed in the stem tissue.

Table 1. Mean lesion area (±s.e.m.) developed on *B. baxteri* after six weeks by six isolates of *P. megasperma* from the FRNP

Isolate	Mean lesion areas (sq. mm)
SEB 250	470 (120)a
SEB 251	690 (125)a
SEB 201	1375 (230)b
SEB 242	765 (125)a
SEB 209	1000 (135)b
SEB 234	925 (100)b

The data presented in Table 1 summarises the total mean lesion area quantified after six weeks. The ANOVA identified that there were significant differences between isolates. Isolates SEB 250 and 251 produced significantly smaller lesions that either SEB 201, 209, and 234.

Variation in radial growth rate

A 6 mm mycelial plug was taken from the actively growing edge of each fungal culture and placed at the center of five replicate CMA plates containing 12 ml of agar. Colony diameter was measured on 4-day-old plates grown at 25°C in the dark and converted to radial growth (mm/day). This experiment was repeated three times. The data was analyzed as a Randomized Complete Block design using ANOVA. *Post-hoc* comparisons to identify significant differences between means was carried out using Fisher's Protected LSD test.

For the *P. megasperma* isolates examined, the mean radial growth rate varied between 2 and 7 mm/day. The majority of the isolates tested had growth rates between 3 and 5 mm/day. Only two isolates had growth rates faster than 6.0 mm/day, and the slowest growing isolates both had growth rates between 2 and 2.5 mm/day.

Variation in oogonium diameter

Mean oogonium diameters were determined from measurements of 30 mature oogonia from 10-day-old cultures growing on 10% V8 juice® agar (uncleared) incubated at 19°C in the dark (Fig. 1). From our studies, it is apparent that radial growth rate varies continually within this species complex

Patterns of isozyme variation

We undertook isozyme analysis (using Cellulose Acetate Plate Electrophoresis of mycelium) of 79 *P. megasperma* isolates from diseased plants from s-w Australia, three from South Australia, and six isolates from overseas
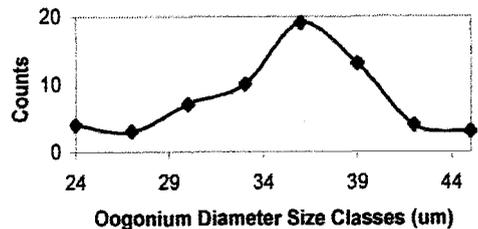
Fig. 1. *P. megasperma* mean oogonium diameters

which represent the six putative taxa within the *P. megasperma* complex (Hansen *et al.* 1986). On each resultant zymogram, each allozyme band was given a designation relative to the fastest anodally moving band. In this way multi- locus genotypes (so called 'isotypes'),
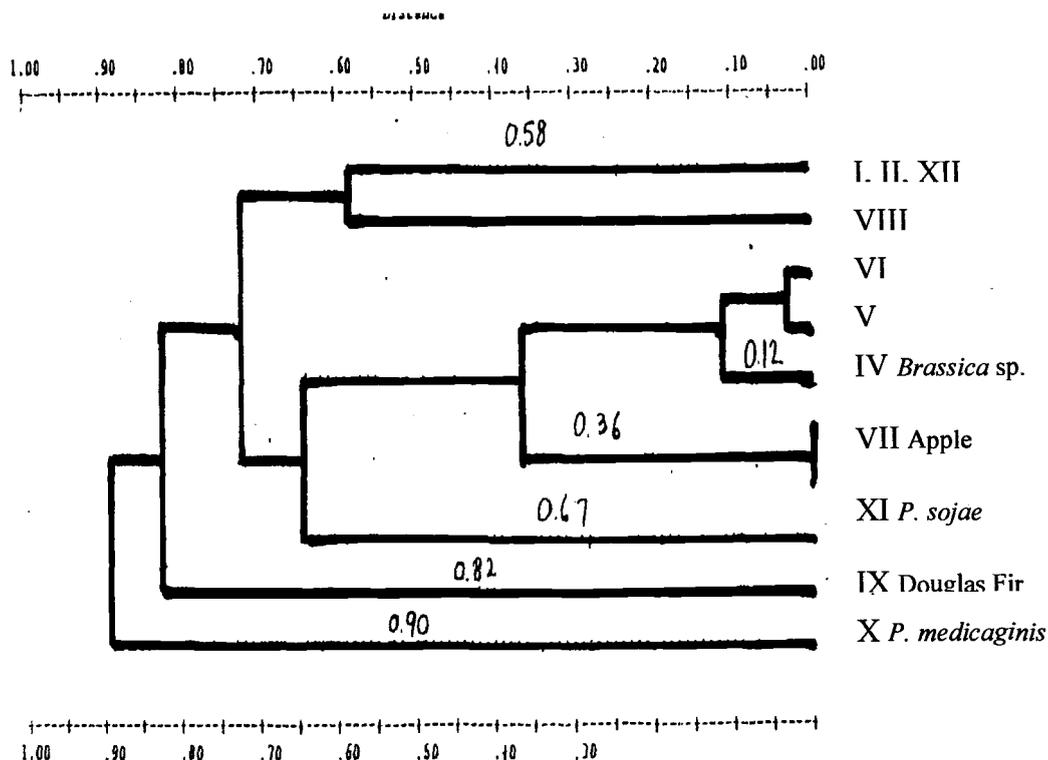
Fig. 2. Phenogram comparing *P. megasperma isolates* from south-west Australia with overseas isotypes and *P. medicaginis* and *P. sojae*

for each isolate were constructed. Twelve putative loci were identified, and genetic distances were determined according to Rogers (1972). Unweighted pair groupings with arithmetic averaging (UPGMA) phenograms were constructed using the computer program BIOSYS-1 (Swofford and Selanger 1981).

Twelve discrete Isotypes were characterised among the *P. megasperma* isolates retrieved from diseased s-w Australian bush. The majority of the isolates screened (approx. 70%) belonged to Isotype I. Isotype II was the next most represented: comprising 6% of the isolates. Six of the Isotypes were monotypic, i.e. we had only one representative of Isotypes: III, VI, VIII, IX, X and XI. Eight of our s-w Australian isolates of *P. megasperma* had the same zymogram as that of P 471 (from apple, California, USA). One of the South Australian isolates from soil had the same zymogram as P 452 from *Brassica* (UK). The remaining overseas isolates (i.e. P 450 = III = *P. trifolii*, P 439 = IX = Douglas Fir, P 484 = X = *P. medicaginis*, and P 445 = XI = *P. sojae*), did not have any homologues from the native bush of s-w Australia.

Fig. 2 describes the association between WA and overseas isolates. The isolate

representing *P. medicaginis* (i.e. P 484), designated as Isotype X, was a genetic distance of 0.90 from the other 78 isolates, and formed a convenient out-group. The overseas isolates from Douglas Fir, *P. sojae*, Apple and *Brassica* sp. segregated from the bulk of the WA isolates (Figure 2). Recent evidence from Cacciola *et al.* (1996) and Costa *et al.* (1996) suggest that there may be at least two discrete taxa amongst these four overseas Isotypes. WA isolates belonging to Isotypes I, II, XII, and VIII segregated-out from the other isolates.

Oogonium diameter varies continuously within an Isotype?

Kuan and Erwin (1980) argued for a broad species concept of the species *P. megasperma* complex, interpreting oogonial size distribution as a continuum within a single taxon. The frequency line of oogonium size classes graph presented in Fig. 1 roughly equates to a normal "bell-shaped" curve. From our results, it is suggested that within the *P. megasperma* complex, morphological variation is continual within a genotypically-defined taxon, and conversely, a morphologically-defined taxon, may encompass a number of genotypically discrete taxa.

146

Conclusions

It is now well established that *P. megasperma* comprises a species complex (e.g. Cooke *et al.* 2000). The recent works of Cacciola *et al.* (1996) and Costa e*t al.* (1996) suggests that further dissection and reclassification be being proposed. Our present study would have benefited from a larger sample set. In this way, we could have quantified more of the variation that exists within the "populations" of *P. megasperma* sub-groups. As it stands, we have found that radial growth rate and mean oogonial diameter vary continuously within isozymically-defined taxa of the *P. megasperma* biological species complex.

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Pathogenicity of *Phytophthora* species on *Quercus* seedlings

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Abstract. *Phytophthora* species are soilborne pathogens often associated with several declining oak woods in Europe and Mediterranean regions as well as in most temperate forests of North and South America and in a few tropical forests. Some *Phytophthora* species associated in Italian oak woods were tested for pathogenicity by inoculation at the collar, on seedlings of *Q. ilex. Q. frainetto, Q. pubescens, Q. cerris* and *Q. robur*. The following ten isolates were used: 1 of *P. cactorum*, 1 of *P. cambivora*, 2 of *P. cinnamomi*, 1 of *P citricola*, 1 of *P. gonapodyides*, 3 of *P. quercina* and 1 of *Phytophthora* sp. The evaluation of infection was based on stem lesion length, and *Phytophthora* re-isolation, 3 months after inoculation. The *Phytophthora* species were differently aggressive towards the five oak species. *P. cambivora* and *P. cinnamomi* were the most pathogenic, *P citricola* and *P. gonapodyides* moderately pathogenic and *P. quercina* generally not pathogenic. In addition the five oak species showed a differential susceptibility towards the *Phtyophthora* species : *Q. ilex* was the most susceptible, followed by *Q. robur*, while the other three oak species were similar.

Introduction

Since the early 1980s a severe oak decline has been reported in different European and Mediterranean regions (Siwecki and Liese 1991; Luisi *et al.* 1993; Jung *et al.* 1996; Gallego *et al.* 1999). Oak decline is a complex syndrome characterised by a gradual, general loss of vigour of the plants, due to the interaction of a number of biotic and abiotic factors, which often ends in the death of trees (Manion 1981; Manion and Lachance 1992). Two types of biotic aggressors are often thought to play an important role in this syndrome: leaf aggressors (herbivore insects and leaf pathogens) able to completely defoliate trees, and soilborne root pathogens (Guillaumin *et al.* 1985; Brasier 1996). Among the soilborne root pathogens several *Phytophthora* species, frequently occurring in the soil of oak stands, were considered as contributing factors or responsible for tree decline (Jung *et al.* 1996; Jung *et al.* 2000). In order to evaluate the relationship between the presence of Phytophthoras in soil and the damage on host, it is important to know their pathogenicity on oak. Pathogenicity of some of the *Phytophthora* species present in oak ecosystems is quite well known as demonstrated on many occasions, i.e. *P. cinnamomi* Rands on many host species (Zentmyer 1980; Robin *et al.* 1998). Recent studies carried out in Italian oak woods revealed the occurrence of several *Phytophthora* species (Vettraino *et al.* 2001). The aim of this research was to evaluate the pathogenicity of some *Phytophthora* spp. isolated in Italian oak woods and the susceptibility of different oak species to those *Phytophthora* isolates.

Materials and Methods

Pathogenicity tests were carried out in greenhouse conditions (temperature 25 ± 1 °C, relative humidity 75 ± 5 % and natural lighting) on 3 year-old plants of *Q. ilex* L., *Q. frainetto* Ten., *Q. pubescens* Willd., *Q. cerris* L. and *Q. robur* L. in two different physiological stages: swollen and open buds. Before the inoculation, seedlings were kept in greenhouse conditions for about 20 days.

Eight isolates of *Phytophthora* spp. from Italian oak woods and 2 reference isolates of *P. cinnamomi* (P382) and *P. quercina* sp. nov. (QUE67) were used in these pathogenicity tests (Table 1). Ten-day-old cultures grown on multivitamin agar were used as inoculum.

For each oak species and physiological stage, 66 plants were inoculated (6 for each isolate and 6 as a control; 660 plants in total). The inoculation technique was the following: an U-shaped cut (about 0.5 x 2 cm) was made aseptically at the collar, removing the bark, and a piece of agar culture (about 0.5 cm^2) was inserted between the bark and the cambial zone (sterile agar medium was used as a control) and fixed with tape. The inoculation site was covered with a sterile wet wad of cotton (to avoid rapid dehydration of the pathogen), Parafilm and aluminium sheet, all of which were removed 20 days later (Robin 1992) (Fig. 1). The evaluation of infection, based on the length of stem discoloration on the cambial

Table 1 - Isolates of *Phytophthora* spp., from different species and locations, used for pathogenicity tests

Phytophthora species	Isolated from	Site
P. cinnamomi (P 382)	*Nothofagus procera*	Surrey (UK)
P. quercina (QUE 67)	*Quercus petraea*	Bavaria (Germany)
P. cinnamomi (CIN)	*Q. frainetto*	Ferrandina (South Italy)
P. cactorum (CAC)	*Q. cerris*	Deliceto (South Italy)
P. cambivora (CAM)	*Q. frainetto*	Campana (South Italy)
Phytophthora sp. (PHY)	*Q. robur*	Cornuda (North Italy)
P. quercina (QUE1)	*Q. ilex*	Cala Violina (North Italy)
P. quercina (QUE 2)	*Q. ilex*	Colognole (North Italy)
P. gonapodyides (GON)	*Q. cerris*	Monte Rufeno (Central Italy)
P. citricola (CIT)	*Q. cerris*	Monte Rufeno (Central Italy)

Fig. 1. Inoculation technique: U-shaped cut at the collar of *Quercus* seedling (top left),inoculum secured with tape (top right) and covered with aluminium foil (bottom).

surface after removing the bark (Robin and Deprez-Louston 1998).

Results

During infection assessment, besides cambial discoloration, other symptoms were also noticed: partial or total wilting of the crown and sprouting of epicormic shoots, at times partially or totally wilted as well. Moreover, some *Q. ilex* seedlings inoculated with the 2 *P. cinnamomi* isolates (P 382 and CIN) and with the *P. cambivora* (Petri) Buisman isolate were dead, since the infection completely girdled the stem. On some *Q. cerris* plants inoculated by the 2 *P. quercina* Italian isolates (QUE1 and QUE2), the infection was limited at the inoculation site (Fig. 2).

Pathogenicity of the different *Phytophthora* species on the five tested oak
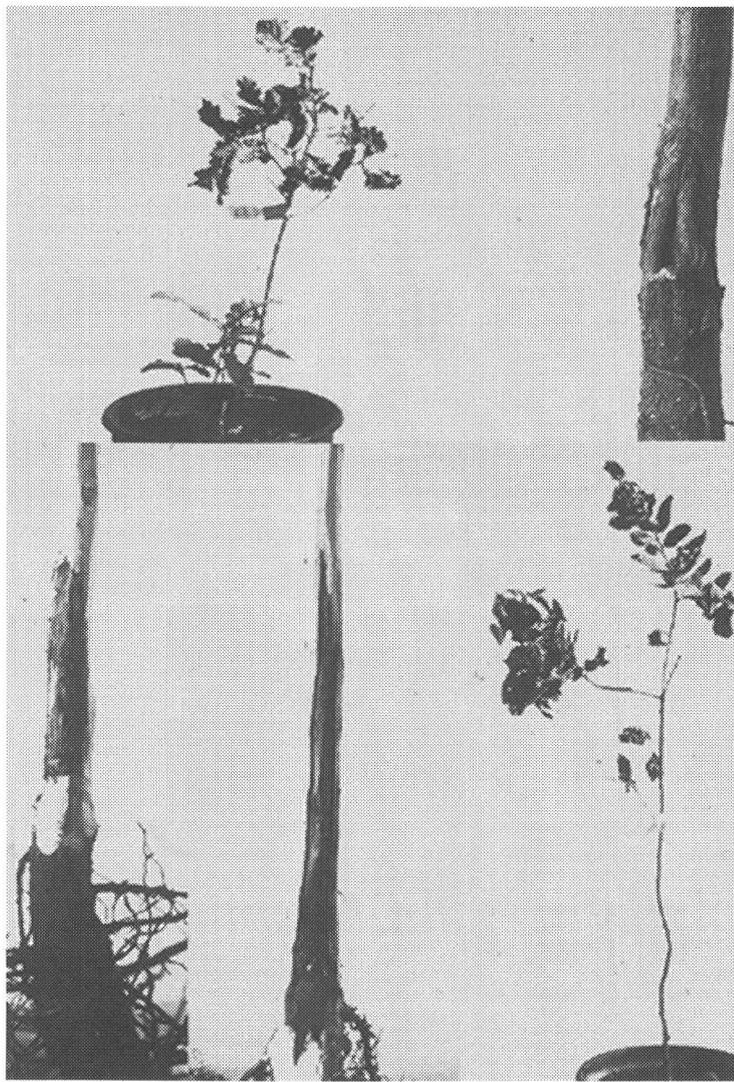
Fig.2. Symptoms observed on oak seedlings, 3 months after inoculation with *Phytophthora* species.: wilted crown and sprouting epicormic shoots on *Q. pubescens* inoculated with *P. cinnamomi* (top left), healed inoculation wound on control oak seedling (top right), stem discoloration on *Q. robur* inoculated with *P. cinna*momi (bottom left and centre), dead *Q. ilex* seedling inoculated with *P. cambivora* (bottom right).

Table 2 - Mean length of stem lesion on *Quercus* spp. seedlings inoculated with 10 *Phytophthora* isolates in two different physiological stages

Oak species	Physiological stage of buds	*Phytophthora* isolates									
		P 382	QUE 67	CIN	CAC	CAM	PHY	QUE 1	QUE 2	GON	CIT
Q. cerris	Open	4.42 A[1]	0.00 A	**6.67 A**	1.00 A	2.42 A	0.00 A	0.00 A	0.00 A	1.58 A	1.83 A
	Swelled up	4.17 A	0.67 A	**1.67 B**	3.42 A	2.58 A	1.92 A	0.00 A	0.00 A	3.25 A	2.67 A
Q. frainetto	Open	4.50 A	0.42 A	3.83 A	1.17 A	3.25 A	0.50 A	0.42 A	0.50 A	1.83 A	2.25 A
	Swelled up	3.33 A	0.00 A	2.67 A	2.83 A	4.00 A	1.58 A	2.50 A	2.08 A	4.08 A	3.83 A
Q. ilex	Open	11.75 A	0.00 A	13.42 A	1.25 A	17.50 A	0.00 A	0.00 A	1.42 A	9.17 A	13.92 A
	Swelled up	17.67 A	0.58 A	14.50 A	3.58 A	11.08 A	0.83 A	0.00 A	1.75 A	6.75 A	5.75 A
Q. pubescens	Open	**3.92 A**	1.08 A	3.33 A	**0.75 A**	3.75 A	0.50 A	0.00 A	0.50 A	3.17 A	4.00 A
	Swelled up	**6.92 B**	2.50 A	4.42 A	**3.50 B**	4.50 A	0.92 A	0.42 A	1.08 A	2.83 A	3.83 A
Q. robur	Open	5.08 A	1.25 A	4.08 A	1.17 A	4.00 A	2.50 A	1.08 A	**0.33 A**	2.00 A	4.08 A
	Swelled up	5.67 A	0.58 A	4.50 A	3.50 A	5.83 A	1.00 A	1.75 A	**2.17 B**	2.33 A	6.00 A

1) On each column, for each oak species, means followed by the same letter do not differ significantly at P = 0.05, according to the Duncan's test
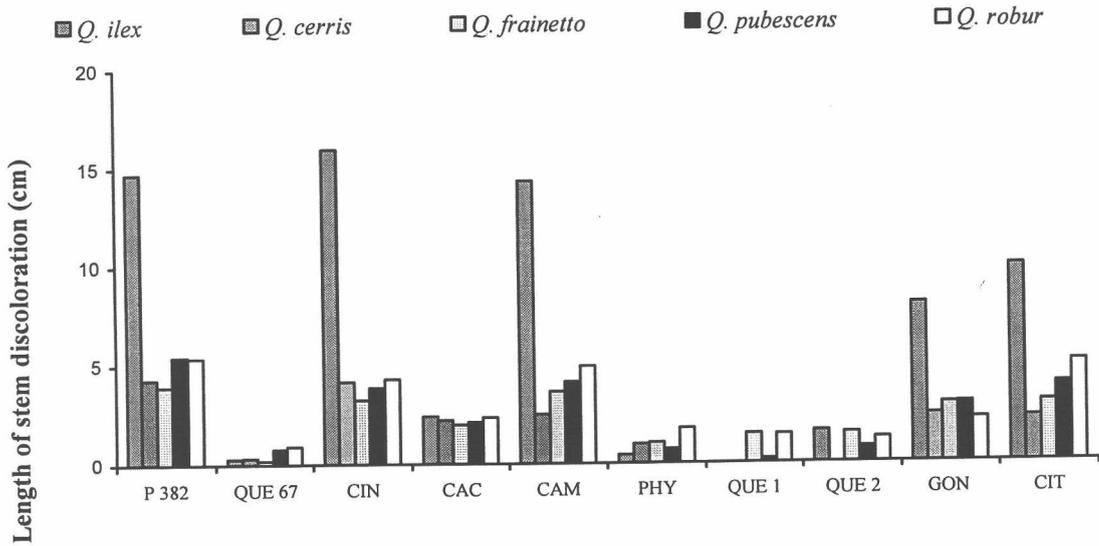
Fig. 3. Mean length of stem discoloration on *Quercus* spp. seedlings inoculated with 10 *Phytophthora* isolates

Table 3 - Percentage of *Phytopthora* spp. reisolated from oak seedlings 3 months after inoculation

| *Phytophthora* spp. | Oak species | | | | | Mean |
	Q. cerris	*Q. frainetto*	*Q. ilex*	*Q. pubescens*	*Q. robur*	
P. cactorum	25	15	30	25	30	25.0
P. cambivora	28	32	90	55	60	53.0
P. cinnamomi	73	50	95	60	65	68.0
P. citricola	90	85	75	70	85	81.0
P. gonapodyides	61	62	70	43	70	61.2
P. quercina	0	0	0	0	0	0.0
Phytophthora sp.	6	4	0	0	10	4.0

species was as follows: *P. cinnamomi* and *P. cambivora*: high; *P. citricola* Sawada and *P. gonapodyides* (Petersen) Buisman : mean; *P. quercina*: generally none. In addition, among oak species *Q. ilex* proved to be the most susceptible. Data of the remaining species do not allow difference distinction in susceptibility (Fig. 3). Sizes of discoloration obtained in the 2 different physiological stages (swelled up and open buds) were submitted to variance analysis and their mean values compared with Duncan's test (SAS, 1996), but no statistically significant differences were observed except in four out of fifty combinations (Table 2).

The most frequently reisolated *Phytophthora* species were: *P. cambivora* and

P. cinnamomi (both the most pathogenic as well), and *P. citricola* and *P. gonapodyides* (both moderately pathogenic). *Phytophthora quercina*, generally not pathogenic in stem inoculation, was never reisolated (Table 3).

Discussion

The results concerning the symptoms observed in this experiment on the pathogenicity of 7 different *Phytophthora* spp. and on the susceptibility of 5 oak species to them, agree with those reported by other authors (Robin and Deprez-Louston 1998; Jung *et al.* 1996). Moreover, differences in pathogenicity of *Phytophthora* species on oaks found in this study, seem to match very well with those obtained by Brasier and Kirk (2001) on *Q. robur*, though they inoculated live logs

151

instead of young stems. Conversely, some authors (Jung *et al.* 1996; Jung *et al.* 1999; Delatour 2001) reported *P. quercina* to be the most aggressive species on *Q. robur*, inducing more than 50% of root damage, though they inoculated the fungus at the soil; this may indicate that the aggressiveness of *P. quercina* is more specific on fine roots rather than on wood tissues. The high susceptibility of *Q. ilex* compared to other tested oak species, was also observed by Paoletti (see Delatour 2001), even though she inoculated (at the soil) only *Q. ilex* and *Q. robur*.

On the basis of their high pathogenicity on different oak species, assessed by various authors, *P. cinnamomi* and *P. cambivora* were indicated as "primary" agents in the decline of pure and mixed oak woods in various Countries (Brasier 1993; Brasier *et al.* 1993a; Robin *et al.*, 1998). Moreover, Brasier *et al.* (1993b) assumed that *P. cinnamomi* can be associated with the fast mortality of oaks occurring in Italy and also in Tunisia and Morocco. *Phytophthora citricola* and *P. cactorum* (Leb. E Cohn) Schröeter, instead, resulted variously pathogenic on *Quercus* spp. (Mircetich *et al.* 1977). The pathogenicity of *P. gonapodyides* has been demonstrated on oak seedlings (Jung *et al.* 1996) and on other hosts species (Erwin and Ribeiro 1996), but little is still known about its pathogenicity on oaks in wood. However, since the aggressiveness of *Phytophthora* species is strongly influenced by environmental conditions and other biotic factors, the only occurrence of *Phytophthora* species in soils of declining woods and their assessed pathogenicity on *Quercus* spp., in controlled conditions, seem not to be sufficient to define their role on decline.

Acknowledgements

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153

Survival of *phytophthora cinnamomi* in plant material under different soil and moisture conditions

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Abstract. Soil moisture and the type of organic matter colonised by *Phytophthora cinnamomi* significantly affected long-term survival of the pathogen. *Banksia grandis* stem pieces, and root tips of *Eucalyptus marginata* (jarrah) colonised with *P. cinnamomi* were placed into pots filled with soil from the jarrah forest or an adjacent rehabilitated bauxite mine site in the south west of Western Australia. The soil was either maintained at container capacity, or allowed to dry-out slowly from container capacity. Samples were harvested over a 210-day period and assessed for *P. cinnamomi* survival. *P. cinnamomi* was recovered after 210 days from banksia stems (98% colonisation) and eucalypt root tips (45% colonisation) from both soil types when the soil was maintained at container capacity. Howeve when the soils were allowed to dry, the pathogen was not recovered after 112 days from either banksia stems or eucalypt roots. Soil origin did not influence *P. cinnamomi* survival for either inoculum type. These findings indicate that under moist conditions the pathogen can survive in small pieces of organic matter for extended periods of time.

Introduction

The *Eucalyptus marginata* (jarrah) forest extends throughout the southwestern corner of Western Australia and is characterised by a Mediterranean climate, typified by long dry summers and cool wet winters (Dell and Havel 1989). The prolonged dry conditions over summer are not favourable to the soil borne plant pathogen *Phytophthora cinnamomi*, which requires a warm, moist environment for optimal growth (Shea 1975; Zentmyer 1980; Shearer and Tippett 1989). Although *P. cinnamomi* may not survive freely in soil for long periods under adverse summer conditions it can survive such conditions saprophytically within dead organic matter (Zentmyer and Mircetich 1966; Shea 1979; Weste and Vithanage 1979; Old *et al.* 1984; Schild 1995). Soil moisture, chemistry, texture, aeration and microbial composition significantly influence the pathogen's survival (Weste and Vithanage 1979; Halsall 1982).

In a modified ecosystem such as the re-habilitated bauxite minesites in the jarrah forest it is likely that, although the rehabilitation processes aim to reproduce the botanical diversity of the surrounding jarrah forest, *P. cinnamomi* may respond differently in the rehabilitated soils compared to the original undisturbed soils (Colquhoun and Hardy 2000). Indeed, *P. cinnamomi* affects floral diversity less in rehabilitated bauxite pits than in infested neighbouring jarrah forest (Colquhoun and Hardy 2000). The current study compared the survival capacity of *P. cinnamomi* in jarrah forest and rehabilitated bauxite mine soils. The ability of *P. cinnamomi* to survive in fine roots of *Eucalyptus marginata* and in woody stems of *Banksia grandis* as well as the effect of different moisture conditions on the inoculum sources were examined.

Material and methods

Experimental Design

A single *P. cinnamomi* colonised *B. grandis* stem plug or five *P. cinnamomi* colonised *E. marginata* root tips were placed into pots of either jarrah forest or mine site soil. Soil moisture treatments were imposed where the soils were either maintained at container capacity or allowed to dry out naturally from container capacity. The pots were positioned at ambient temperature (23 ± 4°C) in a complete randomised block design where each block contained 108 pots, consisting of three replicates for each treatment for each harvest. Samples from each treatment were harvested periodically up to 210 days after inoculation and tested for survival of the pathogen.

Soil collection and preparation

Soil was collected from a jarrah forest Havel 'S' vegetation type (Havel 1975) and an adjacent two-year-old rehabilitated mine site. The Havel 'S' type is characterised by lateritic gravel with a sandy loam to loam matrix and is considered conducive to *P. cinnamomi* (Havel 1975). Soil was collected below the litter layer

to a depth of approximately 30 cm from both sites. The soils were sieved using a 2 mm-diameter sieve. Chemical properties were analysed (SoilWorx, Bibra Lake WA). The soils were baited to confirm that they were *P. cinnamomi* free using the technique described by Hüberli *et al.* (2000).

To determine 'container capacity' for each soil type, three 500 mL pots were filled with 400 g of sieved dry soil and weighed. Water was added until each pot was waterlogged and excess water was allowed to drain (2 mm diameter holes). When the water had stopped draining, the pots were re-weighed and the additional weight indicated 'container capacity'. This was 32% of soil weight for mine soil and 26% for forest soil.

Collection and preparation of tissue types
B. grandis stem plugs

Young *B. grandis* stems of 1-2 cm diameter were cut into 2 cm long plugs. Plugs were rinsed, soaked in distilled water overnight then 100 were placed into each of four 2 L flasks with 200 mL of de-ionised water. The plugs were sterilised for 20 minutes at 121°C on three consecutive days. Once sterilised, the remaining water was drained from the flasks and ten 1 cm^2 blocks of an actively growing culture of *P. cinnamomi* (Isolate MU 97-16) grown on V8 agar (Ribeiro 1978) were distributed uniformly amongst the plugs. The flasks were incubated in the dark at 23°C for six weeks and shaken periodically to ensure uniform colonisation of the plugs.

E. marginata *root tips*

A clonal line of *Eucalyptus marginata* (SS402) susceptible to *P. cinnamomi* was grown for six months in aeroponics chambers (Burgess *et al.* 1998). A zoospore solution, produced following the methods of O'Gara *et al.* (1996) was used to inoculate actively growing roots that were approximately 1 mm in diameter and 200 mm in length. The roots were dipped into the solution for one minute. This inoculation process was repeated after seven days to ensure that the roots were infected. Three weeks after the initial inoculation, the roots were harvested by cutting each root at the root ball. To identify jarrah root sections colonised with *P. cinnamomi* prior to burial in soil, each root was cut into alternating 2 cm and 0.5 cm lengths. The 0.5 cm pieces were plated onto the NARPH agar plates (Huberli *et al.* 2000), incubated at 23°C and

monitored over three days for *P. cinnamomi* growth. Where *P. cinnamomi* grew from a root section, it was assumed that the adjoining 2 cm section of root was also colonised (moving from the root tip toward the stem) and these pieces were used in the experiment. During the 3-day incubation period to test for colonisation, the reserved 2 cm root lengths were kept in moist paper towels at 23 ± 4°C.

Inoculation of soil with roots and woody plugs

Single *P. cinnamomi* colonised *B. grandis* plugs were placed in plastic mesh bags (10 cm x 7 cm with 1 mm diameter mesh) and sets of five *E. marginata* roots were sandwiched between 25 mm x 35 mm pieces of the plastic mesh and held in place using plastic slide frames (50 mm^2 plastic frame). The plugs or roots were then placed into the pots containing 300 g of air-dried soil and covered with a further 100 g of dry soil. De-ionised water was added to the pots over a 12 hour period to bring them to container capacity and pots incubated at 23° ±4° C. The pots maintained at container capacity were watered to weight once a week and were enclosed with plastic bags to minimise evaporation.

Phytophthora cinnamomi *recovery and assessment*

Treatments were harvested at 0, 7, 14, 28, 42, 70, 112, 154 and 210 days after soil inoculation. Three replicate samples from each treatment were assessed at each harvest for *P. cinnamomi* survival and soil moisture content.

At harvest, the plugs were cut longitudinally and then split into ten pieces and plated onto NARPH selective medium to assess the percentage *P. cinnamomi* recovery from each plug. Similarly, each of the five roots in each replicate was cut transversely into half transversely, to give 10 sections that were placed onto NARPH to assess the percentage of *P. cinnamomi* recovery. Where *P. cinnamomi* was not isolated from the plated samples after five days, the samples were removed from the agar, placed in distilled water and baited using *Pimelia ferruginea* leaves (Hüberli *et al.* 2000). The baiting did not yield any further *P. cinnamomi* recoveries during the trial.

Soil moisture content was determined at each harvest by collecting approximately 150 - 200 g of soil surrounding each sample. The soil was weighed, then dried at 102°C and re-

weighed daily until the weight had not changed for 24 hours (usually two days).

Statistical analysis

Data were analysed using the ANOVA module of Statistica (1999 edition, Statsoft Inc., USA). Percentages were arc-sin transformed for analysis. Data were assessed for homogeneity, variation of the mean from the variance and fit to a normal distribution.

Results

Soil type did not significantly (P=0.35) affect the recovery of *P. cinnamomi* from either inoculum type for the duration of the trial despite significant differences in the water content and drying profiles of the soils (Fis. 1 and 2). Since there were no differences in *P. cinnamomi* recovery between the two soil types, the data were combined. Soils held at container capacity maintained 25 – 35 % moisture while soils allowed to dry fell from 30 – 1.5 % moisture (Fig. 1). Moisture significantly (P < 0.01) affected the survival of *P. cinnamomi* in the two soils assessed over 210 days. Soil moisture and pathogen survival were also highly correlated (r = 0.80). Within both of the moisture treatments (pots drying or maintained at container capacity), the percentage of soil moisture proved to be consistent between the two inoculum types as they were not significantly different (P = 0.79). In the soil maintained at container capacity, *P. cinnamomi* was recovered from 98%, and 45% of the woody plugs and roots after 210 days, respectively (Fig. 2). In contrast, when the soils were allowed to dry out, *P. cinnamomi* was not recovered from either inoculum type after 112 days (Fig. 2). In drying soils recovery declined markedly for both plant tissue types after 42 days (Fig. 2).

The source of inoculum (tissue type) had a significant (P < 0.0001) effect on the recovery of *P. cinnamomi* with greater survival in plugs than roots. A large degree of variation in *P. cinnamomi* recovery was evident in the root samples compared to the stem plugs (Fig. 2).

The physical and chemical composition of the two soils varied (Table 1). Mine site soil had lower mineral levels for all of the elements tested, particularly potassium (Table 1). The gravel content for mine soils was also lower than forest soils. Soil pH was within the range for growth and sporelation of *P. cinnamomi* (Zentmyer 1980).

Discussion

The ability of *P. cinnamomi* to survive declined rapidly in drying soils. In contrast, in moist soils it survived for up to 210 days in both fine jarrah roots and woody banksia stems. Similarly Weste and Vithanage (1979) found that *P. cinnamomi* survived for ten months in wet soils compared to less than two months in dry soils. This result is supported by other studies which indicate that *P. cinnamomi* is able to survive in moist conditions in jarrah forest soil for extended periods (Old *et al.* 1984; Shearer and Shea 1987).
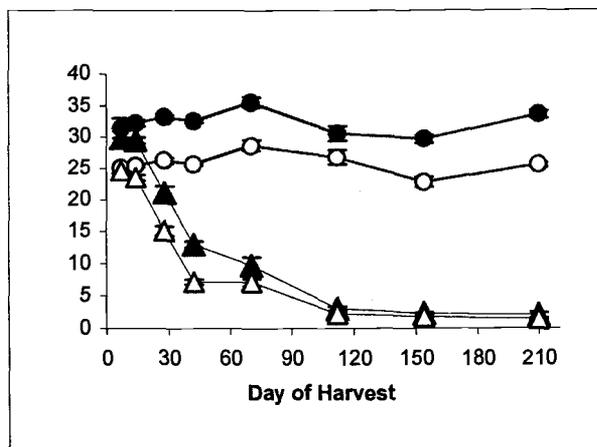

Fig. 1. Water content (%) of soil surrounding *Phytophthora cinnamomi* colonized *Eucalyptus marginata* root tips and *Banksia grandis* woody plugs harvested 0 - 210 days after burial in (●) forest and (O) mine soils maintained at container capacity and (▲) forest and (Δ) mine soils allowed to dry out over 210 days. Bars represent standard error of the mean.
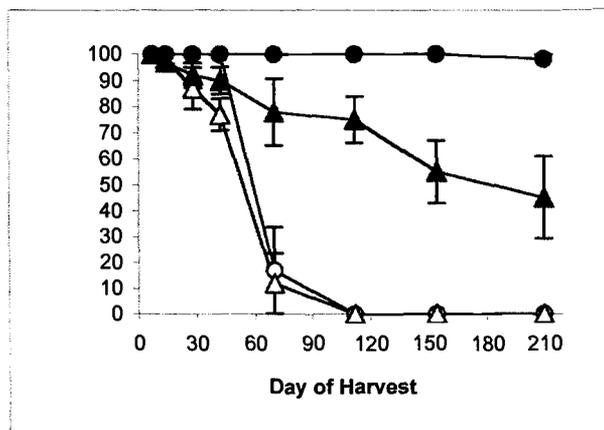
Fig. 2. Recovery of *Phytophthora cinnamomi* (%) from colonised *Banksia grandis* woody plugs in soils maintained at container capacity (●) or allowed to dry out over time (O) and *Eucalyptus marginata* roots in soils maintained at container capacity (▲) or allowed to dry out over time (△) harvested 0 - 210 days after burial. Data from mine and forest soils have been combined. Bars represent standard error of the mean.

Table 1. Physical and chemical Soil properties for the mine and jarrah forest soils

Soil Origin	Nitrate (ppm)	Ammonium (ppm)	Phosphorus (Colwell) (ppm)	Colwell potassium (ppm)	Electrical conductivity	pH in water	pH in calcium chloride
Mine site	1	4	5	28	0.02	6.8	5.7
Jarrah Forest	13	8	7	67	0.06	6.2	5.2

The pathogen responded in the same manner in the two soil types even though they differed in physical and chemical make-up. Soils in rehabilitated bauxite minesites, though taken from areas infested with *P. cinnamomi* before mining, are not highly conducive to *P. cinnamomi* infection (Hardy *et al.* 1996; Colquhoun and Hardy 2000). *P. cinnamomi* commonly causes deaths in areas of impeded drainage in the jarrah forest (Shearer and Tippett 1989). In the rehabilitated areas, improved drainage due to removal of the duricrust during bauxite mining (Colquhoun and Hardy 2000). *P. cinnamomi* commonly causes deaths in areas of impeded drainage in the jarrah forest (Shearer and Tippett 1989). In the rehabilitated areas, improved drainage due to removal of the duricrust during bauxite mining (Colquhoun and Hardy 2000), may be responsible for the reduction in *P. cinnamomi* infestation.

There was more variation in the recovery of the pathogen from the fine roots than the woody stem plugs. Natural colonisation of the roots may have been more 'patchy' than in the woody plugs, which were colonised more uniformly under sterile and constant environmental conditions in the presence of a large amount of inoculum over an extended period.

The size of the two forms of inoculum may also have affected *P. cinnamomi* survival. The stem plugs were significantly larger than the roots and provided better buffering from changes in soil moisture, temperature, and soil microbial activities. Shea *et al.* (1980) also found that isolation of *P. cinnamomi* after host death was higher and more consistent in larger roots than in fine roots.

This study showed that *P. cinnamomi* was able to survive in forest and rehabilitated bauxite mine soils for at least 210 days, particularly in small pieces of woody tissue. Survival was similar in the two soil types, so the

physical and chemical differences between them were not responsible for the differences in severity of *P. cinnamomi* infestation observed between forest and mined areas. However survival was significantly reduced when soil moisture fell below 10 %. Landscaping practices during mine site rehabilitation that minimise waterlogging are therefore possibly the key to the management and control of *P. cinnamomi* in these areas.

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The Reliability of rDNA Internal Transcribed Spacer Sequence Analysis in *Phytophthora* Identification and Taxonomy

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Abstract. The approach to taxonomic and evolutionary issues in the genus *Phytophthora* is being revolutionised by the application of molecular analyses. The internal transcribed spacer (ITS) regions of the ribosomal RNA resolve at an appropriate taxonomic level and the PCR amplification protocols are robust. This, combined with an expanding database of ITS sequences, has resulted in their widespread use in isolate characterisation. Such a molecular approach simultaneously yields objective characters for taxonomic discrimination and sufficient information for phylogenetic studies that identify the nearest known relatives for comparative analysis. Over a dozen new taxa have been discovered over the past few years and ITS sequence analysis has frequently provided the primary support for their status as distinct species. While colony characteristics and morphology are still important, increasingly, the first step is ITS sequencing and a database comparison. While the approach is clearly a powerful one, caution should be exercised. There are many unanswered questions and challenges relating to levels of intraspecific ITS variation, database reliability, discrimination of recently evolved species with identical ITS sequences and the impact of reticulation and hybridisation events on ITS regions. This paper reviews the current status of the SCRI and GenBank ITS sequence databases in the context of such issues.

Introduction

The global significance of *Phytophthora* as a devastating crop pathogen has been recognised (Erwin and Ribeiro 1996) but, with the exception of recently described taxa causing alder mortality, sudden oak death and oak decline, their impact on natural ecosystems remains poorly understood. Of course, all taxa evolved in natural communities and those described to date primarily represent species causing serious plant disease or widespread ecological damage. Recent high profile diseases of trees (e.g. the alder hybrid and sudden oak death) and the actions of the UIFRO working party 7.02.09 have increased awareness of the threats *Phytophthora* poses and the vigilance required to minimise its international movement on planting material. Over the past few years, studies in European forests have yielded previously undescribed taxa (e.g. Jung *et al.* 1999, 2002) and increasing numbers of such studies in natural or semi-natural ecosystems will likely reveal many more taxa.

Traditional methods of *Phytophthora* diagnosis, detection and identification are time consuming, complex and, at times, unreliable. *Phytophthora* primarily causes disease of the roots and stem bases and can be difficult to isolate. In terms of identification, a lack of reliable morphological markers, many of which display considerable plasticity, limits the utility of identification keys. The definitive key to *Phytophthora* identification (Stamps *et al.* 1990)

subdivides the genus into six groups (I-VI) on the basis of sporangial papillum type and the morphology of the developing sexual oospore and details the distinguishing morphological or physiological characters of each taxon. However, such characterisation has, in some cases, resulted in a confused taxonomy to the detriment of both practical control and clear scientific communication.

Recent analyses have utilised the sequence of the Internal Transcribed Spacer (ITS) regions of rDNA to examine the relatedness of a broad range (Crawford *et al.* 1996; Cooke *et al.* 1997; Förster *et al.* 2000) or specific subsets (Werres *et al.* 2001; Mirabolfathy *et al.* 2001; Man In't Veld *et al.* 2002) of *Phytophthora* species. The most comprehensive analysis to date (Cooke *et al.* 2000) examined the main evolutionary trends in the genus and grouped 47 better known, or biologically well-characterised, Phytophthoras into eight ITS clades. These sequences are available in international gene sequence databases and have provided a platform for the descriptions of several recently discovered species (Man In't Veld *et al.* 2002; Jung *et al.* 2002).

The number of *Phytophthora* ITS sequences available in the international databases (e.g. GenBank http://www.ncbi.nlm.nih.gov/Entrez/) is continually expanding. There are in the region of

160 full ITS sequences and over 50 partial or ITS1 only sequences (e.g. Förster *et al.* 2000) currently available. Whilst the ITS1 region is more variable than the ITS2 (Cooke *et al.* 2000), the full-length sequence of approximately 900bp is simpler to work with and allows a more rigorous examination of an isolate's genetic identity. For the purposes of this study only full-length sequences will be considered.

It is acknowledged that responsibility for the quality and accuracy of the data lodged onto sequence database falls to the submitting scientists. In general, sequences accompany a scientific paper and the process of peer review is deemed sufficient quality control. Problems can however arise. The taxonomy of *Phytophthora* is, in places, confused; *P. cryptogea / P. drechsleri* and *P. megasperma*, for example are genetically diverse groups of isolates assembled loosely on the basis of morphology. Prior to the application of molecular methods of species identification such misidentification was inevitable. Misidentified isolates in international culture collections similarly 'muddy the waters'. In addition to the taxonomic issues, data quality is a concern. It is important that sequence data of the highest quality is lodged onto the databases and that timely updates are made as needed.

As costs fall and the hardware and technical skills for PCR and DNA sequencing become more prevalent, groups are relying on molecular characterisation of newly isolated *Phytophthora* species (Hansen and Sutton 1999 and see many articles in this volume). In this article we examine the current status of the *Phytophthora* ITS data in GenBank in terms of its utility and reliability.

Materials and Methods

Complete sequences of the ITS1, 5.8S and ITS2 regions of ribosomal RNA of 339 isolates of *Phytophthora* were assembled from the SCRI database, those of collaborating partners and from GenBank. The sequences were aligned using ClustalW (Thomson *et al.* 1994) using the 'fast and approximate' option and a phylogenetic tree constructed using DNA distance-based methods with the default settings in DNADIST and NEIGHBOR programmes in PHYLIP. The tree was viewed with Treeview (Page 1996) and incorporated into Microsoft PowerPoint as a Windows metafile for further editing.

Results

The phylogenetic tree from the 339 full ITS sequences is shown in Fig. 1. The figure is designed to indicate the overall structure of the principal clades rather than details of the isolates at the tip of each branch. The major lineage furthest from the root comprises 29 primarily non-papillate species in clades 6, 7 and 8. Clades 1-5 are more closely related to each other, forming a cluster of taxa in which the roots of each clade are not so clearly defined.

The number of sequences available from both GenBank and the SCRI database for taxa within each ITS clade is shown in Table 1. Of the 57 taxa, 34 remain represented by the sequence of one or two isolates. Conversely, the ITS sequences of 18 isolates described as *P. drechsleri* are available. The taxa for which GenBank entries either did not cluster with 'type' isolates or displayed unusual levels of variability are indicated (Table 1). In general taxa were identified correctly. However, in the case of *P. megasperma, P. cryptogea, P. drechsleri* and *P. syringae* some of the GenBank sequences grouped within other clades. For other taxa, an unexpected level of intraspecific variation was observed. In some cases isolates defined as a single taxa grouped into several related subclades (e.g. *P. citricola, P. citrophthora, P. capsici, P. cryptogea*) and in other cases, relatively long branches within a species- specific cluster were apparent (e.g. *P. cinnamomi* and *P. glovera*).

A detailed comparison of clade 8 taxa is presented (Fig. 2) as an example of some of the remaining taxonomic challenges. Clades representing *P. syringae, P. lateralis* and *P. medicaginis* are discrete whereas isolates described as *P. megasperma* are interspersed amongst the three lower clades and isolates of *P. cryptogea* and *P. drechsleri* form a loose and, at first sight, confused, assemblage of interrelated taxa. Of the 15 isolates described as *P. cryptogea*, 13 lie within this sub-clade. Another two submitted to GenBank (L76543 and L76538) group within clade 6. Of the 18 isolates described as *P. drechsleri*, 11 lie within this sub-clade. Five form a discrete *P. drechsleri* clade and six others are interspersed amongst *P. cryptogea* isolates. Four sequences submitted to GenBank (AF228093, AF228095, AF228096 and AF087473) group in clade 7b and 3 (L76551 and L76545, and L76547) group within clade 6.

Table 1. List of *Phytophthora* taxa for which ITS sequence data are available.
The species are grouped according to the previously defined clades of Cooke *et al.* (2000) and the number of sequences available in the GenBank or the SCRI database detailed. Several taxa await formal description and are not detailed here. Misplaced taxa, i.e. those that cluster in more than one clade (representing misidentification or a confused taxonomy) are indicated and discussed in the text. [1]The ITS sequence of *P. ipomoeae* is not yet available but has been reported as identical to that of *P. infestans, P. mirabilis* and *P. phaseoli* (Flier 2001).

ITS Clade	Number of sequences in clade GenBank	SCRI	Taxa	Sequences available	Misplaced taxa (GenBank only)
1	20	11	*P. cactorum*	4	
			P. idaei	1	
			P. pseudotsugae	1	
			P. nicotianae	4	
			P. tentaculata	1	
			P. iranica	2	
			P. clandestina	2	
			P. phaseoli	1	
			P. infestans	3	
			P. mirabilis	1	
			P. ipomoeae[1]		
2	22	40	*P. multivesiculata*	1	
			P. citricola	3	var.
			P. inflata	1	
			P. capsici	3	var.
			P. citrophthora	3	var.
			P. glovera	5	var.
			P. colocasiae	1	
			P. botryosa	1	
			P. tropicalis	2	
3	5	13	*P. quercina*	2	
			P. ilicis	1	
			P. psychrophila	1	
4	6	29	*P. megakarya*	1	
			P. palmivora	4	var.
			P. arecae	1	
5	3	3	*P. heveae*	2	
			P. katsurae	1	
6	14	40	*P. humicola*	1	
			P. gonapodyides	3	
			P. megasperma	3	1 (8)
			P. sp 'O' group	1	
			P. sp Asparagus	1	
7	41	20	*P. sojae*	3	
			P. pistaciae	2	
			P. vignae	2	
			P. cajani	1	
			P. sinensis	1	
			P. melonis	6	
			P. cinnamomi	7	variation
			P. uliginosa	1	
			P. europaea	3	
			P. alni	3	
			P. cambivora	3	
			P. fragariae v. *frag.*	1	
			P. fragariae v. *rubi*	1	

8	47	39	P. cryptogea	15	2 (6)
			P. drechsleri	18	4 (7b), 3 (6)
			P. erythroseptica	3	
			P. trifolii	1	
			P. medicaginis	4	
			P. porri	5	
			P. primulae	1	
			P. brassicae	4	
			P. syringae	1	2 (2)
			P. lateralis	2	
			P. ramorum	1	

Discussion

In this study ITS analysis has been extended from 47 (Cooke *et al.* 2000) to 339 *Phytophthora* isolates (Fig 1). Whilst the data adds considerable detail to many of the clades no new lineages of *Phytophthora* have been revealed. Broadly, the tree matches that previously published; isolates cluster into major lineages of more distantly related primarily non-papillate species (Clades 6-8) and a comparatively closely related cluster of semi and papillate taxa (Clades 1-5). Differences such as the fragmentation of clade 3 and the branching pattern of some basal nodes are to be expected in an analysis based on a 'fast and approximate' alignment with no account of transition/transversion ratio or rate homogeneity. A detailed alignment and phylogenetic analysis of over 300 sequences would be prohibitively time consuming and not warranted in this study where the goal was a rapid indication of the clustering of isolates of similar sequence.

This analysis confirms the validity of recent descriptions of *P. ramorum* (Werres *et al.* 2001), *P. europea*, *P. uliginosa* and *P. psychrophila* (Jung *et al.* 2002), *P. brassicae* (Man In't Veld *et al.* 2002), *P. glovera* (Shew *et al.* 1999), *P. tropicalis* (Aragaki and Uchida 2001), *P. pistaciae* (Mirabolfathy *et al.* 2001), *P. quercina* (Jung *et al.* 1999), *P. alni* (Brasier *et al.* 1999; personal communication C.M. Brasier), *P. multivesiculata* (Ilieva *et al.* 1998) and *P. ipomoeae* (Flier 2001) as new species on the basis of both the ITS sequence data and standard behavioural and phenotypic criteria.

Whilst a review of the whole genus is not within the scope of this article, consideration of misplaced taxa or those in need of taxonomic revision will be made.

P. citricola, *P. citrophthora*, *P. capsici* and *P. cryptogea* were examples of taxa in which the isolates grouped into several related subclades. At least 4 distinct ITS types have been defined amongst *P. citricola* isolates. Correspondence between the ITS types and those defined on the basis of morphology, temperature relations (see Balci this volume) has been demonstrated. Other work has identified 5 subgroups with isolates described as *P. citricola* (Oudemans *et al.* 1994) suggesting a re-description is appropriate. The seven sequenced isolates of *P. citrophthora* group in four separate subclades interspersed with isolates of *P. meadii*, *P. botryosa*, *P. colocasiae*, *P. capsici* and even one misidentified isolate of *P. citricola*. *P. capsici* itself forms at least three subclades interspersed with isolates of *P. glovera*, *P. tropicalis* and *P. citrophthora*. Numerous studies on the morphology and genetics (primarily based on isozymes) of these taxa have identified many distinct subgroups within each. Isozymes are appropriate for discriminating amongst closely related taxa but not for reconstructing phylogenetic relationships amongst divergent groups. The ITS sequences are appropriate for such reconstruction but the details of taxa in these clades have yet to be re-examined in sufficient detail for a coherent taxonomic review.

The history of confusion in the identification of *P. cryptogea*, *P. drechsleri* and, to some extent *P. erythroseptica* (reviewed in Mills, Förster and Coffey 1991) is demonstrated, and at least partly explained, in the current analysis (Fig. 2). *P. cryptogea* has a wide host range and since it causes significant problems within the horticultural industry is frequently isolated. Morphologically it is broadly similar to *P. drechsleri* and *P. erythroseptica* and many isolates have thus been misidentified. *P. drechsleri* was originally discriminated on the basis of its ability to grow at 35°C and subsequently other morphologically similar

162
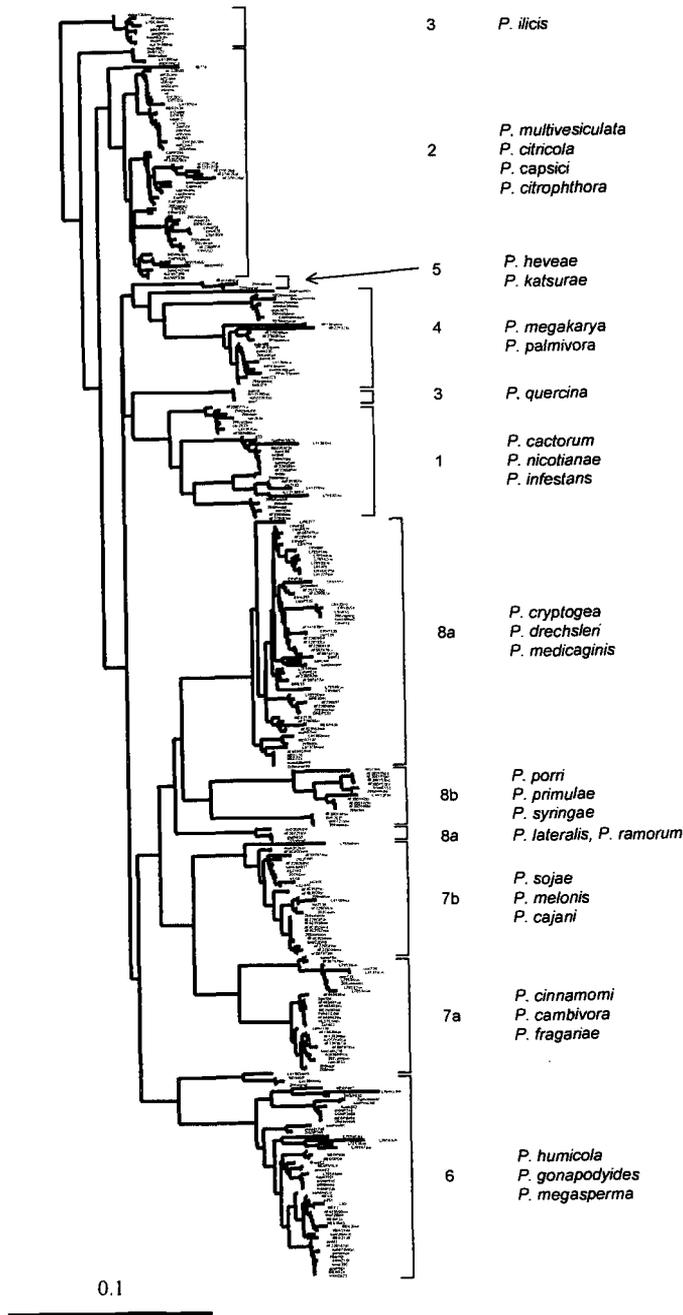
Fig. 1. Phylogenetic tree produced from DNA distance-based analysis of an alignment of the ITS regions of 339 isolates of *Phytophthora*. The main clades are labeled according Cooke *et al.* (2000) and the principal taxa in each clade highlighted. It should be noted that the figure is intended to highlight the overall structure of the tree rather than details of isolates at the tips of the branches. The scale bar represents the number of nucleotide substitutions per site.

```
┌─────── DRE277 P. drechsleri Westringia Australia
│ CRYF80 P. cryptogea
│        ┌ *AF228082  P. erythroseptica Solanum Hong et al
│        ┤ *AF257328 P. erythroseptica Solanum Hong et al
│        └ *AF266797 P. erythroseptica Solanum USA
│ CRYI260 P. cryptogea f.sp. begoniae Begonia Germany CABI
│ ┌ CRYP530 P. cryptogea Begonia Germany
│ │             ─────── CRY1551 P. cryptogea Solanum USA
│ ┤ CRY3009 P. cryptogea Drenth
│ └───────┤CRY1539 P. cryptogea Lycopersicon UK Drenth

│ *AF266796  P. cryptogea Lycopersicon NZ Cooke

│ CRYF10 P. cryptogea

sancryMar2  P. cryptogea Gerbera Cacciola

*AF228100 P. cryptogea Lycopersicon Hong et
*AF141638 P. cryptogea MW1 Hallenberg et al
*AF228099 P. cryptogea Aster Hong et al
├ CRY3010  P. cryptogea Drenth
│   ┌*AF228091 P. drechsleri Lactuca Hong et al
│   ┤── *AF087471 P. drechsleri Lycopersicon Hong et al
│   └── *AF087476 P. cryptogea Gerbera Hong et al
│        ┌─────── SanT2  P. cryptogea Pistacia Italy Cacciola
├ CRY841 P. cryptogea Carthamus Australia Drenth/Dudzinski
│   ┌── CRY754  P. cryptogea soil Australia
│   ┤   ┌── CRY887  P. cryptogea Prunus UK
│   │   ┤   ┌ L41377  P. drechsleri Westringua Australia Drenth
│   │   └   └ L41376 P. cryptogea Soil Australia Drenth

├ CRYP521  P. cryptogea Douglas fir USA
│ CRYF89  P. cryptogea
│├ *AF228101 P. drechsleri Hong et
│└─── Dimorfo  P. cryptogea Cape marigold Italy Cacciola
│ ┌ *AF087475 P. cryptogea  Brassica Hong et al
│ ┤── *AF087477  P. cryptogea Gerbera Hong et al
│ └ *AF228092 P. drechsleri Angelica Hong et al
├ CRYP524  P. cryptogea Juglans USA
│      ─────── CRY843  P. cryptogea Soil Australia
│ DRE33  P. drechsleri Cyamopsis Australia Drenth
│      ┌─ DRE3041 P. drechsleri Solanum Argentina Frezzi/Drenth
│      ┤ ┌ *AF228098 P. drechsleri Lycopersicon Hong et al
│      │ ┤ *AF266798 P. drechsleri Beta USA Brasier
│      │ ┤── *AF228097 P. drechsleri Beta Hong et al
│      └ └ DREP533  P. drechsleri Lycopersicon Italy
├── *AF228090 P. cryptogea Gerbera Hong et al
│ ┌── MEG2136  P. megasperma Douglas fir Drenth
│ ┤ Ma402 P. sp  Sainfoin Iran Mirabolfathy
│ └── MEGP439 P. megasperma DF Douglas fir USA
│ ──── *L41379 P. medicaginis Medicago Australia Drenth
│ Man408 P. medicaginis Medicago Iran Mirabolfathy
│ *AF266799  P. medicaginis Medicago Australia
│ MED701 P. medicaginis Medicago Australia
│       ┌── *L41380 P. megasperma Cicer Spain
│       ┤ MEG2137 P. megasperma Syringa Drenth
│       └── *AF266800 P. trifoli Trifolium
 0.01
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Fig. 2. Phylogenetic tree produced from an alignment of the ITS regions of *P. cryptogea*, *P. drechsleri*, *P. erythroseptica* and closely related taxa. The sequence identity or GenBank accession number, taxa name, host and source of the isolate are detailed. The sequence for isolates marked with an asterisk are available on GenBank. The scale bar represents the number of nucleotide substitutions per site.

isolates with such ability were defined as *P. drechsleri*. This confusion has been resolved and these isolates are now described as *P. meloni sinsensis* or *P. cajan* (see Cooke *at al.* 2000) However the problems of *P. drechsleri* isolates in clades 7b and 6 and a scattering amongst the *P. cryptogea* isolates indicates the need for a comprehensive review. The five isolates of *P.*

drechsleri that form a discrete clade include an isolate with the same isozyme profile as an authentic isolate (Mills *et al.* 1991). This is supported by RFLP data with random genomic DNA probes (F. Panabieres INRA Antibes, pers. comm..) and it is thus likely that this represents the distinct species *P. drechsleri* whereas other isolates are in fact *P. cryptogea*. The closely

164

related subclades of *P. cryptogea* correspond to isozyme groups defined by Mills *et al.* (1991).

In other cases, within a species-specific cluster, long tips to branches were apparent (e.g. within *P. cinnamomi* and *P. glovera*). Examination of these sequences reveals many single base pair changes across the ITS regions. In some cases, such changes were also observed within the 5.8S region. Intraspecific changes are uncommon within the ITS1 and ITS2 regions of many taxa (e.g. *P. infestans*, *P. fragariae*, *P. nicotianae*); thus changes within the highly conserved 5.8S region are very likely to be due to errors in the editing of poor quality sequence data. When used merely for species identification, the occasional erroneous base pair may not be important; a correspondence of the ITS sequence data, morphology and the plant host and disease symptoms would generally be regarded as sufficient basis for confirming an isolate's identity. However, verified intra and interspecific differences of only a few bases have been confirmed amongst other taxa. Within clade 6 for example, a range of closely related taxa that differ in mating system, temperature relations and colony morphology have been identified (CM Brasier pers. comm.). Similarly, the discrimination amongst *P. citricola* and *P. cryptogea* subgroups amounts to a few base pairs. It is conceivable that ITS sequences will form part of a species description and an accurate account of the 'reference sequence' and variants thereof will be important not only for taxonomic but perhaps for legal/quarantine purposes. The authors thus urge rigorous examination of sequence data prior to submission. Although not a requirement for GenBank submission, full isolate details (e.g. a minimum of host plant, country and an international culture collection accession number) should also be encouraged.

With increasing numbers of surveys for Phytophthoras in forests and wild ecosystems and the widespread use of ITS as a tool for examining relatedness and species identification the accuracy of the international sequence databases is increasingly important. In this study we have confirmed that, broadly speaking, the 160 submitted sequences are accurate and provide a valuable resource to scientists in the field. Sequence data offers a verifiable, objective and reliable measure of isolate identity and should prevent misidentification. There are however, errors in unpublished sequences and, as always, caution should be exercised in relying on GenBank sequences without reference to the associated publications. In addition, many taxa remain represented by only one or two isolates. For many taxa, detailed studies of both ITS sequence data and conventional morphological and behavioural criteria of representative sets of isolates are still required. Such studies will allow a re-examination of the status of many taxa and a long overdue tranche of taxonomic revision will no doubt ensue.

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The effect of phosphite on meiosis and sexual reproduction

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Abstract. The fungicide phosphite was found to reduce pollen fertility in Australian and exotic species. In the perennial *Dryandra sessilis* the reduction was evident for a year after treatment with 2.5-10 gL⁻¹ phosphite sprayed to run off. *Pterochaeta paniculata*, an annual, showed reduced pollen fertility in flowers that opened 16-30 days after spraying. The horticultural species *Petunia hybrida* and *Tradescantia virginiana* also displayed reduced pollen fertility after phosphite treatment. Pollen mother cells of *Tradescantia* had a significant percentage of abnormal first and second divisions and micronuclei in the microspores for up to one month after spraying. There was evidence that phosphite induced premature tapetum breakdown in *Petunia* but not *Tradescantia*. The percentage of abnormal meiotic cells and the frequency of premature tapetum breakdown appeared insufficient to account for the high levels of pollen infertility observed after phosphite treatment.

Introduction

Phosphite is the most cost effective, widely used fungicide for control of *Phytophthora* in natural ecosystems and horticultural crops (Coffey and Bower 1984; Wicks and Hall 1988; Ouimette and Coffey 1989; Guest and Grant 1991; Shearer 1994). Apart from providing relatively long-term control of the pathogen (Shearer *et al.* 1991) it is noted for its generally low phytotoxicity on vegetative parts of plants (Barrett and Grant 1997). However, little is known about its effect on sexual reproduction, though several other fungicides, including the well known Benlate (active ingredient Methyl (butylcarbamoyl)-2-benzimidazole carbamate), have been shown to cause aberrant pollen development, reduced pollen germination and altered patterns of synthesis of tapetal proteins (Church and Williams 1977; Ries 1978; Gentile *et al.* 1978; He and Wetzstein 1994; He *et al.* 1996). It is necessary to understand the effects of phosphite on sexual reproduction as the compound is an important tool in the fight to conserve rare plant species threatened with *Phytophthora*. Consequently, we have examined the effect of phosphite on a number of annual and perennial species in jarrah (*Eucalyptus marginata*) forest and from northern sandplains Kwongan vegetation (Fairbanks *et al.* 2001, 2002a). Detail will only be given of the effect of phosphite on pollen of a typical annual species *Pterochaeta paniculata* and a perennial *Dryandra sessilis*

and in the model species *Petunia hybrida* and *Tradescantia virginiana*.

Methods

Experimental design

Plants species utilised in the field were in the jarrah forest at Jarrahdale 48 km SE of Perth, Western Australia, from the northern sandplain at Eneabba 250 km north of Perth, or, in the case of the horticultural species, plants were grown in the glasshouse at Murdoch University. Phosphite treatments involved spraying foliage to run off with the stated concentrations of phosphite (Foli-R-Fos 400. Unitec, Australia, mono-di-potassium phosphite) mixed with 0.25% synertol oil (Organic Crop Protectants, NSW, Australia) to enhance adhesion of droplets.

Pollen fertility was assessed by exposure of pollen grains to fluorescein diacetate (Widhlom 1972) and pollen germination by sowing pollen on nutrient medium (Alexander and Ganeshan 1989). As both sets of data gave similar results, only pollen fertility will be presented here.

Pollen assessment of native species

Dryandra sessilis is a member of the Proteaceae, a tall sclerophyllous shrub with sessile flowers in terminal clusters. Plants were sprayed with 0, 2.5, 5 and 10gL⁻¹ phosphite in winter when they were in flower. There were 5

plants per concentration. Pollen fertility was assessed 6 weeks later then one and two years later. The phosphite content in shoot tips, of 2 plants per concentration, 1 and 6 weeks and one year after treatment was assessed using HPIC analysis (Roos *et al*. 1999).

Pterocheata paniculata was chosen as an example of an annual understorey species. It is a small composite reaching 18 cm high and flowers between July and November. Ten plants per concentration were sprayed with 0, 2.5, 5 and 10gL^{-1} phosphite when vegetative, and a second set of replicates when the inflorescences had initiated but before anthesis. Pollen fertility was assessed 4 to 30 weeks after treatment.

Pollen assessment of model species

The effect of 0-20 gL^{-1} phosphite on pollen fertility of the horticultural model species *Tradescantia virginiana* (6 plants per concentration) and *Petunia hybrida* (8 plants per concentration) was examined by spraying potted plants in a glasshouse and examining pollen fertility 1 -28 days later.

Effect of phosphite on pollen mother cell meiosis

Pollen mother cell meiosis was examined in flower buds of the phosphite treated *Tradescantia* (described above). Buds were fixed in alcohol:acetic acid (3:1) for 24 hours and then transferred to 70% alcohol and stored at 4°C. Pollen mother cells and microspores were stained in 2% aceto-orcein.

Effect of phosphite on tapetum development in model species

The stages of tapetal maturation were examined in buds of *Petunia hybrida* and *T. virginiana* after spray to run-off of 0, 10 or 20 gL^{-1} phosphite. Buds were removed 7 days after treatment and classified into different size classes (Fairbanks 2001), fixed in 3% gluteraldhyde in 0.025 M phosphate buffer (pH 7), dehydrated through 30-100% acetone and embedded in Spurr's epoxy resin (Spurr 1969). Resin blocks were sectioned at 1.5 μ and stained in 1% methylene blue and 1% azur II in 1% borax for 5 mins (Richardson *et al*. 1960).

Results

Effect of phosphite on pollen fertility in native plants

Fertility of pollen in flowers of *D. sessilis* that opened 6 weeks after spraying was reduced and the effect was still marked in flowers one year later (Fig. 1). Two years after spraying pollen fertility was normal. One week after spraying there was 218.7 (± 2.2) μg g^{-1} phosphite in the shoot tips. This dropped to 68.4 ± 68.4 μg g^{-1} after 6 weeks, and no phosphite was detected after one year. No phosphite was detected in the control plants.

Pterochaeta paniculata sprayed when vegetative showed a reduction in pollen fertility when they flowered 16-30 weeks later. Plants sprayed when inflorescences were in bud were also affected (Fig. 2). Phosphite was more phytotoxic to plants in the vegetative condition, and at 10gL^{-1} killed 15% of the plants.

*Effect of phosphite on pollen fertility of model species*

Phosphite was shown to have a similar effect on pollen fertility of the two horticultural species. There was a depression in *Tradescantia* for up to 2 weeks while in *Petunia* pollen fertility was zero in most tests for up to 3 weeks (Figs. 3,4).

Effect of phosphite on pollen mother cell meiosis

Pollen mother cell meiosis in *Tradescantia* revealed significantly (P <0.05) more abnormal meiotic cells in plants treated with 10 or 20 gL^{-1} phosphite than in control anthers. The types of abnormalities observed included bridges and stickiness, lagging chromosomes in the 1st and 2nd divisions of meiosis and micronuclei in the uninucleate microspores (Fairbanks *et al*. 2002b). There were more abnormal cells in 1st and 2nd division of meiosis for up to a week after spraying and more abnormal microspores for up to 1 month after spraying (Table 1).

Effect of phosphite on tapetum development in model species

In *Tradescantia* the tapetal disintegration is of the amoeboid type. There was no difference between the timing of the stages of disintegration in control and phosphite treated plants. *Petunia* tapetal cells disintegration is of the glandular type (Fairbanks 2001). Amongst buds 12mm long, those from the control plants had 53% with intact tapetum and 47% with partially disintegrated tapetum. The proportion was similar in the plants treated with 10gL^{-1} phosphite while in buds from plants treated with 20gL^{-1} phosphite 55% had partially degenerated tapetum and in 45% of buds the tapetum had degenerated entirely.
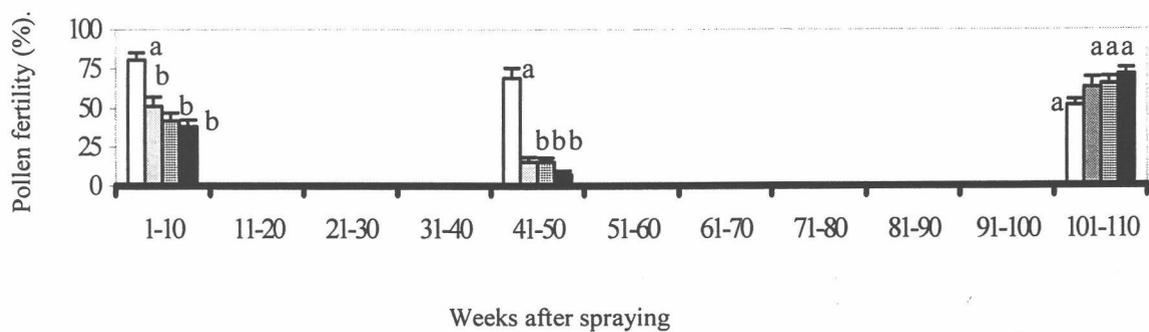
Fig. 1. Pollen fertility of the perennial *Dryandra sessilis* after application of
□ 0, ▨ 2.5, ▨ 5, ■ 10 gL^{-1} phosphite in winter 1997. Bars indicate standard errors of means. Bars within the same assessment, with the same letters are not significantly different.
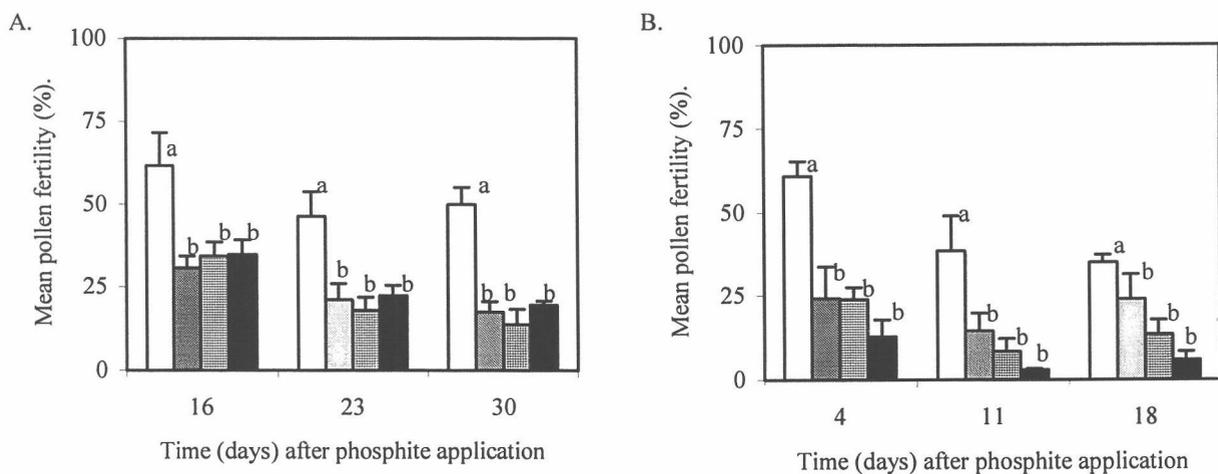
Fig. 2. Pollen fertility of the annual *Pterocheata paniculata* sprayed with □ 0, ▨ 2.5, ▨ 5, ■ 10 gL^{-1} phosphite when (A) vegetative and (B) between flower initiation and anthesis. Bars indicate standard errors of means. Bars within the same assessment, with the same letters are not significantly different. Figure from Fairbanks *et al*, 2001a with permission.
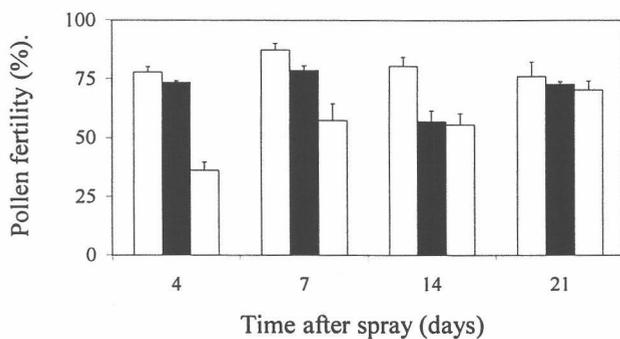
Fig. 3. The effect of phosphite sprayed at □ 0, ■ 10, □ 20 gL^{-1} on pollen fertility of *Tradescantia virginana*. Bars indicate standard errors of means.

169
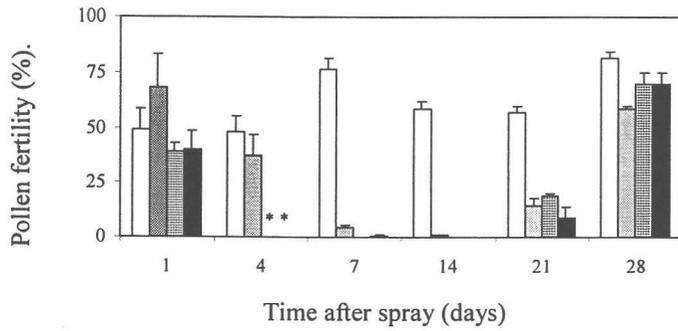
Fig. 4. The effect of phosphite sprayed at □ 0, ▨ 2.5, ▧ 5, ■ 10 gL⁻¹ on pollen fertility of *Petunia hybrida*. * no pollen available. Plants treated with 2.5 gL⁻¹ phosphite had 1% pollen fertility at day 14. Plants treated with 5 and 10 gL⁻¹ phosphite had 0% pollen fertility at days 7 and 14. Bars indicate standard errors of means.

Table 1. Effect of phosphite on *Tradescantia virginiana* microspore meiosis up to 28 days after treatment with phosphite applied as 0, 10 and 20 gL⁻¹ spray to run-off.

Days after treatment	Phosphite con. (gL⁻¹)	% Abnormal Cells[A]		
		Division 1	Division 2	Uninucleate microspores (micronuclei)
4	0	1.5ᵃ	1.6ᵃ	2.0ᵇ
	10	6.6ᵇ	7.0ᵇ	5.6ᵃᵇ
	20	8.7ᵇ	6.9ᵇ	7.0ᵇ
7	0	1.8ᵃ	1.3ᵃ	4.6ᵃ
	10	11.1ᵇ	7.8ᵇ	9.3ᵃᵇ
	20	12.2ᵇ	13.0ᵇ	20.1ᵇ
14	0	3.3ᵃ	3.1ᵃ	3.1ᵃ
	10	3.8ᵃ	3.1ᵃ	6.8ᵃᵇ
	20	4.9ᵃ	4.6ᵃ	8.6ᵇ
21	0	5.5ᵃ	2.7ᵃ	3.4ᵃ
	10	8.0ᵃ	3.0ᵃ	3.6ᵃ
	20	13.0ᵃ	2.7ᵃ	8.5ᵇ
28	0	3.1ᵃ	2.6ᵃ	3.0ᵃ
	10	4.0ᵃ	2.5ᵃ	6.1ᵇ
	20	3.7ᵃ	3.2ᵃ	4.7ᵃᵇ

[A]Three to 14 meiotic anthers per treatment were analysed. Means followed by different letters are significantly different at the 95% confidence level dependent on harvest time, SE in parenthesis.

Table 2. Species showing a reduction in pollen fertility after treatment with at least one concentration of phosphite (2.5 to 20 gL⁻¹). Data from Fairbanks 2001.

Location	Habit	Family	Species
Jarrah forest	Perennial	Proteaceae	*Dryandra sessilis*
		Proteaceae	*Adenanthos barbiger*
		Rhamnaceae	*Trymalium ledifolium*
		Sterculiaceae	*Lasiopetalum floribundum*
		Rutaceae	*Boronia cymosa*
		Euphorbiaceae	*Phyllanthus calycinus*
	Annual	Asteraceae	*Pterocheata paniculata*
		Asteraceae	*Hyalosperma cotula*
		Asteraceae	*Podotheca gnaphalioides*
Northern sandplain	Perennial	Myrtaceae	*Eremaea astrocarpa*
		Dilleniaceae	*Hibbertia hypericoides*
		Polygalaceae	*Comesperma calymega*

170

Discussion

Treatment of plants with phosphite caused a reduction of pollen fertility in several annual and perennial species from both the Australian native flora and horticultural species. These data supported information from other species showing that the effect was widespread (Table 2). A reduction in pollen fertility of variable extent and duration has been recorded for a number of perennial and annual species belonging to Asteraceae, Dilleniaceae, Euphorbiaceae, Goodeniaceae, Myrtaceae, Papilionaceae, Polygalaceae, Proteaceae, Rhamnaceae, Rutaceae and Sterculiaceae families, from the jarrah forest and the northern sand plain (Fairbanks *et al.* 2001, 2002a).

Meiotic abnormalities observed in first and second division as a result of phosphite would have caused abnormal microspores and contributed to the reduction in pollen fertility. Tapetum development and disintegration is precisely timed during normal pollen maturation and early tapetum disintegration is a well-known cause of pollen sterility in several species with male sterile flowers (Echlin 1971; Izhar and Frankel 1971). In the species with a glandular-type disintegration of the tapetum, premature disintegration of the tapetum may also contribute to pollen infertility. No effects on tapetum were observed in the species with an amoeboid –type disintegration. The extent of the damage to pollen cannot be fully accounted for by the percentages of abnormal meiotic cells or the frequency of early tapetum breakdown. The mechanisms active in *D. sessilis* must be effective after one year, even in the absence of detectable levels of phosphite in the shoot tips.

Our results suggest that caution should be exercised in applying phosphite to rare and endangered species and that spraying just before or during the flowering season should be avoided.

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172

Simultaneous detection and identification of *Phytophthora cinnamomi* and *P. cambivora* direct from French chestnut grove soils by multiplex PCR

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Abstract. Phytophthora cinnamomi and *P. cambivora* are the most pathogenic species associated with chestnut (*Castanea sativa*) decline in Europe. Mapping their incidence and distribution from French nursery and plantation soils may offer valuable information for limiting spread, especially by commercial routes. As conventional biological baiting and taxonomic confirmation is often unreliable and slow, we have focused on the development of a multiplex PCR approach for the simultaneous detection of both species direct from soil. Pre-existing and novel primers, based on ITS sequences, have been evaluated for their specificity and use in a multiplex PCR capacity. Coupled with a modified mechanical DNA lysis procedure, both species have been successfully detected, individually or together, in artificially seeded soils using serial dilutions and/or polyvinylpolypyrrolidone chromatography purification to deter PCR inhibitors. Only *P. cinnamomi* was detected in naturally infected chestnut grove soils. Levels of detection are comparable to other *Phytophthora* species where PCR based diagnostic systems have been reported.

Introduction

Ink disease on chestnut (*Castanea sativa* Mill.) in Europe is caused by two soilborne pathogens, *Phytophthora cinnamomi* and *P. cambivora*. Symptoms include root and extensive collar rot lesions near the base of the trunk, from which inky fluid exudates often flow, associated with general dieback of the crown including eventual overall decline. The disease has been reported in France since the beginning of the 20th century (Crandall *et al.* 1945) with a probable introduction from Spain into the French Basque region during the 1860s (Grente 1961). The disease is generally considered widespread, infecting both nursery material and adult trees from naturalised forest stands, and remains problematic. A dramatic resurgence of the disease has recently been reported from Italy (Anselmi *et al.* 1996; Vettraino *et al.* 2001). Invariably infection causes direct loss of yield. In severe situations epidemics can limit the establishment of new groves (Vettraino *et al.* 2001), or make existing plantations uneconomic, often resulting in their abandonment or entire removal.

Conventional baiting methods for these species remains slow and are generally considered both unreliable and insensitive. Further, isolation frequencies of both species using these approaches are often low and erratic, and appear influenced by environmental conditions such as air temperature under which baiting is carried out (unpublished results of this laboratory). Despite their economic importance as destructive pathogens, aspects of their basic ecology, including spread and epidemiology within temperate forest soils remains poorly understood (Hansen and Delatour 1999). Mapping their incidence and distribution from French nursery and plantation soils may offer valuable information for limiting spread, especially by commercial routes, and may help form the basis of possible integrative management strategies for this disease.

Alternative methods for improved detection of plant pathogens, based on the polymerase chain reaction (PCR) have become firmly established in the repertoire of techniques available for plant disease diagnosis (Henson and French 1993), including *Phytophthora* spp. (Nechwatal *et al.* 2001; Winton and Hansen 2001) offering higher levels of specificity and sensitivity than most previously described conventional techniques.

In light of this, and the limitations of biological baiting as outlined above, the aim of this study was the development of a multiplex PCR system for the fast, sensitive, simultaneous detection of both species direct from chestnut grove soils for improved ecological studies. Primer sets were designed to regions of inter-specific ITS sequence divergence between both species and other

phylogenetically close *Phytophthora* species, particularly those of forestry importance, and used in a two-step nested approach, using universal ITS primers (White *et al.* 1990) in first round amplification. For practical applications, we have incorporated a fast mechanical lysis procedure for DNA extraction from naturally or artificially infected chestnut grove/nursery soils. We further evaluate the multiplex PCR over conventional baiting.

Materials and Methods

Fungal isolates, culture conditions and DNA preparation

Details of all authenticated *P. cinnamomi* and *P. cambivora* isolates, including other *Phytophthora* species, used in this study are documented in Table 1. Isolates were maintained on V8-juice agar at 20°C. Additional fungal species included for primer specificity testing were maintained according to their specific requirements. High molecular weight fungal (including plant) DNA was prepared from young mycelial growth using the Puregene® DNA isolation kit (Gentra Systems, Minneapolis, USA), quantified spectrophotmetrically (Sambrook *et al.* 1989), and stored at -20° C.

PCR conditions and electrophoresis

Internal transcribed spacer (ITS) regions were amplified with primers ITS 5 and ITS 4 (White *et al.*, 1990, see Table 2) as follows; 5 ng template DNA, each primer to 0.1 mM, 200 µM of each dNTP (Amersham Pharmacia Biotech), 1 Unit Red GoldStar™ DNA polymerase (Eurogentec, Belgium), 1 x PCR reaction buffer, 1.5 mM $MgCl_2$ (all Eurogentec, Belgium) in 25 µl total volumes. Cycling conditions, using a Perkin-Elmer 9700 thermal cycler (Perkin-Elmer, Norwalk, USA), comprised an initial denaturation (3 min at 94°C) followed by 30 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C with a final post extension of 72°C for 10 min. Direct PCR with species-specific primer pairs (Table 2, Fig. 1) were identical to above, except higher annealing temperature of between 60° and 64° C were used. For multiplex use, each primer set was added in equal 0.1 mM quantities and subjected to the same cycling regime as above. For nested applications, a 5 µl aliquot of a 50^{-1} pre-amplification product dilution generated with ITS4 and 5 (White *et al.* 1990) was used as template. All components were dispensed with aerosol resistant tips in a designated laminar flow cabinet with appropriate controls in all experiments. All experiments were conducted at least in duplicate.

PCR amplicons were assessed by electrophoresis in 15 g/l agarose (Euromedex, France, 0.5 X TBE (89 mM Tris-borate, 2 mM EDTA at pH 8.0) gels. A UV transilluminator (Bioblock Scientific) with the BIO-CAPT MW computer software was used for image documentation following ethidium bromide staining,

DNA sequencing and primer design

ITS products from additional *Phytopthora* species were commercially cycle sequenced (MWG Biotech AG, Ebersberg, Germany) and the identity confirmed by BLAST database searches (http://www.ncbi.nlm.nih.gov/BLAST/). Details and database accession numbers of these and all sequences used in this study are given in Fig. 1. Full length ITS sequences of other *Phytopthora* species were selected from the databases for direct ClustalW (Thompson *et al.* 1994) multiple sequence comparisons with *P. cinnamomi* and *P. cambivora* sequences (Fig. 1) based on two criteria; known pathological or ecological presence within forest ecosystems (including chestnut stands) and the more recent *P.* 'europaea' taxon, and their ITS phylogenetic positions as defined by Cooke and Duncan (1997) and Cooke *et al.* (2000) (see Fig. 1 for species and sequence details). To maximise the amount of sequence input data available for enhanced primer selection, consensus ITS sequence was obtained for each species, incorporating intra-specific nucleotide base variability employing standard IUPAC nucleotide base nomenclature codes. Resulting consensus sequences were then arranged according to their similarity to *P. cinnamomi* and *P. cambivora*, according to Cooke *et al.* (2000) and aligned using ClustalW (Thompson *et al.* 1994). Areas exhibiting inter-specific sequence divergence (but intra-specific conservation), particularly between the most closely related species (*P. cinnamomi, P. cambivora* and *P. fragariae* var. *fragariae/rubi*) were selected. Following experimentation with previously reported primers and earlier designs from this study (Table 2), two sets of species-specific primer pairs (PciF2/PciR2, and PcaFshort/PcaR, see Table 2), exhibiting maximum 3' specificity to *P. cinnamomi* and *P. cambivora*, respectively, were designed using the GeneFisher (version 1.22, http://bibiserv.techfak.uni-bielefeld.de) and PRIMER software (version 0.5, Whitehead Institute) programs following the general primer design concepts as outlined by

Table 1. Origins of authenticated *P. cinnamomi* and *P. cambivora* strains, and other *Phytophthora* reference species used in this study, including specificity of primer combinations PciF2/PciR2 and PcaFshort/PcaR, respectively.

Species	Isolate	Source	Host	Origin	ITS	*P. cam*	*P. cin*
P. cinnamomi	9	INRA[a], Bordeaux	*Quercus rubra*	France	+	-	+
P. cinnamomi	571	INRA, Bordeaux	*Quercus rubra*	France	+	-	+
P. cinnamomi	578	INRA, Bordeaux	*Quercus suber*	France	+	-	+
P. cinnamomi	582	INRA, Bordeaux	*Quercus ilex*	France	+	-	+
P. cinnamomi	639	INRA, Bordeaux	*Castanea sativa*	France	+	-	+
P. cinnamomi	680	INRA, Bordeaux	*Castanea sativa*	UK	+	-	+
P. cinnamomi	299	LNPV[b]	*Castanea sativa*	France	+	-	+
P. cinnamomi	469	LNPV	*Castanea* sp.	France	+	-	+
P. cinnamomi	590	FARS[c]	N/A	UK	+	-	+
P. cinnamomi	244	U. California[d]	*Persea americana*	Hawaii	+	-	+
P. cinnamomi	247	U. California	*Eucalyptus* sp.	Australia	+	-	+
P. cinnamomi	251	U. California	*Persea americana*	New Guinea	+	-	+
P. cinnamomi	248	U. California	*Vitis vinifera*	South Africa	+	-	+
P. cinnamomi	309	U. California	*Erica cornea*	Germany	+	-	+
P. cinnamomi	322	U. California	*Persea americana*	USA	+	-	+
P. cinnamomi	312	N/A	*Camellia* sp.	USA	+	-	+
P. cinnamomi	321	N/A	*Rhododendron* sp.	China	+	-	+
P. cambivora	599	INRA, Bordeaux	*Quercus* sp.	France	+	+	-
P. cambivora	661	INRA, Bordeaux	*Quercus* sp.	France	+	+	-
P. cambivora	450	LNPV	*Castanea* sp.	France	+	+	-
P. cambivora	463	LNPV	*Castanea* sp.	France	+	+	-
P. cambivora	626	U. Tuscia[e]	*Castanea sativa*	Italy	+	+	-
P. cambivora	627	U. Tuscia	*Castanea sativa*	Italy	+	+	-
P. cambivora	480	N/A	*Castanea sativa*	Italy	+	+	-
P. fragariae var. *rubi*	666	CTIFL[f]	*Rubus* sp.	France	+	'+'	-
P. fragariae var. *fragariae*	277.95	INRA, Bordeaux	N/A	France	+	'+'	-
P. fragariae var. *fragariae*	294	INRA, Bordeaux	N/A	France	+	'+'	-
P. 'europaea'[g]	2AE2	INRA, Nancy	*Quercus robur*	France	+	'+'	-
P. 'europaea'	608	INRA, Bordeaux	*Quercus robur*	France	+	'+'	-
P. cactorum	665	CTIFL	*Fragaria* sp.	France	+	-	-
P. cryptogea	629	INRA, Bordeaux	*Castanea sativa*	France	+	-	-
P. cryptogea	670	INRA, Antibes	*Anemone* sp.	France	+	-	-
P. citricola	671	INRA, Bordeaux	*Rhododendron* sp.	France	+	-	-
P. citricola	481	N/A	*Castanea sativa*	Italy	+	-	-
P. gonapodyides	589	INRA, Nancy	*Quercus robur*	France	+	-	-
P. palmivora	437	N/A	*Vanilla planifolia*	N/A	+	-	-
P. drechsleri	436	N/A	N/A	N/A	+	-	-
P. capsici	439	N/A	*Cucumis melo*	N/A	+	-	-
P. megasperma	668	INRA, Antibes	*Lycopersicum* sp.	France	+	-	-
P. megasperma	667	INRA, Nancy	*Quercus* sp.	France	+	-	-
P. parasitica var. *nicotianae* A2	448	N/A	*Banksia* sp.	Australia	+	-	-

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[g] Undescribed species, related to *P. cambivora*.
[h] Undescribed inter-specific hybrid involving *P. cambivora* and *P. fragariae* like species.
N/A, Not available.

175

Table 2. Characteristics and origins of primers used in this study.

Primer[a]	Sequence (5'-3')	Tm (°C)	Sources
ITS 4	TCC TCC GCT TAT TGA TAT GC	53.0	White *et al.* (1990)
ITS 5	GGA AGT AAA AGT CGT AAC AAG G	69.0	White *et al.* (1990)
PCI 1 (F)[b]	CGT GGC GGG CCC TAT CAC TG	66.0	Lee *et al.* (1993)
PCI 2 (R)	GCC TCC ACA ACC AGC AAC GT	61.9	A. Baudry (pers. comm.)
PciF2 (F)	GGA ACT GAG CTA GTA GCC TC	59.9	This study
PciR2 (R)	CAA TTG AGA TGC CAC CAC AA	55.8	This study
PCA 3 (F)	GTG ACG TAG GTT CAT CTG CT	57.8	Schubert *et al.* (1999)
PCA 4 (R)	GTG ACG TAG GCC AAA ATA ACA	56.1	Schubert *et al.* (1999)
PcaF (F)	CCC ACT TAG TTG GGG GCT AGT C	63.8	This study
PcaFlong	CAC TTA GTT GGG GGC TAG TCC C	63.1	This study
PcaFshort	CAA CCC ACT TAG TTG GGG GCT A	64.3	This study
PcaR	TCC CCA CAG TAT ATT CAG TAT TCA	56.8	This study

[a]'F' and 'R' signifies forward and reverse, respectively.
[b]Oligonucleotide hybridisation probe sequence '*P. cinnamomi*' as reported by Lee *et al.* (1993).
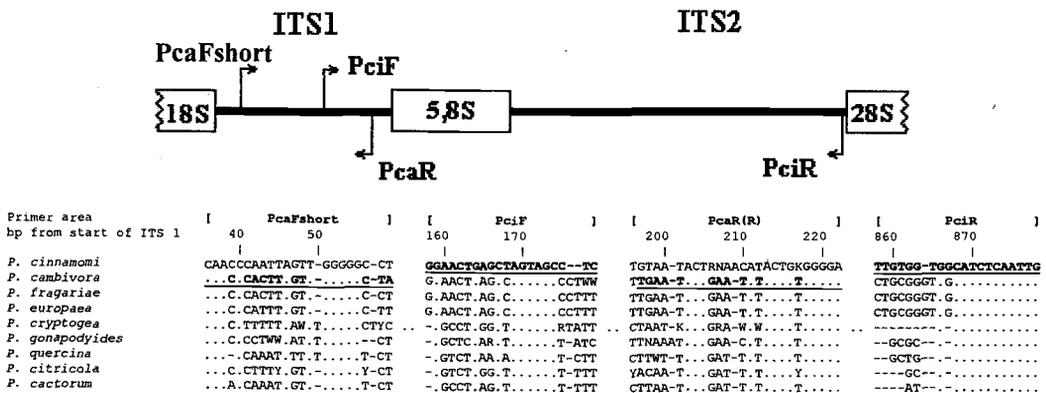
Fig. 1. Species-specific primers PciF2 and PciR2, and PcaFshort and PcaR, designed from areas of inter-specific ITS nucleotide divergence detected from multiple alignment analysis of *P. cinnamomi* and *P. cambivora*, and selected *Phytophthora* species (1-9) consensus sequences. Standard IUPAC nucleotide base nomenclature codes were used for the integration of intra- and inter-specific nucleotide base variability for the final consensus sequence alignment. All currently available ITS sequences from the databases were used, including those generated in this study, and gifted (*P. 'europaea'*, 2ae2).

(Accession numbers used for *P. cinnamomi* are AF087478, AF266764, AF242795, AF242820, AJ344545[a], AJ344546[a], L41374, Y08656, Y08657; *P. cambivora*, AF087479, AF139369, AF266763, AF242794[b], AF242819[b], AJ007040, AJ344547[a], AJ344548[a], Y08654[b], Y08655[c]; *P. cactorum*, AF087480, AF228072, AF266772, AF242784[b], L41357, Y08652, Y08653; *P. citricola*, AF228080, AF242786, AF242792, AF242796, AF266788, AJ007370, L41375, Y08658[b], Y08659[c]; *P. cryptogea*, AF087475, AF087476, AF087477, AF228090, AF228099, AF228100, AF228101, AF266796, L41376; *P. fragariae* var. *rubi*, AF266761, AJ344549[a]; *P. fragariae* var. *fragariae*, AF266762, AF139370; *P. gonapodyides*, AF266793, AF228102, AJ007368; *P. quercina*, AJ007369, AJ131986; *P.'europaea'*, 2ae2[d]).

[a]Full length ITS 1/2 and 5.8S sequence generated in this study.
[b]ITS 1 region only.
[c]ITS 2 region only.
[d]Putative full length ITS 1/2 and 5.8S sequence of *P. 'europaea'* kindly provided by C. Delatour, INRA, Nancy.

Dieffenbach *et al.* (1995). Predicted amplification size products for *P. cinnamomi* and *P. cambivora* were 686 and 172 bp, respectively. Primers were synthesised by Invitrogen (UK). Species specificity of PciF2/PciR2 and PcaFshort/PcaR was assessed by direct, nested, and nested multiplex PCR against closely related species (Table 1), and a broad spectrum of fungal and bacterial species including *Pythium aphanidermatum*, *Py. intermedium*, *Py. irregulare*, *Py. sylvaticum*, *Py. ultimum*, *Acremonium strictum*, *Armillaria gallica*, *A. mellea*, *Fusarium lateritium*, *Nectria radicicola*, *Trichoderma* sp., *Agrobacterium tumefasciens* and *Bacillus subtilis*, including plant DNA (*C. sativa*). The primer pair ITS4 and ITS5 (White *et al.* 1990) was used to verify that extracts were of amplification quality. Sensitivity of the conventional direct, nested and nested multiplex assays were assessed by testing 10 fold serial dilutions of *P. cinnamomi* (isolate 9) and *P. cambivora* (isolate 450) genomic DNA, individually and mixed, respectively.

Preparation of zoospores and artificial inoculation of control soil for sensitivity testing

Zoospores of *P. cinnamomi* (isolate 9) and *P. cambivora* (isolate 450) were obtained using the method of Robin (1991) using *Phytophthora* free soil sourced from a single location within the INRA, Bordeaux Research Centre (routinely and consistently tested as negative for any *Phytophthora* spp. in numerous studies over many years). Individual 10 g samples of *Phytophthora* free sieved soil (as above) were individually spiked with a serial dilution of zoospores (1000, 500, 100, 50, 10, 5, 1 zoospores/g soil) prepared from each species, including equal number of both (for multiplex assessment). Extracts were assessed individually through single nested PCR with the primer set specific for the inoculated species, and by nested multiplex PCR for the mixed species material. Samples of soil without any added zoospores and extraction buffer without soil were also tested in these experiments as negative controls. Positive controls were included in all tests.

Detection of Phytophthora *spp. in chestnut grove soils*

Soils from pre-selected sites (Table 3), as part of a large scale chestnut decline survey from across France and the UK, with or without decline symptoms present, were used to test the molecular detection method. Typically, soils were sampled from under 10 trees per site. A general visual assessment of tree health status at each site, as an indication of the presence of *Phytophthora*, was made according to a general arbitrary Decline Index (DI) formula (modified from Vettraino *et al.* (2001), where declining trees were characterised by an index ≥ 3 (considered infected with the chance of conventional recovery relatively high). Conventional detection of *Phytophthora* from soil was carried out following the method described by Robin *et al.* (1998) using *C. sativa* leaf disks as bait and PHARby selective medium (Robin 1991). Isolates were identified by colony and morphological criteria with authenticated reference strains (see Table 1) and published species descriptions (Stamps *et al.* 1990; Erwin and Ribeiro 1996). In addition, PCR-RFLP analysis (Cooke and Duncan 1997) and diagnostic PCR with species-specific primers for *P. cinnamomi* and *P. cambivora* (this study) were employed.

High molecular weight DNA was extracted from individual 10 g samples from each of the eleven chestnut grove soils and *Phytophthora* free control soils, to which *P. cinnamomi* and/or *P. cambivora* was also added, following a modification of the procedure of Cullen and Hirsch (1998) as described by Langrell (2003). Soil DNA extracts were purified through water-insoluble polyvinylpolypyrrolidone (PVPP; Sigma) based on the method previously described by Berthelet *et al.* (1996) and Cullen and Hirsch (1998) prior to single nested or nested multiplex PCR assessment as described above.

Results

All *P. cambivora* ITS sequences examined revealed complete sequence homogeneity, regardless of host or geographic origin, as opposed to limited intra-specific nucleotide variability observed within *P. cinnamomi*, albeit derived from isolates of much wider geographical and host provenance. From alignment inspection, previously reported primers PCI 1(F) (Lee *et al.* 1993) and PCI 2(R) (Baudry *et al.* 2001) exhibited low or insufficient levels of specificity for *P. cinnamomi*, particularly at their 3' ends, and showed cross species amplification with other closely related taxa. Both primers were subsequently rejected for further analysis. Consequently, primer pair PciF2 and PciR2 (Table 2 and Fig. 1) were designed and generated a single predicted sized product of 686 bp (e.g. see Fig. 2, lanes 2-4) from all 17 isolates of *P. cinnamomi* (representing 9 host genera, from 8 countries spanning 5 continents), but not *P. cambivora* (e.g. see Fig.

2, lane 10), other closely related *Phytophthora* (Table 1) or other test species, including *C. sativa* DNA using either direct or nested PCR (internal to ITS5 and ITS4).

In *P. cambivora*, sufficient interspecific ITS sequence divergence was located in at least one location for the design of three forward candidate specific primers. Primers PcaF(F), PcaFlong and PcaFshort were designed and synthesised for evaluation with a single reverse primer (PcaR), offering selection against *P. cinnamomi*. Primer combinations PcaF and PcaR, and PcaFlong and PcaR both amplified *P. fragariae* var. *fragariae/rubi* and *P. 'europeae'*, at high stringency in direct PCR. By contrast, PcaFshort and PcaR appeared species-specific, yielding a predicted PCR sized fragment of 172 bp (e.g. see Fig. 2, lanes 5-7) from all 7 *P. cambivora* isolates (representing 2 host genera, from 2 European countries) but not other closely related (Table 1) or test species as described above for *P. cinnamomi* (e.g. Fig. 2, lane 11), and were subsequently selected for routine use. However, following nested PCR, PcaFshort and PcaR also resulted in nonspecific amplification of *P. fragariae* var. *fragariae/rubi* and *P. 'europeae'*, despite stringent annealing conditions (64° C). Incorporation of touchdown parameters to the cycling conditions restored complete specificity to the nested approach (data not shown). Sensitivity of individual and nested multiplex PCR resulted in detectable product down to 25 and 100 fg for pure DNA of *P. cinnamomi* and *P. cambivora*, respectively.

Using the mechanical lysis procedure, good quality high molecular weight total DNA was consistently extracted from a wide range of chestnut grove soils, from across France with yields in the order of approximately 20 µg/g. The purity of the extracts varied with different soils (as assessed by pellet colour). First round ITS amplification with universal primers ITS 5 and ITS 4 was successful with all soil DNA extracts tested in this study (due to eukaryotic soil microflora), but only following PVPP spin-column purification (this step also acted as a simultaneous pre-check of template quality prior to nested multiplex PCR).

Clear unambiguous bands of the correct size were consistently observed down to levels of 500 zoospores/g of individually spiked air dried soil for either species following individual nested PCR, and as low as 100 and 50 zoospores/g soil employing the

multiplex system for *P. cinnamomi* and *P. cambivora*, respectively. Control soils, including extraction buffer without soil, did not yield any amplification signals.

Individual and multiplex nested assays for *P. cinnamomi* and *P. cambivora* were conducted on DNA extracted from 11 natural soils from 8 sites from 7 regions across France (Table 3). Of the 11 natural soils tested 4 proved positive for *P. cinnamomi* (in each case individual and multiplex results concurred) and further corresponded with positive biological baiting. *P. cambivora* was not detected by either approach. Based on the limited sample number and number of positive samples detected, no simple correlation between site calculated DI and species detection, by either method, was apparent. No other *Phytophthora* species were recovered/noted through biological baiting. Each control soil yielded expected results, irrespective of method used, as shown in Table 3.

Discussion

Conventional *Phytophthora* detection through biological baiting, followed by morphological species confirmation is often slow, time consuming and prone to taxonomic ambiguity. Further, the frequency of isolation is often exceptionally low, with levels of recovery generally considered inadequate for any meaningful ecological interpretation or host-pathogen relationships to infer. In view of this, we postulated the development of a multiplex PCR based assay for the simultaneous detection of both *P. cinnamomi* and *P. cambivora* direct from total soil DNA extracts would lead to improved detection frequencies, circumventing the inherent problems associated with biological baiting as described above. Although other PCR diagnostic systems have been reported for *P. cinnamomi* and *P. cambivora* previously (Coelho *et al.* 1997; Schubert *et al.*, 1999, respectively), neither have been used for the detection of either species direct from soil, rather confirmation of species identity of isolates already obtained via conventional baiting approaches.

Based on re-evaluation of interspecific ITS sequence divergence, two novel sets of primers, PciF2 and PciR2, and, PcaFshort and PcaR (Table 2), species-specific for *P. cinnamomi* and *P. cambivora*, respectively, were designed from integrating ITS sequence data representing phylogenetically close *Phytophthora* species of

Table 3. Soil origin and comparative results for *P. cinnamomi (P. cin)* and/or *P. cambivora (P. cam)* by the biological baiting and plating isolation technique and PCR methods.

Code	Region	DL[a]	DI[b]	Biological baiting		ITS 5/4[c]	PciF2/ PciR2[d]	PcaFshort /PcaR[e]	Multiplex[f]
				P.cin	*P. cam*				
Le Bey 1	Dordogne	O	0	-	-	+	-	-	- -
Le Bey 2	Dordogne	N	0	-	-	+	-	-	- -
Le Bey 3	Dordogne	C	3	+	-	+	+	-	+ -
Doissat	Dordogne	N	1	+	-	+	+	-	+ -
Colognac	Gard	O	1	-	-	+	-	-	- -
Cestas	Gironde	G	4	-	-	+	-	-	- -
Lucbardez	Landes	N	3	+	-	+	+	-	+ -
Redon	Loire-Atlantique	N	0	-	-	+	-	-	- -
St. Lézin	Maine-et-Loire	N	2	+	-	+	+	-	+ -
Gonfaron 19	Var	O	2	-	-	+	-	-	- -
Gonfaron 25	Var	O	2	-	-	+	-	-	- -
Controls									
P. cinnamomi	N/A	N/A	N/A	+	-	+	+	-	+ -
P. cambivora	N/A	N/A	N/A	-	+	+	-	+	- +
Negative	N/A	N/A	N/A	-	-	-	-	-	- -

[a] Domestication level (O, orchard, C, coppice, N, naturalised plantation, G, garden).
[b] Decline index of site from where soil had been sampled (where 0 = indicates healthy tree(s), to 5 = dead tree(s)).
[c] Amplification of purified soil extracts with universal primers ITS 5 and 4 (White *et al.* 1990).
[d] Nested PCR amplification of purified DNA soil extracts with *P. cinnamomi* primers PciF2 and PciR2 only.
[e] Nested PCR amplification of purified DNA soil extracts with *P. cambivora* primers PcaFshort and PcaR only.
[f] Nested multiplex PCR of purified DNA soil extracts with PciF2 and PciR2, and PcaFshort and PcaR together.
N/A, Not applicable.
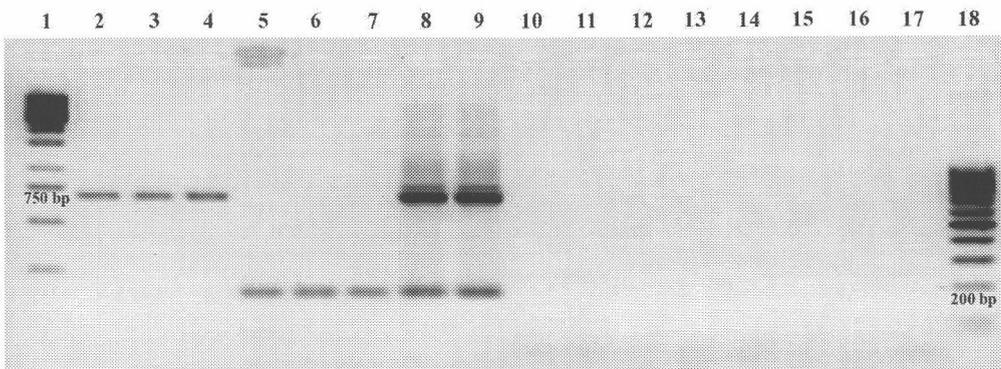
Fig. 2. Direct and multiplex PCR comprising PciF2/PciR2 and PcaFshort/PcaR (Tm 64°C).
Lane 1, 1 Kb marker (Invitrogen), note 750 bp marker fragment, lanes 2 to 4, *P. cinnamomi* strains 639, 299 and 247 (direct with PciF/PciR), 5 to 7, *P. cambivora* strains 599, 450 and 626 (direct with PcaFshort/PcaR), 8 and 9, equal mix of *P. cinnamomi* (strain 639) and *P. cambivora* (450) DNA (multiplex PCR), 10, *P. cambivora* strain 599 with primers PciF2/PciR2, 11 *P. cinnamomi* strain 639 with primers PcaFshort/PcaR. Multiplex specificity confirmed against (lane 12) *P. fragariae* var. *fragariae*, 13, *P. 'europeae'*, 14, *P. citricola*, 15, *P. cactorum*, and 16, *P. gonapodyides*. Lane 17, negative control (sterile distilled water). Lane 18, 100 bp marker (Invitrogen), note 200 bp marker fragment. Specificity confirmed against other species detailed in Materials and Methods not shown.

forestry importance (Fig. 1). Although each individual set exhibited complete species-specificity in direct PCR against other *Phtophthora* species detailed in Table 1, and other non-*Phytophthora* test species, nested PCR, against first round template generated with ITS 5 and 4, revealed some unexpected, cross-species amplification of PcaFshort and PcaR with *P. fragariae* var. *fragariae/rubi* and an undescribed *P. 'europeae'* taxon (related to *P. cambivora*), despite exploitation and incorporation of inter-specific nucleotide divergence at or near their 3' ends. Efforts to eliminate this cross-reactivity are in progress.

Direct PCR, using single individual species-specific primer pairs PciF2 and PciR2, and, PcaFshort and PcaR gave detectable amplification signal down to approximately 20 pg for *P. cinnamomi* and *P. cambivora*, respectively. These levels were increased to 25 and 100 fg for *P. cinnamomi* and *P. cambivora*, respectively, employing a nested approach (either individually or in multiplex format).

Species detection from total soil DNA extracts was only possible following post-extraction PVPP purification and nested PCR of serially diluted pre-amplified first round total rRNA template with universal primers ITS 4 and ITS 5 (White *et al.* 1990). The reason for this phenomenon remains unclear as both universal primers, and species-specific primer sets, each target the same tandemly repeated rRNA molecule.

The levels of sensitivity reported here for zoospores in artificially inoculated *Phytophthora* free soils (100 and 50 for *P. cinnamomi* and *P. cambivora*, respectively) compares well with other PCR assays reported for other *Phytophthora* species (e.g. 5 and 300 for *P. quercina* and *P. citricola*, respectively (Nechwatal *et al.* 2001), and 200 for *P. lateralis* (Winton and Hansen 2001), particularly considering the nature and complexity of the soil matrices from which extracts were prepared.

When compared with the natural grove soil data presented here, the multiplex PCR detection method exhibited a similar detection frequency to the baiting and plating isolation technique. Further work will concentrate on quantitatively evaluating the multiplex PCR method over the biological baiting approach with particular reference to correlation with DI values.

The PCR technique described here is robust, fast and species-specific, requiring less than one working day to obtain a result as opposed to several weeks, taxonomic ambiguities and low recovery frequencies associated with biological baiting. As the primer sequences described here were designed from consensus sequence data from isolates of extreme host and global geographic provenance and that the DNA extraction system was successful across a wide range of soil types, suggests this diagnostic system, used either in individual nested, or multiplex formats, may be directly transferable for use as effective reliable ecological research tools against both species in other forest ecosystems from different parts of the globe where *P. cinnamomi* and/or *P. cambivora* are problematic.

In addition to use as a research tool in mapping the incidence and distribution of these species from chestnut grove soils, and in generating basic ecological data for formulating disease management strategies, this system, with further development, could, theoretically, help form the basis of an integrated diagnostic test in support of chestnut tree certification schemes, helping to raise the health status of young nursery material.

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[31]P NMR in vivo spectroscopy of *Phytophthora palmivora* mycelia

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Abstract. Potassium phosphonate is an effective and selective inhibitor of many plant diseases caused by the fungal genus *Phytophthora*. The biochemistry of *Phytophthora* differs in many ways from that of the true fungi. In particular, unlike true fungi, *Phytophthora* contains little or no long-chain polyphosphate but an abundance of the short-chain, acid-extractable polymer . [31]P NMR spectroscopy was used to examine changes in phosphorylated metabolites within living mycelium of the plant pathogen, *Phytophthora palmivora* over twenty-four hours. Mycelium raised in conventional, defined growth medium afforded spectra consisting of relatively narrow peaks, some of which were superimposed on broader resonances attributed to pyrophosphate and short-chain polyphosphates. Treatment of *Phytophthora* mycelium with potassium phosphonate over a period of days is known to induce formation of isohypophosphate and increase levels of a phosphate ester and of pyro- and tripolyphosphate. The *in vivo* NMR spectra displayed increases in the phosphate ester and isohypophosphate signals within 6 hours of phosphonate addition. However, corresponding increases in the pyro- and polyphosphate regions were absent and the intensity of one of the polyphosphate signals decreased.

Introduction

Phosphonate (Phi), an isostere of phosphate (P_i), provides effective protection for plants against many species of pseudofungi belonging to the genus *Phytophthora* (Griffith *et al.* 1992). The site of action of phosphonate is not known. We have attempted to identify the site(s) of Phi action within members of the genus *Phytophthora* by following the changes in concentrations of metabolites after treatment of the organism with phosphonate. These studies established that addition of Phi to *P. palmivora* grown under limiting P_i (0.1 mM) conditions resulted in altered cell wall composition (Dunstan *et al.* 1990), a decrease in the level of nucleotide phosphorus and an increase in the level of pyrophosphate (PP_i) (Griffith *et al.* 1990). Parallel studies using [31]P NMR identified very short chain polyP as a characteristic of the genus (Niere *et al.* 1994). These [31]P NMR studies also showed that the distribution of phosphorus between the various metabolite pools was altered when various *Phytophthora* spp were grown for several days in the presence of Phi at concentrations sufficient to reduce growth by approximately 50%. As free P_i and PP_i concentrations increased, the mean chain length of polyP shortened and a new metabolite, identified as isohypophosphate (the phosphate-phosphonate anhydride analog of PP_i), was observed.

These past studies examined changes which developed slowly, over a period of days, as the mycelium passed from log to stationary phase in the presence of Phi. In this paper we use in vivo [31]P NMR to examine short-term changes induced by Phi.

Materials and methods

Reproducibility

All data presented in this paper are based on at least two experiments except where indicated. The data presented are representative.

Strains

P. palmivora P376 originated from the *Phytophthora* culture collection at the University of California, Riverside and was obtained from Professor Michael Coffey. This strain was selected due to its sensitivity to phosphonate (Niere *et al.* 1990).

Media and growth conditions

Mycelia were grown in 500 mL conical flasks in 150 mL of liquid modified (Fenn and Coffey 1984) Ribeiro medium (dLPR, 0.1 mM P_i) prepared by omitting Mn from the microelement solution. Omission of Mn gave in vivo NMR spectra with narrower peak widths and improved signal-to-noise. It had no effect on mycelial growth rate or on the NMR spectra of acid extracts, indicating that the organism could still access sufficient Mn to sustain normal growth in dLPR media. Zero-phosphate medium (dZPR) was prepared by

omitting phosphate from the dLPR medium. Media were adjusted to pH 6.2 with KOH prior to autoclaving for 15 mins at 121 °C and 18 p.s.i. Thiamine, separately autoclaved, was added aseptically prior to inoculation. Mycelium for inoculation was propagated on dLPR medium to which agar (2% w/v) had been added. Each flask was inoculated with three pieces of agar (5 mm diameter). The flasks were then incubated in the dark without shaking at 26 °C.

Mycelium destined only for PCA extraction was removed from the agar inoculum plugs, collected by vacuum filtration on a Whatman GF/A glass-fibre filter, washed three times with ice-cold water (3 x 15 ml), frozen immediately in liquid nitrogen, freeze-dried and stored in a sealed sample tube at -20° C until extraction.

Mycelium used during in vivo NMR experiments was collected aseptically by filtration through a sterile nylon sieve, placed in a sterile petri dish containing culture medium and detached from the agar inoculum plugs. Normally eight flasks of 3-day cultures or six flasks of 4-day cultures provided sufficient mycelium to fill the receiver coil volume of the NMR tube. This material typically yielded 50-100 mg dry wt of mycelium.

In vivo NMR experiments
Small pieces of mycelium were positioned quickly and gently in a 10 mm diam NMR tube to a depth of 5 cm (1 cm above the top of receiver coil) and the reference capillary tube was positioned along one side. The perfusion apparatus was similar to that described by Lee and Ratcliffe (1983). All [31]P NMR spectra were run in 10 mm NMR tubes on a Bruker AC-200F spectrometer ([31]P operating frequency 81 MHz) at 294±1 K. Acquisition parameters for in vivo spectra were: 65 ° pulse angle, 2 K data points, 0.17 s acquisition time, 0.52 s relaxation delay. Composite pulse decoupling was used with the decoupler gated off during the relaxation delay. FIDs were zero-filled to 8 K and line broadening of 10 Hz was used prior to Fourier Transformation. Spectra were usually accumulated over 2 h (10,300 transients). NMR acquisition and processing of extract spectra were as described previously (Niere *et al.* 1994). Where appropriate, estimates of changes in different P-metabolite pools in vivo were based on changes in relative peak intensities. Chemical shifts in all spectra were referenced to an aqueous methylene

diphosphonic acid (1 mm capillary tube) signal set at 16.85 ppm downfield from 85% H_3PO_4. Peaks in extract spectra were assigned by reference to published chemical shifts (Navon *et al.* 1979). Peaks in the in vivo spectra were assigned on the basis of chemical shifts reported in the literature, behaviour of the peaks under different experimental conditions and by comparison with spectra of corresponding PCA extracts.

Results and Discussion
Although the signal to noise limitations of the instrumentation prohibited observation of transient metabolite perturbations, many changes, persisting over longer time scales, were observed when environmental conditions were varied.

Interpretation of ^{31}P NMR in vivo spectra of P. palmivora
The [31]P NMR spectrum of 3 day old *P. palmivora* mycelium in oxygenated dLPR culture medium is shown in Fig. 1. The two overlapping peaks labelled 1 and 2 at +4.9 and +4.4 ppm were similar to those reported by Navon *et al.* (1979) for *Saccharomyces cerevisiae* grown in glucose-containing medium and were assigned to sugar phosphates and other phosphomonoesters. The chemical shift of peak 3 (+2.7 ppm) was similar to that of the peak assigned to cytoplasmic P_i in other micro-organisms (Navon *et al.* 1979).

Peaks 4, 5 and 6 were always conspicuous features of in vivo spectra, but presented difficulties in assignment. Peak 4 was assigned in part to external P_i on the basis of changes in its chemical shift and intensity as external pH and P_i concentrations were altered. However, since peak 4 persisted when phosphate-free medium was used as the perfusate, part of this peak may result from P_i located in an acidic membrane-bound compartment such as the vacuole ($P_{i,vac}$). The chemical shifts of peaks 5 and 6 and the lack of corresponding peaks in the spectra of acid-extracts suggest that they may be due to relatively mobile phosphodiester components of the cell wall.

Peaks 7, 9 and 12 were assigned to the g, a and b phosphates of NTP on the basis of their chemical shifts (-5.0, -8.3 and -18.9 ppm). The a- and b-P of NDP can also contribute to peaks 7 and 9. Peaks 10 and 11 were assigned to NDPG and NAD may also make some contribution to peak 10 (Nicolay *et al.* 1983).
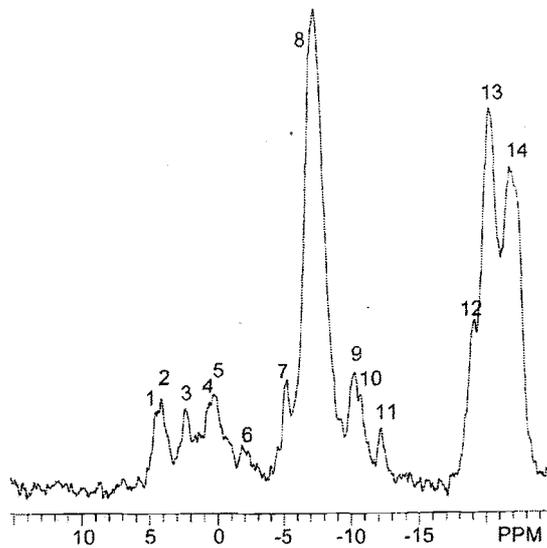
Fig. 1. ^{31}P NMR spectrum of 3 d old *P. palmivora* mycelium perfused with oxygenated dLPR culture medium. The spectrum was collected over 2 h, acquisition beginning 2 h after insertion of the mycelium into the NMR tube. Peak assignments: 1 & 2, monophosphodiesters; 3, cytoplasmic P_i; 4, $P_{i,}$ in the perfusate + unknown; 5 & 6, unknown; 7, □-P of NTP & □-P of NDP; 8, terminal P of polyP and PP_i; 9, □-P of NTP and NDP; 10, P of NDPG and NAD; 11, P of NDPG; 12, □-P of NTP; 13, penultimate P of polyP; 14, middle P of polyP (DP>4).
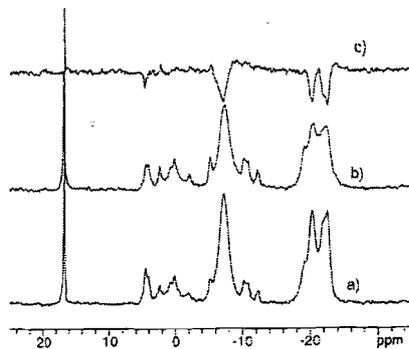
Fig. 2. ^{31}P NMR spectra of *P. palmivora* mycelium grown in dLPR and perfused with oxygenated dZPR. a) t = 1-3 h; b) t = 19-21 h; c) difference between a) and b)

The intense signal (peak 8) centred at -7.4 ppm was assigned to pyrophosphate and terminal P nuclei in polyphosphate (polyP$_{term}$) while peaks 13 and 14 at -19.9 ppm and −21.5 were assigned to penultimate and middle P nuclei in polyP chains (polyP$_{pen}$ and polyP $_{mid}$). The prominence of the polyP$_{term}$ and polyP$_{pen}$ peaks confirms the presence of substantial mounts of unusually short polyP in living *Phytophthora* mycelium. This is not a result of growth in low-phosphate media - the mean chain length of NMR-visible polyP remains small even during growth in high phosphate (10 mM P_i) media, with the polyP$_{term}$ peak

remaining a major feature of the in vivo spectrum (J. Niere, unpublished results). This is in marked contrast to the in vivo NMR spectra of other polyP-containing organisms (Elgavish and Elgavish 1980; Sianoudis *et al.* 1986; Bourne 1990; Bental *et al.* 1991; Ashford *et al.* 1994;) where the polyP$_{term}$ peak is absent or very much smaller than the dominant polyP$_{mid}$ peak.

Effect of phosphonate

The use of dZPR as perfusate during the Phi experiments was necessitated by the

184

very slow uptake of Phi (0.25 mM) from culture medium containing P_i at levels (< 0.1 mM) well below that required for optimum growth rates (Griffith *et al.* 1993). In order to differentiate the effects of P_i deprivation from those of Phi exposure, additional in vivo NMR experiments were run using dZPR perfusate. In these experiments, sugar phosphate peak intensities decreased slightly over eighteen hours but cytoplasmic Pi levels were sustained over the period of the experiments. PolyP peak intensities remained constant for several hours and then began to decrease slowly after 10 ± 3 h. The decreases in the polyP$_{term}$, polyP$_{pen}$ and polyP$_{mid}$ peaks were similar, indicating that the mean chain length of NMR-visible polyP remained essentially constant . The changes after 21 h in dZPR are shown in Fig.2. The substantial delay (not shown) prior to decreases in NMR-visible polyP levels suggests that P_i requirements (including maintenance of cytoplasmic P_i levels) may have been initially met by utilisation of NMR-invisible P pools.

When Phi (0.25 mM) was introduced into the perfusate, several clear changes were observed. The rate of uptake of Phi and the onset of these changes varied from experiment to experiment. In the experiment depicted in Fig. 3, two overlapping peaks at +3.5 and +3.7 ppm, assigned to Phi by their chemical shifts, were observed within 2 h of addition. Preliminary experiments indicated that Phi in the culture medium contributed to the +3.5 ppm peak whereas the 3.7 ppm signal was due to Phi within the mycelium.

The phosphomonoester peak at +4.9 ppm began to increase within three hours of phi exposure. The chemical shift of this peak in the well-resolved PCA extract spectrum (not shown) was very close to that of glucose-6-phosphate.

The increase in the phosphomonoester peak, was accompanied by the appearance of a new peak at -3.8 ppm. It had the same chemical shift as the H-P isohypophosphate signal (IHP) previously observed in spectra of PCA extracts from phosphonate-treated mycelia (Niere *et al.* 1990). Although IHP contains two different P nuclei, their chemical shifts are within 1 ppm of one another. Given their proximity and the P-O-P coupling constant of 17 Hz (Niere *et al.* 1990), these nuclei would be expected to produce a single peak in the in vivo spectrum.

The intensities of the glucose-6-phosphate and IHP peaks in the in vivo spectra followed the intensity of the Phi signal, increasing as it increased, and stabilising when it stabilised. In those cases where Phi was assimilated rapidly and in large amount, the increases in glucose-6-phosphate and IHP were also more pronounced. Where uptake of Phi was slow, accumulation of glucose-6-phosphate appeared to precede that of IHP.

The changes in the polyP peak intensities were different from those induced by lack of external P_i. The difference between the spectrum acquired prior to Phi addition and the spectrum acquired at16-18 h exposure to Phi, is shown in Fig 4. The polyP$_{term}$ peak (-6.9 ppm) decreased slightly and moved to a slightly lower frequency, but since this peak also broadened slightly, its area remained essentially constant. Peaks due to other P nuclei in polyP chains decreased markedly. Phosphorus stored in the middle of polyP chains (polyP$_{mid}$ at -21.5 ppm) declined more than phosphorus located closer to the end of the chain (polyP$_{pen}$ at -19.9 ppm), indicating a decrease in mean polyP chain length. These decreases in polyP signals lagged behind the changes in intracellular Phi, glucose-6-phosphate and IHP levels, and continued after levels of the other metabolites had stabilised.

Comparison of the NMR spectra of PCA extracts of Phi-treated mycelia and mycelia harvested at t = 0 h (not shown), showed that, despite the decrease in some NMR-visible polyP peaks in vivo, levels of phosphorus stored as pyrophosphate and tripolyphosphate had *increased* slightly during 18 h exposure to Phi. This indicated that the very large increases in pyrophosphate and tripolyphosphate previously observed in mycelium grown in LPR in the presence of phosphonate (Niere *et al.* 1990) are initiated but not completed in the short term following Phi exposure. It is also possible that these very large increases may depend on the presence of an external source of Pi which was necessarily absent during the short-term in vivo experiments. The lack of change in the intensity of the in vivo polyP$_{term}$ peak may be due to small increases in the contributions of pyrophosphate and tripolyphosphate being off-set by decreases in levels of longer chain polyP.

In conclusion, polyphosphate, initially reported (Chilvers *et al.* 1985) to be nonexistent in *Phytophthora*, has been shown
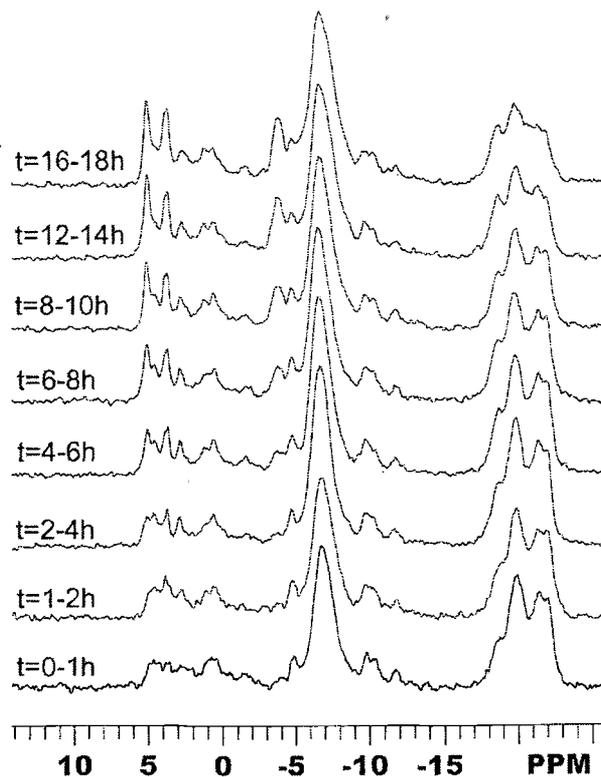
Fig. 3. ^{31}P NMR spectra of *P. palmivora* mycelium grown in dLPR and perfused with oxygenated dZPR to which phosphonate (0.25mM) was added at t = 0 h.
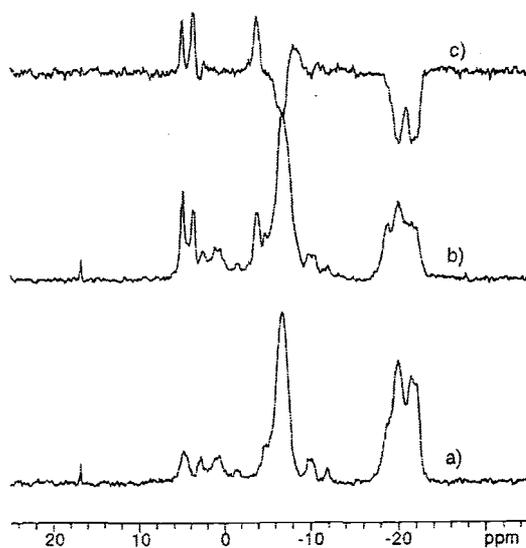
Fig. 4. ^{31}P NMR spectra of *P. palmivora* mycelium grown in dLPR and perfused with oxygenated ZPR. Phosphonate was added to the perfusate at t = 0 h. a) t = -2-0 h; b) t = 16-18 h; c) difference between a) and b).

to be a major phosphorus metabolite in living *Phytophtora* mycelia. We have shown that the polymer can be used by *Phytophthora* to sustain free phosphate levels under conditions of phosphate deprivation. In the absence of external phosphat, phosphonate is taken up rapidly and and increases in levels of a sugar phosphate and production of IHP occur within a few hours. Perturbations in polyphosphate levels and mean chain lengths become apparent some hours later. It is still unclear whether IHP itself plays an integral part in phosphonate action and disruption of polyphosphate metabolism or whether it is simply a harmless by-product formed when phosphonate is metabolised.

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Advances in technology for detection of plant pathogenic microorganisms

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Abstract. DNA diagnostics are developing at a rapid pace being fuelled by the needs of the medical diagnostic and genomics industries. The genomics industry is based on high throughput analysis of genomes in the search for DNA markers linked to valuable traits. The industry requires analysis of thousands of samples and a major criterion is that the cost of analysis must be kept low. The development of high throughput facilities based on robotic workstations, solid state PCR and MALDI-TOF analysis makes this feasible with a cost of less than $1 per sample. In parallel, microarray technology has developed to the stage where it is possible to screen entire genomes for genetic defects instantaneously. This is increasingly being applied in human and veterinary medicine and in other areas such as environmental health, water analysis, certification of transgenic foodstuffs, and in the food industry where instant genotyping or pathogen profiling is a priority. It is inevitable that these technologies will impact on plant pathology diagnostics. Already in Australia, and other parts of the world we are seeing the establishment of commercial high throughput services offering pathogen profiling of soil samples using either DNA probe technology or MALDI-TOF analysis.

Introduction

Plant disease causing microorganisms (bacteria, fungi, viruses) are a major limitation on the productivity of plant production in agricultural ecosystems. A major cost of plant production is the cost of management of plant diseases through the application of fungicides, preventing the spread of disease by quarantine, and by the breeding of resistant varieties. Diseases have the potential to wipe out entire industries with very serious economic consequences. In natural ecosystems diseases can reduce biodiversity of plant species and consequently of animal species which depend on the plants. Plant deaths from disease may also raise the watertable leading to salt degradation of the land.

Diagnostics is a major weapon in the fight against plant-disease causing microorganisms. The ability to detect the pathogen at an early stage in disease development allows the timely implementation of disease control outbreaks such as the application of fungicides or removal of the infected material. Quarantine is an important component of disease management and, in a physically isolated country like Australia, has been successful in keeping out a number of serious plant diseases. However the increased movement of plant germplasm and soil both within, and between, national borders in recent years has increased the potential for the spread of plant diseases. Pathogens are carried as contaminants in asymptomatic material such as seeds, or fruit or in products derived from plants. The problems are exacerbated by the increased number of people travelling by air bringing with them food and other material such as wooden ornaments which may harbour pathogens. This means that there is a need for greater vigilance by quarantine services coupled with a need for more effective methods for detection of pathogens.

Traditionally pathogens are detected either by plating samples on microbial growth media, or where appropriate by planting seeds (under quarantine conditions) to see if any diseased plants grow. The problems associated with these are (a) they take some considerable time and resources, and (b) the pathogen may not grow or even cause disease despite being present. It may not be until after the importation has been approved, and a particular set of environmental and/or physiological conditions occurs that the disease manifests itself.

An alternative approach would be to detect the DNA of the pathogen. This detection method is independent of environmental conditions or of the growth stage of the pathogen. In recent years there has been a tremendous increase in the application of molecular biology techniques to the detection of phytopathogenic microorganisms in asymptomatic plant material (Bej and Mahbubani 1992; Bonants, *et al.* 1997; Dunn, *et al.* 1995; Ersek *et al.* 1994; Kappe *et al.* 1996; Navot *et al.* 2001; O'Brien 1996; Ristaino *et al.* 1998; Schots *et al.*

1994; Skinner 1992; Varma and Kwon-Chung 1992; Wiglesworth *et al.* 1994). This is based on results showing that such tests are sensitive, rapid and highly specific. There have been numerous reports in the scientific literature on the development of DNA probes, or more commonly PCR tests for detection of phytopathogenic bacteria, fungi and viruses.

PCR Tests

The most common type of molecular test developed is based on the Polymerase Chain Reaction (PCR) (Schots *et al.* 1994). This requires a DNA sequence that is specific to the pathogen. Such sequences exist in all organisms, usually as the sequences between genes where there is no selective pressure either for or against nucleotide substitutions. These sequences can be used to differentiate not only species, but also subspecific groups, eg., pathotypes.

The PCR Process

In the PCR reaction, a specific target DNA sequence 200-1000 base pairs long is amplified several billion times. Amplification starts with heat denaturation of the DNA to separate the strands (usually at approx 92^0C), followed by the annealing of short (15-20 bases) oligonucleotide primers to sites flanking the

target sequence (temp range 40 to 60^0C) (Fig. 1). The primers are extended by a thermostable DNA polymerase (at 72^0C) so that we now have four strands of DNA. We initiate a new cycle by heating the DNA to separate the strands, and the process repeats. At each cycle the number of DNA strands doubles and after 35-40 cycles there are enough copies so that we can see the DNA on a gel or by some other means. Each stage in the process (primer annealing, extension and separation of the strands) occurs at a different temperature which is achieved by carrying out the reaction in a thermalcycler which is programmed to go to specific temperatures in a defined sequence.

Usually the products are analysed by electrophoresis on agarose or acrylamide gels although alternative methods for analysis exist. One of these is the Taq Man system developed and marketed by Applied Biosystems. In this process extension of the primers by DNA polymerase generates fluorescence. The amount of fluorescence increases with each cycle. This allows us to follow the product accumulation after each cycle. This is called "real time PCR" and can be used to quantify the amount of template in the starting material.
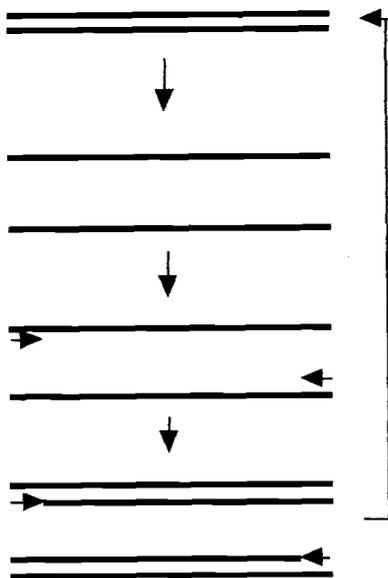
The DNA is heated to separate the strands.

Reducing the temperature allows the primers to anneal to sites flanking the target.

Raising the temperature to 72 degrees allows DNA polymerase to extend the primers.

On completion of the cycle the number of DNA strands is doubled.

Fig. 1. The PCR process
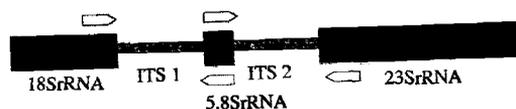
Fig. 2. Organization of the ribosomal RNA (rRNA) genes. The arrows indicate primers used for amplification of the ITS regions.

The majority of PCR diagnostic tests for fungal pathogens focus on the Internal Transcribed Spacers (ITS) of the ribosomal RNA (rRNA) genes (Fig. 2). The reasons for this are threefold: (i) is that the sequences at the ends of the small subunit (SSU) and large subunit (LSU) genes are conserved and so we can use the same primers to amplify the ITS sequences from many different species without first sequencing the genes (Kappe et al. 1996; Medlin et al. 1988). From the sequence of the amplified ITS regions we can develop species specific primers. (ii) because nucleotide substitutions within the ITS region are phenotypically neutral, the sequences are highly variable and hence a good source of species specific probes. (iii) The rRNA genes are multicopy, being repeated hundreds of times in bacteria and thousands of times in eukaryotic nuclei. The high copy number makes them easy to detect.

An advantage of PCR detection tests is that they are extremely sensitive. A good example of this is the generation of an individual's DNA fingerprint from the saliva left on a cigarette butt. Similarly in plant pathology, a minute fragment of a root, stem or leaf can be enough to carry out analysis. A caveat is that the distribution of the pathogen within the sample may not be uniform, and hence we may fail to detect it.

Application of PCR detection tests in commercial diagnostics

From a research point of view PCR has provided a tremendous boost to the study of plant pathology. It has revolutionised our ability to probe pathogen population structure and to investigate the distribution of the pathogen in natural ecosystems and in plants.

Despite the advantages of PCR tests in terms of sensitivity specificity and rapidity, these tests have not moved out of the research laboratory into commercial application. There

are very few commercial diagnostic operations based on PCR. Those that are tend to be government funded institutions which are often not working on full cost recovery, or commercial operations which deal with large numbers of samples (high throughput facilities). The main factor limiting the adoption of PCR detection test by industry is the cost of the process. At the current state of development it is an expensive process which in addition requires highly skilled labour and expensive equipment.

The major cost component of PCR analysis is labour. The reagents used for amplification comprise only a minor cost component by comparison. The processes of extracting DNA setting up PCR reactions, and analysis of the PCR products by gel electrophoresis are time consuming. By replacing some of these steps with less labour intensive procedures the costs per sample can be decreased considerably (Fig. 3).

New developments in PCR diagnostics

Robotic workstations.

The development of automated procedures for extraction of DNA from samples is one approach which has been intensively investigated in recent years as a means of overcoming this limitation on the commercial application of PCR diagnostics. The manipulations involved in DNA extraction are carried out by robotic workstations which handle hundreds of samples simultaneously (Ranjard, et al. 2001). They can carry out procedures such as extraction of DNA, set up of PCR reactions, loading of the reactions into the thermalcycler, and loading the PCR amplification products onto a gel or into some alternative form of analysis. Robotic workstations enable high throughput facilities to process in the region of 10,000 samples per day with relatively little labour input. This greatly reduces the cost per sample.
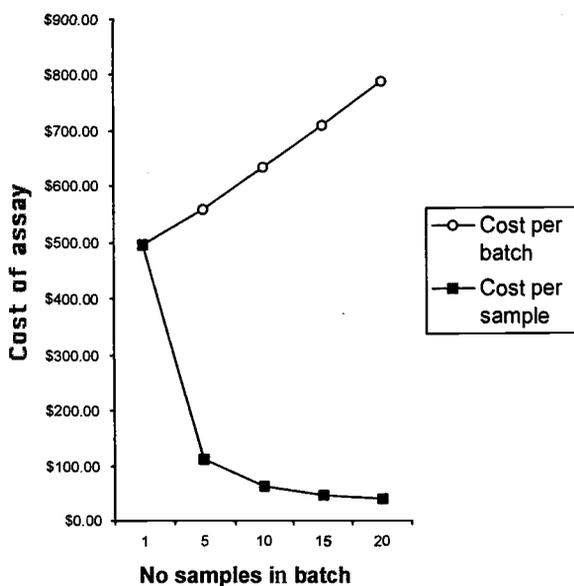
Fig. 3. Effect of sample numbers on costs of PCR analysis. As the number of samples increases the costs per sample decreases.

Mass spectroscopic analysis of DNA.

Another labour intensive component in PCR analysis is analysis of the PCR product by gel electrophoresis. Pouring, loading, running and imaging the gels is time consuming. This problem can be overcome by using real time PCR systems such as the Taq Man system (see above) or more popularly with high throughput facilities, mass spectroscopy.

There are different types of procedures for mass spectroscopic analysis of DNA but the most common and popular is designated Matrix, Assisted Laser Desorption Ionisation-Time of Flight (MALDI-TOF) mass spectroscopy (Bray *et al.* 2001; Monforte and Becker 1997). In this procedure DNA is mixed with a matrix and immobilised on a solid support. The mixture is irradiated with a laser which vaporises the DNA. Disintegration of the DNA is avoided by use of an appropriate matrix which absorbs the initial energy of the laser and transfers it to the DNA. The vaporised DNA under the influence of an electric field then flies down a tube to a detector at the end. The time taken to traverse the tube depends on the *mass* of the DNA and is typically of the order of milliseconds. Batches of 800 samples can be loaded into the MALDI-TOF for analysis. In addition to the speed and automation, another advantage of MALDI-TOF is that very little DNA is required for analysis. This means

that only 5-10 cycles of PCR are required instead of the 30-40 cycles normally required.

On site analysis by PCR.

A drawback of the high throughput facilities for PCR diagnostics is that samples have to be transferred to them for analysis. This inevitably means a delay in getting the result. There are situations in which it would be extremely useful to be able to carry out an analysis on the spot eg., testing the nutrient fluid in hydroponics facility for the presence of a pathogen. Advances in microfluidics are making on site detection by PCR in very short time frames a reality. Microfluidics is the miniaturisation of separation and assay techniques such that multiple procedures can be carried out on a single chip made of glass or other polymers (Lemieux *et al.* 1998). Channels etched into the surface of the chip are used to deliver reagents to and from a reaction chamber. Processes such as electrophoresis can also be carried out on a chip and combined with DNA extraction procedures.

Reactions such as PCR amplification can be carried out with the reagents immobilised on the surface of the chip (solid state PCR). Because such small volumes (nL) are required mixing occurs very quickly, as does temperature ramping (shifting between temperatures) due to the very efficient transfer of heat. This means

191

that PCR amplification can be completed in minutes as compared to the 2-3h required for conventional PCR.

Belgrader *et al.* (1999) have described a microfluidic system for extraction of DNA from bacteria and amplification and analysis of the product within 7 minutes. In another study Hofgartner *et al.* (1999) compared microchip analysis of PCR products from herpes simplex encephalitis virus with the more conventional gel retardation assay. They reported that microchip analysis took less than 110 seconds per sample compared to 18h for the conventional assay and that the level of sensitivity and specificity was comparable to the established method.

Pathogen profiling

Pathogen profiling is the term used for the process of detecting multiple pathogens in a sample. Unlike the cases we have been considering above which involve a test for single pathogens, pathogen profiling enables us to detect multiple pathogens simultaneously. This is made possible through the use of microarrays. Mircoarrays are DNA chips with oligonucleotide sequences immobilised on the surface in a defined pattern (array) such that we know the position of each sequence (Fig. 4). The sequences are derived from genes (usually the rRNA genes) of the organisms we are trying to detect. DNA is extracted from the sample and hybridised to the immobilised sequences on the chip. The extent of hybridisation to each target sequence is determined by labelling the DNA. Using a microarray scanner we can measure the amount of DNA hybridised to each target sequence on the filter. How many pathogens can be detected on a single chip? Microarrays containing 10,000 sequences cm^2 are often used for gaining a profile of gene expression in a single cell, or for genotyping, although much higher density arrays have been described (Lemieux *et al.* 1998). Although this number if certainly greater than would be required for plant pathology it demonstrates the potential of the technique for detecting multiple pathogens. For plant pathology the arrays would be much simpler consisting of perhaps less than one hundred sequences. This makes them cheaper to construct.
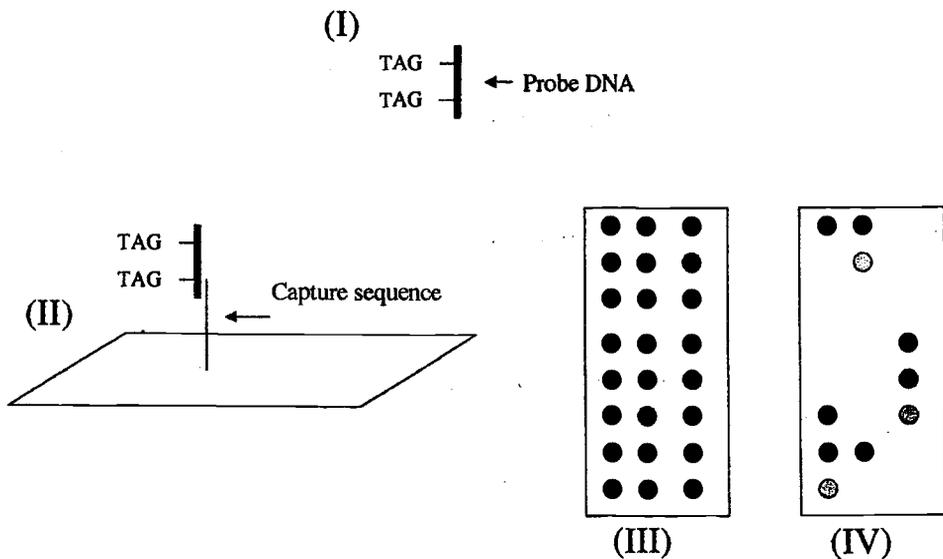
Fig. 4. Detection of pathogens using microarrays. The probe DNA is extracted from the assay sample and labelled with a fluorescent tag (I). The labelled DNA is hybridised to a chip on which the capture sequences are immobilised (II). Usually the chip has multiple capture sequences immobilised in a defined pattern (array) (III). From the amount of fluorescence on each spot of the array we can determine what pathogens are present in the sequence and their amount (IV).

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Aerial application of phosphite to protect endangered Western Australian flora

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Abstract. Research into the trunk injection of phosphite, a buffered solution of phosphorous acid (H$_3$PO$_3$), to protect native plants against *Phytophthora cinnamomi* disease was first carried out in Western Australia in the late 1980's. Initial aerial application trails took place near Albany in autumn 1993 at one fifth of the present standard application rate because of concerns about possible phytotoxicity. The current standard rate for aerially applied phosphite is 24 kg/ha^{-1} of 400g/kg^{-1} formulation together with a surfactant or wetting agent at 0.3% (v/v). Symptoms of phytotoxicity at thres rates are minor. Generally, the chemical is applied as two treatments in autumn, about one month apart. The first broadscale aerial application of phosphite took place in 1997 and since then about 640 ha of native vegetation at more than 50 sites has been treated, mostly to protect endangered flora. Several sites near Albany received their third application this year. Spraying is normally done in autumn to take advantage of calm, clear weather in the Stirling Ranges where most aerial application takes place. Recent research has indicated for some species at least, that autumn application may lead to greater foliar uptake of phosphite. Monitoring indicates that aerial phosphite treatments will need to take place every two years and in some cases, where only a few wild plants are left, annual applications may be needed. The area of most urgent research need is the interaction between phosphite, wildfire and regeneration of rare flora to determine optimum application rates and frequency.

Introduction

The aerial application of phosphite to protect plant species threatened by the soil-borne pathogen *Phytophthora cinnamomi* is an accepted management procedure in Western Australia alongside the 'traditional' management procedures which revolve around prevention of spread of the disease (Hardy *et al.* 2001). This paper summarises the history and methodology of phosphite use in this State and also examines recent research and challenges for the future use of the chemical for conservation purposes.

Background to phosphite use in Western Australia

Research into trunk injection of phosphite, a buffered solution of phosphorous acid (H$_3$PO$_3$), to protect native plants against *P. cinnamomi* disease was first carried out in Western Australia in the late 1980's. While the use of phosphorous acid and related fungicides have been widely used in horticultural situations since 1977 (Cohen and Coffey 1986), it was first used for the control of *Phytophthora* species in native plants by the Department of Conservation and Land Management in Western Australia in the late 1980's (Shearer and Fairman 1991). Early trials involved trunk injection of the chemical into *Banksia* and *Eucalyptus* species followed by application using backpack sprayers in the early 1990's. However, it was soon realised that to effectively control infestations of more than a hectare or two, the chemical needed to be applied by aircraft.

Initial aerial application trials took place near Albany on the south west coast of Western Australia in autumn 1993 using a Cessna AgWagon fitted with a Micronair spraying system to spray *Banksia* shrubland partially infested by *P. cinnamomi*. Droplet size ranged from 100-500 µm with 70% of droplets being in the range of 100-300 µm. While this was a somewhat lower VMD (volume mean diameter) than is usually recommended smaller drops were preferred in order to penetrate the dense canopy of the early target sites. Droplet penetration measurements showed that water-sensitive papers laid under the trees intercepted only 20% fewer drops than papers attached to the tops of trees (Komorek and Shearer 1996). Because most aerial spraying nowadays is carried out in the Stirling Ranges near Albany in winds below 10 – 15 km hr^{-1} an aircraft fitted with CP nozzles producing a larger droplet VMD (250-350 µm) is used. Following on from research trials the chemical has generally been applied in two treatments 3 to 6 weeks apart. Two applications of 50% of the dose, rather than one application of 100%, was thought to result in greater uptake of the chemical as well as minimising the risk of phytotoxic affects.

The first aerial application trials used 8.5% and 10% phosphite applied at 26-30 L ha^{-1} because of concerns about possible phytotoxicity. However, these rates proved too low and gave less than 18 months protection to plants. Further trials using higher

concentrations (up to 40%) and application rates of up to 60 L ha^{-1} showed that longer protection could be ensured (up to 3 years) with minimal phytotoxic symptoms.

The first broadscale aerial application of phosphite took place in 1997, since then about 640 ha of native vegetation at more than 50 sites has been treated, mostly to protect endangered flora. Several sites near Albany received their third application this year (2001). Aerial spraying sites range in area from less than one hectare up to 35 ha. Spraying is normally done in autumn to take advantage of calm, clear weather in the Stirling Ranges where most aerial application takes place.

Current operational methods

The current standard rate for aerially applied phosphite is 24 kg ha^{-1} of 400 g kg^{-1} formulation together with a surfactant or wetting agent at 1% to 2% (v/v). Results of recent monitoring indicate that aerial phosphite treatments need to take place at least every two years and in some cases, where only a few individuals of a threatened species are left in the wild annual applications may be needed. Until further trial results are available post-fire seedling regeneration will be sprayed at 6 kg ha^{-1} as a single application.

Great variability in site and species characteristics contribute to making it very difficult to predict how long the protection will last from an application of phosphite. Phosphite uptake may be influenced by species composition, canopy cover and dilution post-spray depends on growth rates. Site factors such as soil and climate may also be important.

Recent research

Research into phosphite application methodologies is ongoing. Recent research has looked at spray adjuvants, time of spraying, season of spraying and whether a treatment in one or two applications gives better uptake. The spray adjuvant trial produced enough evidence to support a switch from Synertrol Oil, based on food grade canola oil (832 g L^{-1}) (Organic Crop Protectants Ltd.) to BS1000 (alchohol alkoxylate 1000 g L^{-1}) (Crop Care Australasia Pty Ltd) a non-ionic surfactant (more suitable for a water-soluble product such as phosphite), though the differences in phosphite uptake between the four types of spray additives or surfactants trialed were small (S. Barrett, unpublished). This study, as with several other studies, illustrated that there is a wide variability (up to

a factor of 20) in phosphite uptake between species.

Preliminary results from a trial carried out in *Banksia* woodland at Gull Rock looking at season of application showed that for one of the four species studied uptake of phosphite in late autumn (April) was significantly higher than in early spring, late spring and early autumn. The same trial found no significant difference in uptake when phosphite was applied in two 'doses' (as is the general practice) when compared to a single 'dose'. When sampled two weeks after application two of the four species had significantly higher root phosphite levels in autumn than in spring (R Smith, unpublished). However, application in two doses will still be desirable in those situations where a single application might lead to phytotoxic effects.

More work needs to be done on the timing and frequency of phosphite application in a range of vegetation types. Another area of urgent research need is the interaction between phosphite, wildfire and regeneration of rare flora. Most of the phosphite spray sites in the Stirling Range were burnt in spring 2000 and whereas most applications to date have been at least 6 years old for the next several years we will be spraying mostly seedlings or young juvenile plants. Ideally we would carry out several years of research to determine the appropriate rates and frequencies of phosphite application for these seedlings. However, we will not be able to wait for this research to give us the answers because *Phytophthora cinnamomi* will begin causing mortality of these seedlings almost immediately.

The use of phosphite has been shown by the use of 'control' quadrats to be successful in slowing the rate of spread of *P. cinnamomi* and increasing the percentage survival of threatened plant species in Western Australia (Barrett 1999). These encouraging results have led to an ongoing program of aerial phosphite application where the plant species most threatened by *P. cinnamomi* root-rot disease are sprayed every one or two years. However, because of uncertainties about the optimum dosage, time and frequency of treatment with phosphite given the large variability in such factors as species uptake and site characteristics research into application methodologies will continue 'hand-in-hand' with the aerial spraying program for several years.

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Breeding Port-Orford-cedar for resistance to *Phytophthora lateralis*: current status & considerations for developing durable resistance

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Abstract. A non-native root disease, caused by *Phytophthora lateralis*, is causing widespread mortality throughout the range of Port-Orford-cedar (*Chamaecyparis lawsoniana*). In 1997, the Forest Service and Bureau of Land Management in collaboration with Oregon State University initiated an operational breeding program for resistance. Over 1000 parent trees ranked high in a branch lesion test are being evaluated further. The frequency of resistant candidates is low, and depends on the criteria used to define a candidate as resistant. Large differences in survival among families occur, often varying from 0% to 100%. The oldest field tests indicate good survival through 12 years for rooted cuttings and seedlings of top parents. Some results from crossing suggest a major gene for resistance, but some differences exist among the different types of tests, especially for one of the more resistant families. The number and types of resistance mechanisms are unknown, and may be difficult to discern without diagnostic races of the pathogen. Breeding in Port-Orford-cedar can be done at a very early age, favouring the development of increasing levels of resistance. Management activities that reduce the spread of the pathogen, the size of the pathogen population, and the introduction of new strains will aid in developing durable resistance.

Introduction

Since 1952, an introduced root pathogen, *Phytophthora lateralis* Tucker & Milbrath, has spread throughout much of the native range of Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) killing large and small trees. Even earlier, it spread through the horticultural industry in the Pacific Northwest where Port-Orford-cedar had been a valuable ornamental species. Efforts are being made to stem the further spread of the disease in southwestern Oregon and northwestern California (Goheen *et al.* 2000). The development of resistant populations offers the opportunity to use this species in restoration and reforestation plantings as well as in horticulture.

The existence of genetic resistance to *P. lateralis* has been known since the late 1980's (Hansen *et al.* 1989; Sniezko and Hansen 2000; Sniezko *et al.* 2000). Following small-scale selection efforts in the early 1990's, the USDA Forest Service and USDI Bureau of Land Management began a joint operational selection and breeding program in 1997 (Sniezko and Hansen 2000). The BLM and Forest Service have coordinated the phenotypic selections that have been made throughout much of the species range on a wide variety of ownerships, including National Forests, BLM Districts, Redwood National Park and private lands. Over 9700 selections have undergone initial evaluation using a branch dip technique (Bower *et al.* 2000). Rooted cuttings from the top parent trees selected from this initial branch dip screening are established in a breeding arboretum at the Forest Service's Dorena Tree Improvement Center in Cottage Grove, Oregon. These selections undergo further testing and are used in breeding.

Despite the relatively small natural range of Port-Orford-cedar, the species has moderate levels of genetic variation (Kitzmiller and Sniezko 2000; Zobel *et al.* 2001). For breeding purposes the range of Port-Orford-cedar has been divided into six breeding blocks with elevation zones based on early results of common garden tests (Jay Kitzmiller and Jim Hamlin, personal communication).

P. lateralis is a root pathogen that causes infection mainly through unsuberized growing tips of small rootlets, particularly in cool, wet environments. Primary vectors of spread include vehicles and animals.

The presence of a low frequency of resistance in natural populations of Port-Orford-cedar, a high number of phenotypic selections, and early flowering potential in this species (Elliott and Sniezko 2000) make us cautiously optimistic about the potential of developing durable resistance. Breeding efforts are underway and the first operational

containerised seed orchards have been established. Objectives of the program include of Port-Orford-cedar while maintaining adaptability, genetic variation and growth potential. With evidence for at least one type of strong resistance, and operational seed orchards in some breeding zones set to produce good cone crops in fall 2002, it is an opportune time to reflect on the status of the program, the utilization of this resistant material and the potential for developing durable resistance.

Current Status
Overall
More than 1000 of the initial field selections passed the first phase of selection using a branch dip inoculation technique. Most of these selections are currently in the breeding arboretum at Dorena Tree Improvement Center, and rooted cuttings from these trees are now being tested to confirm resistance.

A breeding program is in progress to help confirm inheritance patterns and provide families to make selections for the operational program. Identification and evaluation of mechanisms of resistance is also underway. Provenance tests have been established to help gather long term data on adaptability and seed source movement to help refine the breeding block guidelines established by seedling common garden tests. Field tests are being established to validate screening tests, examine the durability of resistance and provide demonstrations of resistance. Seed orchards are being organized by breeding zone as information from resistance screening becomes available. Techniques have been refined to induce seed production at an early age which will shorten the time to complete breeding cycles and start the production of resistant seed. Resistant seed from the first cycle of selection should be available in fall 2002 for some breeding zones.

Testing Method
Several methods have been used to assess resistance in Port-Orford-cedar, and the results vary somewhat depending upon the testing method (Table 1). In the initial testing phase (selections from natural stands) branch test techniques were used to quickly assess thousands of trees (Bower et al. 2000), but the correlation with results from root test techniques has only been weak to moderate (Sniezko et al., this proceedings; also, unpublished data). As the breeding program proceeds, a root test will likely be utilized and therefore only root testing techniques will be

developing durable resistance for populations

discussed here. Table 1 presents an overview of results of first-year mortality from a 2000 test of 44 open-pollinated seedling families involving three testing methods: a greenhouse test (using a root dip technique), a raised bed test, and a field test. Seed for the tests were collected from a containerized greenhouse orchard. In the 2000 testing, all three methods provided good differentiation among seedling families, with each test showing a wide range in percent mortality. Also noted in Table 1 is the percent mortality of rooted cuttings of the resistant control (CF1) in three adjacent tests.

All three methods have their potential advantages. The greenhouse test (involving root dip inoculation) is relatively quick, inexpensive, and can handle a large number of seedlings and rooted cuttings, but provides only short-term testing (usually one year). The raised bed test is intended to more closely mimic field test results but to provide a more convenient and controlled environment; seedlings can be evaluated for two or three years. The field test lends itself best to long-term evaluation and monitoring for changes in virulence of the pathogen or changes in the environment that may affect the resistance of the host; it also has the best potential for the

Table 1. Variation among families in *P. lateralis* mortality from three test methods

| Parent | Test Location | | |
	GH Root Dip	Raised Bed	Camas Valley
7490	0	38.9	8.3
510005	25.0	33.3	0
CF1	50.0	50	25.0
117499	83.3	66.7	50
510044	66.7	75.0	75
70102	100	91.7	100
44 families[1]	67	77	59
CF1 r.c[2]	0	0	0

[1] Mean of 44 open-pollinated families
[2] Rooted cutting 'Control' in adjacent test at all 3 locations

demonstrations for land managers and the public. However, field sites are less controlled and adequate levels of infection needed for resistance testing may take several years or more.

There appears to be reasonable agreement among the results of the three testing methods (Table 1). However, although it is still among the top families in all tests, family 117490 has noticeably more mortality in the raised bed test. This was the only family with no mortality in the greenhouse, but it had 39 percent mortality in the raised bed test. The greenhouse test uses a root dip inoculation in which the pathogen infects the seedlings at the bottom of the root system and travels the length of the roots to the root collar before mortality occurs. In the raised bed test, inoculum would be present throughout the soil and could potentially infect seedlings throughout the root system. In other greenhouse testing, 117490 has shown little or no mortality across a range of control crosses, and no other parent has performed as consistently outstanding in seedling progeny (unpublished data). Whether this is an indication of a major gene for resistance is being investigated; if so, it appears the resistance is somewhat relative to the test environment.

Another interesting point from Table 1 is the presence of no mortality in rooted cuttings of the high resistant control (CF1) across all three testing methods. The root system of rooted cuttings (or at least CF1) may differ enough from seedlings that infection is more difficult; or CF1 may have a different type of resistance than 117490. Additional data on how these three testing methods relate is currently being compiled and summarized.

Long-term field results

Current data suggests that *P. lateralis* has only been in the native forests since around 1952, and resistance was only generally acknowledged to exist in the late 1980's. Data from several small plantings established in the late 1980's and early 1990's indicate that seedlings or rooted cuttings from resistant families or parents that survive through the first year or two are generally still alive 5 to 10 years later (Sniezko and Hansen 2000). In addition, field visits to some of the first selections such as 510015 show that they are still alive 10 years later despite being surrounded by dead trees (Chuck Frank, pers. comm..). Given these factors the evidence to date gives room for cautious optimism in developing resistant populations.

Knowledge of P. lateralis

The lack of much known genetic variability at the molecular level (McWilliams 2002), and relatively slow rate of spread of this pathogen are encouraging from a resistance program standpoint. There is some variability in growth of *P. lateralis* isolates in lab culture, but it is unknown at this point what ramifications this might have on resistant populations. The origin of *P. lateralis* is unknown and it is possible that more virulent strains may exist. More information on this pathogen and its evolutionary potential should allow us to optimize resistance breeding options (McDonald and Linde 2002).

No strains of *P. lateralis* virulent to the most resistant Port-Orford-cedar selections are known to exist, but this may be partly due to the low frequency of high resistant trees in the forest, a relatively low degree of monitoring, and the relatively recent initiation of a resistance program. Further work to examine variability in virulence is being planned. The raised bed test allows us to maintain a small, high hazard environment that can be repeatedly used for testing resistant trees. The large number of *P. lateralis* spores available and the high frequency of resistant genotypes being tested in the raised beds should make this an ideal site for monitoring the potential for increased virulence in the pathogen population.

Resistance to P. lateralis

The available information on resistance in Port-Orford-cedar is increasing rapidly with results from recent tests. Most of the information to date has been compiled from the relatively few resistant selections made before 1991 (Sniezko and Hansen 2000; Sniezko and others, this proceedings) and before the large number of phenotypic selections were made for the operational program that began in 1997. Until recently, only rooted cuttings or seedling families from a few parents such as CF1, 510015, and 117490 were known to exhibit 'strong' resistance (relatively high survival in testing relative to other parents). Information on the nature of this strong resistance (number of mechanisms, inheritance, durability) is limited, but further examinations are underway. The conservative assumption that will be used for planning is that this strong resistance is from one major gene (one R gene) and is the same among all families showing relatively high survival in tests. This assumption may help optimize planting strategies and avoid some potential pitfalls of utilizing resistance or managing the resistance program (Pink 2002; McDonald and Linde 2002). *P. lateralis* spreads in water and with soil movement. Even if a virulent strain arose at one site the

new strain would spread only slowly as long as forest sanitation measures are maintained. However, current and future investigations should provide more information.

A preliminary examination of recent testing results indicates that there may be at least one type of partial resistance as indicated by mortality rate differences among seedling families or rooted cuttings of different parents. Further work is underway to confirm these differences. This type of resistance could be an important component for low hazard sites or for use in conjunction with other resistance in developing durable resistance. However, if it does exist, further breeding would likely be needed to make this partial resistance more useful, particularly on higher hazard sites.

Resistance is an important management tool in maintaining Port-Orford-cedar in areas at risk for *P. lateralis*; however, other management practices that minimize the spread of the pathogen or reduce site hazard may greatly increase the potential durability of resistance.

The operational containerized seed orchards at Dorena contain a mixture of parent trees, many of which have at least the strong type of resistance. These orchards will provide a mixture of genotypes for planting. Validation plantings from this seed can be established to assess the increase in resistance under field conditions over the use of seed from general forest collections. The orchards can be easily modified yearly to include the latest selections and any different resistance mechanisms as new information becomes available.

Use of resistant trees (Maintaining resistance)
There is currently a reluctance to grow Port-Orford-cedar in the Pacific Northwest, because of the disease. Potential applications for resistant trees include restoration plantings, general reforestation and ornamental use. Resistance is potentially a great tool in the management against this exotic pathogen, and the way in which it is utilized may influence its durability. Port-Orford-cedar is a long-lived tree species and a primary goal is to obtain resistance that will maintain the species over time in ecosystems already infested with *P. lateralis*. Crop breeders often work with a lack of information about variability and race structure of the pathogen, focus on major gene resistance, and are constrained by a focus on crop uniformity (Pink 2002). It is intended that the *P. lateralis*

resistance program for Port-Orford-cedar will differ radically in several ways from conventional resistance programs associated with crop or horticultural breeding and poplar tree breeding. Among these differences: resistance is the main focus of the program, breeding zones have been established to help ensure long-term adaptability, and diverse populations of genotypes will be utilized in most plantings. However, the number and type of resistance mechanisms may be difficult to discern without diagnostic races of the pathogen.

Forest sites vary greatly in their relative disease hazard and there may be cases where seedlings with a different array of resistance mechanisms could eventually be planted. Planting resistant material of any nature is likely to reduce the incidence of disease since the frequency of suitable hosts will be less. Management activities that reduce disease hazard would also help lessen the likelihood of pathogen evolution.

The size and density of a planting can influence pathogen evolution. However, most plantings on federal lands are likely to be small and Port-Orford-cedar is generally mixed with other species. These lower density plantings should also help reduce the overall selective pressure on the pathogen and help maintain resistance. Most ornamental plantings will also be small. Port-Orford-cedar plantations established by private landowners will be larger, but still relatively small and this should help minimize the development of virulent strains.

A combination of factors makes this one of the fastest moving resistance programs for forest trees in the world. In theory, the current federal program allows consideration of long-term as well as short-term needs. This should allow us to continue beyond the early dramatic resistance we currently see and toward ensuring durable resistance. Ultimately, support from federal and nonfederal land managers will help decide the success of the breeding program.

In summary, due to the tremendous cooperative efforts of many people in making field selections and evaluating them, the relatively low evolutionary potential and slow spread of the pathogen, management protocols for reducing inoculum levels and slowing the spread, and the biology of Port-Orford-cedar, great early advances have been made in increasing our knowledge of resistance.

Resistant seedlings should be available in the near future. The next five years have the potential for greatly increasing our knowledge of the underlying mechanisms and inheritance of resistance, more fully developing the resistant base populations for each breeding zone, and beginning restoration and reforestation efforts of Port-Orford-cedar.

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Variation in *Phytophthora lateralis* resistance among 29 seedling families of *Chamaecyparis lawsoniana*

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Abstract. In 1999, 29 seedling families of Port-Orford-cedar, *Chamaecyparis lawsoniana*, (15 full-sib, 14 wind-pollinated) were planted in raised beds containing *Phytophthora lateralis* and at a field site. Mortality was observed for one year. Parent trees had been selected in 1989 and 1990 in natural stands with moderate to heavy *P. lateralis* infestation, and had been previously evaluated with a preliminary branch inoculation technique. Significant family differences exist among families in the raised bed test, with family means ranging from 0 to 100% mortality (mean = 58%). Several families had higher survival than the resistant controls. Mortality at the field planting was low. A significant correlation (r=0.60) was found between the 1990 branch inoculation scores of parents and mortality of seedling families from those parents. Selections from within the top ranked families will be added to the breeding arboretum and will be ready for breeding within a few years. The raised bed offers several potential advantages over field trials for evaluating resistance.

Introduction

Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) is an important component of many forest ecosystems in southwest Oregon and northwestern California. An introduced pathogen, *Phytophthora lateralis* Tucker & Milbrath, has spread throughout much of the range and has caused substantial mortality of large trees as well as seedlings, especially in riparian areas. Reforestation or restoration of Port-Orford-cedar in many areas will depend upon reducing the site hazard or the production of resistant seedlings. The use of resistant populations of Port-Orford-cedar would be the most efficient method to achieve restoration objectives.

Initial evaluations of Port-Orford-cedar indicated that there was little resistance to *P. lateralis*, but a subsequent study indicated otherwise (Hansen *et al.* 1989). A 1996 test of 345 seedling families from throughout the range of the species confirmed the presence of resistance, but also indicated that the frequency of strong resistance appears to be low (unpublished data).

An operational breeding program for resistance began in 1997 using previously evaluated parents and greatly expanding the number of parents to be screened (Sniezko and Hansen 2000). As part of this program, validation testing is done at several sites for a subset of families to establish the relationship with various greenhouse screening techniques and to determine the durability of resistance in the field. One of the components of a resistance program is the ability to efficiently evaluate trees for resistance. Field trials are expensive, they often involve hit-and-miss infection, the moderate levels of infection needed to clearly discern family difference may take several years or more, and it can be difficult to confirm the cause of mortality unless inspections are frequent. Several potential alternatives to field testing are available for testing Port-Orford-cedar, but refinement of techniques and field validation is needed. A new test method examined here uses inoculated raised beds as surrogates for field sites to test early survival under moderate to high infection levels.

This paper examines early seedling family differences in lesion progress and mortality in a field test and a raised bed test. Also examined is correlation of results from a preliminary branch inoculation test of parent trees with the results of these two trials of seedling families.

Methods

Twenty-nine seedling families from parent trees previously evaluated for *P. lateralis* resistance were used to establish two plantings: (1) a raised bed test at Oregon State University (OSU), and (2) a field test in southern Oregon at Camas Valley (CV) on the Bureau of Land Management's Roseburg District. Seed was sown in March 1998 in 164 cm^3 tubes in a greenhouse at Dorena Tree Improvement Center. In mid to late January

trees were planted in three raised beds at OSU and at the Camas Valley field site. The raised beds at OSU had been inoculated in 1998 for an earlier trial using two *P. lateralis* isolates; the field site had recent tree mortality from *P. lateralis* and had been used for seedling disease testing in 1998.

The 29 families consisted of 15 full-sib families and 14 open-pollinated families, and represented 21 parents (Table 1). Nineteen of the 21 parent trees originated from four USDA Forest Service Ranger Districts in southwest Oregon and northwest California (Fig.1); the other two parents were from a Coos County site (the two CF controls). In several previous tests, three parents (CF1, CF2, and 510015) and their seedling offspring were consistently among the highest for resistance (Sniezko and Hansen 2000; Sniezko *et al.* 2000). Seedling families of these parents are used here as high resistance controls. Three selfs and one reciprocal cross were among the 15 full-sib families (Table 1) A parent was represented in one to five crosses as either a female or male. Twelve of the open-pollinated families were from a containerized breeding orchard at Dorena, while two were from seed collections in the forest. Seven parents represented by full-sib families were also represented by open-pollinated families (Table 1). In an earlier 1989 and 1990 evaluation of 193 parents using a branch inoculation technique there was a wide range in branch lesion length (BLL) among 19 of the parents represented by seedling families here (Table 1). The 193 parents screened in 1989/1990 were among the first phenotypic field selections by the USDA Forest Service and were selected in areas of moderate to high disease hazard. Control crosses were made among a subset of these parents and represent varying resistance backgrounds (as determined by preliminary BLL technique). These were among the first full-sib families tested for resistance.

Both sites were established using a randomized complete block with six replicates. Each replicate consisted of 2 to 4 seedlings per family (an average of 3.3) in a non-contiguous plot. Two replicates were established per raised bed at OSU. Seedlings from three replicates at each site were lifted early in 1999 (March 31st at OSU, April 26th at CV) to examine root lesion development; the remaining three replicates were left to assess seedling mortality. Mortality at OSU was monitored weekly through October 15, 1999; the CV site was assessed twice in 1999 (June and September). For the 15 full-sib families, seedlings from three replicates at each site were top-clipped just prior to planting to established rooted cuttings for potential breeding use.

Test methods

In the 1989 and 1990 branch test, large branches collected from trees selected in natural stands were infected by inserting mycelium in a wound and scoring branch lesion length (BLL) three weeks after inoculation. For the seedling families in 1999, root lesion score (LS) represents an early evaluation of disease progress and was evaluated by removing seedlings from the soil in three of the six replicates and rating them into six classes based upon the percent of length of root with noticeable infection (Table 2). For three replicates at OSU and CV mortality was recorded at time of initial browning of foliage. At OSU, surviving seedlings were lifted and visually checked for root infection; at CV, seedlings were left for future evaluations.

Analyses

Variables included in the analyses of variance included total mortality (percent) through the last assessment in 1999 and root lesion score (LS). The analysis was done by site using plot means for the 29 families (arcsin square root transformation was used for percent mortality). The 18 female parent means from seedling families tested at OSU and CV (means of one to three families per parent) were used in correlations with the 1990 branch lesion length (BLL) from eighteen selections made in the forest. BLL for two of the 18 parents were from a 1989 test, the other 16 were tested in 1990. These 18 parents represented a range of areas in northern California and southwestern Oregon (Fig. 1). The CF1 and CF2 controls were excluded from these correlations (not part of the 1989 and 1990 field selections), and one parent was used only as a male.

Results

Raised Beds - OSU

For lesion score (LS-OSU) there were significant differences among replicates (p=.02), but not among families (p=.12). Family means ranged only from 2.1 to 3.2, and
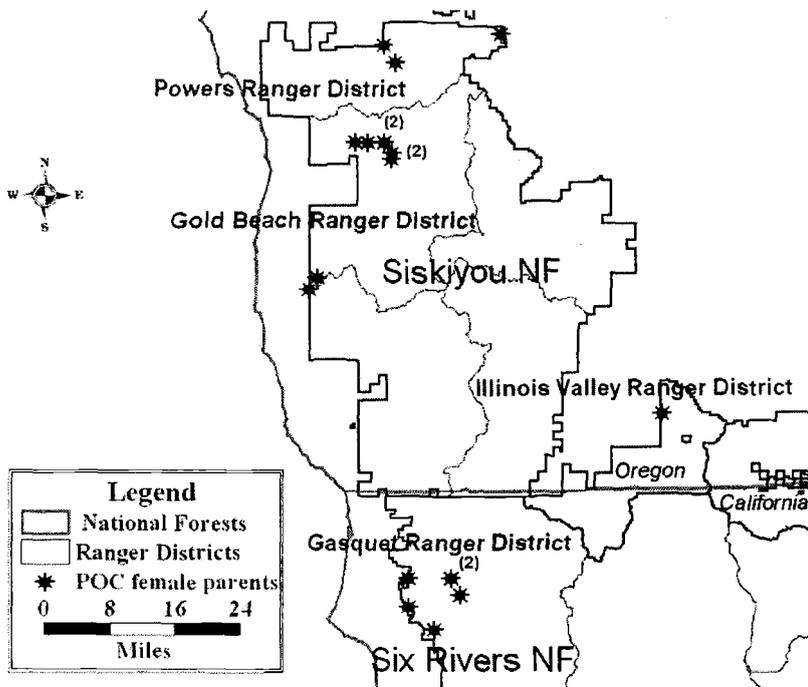
Fig. 1. Locations of 18 of the female parents used in 1989/1990 branch lesion testing and 1999 seedling tests.

replicate means from 2.5 to 2.9. For percentage mortality, differences in family means were highly significant (P=0.01), but replicate means were not significantly different (p=0.08, replicate means ranged from 48% to 66%). The overall mortality in the raised beds was moderate (58.2%) and family mortality varied from 0 to 100% (Table 1), with 9 of the 29 families having less than 50 percent mortality. The 14 open-pollinated families varied from 33.3 to 83.3% mortality; the full-sib families from 0 to 100%. Three of the four families with mortality >80% involved parent 117344; however, this parent was also involved in two crosses that had among the lowest mortality, including one full-sib family with no mortality. Three of the top ranking full-sib families involved parent 117490, including the one full-sib family with no mortality. The four families which included the three resistant control parents (CF1, CF2, and 510015) each had 30.6 to 50.0% mortality, but were not the top performers (Table 1). The family mean correlation between lesion score and mortality at OSU (r=0.29) was non significant (Table 3).

Field Site –Camas Valley (CV)

For lesion score, replicate means were low, ranging only from 0.55 to 1.10, but were significantly different (p=0.04). Families were not significantly different for LS (p=0.64),

ranging from 0.3 to 1.5. For percent mortality, there were highly significant differences among replicates (p<0.01; replicate mortality ranging from 5.5% to 31.0%), but not among families (p=0.12; family mean mortality ranging from 0 to 50%; 15.4% overall mean mortality). The three high resistant parent controls did well for LS and mortality percent, except for parent 510015 (50% mortality), which unexpectedly ranked last for mortality percent in this test (while ranking among the top five families for LS). No other family had >33% mortality at this site, and eight of the 29 families had no mortality.

The correlation of mortality percent and lesion code was significant and negative (Table 3). However, this correlation was principally due to family 510015, one of the high resistance control families, that had unexpectedly poor survival on this site in the three replicates scored for mortality but did well in the three replicates where lesion score was recorded. The correlations among CV and OSU tests were non significant (Table 3).

Correlations with 1990 branch lesion length (BLL)

The correlations of OSU Mortality (r=0.60) and LS (r=0.49) with BLL were significant, while the correlations between CV

Table 1. Percent mortality in 1999 raised bed test at OSU for 29 families by female and male parent, and parental branch lesion length from 1989/1990 testing

Female	BLL[1] (mm)	Male 117334[2]	117344	117490	117499	117502	117503	510008	510044	510049	CF2	OrchOP[3]	WildOP[4]
117335	12.7	58.3	—	—	—	—	—	—	—	—	—	75.0	—
117341	20.4	—	—	—	—	—	—	—	—	—	—	69.4	—
117344	82.4	—	—	—	—	—	—	—	83.3	—	—	66.7	—
117490	13.1	—	0	—	—	—	—	—	—	—	30.6	55.6	—
117499	95.6	—	—	—	91.7	—	—	—	—	—	—	77.8	—
117502	51.2	—	—	—	—	44.4	—	—	—	—	—	—	—
117503	13.8	—	—	—	—	—	52.8	—	—	—	—	55.6	—
117650	17.6	—	—	—	—	—	—	—	—	—	—	77.8	—
118051	102.1	—	—	—	—	—	—	—	—	—	—	83.3	—
118562	37.8	—	—	—	—	—	—	88.9	—	—	—	—	—
118567	21.7	—	—	—	—	—	—	—	—	—	—	33.3	75.0
118573	9.5	—	33.3	—	—	—	—	—	—	66.7	—	33.3	—
510005	20.3	—	—	—	—	—	—	22.2	—	—	—	—	—
510008	34.4	—	—	33.3	—	—	—	—	—	—	—	—	—
510015	19.4	—	—	—	—	—	—	—	—	—	—	—	33.3
510041	50.5	—	80.6	—	—	—	—	—	—	—	—	—	—
510044	79.2	—	100	—	—	—	—	—	—	—	—	—	—
510049	23.0	—	—	66.7	—	—	—	—	—	—	—	—	—
CF1	—	—	—	—	—	—	—	—	—	—	—	50.0	—
CF2	—	—	—	—	—	—	—	—	—	—	—	50.0	—

[1] Branch lesion length (mm) from 1989/1990 testing
[2] BLL for 117334, which was used only as a male parent, was 32.2.mm
[3] orchOP = orchard open –pollinated
[4] wildOP= open-pollinated in the forest

Table 2. Lesion scale used for OSU and Camas Valley 1999 testing

Lesion Score	Percent of root length with infection
0	0
1	1 to 5
2	6 to 33
3	34 to 60
4	61 to 99
5	100 (or infected at root collar)

Table 3. Correlations (and significance levels in parenthesis) among sites and testing types for OSU raised bed and Camas Valley field site and parental scores from 1990 branch lesion length test (BLL).

	CV lesion score	OSU % mortality	OSU lesion score	BLL[1]
CV % mortality	-0.42 (.02)	0.19 (.33)	0.17 (.36)	0.19 (.44)
CV lesion score	1	-0.03 (.88)	0.15 (.42)	-0.37 (.13)
OSU % mortality		1	0.29 (.12)	0.60 (.01)
OSU lesion score			1	0.49 (.04)

[1] 18 parents represented in all three tests (female means for OSU and CV crosses, clonal means for BLL) were used for the correlations in this column. Correlations in other columns are scores from all 29 families
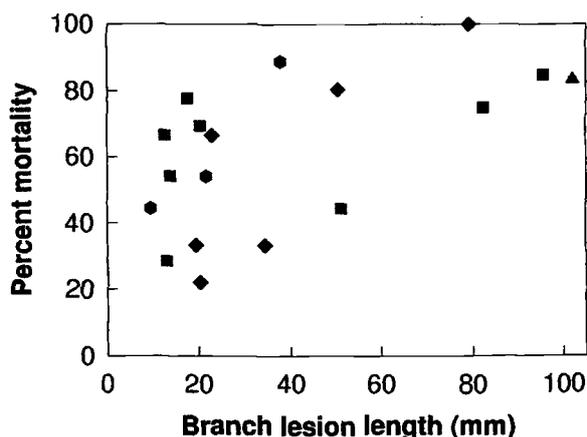
Fig. 2. Branch lesion length of 18 parents vs. percent mortality in raised beds for 18 seedling families (female mean basis). Parent trees are from four ranger districts: ■Gold Beach, ▲Illinois Valley, ●Powers, and ◆Gasquet

CV traits and BLL were non-significant (Table 3). Four of the 18 parents evaluated in 1990 for BLL had much higher means than the other 14 parents; these families also had among the highest mortality at OSU (Fig. 2) as well as some of the highest values for OSU lesion score.

Discussion

One year after planting, the mortality at the Camas Valley site was too low (15.4%) and variable to provide significant family differentiation and future mortality at this site will be monitored. The early assessments at CV and the raised bed using root lesion score also did not provide significant differences among families and offered no clear advantages for general assessment over assessing mortality in the raised beds at OSU. However, there were significant family differences in mortality in the raised beds at OSU in the first year of exposure to *P. lateralis*. In this test no parent was outstanding in all its crosses, and this may be partially influenced by the low number (<13) of seedlings per family. The mean mortality (37.3% dead) of the five families involving parent 117490 was much lower than mean of all 29 families (58.2%). Despite a wide range in family means for mortality at OSU there was only a relatively narrow range in lesion score at OSU and no families had LS<2.1 (not even the one cross that later showed no mortality; this family had the fifth lowest LS). This may be due to the difficult and subjective scoring on root infection and the tendency to primarily to confirm resistance determined from tests as well as for long term validations,

not assign zeroes for any tree (Wendy Sutton, personal communication). These factors would lower the correlation with mortality. At OSU, 28 of the 29 families had at least one surviving seedling. When the roots of the surviving seedlings in the remaining three replicates were examined it was noted that most of the surviving seedlings had little or no observable infection. This raised bed trial was terminated after one year, but subsequent studies are examining whether additional mortality occurs in a second year.

The moderate correlation between the early 1990 branch lesion test and the current raised bed test are encouraging. It appears that this technique may be useful in quick screening of new parents from the field and would eliminate some of the families that would have poorest survival under the raised bed testing (although some of these families could conceivably still be useful for other partial resistance traits).

Test results will vary somewhat by test method and environment (Sniezko and Hansen, this proceedings). Currently several methods, including field plantings, are available for testing resistance. One objective of this trial was to examine whether testing in raised beds would mimic field test results, at least for short-term testing. At the current time, a more definitive answer is pending due to the low infection level in the field. Testing in fairly remote, heterogeneous field sites has many disadvantages and will likely be used and as demonstrations. However, there is good mortality and family differentiation in the

206

raised beds and this may represent a moderately high hazard site. Several other trials currently under analyses will look further at this relationship.

Genetic variation in resistance to *P. lateralis* is evident, but the mechanisms of resistance and their inheritance are currently unknown and under investigation. More rigorous examination of testing methods began in 2000 and analyses of the results are underway. In addition to the raised bed and field tests, a greenhouse (GH) test involving root dip inoculation was included. The GH method examines resistance where the pathogen spreads from the root tip toward the root collar from a one time inoculation; the raised bed may mimic some higher hazard field conditions where inoculum is present throughout the root zone and infection with *P. lateralis* may occur at varying points in the root system, including near the root collar

Only one family at OSU (the highest mortality site) had 100% mortality. Rooted cuttings of surviving seedlings from each of the full-sib families have been placed in the breeding arboretum for future evaluation.

In a 1996 greenhouse test of randomly selected trees from throughout the range of Port-Orford-cedar, many families had complete or nearly complete mortality (unpublished data) and there was only a very low correlation between root dip and stem dip inoculation techniques. Results as reported here from phenotypic selection in areas of moderate to high disease hazard show a moderate positive correlation between a quick branch inoculation technique used to evaluate phenotypic selections from the forest and seedling mortality in a raised bed test. A modified branch inoculation technique has been used in the first phase of the operational disease screening that started in 1997, with over 10,000 selections from natural stands screened to date (see Bower *et al.* 2000 for some details).

It is very encouraging to see the widespread differences among families in mortality in the raised bed test. Some families show excellent survival with this new screening method and more comprehensive tests already in progress should help better evaluate the potential of parents such as 117490 (which generally did better than existing high resistance controls). Some of the tests are also examining mortality rate (there was a 76 day range in days-to-mortality among the 28 family means in this trial (unpublished data)), which may be a useful type of partial resistance, especially coupled with further breeding. Rooted cuttings of many of the surviving seedlings are available and will be included in the breeding arboretum or used for further testing.

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Progress in selection and production of jarrah (*Eucalyptus marginata*) resistant to *Phytophthora cinnamomi* for use in rehabilitation plantings

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Abstract. Resistance to *Phytophthora cinnamomi* in jarrah (*Eucalyptus marginata*) is under strong genetic control. It has high heritability, is probably polygenic, and is durable in field trials. Seedlings from healthy mother trees, either growing on long-term dieback sites or from a provenance collection made across the range of the species, were grown in the glasshouse and resistant seedlings selected from inoculation trials. These were micropropagated by tissue culture. The resulting clonal lines were planted in field validation trials on dieback-affected sites and soil at the base of the plants was also inoculated with *P.cinnamomi* to test survival and growth of resistant plants. In spite of some drought deaths, survival of most resistant lines has been high. Some 45 unrelated resistant lines have been selected. However, due to high costs of production and establishment problems in forest sites, it is not feasible to use the clonal jarrah directly in large-scale operational plantings. Clonal seed orchards are now being planted to supply seed of resistant jarrah for use in the rehabilitation of dieback-affected forest and plantings on cleared land.

Introduction

Phytophthora dieback (jarrah dieback), caused by *Phytophthora cinnamomi* Rands (Podger 1972), is the most serious and widespread disease of the Western Australian jarrah (*Eucalyptus marginata* Donn ex Sm.). In this paper we review the work that showed that resistance to *P. cinnamomi* in jarrah is under strong genetic control (Stukely and Crane 1994). We then describe the screening program undertaken to select a large number of genetically unrelated, but resistant, lines of jarrah, the field validation trials, and the establishment and use of seed orchards. The overall strategy followed in the program is outlined in Fig. 1. This project began in 1984 in parallel with a selection program for *Pinus radiata* D.Don with resistance to *P.cinnamomi* (Butcher *et al.* 1984).

Demonstration of resistance

Seed was collected from jarrah trees on a variety of sites, some of which were infested with *P.cinnamomi.* Seedling "families" (half sibs) derived from each of sixteen mother trees were grown and subjected to inoculation with *P.cinnamomi* in glasshouse and field trials (Stukely and Crane 1994).

Glasshouse trials

In the glasshouse, plants were either stem-inoculated or the soil was inoculated with plugs of infected wood.

Soil inoculation trials involved the introduction of four isolates of *P.cinnamomi* into the potting mix by way of infected plugs cut from branches of *Pinus radiata* (Butcher *et al.* 1984). Isolates were all of the A2 mating type and had been shown to be pathogenic to jarrah seedlings in a pilot trial: 251N12 (isolated from
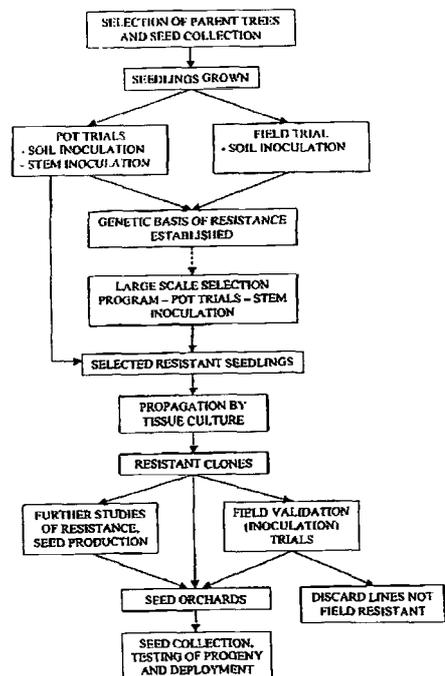

Fig. 1. Strategy followed in the selection, testing and deployment of dieback resistant jarrah.

from *Pinus radiata*), Sc 381 (*Allocasuarina fraseriana* (Miq.) L. Johnson), DCE 210 (*E.marginata*) and 480R1 (*Banksia* sp.). These trials were conducted over a full summer-autumn period, with low seedling mortality taken to indicate family resistance.

Stem inoculation trials involved the insertion of *P.cinnamomi* inoculum (isolate Sc 72, IMI 264384) into a surface incision on each seedling stem. The resulting lesions were measured over 14 days, with short lesions indicating seedling resistance.

Conditions in the glasshouse trials were highly favorable to the pathogen, and also to seedling growth. The trials were carried out in summer; soil was kept moist, and cooling on hotter days regulated the ambient temperature to a maximum of about 32C.

Field trial
A field trial was conducted on a cleared forest site already infested with *P.cinnamomi*, and four additional isolates of the fungus (as listed above) were introduced into the soil around each seedling by way of infected pine branch plugs. Low mortality was again taken to indicate family resistance.

There was a large degree of variation in response between the jarrah families in all trials, ranging from highly resistant to highly susceptible. Resistant plants were not immune to infection, but were able to restrict the growth of the pathogen sufficiently to survive and grow well. The performance of families, particularly those at the resistant and susceptible ends of the range, was consistent under the different treatment regimes and between the glasshouse and field trials.

The resistance of jarrah to *P.cinnamomi* has high heritability (0.43 at individual seedling level; 0.74 – 0.85 at family level). The wide range of responses shown among families indicates that the resistance is probably polygenic. Furthermore, it is effective against a combination of *P.cinnamomi* isolates, and is durable in field trials (Stukely and Crane 1994).

Selection program
The stem inoculation of seedlings described above was adopted as the standard screening method to compare the resistance of seedling families derived from individual mother trees in the forest. A standard susceptible family (717) was included in the trials for comparison. Trials were carried out during the summer-autumn.

Seedlings were at least 250mm in height at inoculation. The method became less reliable on plants older than about one year, as reddish-brown surface pigmentation and bark then developed. At this stage the pathogen often preferentially invaded internal tissues so its progress was not visible as a continuous surface lesion (Stukely and Crane 1994).

Seed-lots from a jarrah provenance collection (R. Mazanec, Department of Conservation and Land Management, pers. com.), which covered forest areas affected by and also free of dieback, in both the Northern and Southern jarrah forest regions, were first tested. Between 17% and 56% of these families exhibited some resistance (Fig. 2). More recently, healthy mother trees growing on high-impact dieback sites, mostly located in the Northern jarrah forest and where the disease was known to have been present for over c.25 years, have been sought. Tests of seedling families derived from these trees have shown that they are indeed more likely to have higher levels of resistance (68-84% of families were resistant) (Fig. 2).

An additional level of stringency was applied to the test by maintaining the selected seedlings for at least two months after the fourteen-day measurement period, and discarding any that showed further lesion extension or girdling of the stem. The families with the highest resistance response (ie having the shortest mean lesions) were identified, and the outstanding seedlings from these families selected for clonal propagation.

Propagation of selected genotypes
The infected portion of the stem of each selected seedling was removed when measurements were completed, so that the seedlings could then be propagated and maintained free of the pathogen.

Micropropagation techniques for jarrah were developed by Bennett (McComb *et al.* 1996). These methods were applied to the seedlings showing the highest levels of resistance, and for comparative purposes, to some seedlings that were susceptible. Some 60 lines of jarrah (45 of which are unrelated) with resistance to *P.cinnamomi* have been selected and successfully propagated.
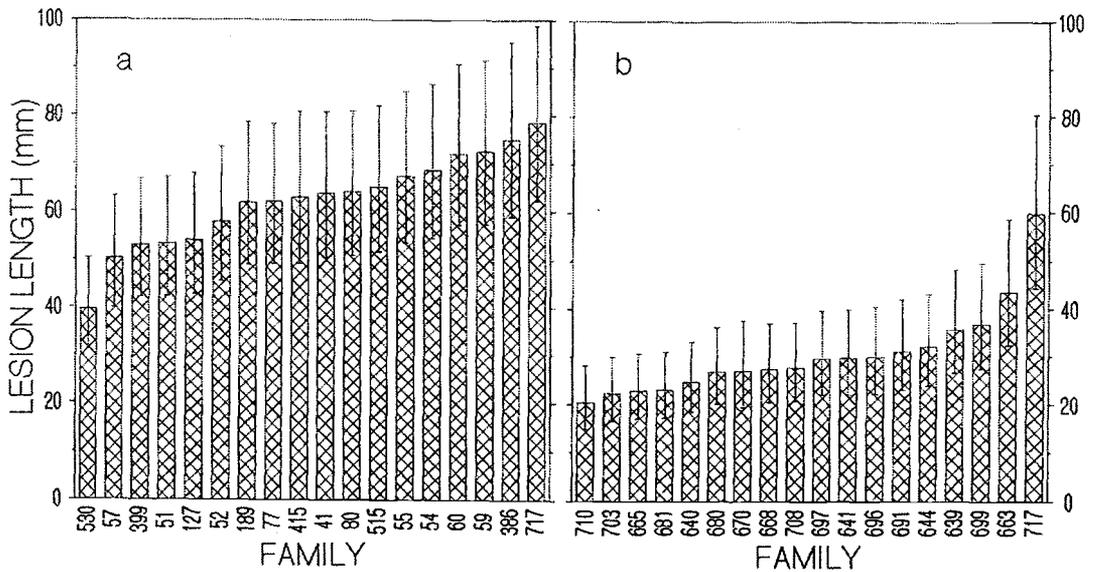
Fig. 2. Mean stem lesion lengths, 14 days after inoculation with *P.cinnamomi*, on seedlings of jarrah families grown under glasshouse conditions. Parent trees were (a) from a provenance collection; (b) survivors on old dieback sites. The standard susceptible family is 717. Bars indicate 95% confidence limits.
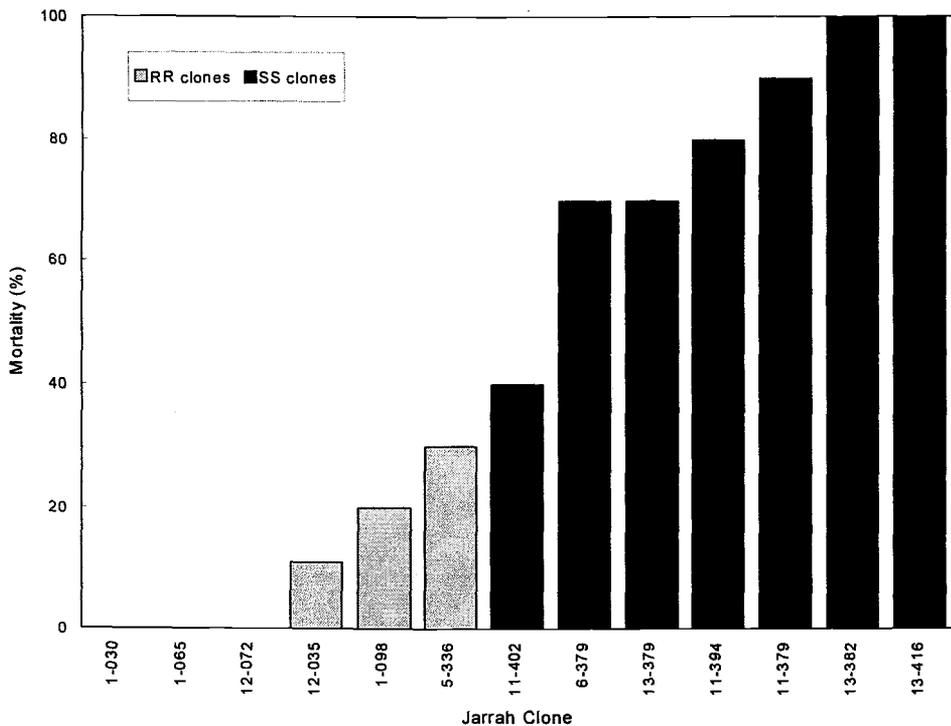

Fig. 3. Mortality of 13-year-old jarrah clones, propagated from seedlings selected as resistant (RR) or susceptible (SS) to *P.cinnamomi*, in a field validation trial.

Field testing of selected genotypes

Tissue cultured clones of resistant seedlings were planted in field validation trials on dieback-affected sites, and soil at the base of the plants was also inoculated with four isolates of *P.cinnamomi* (listed above) to test survival and growth. Susceptible clones were included for comparison. Seventy-four clones were grown in 10 trials. Data for the oldest validation trial are given in Fig. 3.

Survival of most resistant lines has been high (Fig. 3) and they have shown excellent growth, while susceptible lines have had high mortality. Drought deaths in the first summer have been a problem in some trials. These

210

validation trials are an essential component of the selection process, complementing the glasshouse selections and giving a further opportunity for the removal of any inferior lines from the program.

Deployment of dieback resistant jarrah

There is wide variation in the rooting ability of jarrah clones in tissue culture, with successful root production ranging from 0% to c.80% of shoots placed in culture. The performance of individual clones is consistent, and those with poor root production *in vitro* show poor survival on transfer to soil. Such characteristics add significantly to the cost of production of these clones, which are also likely to suffer high mortality after transplanting to the field due to restricted root development.

Due to generally high costs of production of the clones, and establishment problems in some forest sites, it is not feasible to use the clonal jarrah directly in large-scale operational plantings. However, some small-scale plantings have been established in operational forest rehabilitation areas.

Clonal seed orchards are now being planted to supply seed of resistant jarrah for rehabilitation plantings in dieback-affected forest and on cleared land. To maintain genetic diversity, at least 30 unrelated jarrah clones will be included in each production orchard. The orchards will be culled and upgraded as necessary to ensure that only the most resistant available jarrah lines are used. It is expected that due to the high heritability of the resistance to *P.cinnamomi*, the resulting seed crops will carry high levels of resistance and this will be tested in ongoing trials. Methods of maximising seed production in jarrah seed orchards are currently being investigated (M. Wheeler, Murdoch University, pers. com.).

In the forest, small plots of resistant jarrah trees will be established in dieback areas, or those under potential threat; it is hoped that these will cross-pollinate with surviving trees to produce a new generation of resistant seedlings. A forest rehabilitation program using dieback resistant jarrah will enable jarrah to be re-established on sites severely impacted by *Phytophthora* dieback. Dieback resistant jarrah will become an important element in the integrated management of the dieback problem in the jarrah forest. In addition, jarrah is now being included in trials on groundwater recharge sites on agricultural land where increasing soil salinity is a long-term problem. There is some potential for the use of jarrah to lower water tables on appropriate sites in the western part of the Western Australian wheatbelt.

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Management of *Phytophthora* in Natural Ecosystems and Use of Phosphite
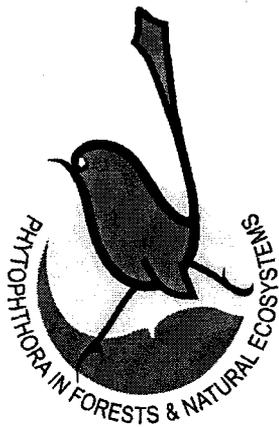
Phytophthora – a plant quarantine disease risk for Northern Territory forest and savanna ecosystems

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Abstract. The horticultural industry has developed rapidly in northern Australia, with centres of expansion focussed on Kununurra, Darwin and Katherine. Rootstock, irrigation management, variety evaluations, seedling assessment and selections are principal areas of research and development. During 1990/91, a survey was conducted of Northern Territory nurseries to determine the incidence of root and collar rots. Four *Phytophthora* species, including *P. cinnamomi* were recovered. Additionally, 13 *Pythium* species were detected. This paper provides a historical review of the occurrence, impacts and distribution of *Phytophthora* species in the Northern Territory. With the prospect of horticulture expanding into remote areas of the Northern Territory, there exists the possibility for the future accidental introduction of *Phytophthora* inoculum in potted plants, and in clods of dirt adhering to agricultural and earthmoving equipment.

Introduction

The horticultural industry has developed rapidly in northern Australia, with centers of expansion focussed on Kununurra, Darwin and Katherine. With the prospect of horticulture expanding into remote areas of the Northern Territory (NT), there exists a risk for the accidental transfer of exotic soilborne root-rot and vascular-wilt diseases. To date, very little of the 'Top End's' native bush has been cleared (i.e. <1%). This has resulted in the bush being kept free of several potentially important soilborne root pathogens. Standard plant quarantine procedures have achieved the level of protection required by the Australian horticultural industry. However, there exists the possibility for the future accidental introduction of *Phytophthora*. The movement of secondhand machinery and earthmoving equipment also presents the opportunity for *Phytophthora* to be transferred in clods of adhering soil.

This paper provides a review of the investigations into the occurrence and impact of *Phytophthora* species in the NT. The paper describes studies in both horticultural and native bush situations in Darwin and also in the more remote part so of the NT, including Gove Peninsula.

The occurrence of *Phytophthora* species in the Northern Territory – 1980's

In 1980 a survey was conducted in the Northern Territory north of the 13° S latitude to establish the occurrence of *Phytophthora* species in the area (Blowes and Pitkethley

1981). Soil and root samples were collected in and around nurseries, avocado orchards and native bush.

P. nicotianae was recovered from diseased citrus trees that displayed root and collar rot symptoms, and from potted avocado seedlings exhibiting foliar dieback (Table 1).

P. cinnamomi was isolated from the roots of avocado trees growing in three orchards. All except one tree were part of a consignment of grafted avocado stock, potted in soil, which was imported from central Queensland. All isolates of *P. cinnamomi* were of the A2 mating type. *P. palmivora* was also isolated from the roots of one avocado tree.

An unidentified *Phytophthora* sp. was isolated from the roots of three avocado trees that showed no obvious symptoms of disease. Additionally two isolates of the unknown *Phytophthora* sp. were collected from soil and fine root samples collected from sites supporting native bush. Termed "Morphodeme NT2" by Shepherd & Garrettson-Cornell in 1983 (Table 2), all of these unknown isolates formed oospores when grown with an A2 strain of *P. cambivora* and two isolates did the same when grown with *P. cinnamomi* (A2). The same isolates were described by Dr D.J. Stamps (C.M.I.) as nearest to *P. drechsleri* – based on the sporangia and high growth temperature. The identity of "NT2" is at present undetermined,

Table 1. Results of 1980 *Phytophthora* survey

Plant samples	No. of trees sampled	Method of isolation	*Phytophthora* spp. retrieved	Mating type
Nurseries	60	Lupin baits	-	-
Diseased samples	43	Lupin baits and direct root plates	*P. nicotianae*	A1 (5 isolates) A2 (2 isolates)
Avocado (9 orchards)	86	Lupin baits and direct root plates	*Phytophthora* sp. *P. nicotianae* *P. cinnamomi* *P. palmivora*	A1 A1 A2 A2
Native vegetation	45 sites	Lupin baits	*Phytophthora* sp.	A1

Table 2. Diagnostic features of *Phytophthora* spp. recovered

Feature	*P. cinnamomi*	*P. nicotianae*	NT2
Culture Morphology	Rose, Irregular, diffuse boundary	Chrysanthemum, fluffy boundary	Rose, definite regular boundary
Growth Rate (mm/day on CMA)	14.1	7.3	16.8
Hyphal Characters	Corraloid	Torulose	Corraloid with hyphal aggregations
Chlamydospores	Abundant in bunch-like clusters	None	None
Sprorangia	Ovoid-ellipsoid	Ovoid-spherical	Globose-obpyriform
Oogonia	A_2 strain only recovered	A_1 and A_2 recovered, oogonia 20-25 μm	A_1 strain only recovered

and no isolates of the fungus are currently available for further isozyme or DNA analysis.

Phytophthora-mediated dieback and death of *Eucalyptus tetradonta* in native forest of Nhulunbuy?

The township of Nhulunbuy is situated on the northeastern extremity of the Northern Territory Arnhem Land Reserve. The town is surrounded by the stringy bark *Eucalyptus tetradonta*. The understorey includes species of *Grevillea*, *Acacia*, grasses and sedges. Other eucalytps present are *E. miniata*, *E. polycarpa*, and *E. foelscheana*.

In July 1981 crown dieback symptoms were observed in all stands of *E. tetradonta* near the town. Symptoms ranged from areas with dieback in branches, to forests with groups of dead crowns, and zones containing only dead trees. The only primary symptoms observed were wilting and death of the fine roots, and the production of adventitious roots from the base of the main trunk (Weste 1983).

Samples consisted of soil containing fine roots taken from trees and understorey shrubs with early dieback symptoms and always adjacent to healthy sites. From the fungi isolated (Table 3) it was concluded that, "the deaths and dieback observed in forests of *E. tetradonta* were therefore almost certainly due to *P. cinnamomi*, either acting alone or in conjunction with environmental stress due to soil infertility and alternate periods of water saturation and water stress" (Weste 1983). The disease is apparently progressed from a fine root pathogen to a root and collar rot. It was also suggested that, 'the pathogen has probably been present in the soil for some 10 years for symptoms in trees to have become so advanced and widespread".

As introduced in the previous section, conjecture remains around the identification of the causal agent. In a recent personal communication from Gretna Weste, she propounded that, "*P. cinnamomi* was only retrieved from horticultural potted plants from the township, and the introductions can be traced to a single shipment of plants potted in soil from central Queensland". Various workers have carried out numerous follow-up surveys, re-sampling the original "diseased" sites. In July 1988, a comprehensive survey of soils and vegetation of the Gove Peninsula (including previously "diseased"

Table 3. Nhulunbuy *Phytophthora* survey

13 sites were sampled and 2 trees excavated

Fungi isolated	No. of sites	No. of plates from trees
P. cinnamomi	9	All
P. cinnamomi and *Pythium* sp.	2	2
Alternaria sp.	1	

NB. Taxonomic identification of *P. cinnamomi* carried out Dr D.J. Stamps – C.M.I. (*G. Weste, pers. comm.*).

sites) failed to retrieve a single *Phytophthora* isolate (P&WCNT 1988). Additionally, in 1992 J. Duff examined six soil samples for the presence of *Pythium* and/or *Phytophthora* species from trees showing foliar dieback symptoms from the native bush of Gove Peninsula. Two fungal organisms were subsequently identified from infected lupin roots: *Pythium myriotylum* and *Phytophthora drechsleri*.

At this stage, the foliar dieback symptoms are not as obvious as first reported. This does not discount the possibility of a cryptic or recalcitrant, soilborne pathogen being pathogenically active at a very low rate. Throughout the historical investigations, no mention has been made of the possible role of defoliating insects and/or foliar pathogens. This "gap in information" may go some way to explaining the significance of this forest-tree issue.

The incidence of *Phytophthora* and *Pythium* species in Northern Territory nurseries – 1990's

During the 1990's two surveys were conducted of Northern Territory nurseries to determine the incidence of *Phytophthora* and *Pythium* species (Duff 1992; Weinert, pers. comm.). Using cotyledon bait tissue, Duff (1992), recovered four *Phytophthora* species including *P. cinnamomi* from Katherine and Darwin nurseries. Additionally, thirteen *Pythium* species were detected; these included an extremely rare species, *Pythium pachycaule* (Table 4). In a similar way, Mathew Weinert (pers. comm.) examined soil samples from nurseries and durian farms in the Darwin area in April 1998 (Table 5). A total of 18 *Phytophthora* isolates were recovered. All isolates were lupin baited from soil samples. Sixteen of the isolates were *P.nicotiana*.

Table 4. Sample and results from 1992 horticultural survey of the Northern territory

Host/substrate	Darwin Region	Katherine Region	Alice Springs Region
Compost heap	*P. cinnamomi,* *P. nicotianae*	*P. cinnamomi*	*Pythium* spp.
Soil/potting mix	*Pythium* spp.	*Pythium* spp.	*Pythium* spp.
Potting media from native shrubs	*Pythium* spp.	*P. nicotianae*	*Pythium* sp.
Potting media from exotic shrubs	*P. cinnamomi,* *P. drechsleri,* *P. nicotianae,* *P. palmivora*	*P. cinnamomi,* *P. nicotianae*	*Pythium sp.*

Table 5. Sample and results from 1998 horticultural survey

Location	Host	UQ Accession	Species
Instant Palms	*Alstonia actinophylla*	4559	*P. palmivora*
Tiwi Gardens	*Hibiscus* sp.	4564	*P. nicotianae*
Various	*Durio zibethinus*	4567-4598	*P. palmivora*

The threat to the Top End's native bush

It has been proposed that "*P. cinnamomi* entered northern Australia with Indo-Malasian floristic elements during Pleistocene-Holocene times, from a centre of origin in the New Guinea/Celebes region" (Shepherd 1975). This theory predicts that the long period of association with *P. cinnamomi*, would have lead to the selection of a predominantly *P.cinnamomi* resistant vegetative assemblage where the disease potential was frequently high. The susceptibility of the dominant and understorey species of native vegetation of the Northern Territory to *P. cinnamomi* infection is not known (Blowes and Pitkethley 1981). Thus

conjecture still exists as to whether *P. cinnamomi* is indigenous to northern Australia or has been recently introduced. Bellgard (2001) expounded the recent introduction hypothesis, and suggested that *P. cinnamomi* could act as a non-specific, primary pathogen if accidentally introduced into water-logged situations with "stressed" plant hosts pre-disposed to infection.

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Addressing Sudden Oak Death in California

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Abstract. Sudden Oak Death, caused by *Phytophthora ramorum*, has killed tens of thousands of oak (primarily *Quercus agrifolia* and *Quercus kellogii*) and tanoak (*Lithocarpus densiflorus*) trees in central coastal California. The dead and dying trees are scattered throughout mixed ownerships in an area where approximately 8 million people live. This complicates management of the disease and necessitates the cooperation of hundreds of organizations, involving thousands of people. To coordinate the response to Sudden Oak Death, the California Oak Mortality Task Force was formed. The task force developed state and federal regulations for the pathogen, as well as programs for monitoring, management, research, education, funding, fire prevention and wood utilization.

Introduction

This paper explains how Sudden Oak Death is being addressed in California. Sudden Oak Death erupted in several heavily populated communities of well-educated, environmentally concerned, outdoor-oriented citizens many of whom are politically well connected. It killed off trees surrounding the homes and properties of influential upper class society. Much of how California responded was driven by their fear and outrage at the rapid collapse of their beloved oak forests. Sudden Oak Death became a media darling and a phenomenon – because its causal agent was new to science, because it caused spectacular mortality in well-heeled urban areas, and because it attacked oak trees that were already in the center of an environmental debate prior to Sudden Oak Death's arrival. This paper focuses not on the science of Sudden Oak Death, but on the social and political aspects of the disease and how that influenced California forestry officials' response to it.

Why was Sudden Oak Death ignored from its "discovery" in 1995 to 1999?

Sudden Oak Death was first reported on tanoak (*Lithocarpus densiflorus*) in 1995 in Marin County (Garbelotto *et al.* 2000). It was generally considered a curiosity, since the trees were exhibiting symptoms (bleeding, wilting, cankers) that did not match any previously known agent, but mimicked bacterial or fungal infection. The forestry community was not concerned since tanoak is considered a weed species and aggressively treated with herbicides throughout Northwest California timberlands. Beetles were attracted to many of the trees so entomologists believed they were the primary agent. Not much was done about the problem which at that time was appearing as widely scattered patches of dead and bleeding tanoaks primarily along the flanks of Mt Tamalpais on watershed and park lands. Dead trees were also popping up along residential neighborhoods that bordered these wildlands and the Marin County Fire Department was removing many of them to widen roads and reduce fire risk. Sudden Oak Death was also present in Santa Cruz County (50 miles south) and in Big Sur (75 miles south of Santa Cruz).

Public outrage and the discovery of *Phytophthora ramorum*

By 1998 there was a tremendous increase in the numbers of dead and bleeding trees and coast live oak (*Quercus agrifolia*) started dying in large numbers. Coast live oak is an evergreen oak with a natural bonsai growth form, a symbol of the natural beauty of California and when it was recognized as dying-off, the public was alarmed and outraged that more was not being done about the problem. Due to the variation in symptom expression, at the time we did not know for sure if the coast live oak and tanoak, and large and small trees were dying due to the same casual agent. By this point so many trees had died that many attributed it to drought or general oak decline, rather than a pathogen (Hagen 1999).

Coastal California experienced several "El Nino" years from 1995 - 1999 with heavy rains that extended into late spring (June). It is still speculation, but we believe that these sustained rainy periods under moderate temperatures allowed the pathogen to spread rapidly and infect tens of thousands of trees in a relatively short period of time.

Concentrations of mortality continued to develop in Muir Woods National Monument and the Marin Municipal Water District lands both in Marin Co. USDA Forest Service, State and Private Forestry, Forest Health Protection applied for emergency money to determine the cause of the problem and what should be done about it. In 1999 there were different perspectives between regional and national forestry professionals. The proposal was ranked very low by the Pacific Southwest Region due to the pressing need for emergency funds to help manage Port-Orford Cedar root disease, white pine blister rust on sugar pine and other forest insects and pathogens. The Washington Office of the USDA Forest Service, was concerned about the threat to the rest of the United States and sensitive to the political upheaval generated by Sudden Oak Death and provided funds. We hired David Rizzo, UC-Davis to investigate the problem. Everett Hansen, Oregon State University was visiting California on vacation and agreed to view the situation with Rizzo and myself. In June 2000 in over 100 degree F. heat we examined several tanoaks and oaks and on his first try Rizzo isolated the new *Phytophthora*, named one year later as *P. ramorum*.

The media reaction to Sudden Oak Death and the new *Phytophthora* was overwhelming. Sudden Oak Death made the front page of the Sunday New York Times, USA Today, the Los Angeles Times, the Chicago Tribune and was carried on several network TV stations. Driving home from work I would learn breaking events from news segments on the radio, such as, hearing Mary Nichols, California Resources Secretary say California was going to eradicate Sudden Oak Death and our new California Oak Morality Task Force was going to do it!

The California Board of Forestry and Fire Protection (that sets forest policy for the California Department of Forestry and Fire Protection) became very interested in Sudden Oak Death and identified it as one of its key issues for 2000. The issue of limiting cutting of oaks on private lands has been raging for over 20 years in California, environmentalists want some protection for oak lands that are being converted into housing tracts and shopping centers for California's growing population and for vineyards for the lucrative wine market. The Board positioned Sudden Oak Death

prominently, partly because here was an oak issue they could redeem themselves on – they could demonstrate their concern about oaks by getting involved with Sudden Oak Death. They invited the USDA Forest Service, Pacific Southwest Regional Forester to speak on addressing Sudden Oak Death, contributing to the Region's making Sudden Oak Death a high profile issue, along with fire prevention and a management plan for the Sierra Nevada forests.

Organizing forestry and agricultural professionals to respond to Sudden Oak Death

In July 2000, we were relieved and excited that we had found the underlying cause of Sudden Oak Death and did not anticipate that it would take more than 6 months to convince the scientific/forestry community and the public that *P. ramorum* was the cause of this tree die-off. Politicians, high level forestry officials and others were not familiar with Phytophthora. The poorly informed press coverage was heavy, as was the amount of frass on the trees, and it was a hard sell to convince people that the primary cause was a pathogen not insects. People were not familiar with Dave Rizzo so that added to their skepticism.

In August 2000, shortly after the *Phytophthora* was recovered the California Department of Forestry and Fire Protection (CDF) and the California Forest Pest Council (CFPC) (a non-profit group that advises the CA State Board of Forestry on forest health matters) both proposed forming an interagency working group to address Sudden Oak Death for the state. To prevent a duplication of effort the groups combined to form the California Oak Mortality Task Force. The task force purpose is to bring together public agencies, non-profit organizations and private interests to address Sudden Oak Death. The goal is to minimize the impacts of Sudden Oak Death on oak forests.

Objectives include:
Assist communities threatened by Sudden Oak Death to maintain a safe and healthy environment;
Develop and maintain an adaptive integrated pest management program for Sudden Oak Death;
Provide information and educational materials;
Identify sources of funding, staffing and other needed resources.

Much to our surprise nearly 200 people showed up at our first general task force meeting.

The interest in Sudden Oak Death has been keen and the California Oak Mortality Task Force has grown to over 65 agencies and more than 800 people. Accomplishing projects quickly in this interagency structure, with a diverse range of opinions continues to be challenging. But we have achieved some of our objectives through our website: www.suddenoakdeath.org, monthly updates, and GIS database.

Our overall strategy is based on research to learn more about the disease that will lead to methods to slow its spread; education so professionals and the public understand what is occurring in the forest; monitoring to understand the impact and distribution of Sudden Oak Death and *P. ramorum*; and regulations to limit human-caused movement of the pathogen. Management activities are being designed and considered for the future: silvicultural controls and resistance programs but more research is needed to understand the oak forests and the epidemiology of the pathogen before they can really be started. Matteo Garbelotto and others are evaluating chemical treatments, primarily for landscape trees. Our approach does not include pathogen eradication except if *P. ramorum* were to appear in a very isolated new area.

The California Department of Food and Agriculture imposed a regulation for *P. ramorum* in May 2001. The regulation prohibits the movement of all known hosts beyond the regulated area – comprised of all the known infested counties. USDA in February 2001 also pledged to impose a parallel domestic and international quarantine for *P. ramorum*. Of particular concern is importation of rhododendron from known infested areas in Europe and the legality of shipping known hosts to states outside of California. Oregon has quarantine against *P. ramorum*, as does Canada and South Korea. USDA Animal Plant Health Inspection Service (APHIS) moved Sudden Oak Death out of their emergency operations in April 2001 since we are not recommending eradication in CA. As of September 2001, APHIS officials continue to assure us that the regulations are pending (Dan Hammon pers. comm.).

Funding: how much is too much?
Much of what the task force was created to do was propel Sudden Oak Death from a local issue to a statewide issue. Marin County in 2000, asked California Governor Gray Davis to declare Marin County a disaster area due to Sudden Oak Death and requested millions of relief dollars. Their request was turned down and indeed the total funding for Sudden Oak Death through October 2000 totaled less than $300,000. As of September 2001, $9 million has been allocated for Sudden Oak Death, over $4 million from the USDA primarily for research, $3.6 million from the State and $1 million from a private foundation.

We have spent many evenings contemplating what is the appropriate response by the USDA and California to Sudden Oak Death and *P. ramorum*. To the $9 million of current funding, an additional $5 million primarily for research has just being proposed by USDA Forest Service Research. The $70 million Boxer Sudden Oak Death bill is still lurking in the US Senate and House. Meanwhile many of the most severely impacted areas of Sudden Oak Death appear to be progressing on the decreasing side of the bell curve that can be drawn to express the development of all forest diseases. New areas are popping up, Southern Oregon and Mendocino County most importantly since both are areas have large acreages in highly susceptible habitat with favorable climates for the pathogen. We expect that Sudden Oak Death will be episodic, in years with heavy, prolonged rainy periods in the spring the disease will flare-up and in dry years it will slowly spread or maintain itself.

I have hosted many high-level forestry officials on tours of Sudden Oak Death this summer. Most pathologists can't believe the number of trees that Sudden Oak Death has killed. They are impressed with its ability to kill so much of the bark so rapidly, and at how many trees it infests in an area. However, general forest mangers are less impressed. Due to the media hype, they expect to see a moonscape, instead they view patchy areas of mostly previously killed (now gray skeleton trees) that do not stand out very well when you view densely wooded stands from a distance. They caution me that if pathologists cry-wolf with Sudden Oak Death, if our poster-child exotic pest fades out and is not very damaging 5 years from now, that our concerns will be ignored in the future.

What we have learned over the past 15 years dealing with forest pathogens and insects is: society is willing to tolerate very high rates of tree mortality and not care about it. Pitch

220

canker, caused by *Fusarium circinatum* is a good example of this attitude. Pathologists have documented that in native and non-native Monterey pine (*Pinus radiata*) stands in urban and forested areas approximately 1/3 of the trees will die. Forest managers and urban foresters for the most part, accept this mortality. There are thousands of dead trees, particularly evident along highways, California's highway department (CALTRANS) removes the trees that are unsightly or dangerous and mostly no one even seems to notice.

In coastal California, in 5 – 10 years the community and forest will adapt to *P. ramorum* and be used to living with it. Millions of tanoaks under majestic redwoods will die but new species will grow in and the redwoods – which people cherish will endure. Mixed-evergreen forests will recover, one-third of the coast live oaks are expected to die but the remaining 2/3 will survive and other species will be favored and grow in. Hopefully, and with some management, exotic weeds will be kept at low levels and the forests will change but remain intact.

The down sound of my optimistic viewpoint of how Sudden Oak Death will play out in coastal California is that funding for research and management of the pathogen will fade away as will interest and concern over it. We believe that a pathogen capable of killing 1/3 of individuals of a tree species deserves continued high level funding and concern, but I am a pathologist, not the head of a forestry agency consumed with unprecedented wildfires, inadequate budgets to maintain forest resource staffing, numerous lawsuits over environmental protection, etc.

What will *P. ramorum* do in new areas? Where will it spread? How will it impact those areas? Those questions are hard to answer. Because of these unknowns adequate levels of research funding for *P. ramorum* are needed to determine its host range, develop easy to use diagnostic tools, etc. There are immediate local needs as well, communities need assistance with training materials, guidelines and best management practices for handling plant debris, removing hazard trees and science-based answers to the questions and concerns of professionals, landowners and homeowners impacted by Sudden Oak Death.

Trying to address a new pathogen impacting a major metropolitan area in California presents many challenges. We continue to move forward in an atmosphere of ambiguity, unknowns, and a shifting political and physical environment.

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Monitoring effectiveness of roadside sanitation treatments to decrease likelihood of spread of *Phytophthora lateralis* in Southwest Oregon, USA

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Abstract. Phytophthora lateralis is an introduced pathogen that causes a severe root disease of Port-Orford-cedar (*Chamaecyparus lawsoniana*) in Southwest Oregon and Northwest California USA. A number of management techniques are employed to reduce the probability of spread of *P. lateralis* on federally managed forests. One of these, the roadside sanitation treatment, involves killing all Port-Orford-cedar in buffer strips 8m wide on either side of selected roads. The objective of the treatment is to either reduce likelihood of vehicles picking up infested soil along roadsides where *P. lateralis* infection already occurs or to remove hosts in the zone of greatest vulnerability to infection in areas where the pathogen has not yet been established. Although roadside sanitation has been widely used, it is controversial with some sectors of the public, and its effectiveness has not been evaluated. We monitored 13 treatment areas for up to 8 years using Port-Orford-cedar seedlings as baits. Each spring, seedlings were planted in the same locations along transects, collected after 6 weeks, and assayed for infection. We found that where Port-Orford-cedar root disease was present and severe along a road, sanitation significantly reduced the amount of inoculum four years after treatment.

Introduction

Port-Orford-cedar (*Chamaecyparis lawsoniana* (Murr.) Parl.) is an ecologically important tree species and the largest member of its genus. It is also an extremely valuable timber tree. Its natural range is limited to southwestern Oregon and northwestern California from just north of Coos Bay south to the Mad River, and from the Pacific Ocean to about 31 km inland with a disjunct population in the Scott Mountains. Although it has a small geographic range, Port-Orford-cedar has a wide ecological amplitude, occupies many different environments, and is a component of more than 90 plant associations. It is a shade tolerant tree, usually occurs in stands with several other conifer species, and prefers but is not limited to wet areas and riparian zones where it is frequently an important source of stream shade. Because its wood is very resistant to decay, Port-Orford-cedar snags and logs serve as long lasting wildlife habitat and downed trees provide critical long-term large woody structure in streams. Port-Orford-cedar also grows well on ultramafic soils and is often the only conifer that attains large size on sites with such soils.

Port-Orford-cedar is affected by an extremely virulent introduced pathogen, *Phytophthora lateralis* Tucker and Milbrath cause of Port-Orford-cedar root disease. The pathogen was first reported affecting Port-Orford-cedar stock in ornamental nurseries in Seattle Washington in 1923, was observed killing trees in landscape settings in the Willamette Valley during the 1940s, and was confirmed in the host's native range near Coos Bay, Oregon in 1952. Subsequently, it has spread widely and has had significant impacts on hosts growing on vulnerable sites throughout much of Port-Orford-cedar's range. The origin of *P. lateralis* is unknown.

P. lateralis is very well adapted for active spread via zoospores in water and passive, long distance spread via chlamydospores in soil that adheres to vehicles. High risk areas for infection are stream courses, drainages, or low lying areas down slope from already-present infection centers or similar areas below roads where new inoculum can be introduced by vehicles in road construction, road maintenance, logging, or traffic flow. The pathogen sporulates, germinates, and grows best when moist conditions prevail and temperatures are between 10° and 20° C. Consequently, most spread occurs in the cool, rainy late fall, winter, and early spring months.

A number of techniques have been developed for use in strategies to manage Port-Orford-cedar root disease. These include road closures, timing access into stands with Port-Orford-cedar during dry, warm weather, washing vehicles before they enter uninfested areas, special care in road building and maintenance,

and featuring Port-Orford-cedar on sites that are unfavorable for the pathogen (upslope situations, convex slopes, areas away from streams and roads, and well-drained microsites).

Another management technique is roadside sanitation. The roadside sanitation approach involves eliminating Port-Orford-cedar in buffer zones along either side of roads. There are two different kinds of objectives for sanitation treatments. They can either be aimed at preventing or reducing new infections along roads that cannot be closed in currently uninfested areas, or at eliminating or minimizing the amount of inoculum readily available for vehicle transport from already-infested roadsides to roadsides in uninfested areas. The key feature of a sanitation treatment with either objective is to create a zone along treated roads where live Port-Orford-cedar roots are absent. Cedars may be killed by girdling, cutting, pulling, or burning. Ideally all Port-Orford-cedars of any size within the sphere of influence of the road are treated. The recommendation now given by the Forest Service is to treat all Port-Orford-cedar in buffer zones extending 8 m above and below a road. Where a road cuts across a steep slope, the top of the cut bank rather than a distance of 8 m is used as the upper edge of the treated area.

Use of the roadside sanitation approach is based on knowledge that *P. lateralis* only infects roots of living hosts. The pathogen can survive for various lengths of time in the roots of dead trees that were infected while alive and subsequently succumbed to the effects of the root disease, but it cannot colonize the roots of already dead Port-Orford-cedars. Therefore, if all living Port-Orford-cedars are killed in an infested area and establishment of new host regeneration can be prevented, amount of inoculum should progressively decrease on the site. Hansen and Hamm (1996) demonstrated that *P. lateralis* can survive in dead infected roots for up to seven years under ideal environmental conditions for the organism; under more typical conditions it probably survives for much shorter times. In theory, if a sanitation treatment that involves killing all hosts and preventing host regeneration is done along an infested road edge, *P. lateralis* inoculum available for vehicles to pick up and carry to other areas should be reduced or eliminated over time. Where a road runs through an uninfested area with Port-Orford-cedar, elimination of live cedar roots in a buffer along the roadside means

that there are no live hosts close to the spots where contaminated soil is most likely to fall off vehicles using the road. Since zoospores, the propagules of *P. lateralis* that would most likely be involved in spread away from the road, are quite delicate, they are unlikely to be able to reach and infect hosts beyond the buffer created in a sanitation treatment. Also, inoculum will not have a chance to build up to high levels in concentrations of live trees close to roads as often happens in roadside areas where sanitation treatments have not been done. Such inoculum buildup appears to greatly increase the likelihood of infection in trees downhill from the site of introduction (Goheen *et al.* 1987, Hansen 1993). Roadside sanitation has been widely recommended for use in Port-Orford-cedar root disease management strategies (Erwin and Ribeiro 1996, Goheen *et al.* 1997, Hadfield *et al.* 1986, Hansen 1993, Hansen and Hamm 1996, Hansen and Lewis 1997, Hansen *et al.* 2000, Harvey *et al.* 1985, Kliejunas 1994, Nielsen 1997, Zobel *et al.* 1985). It has been used extensively on Forest Service lands.

No quantitative monitoring of effectiveness of roadside sanitation treatments has been done to date. Since sanitation treatments are already widely used, are costly, and are controversial with some sectors of the public, forest managers need data on effectiveness of such treatments to make decisions about whether or not they should be continued. The objective of our evaluation was to monitor a sample of known *P. lateralis*-infested roadside areas that had received sanitation treatments and determine if treatments actually resulted in decreases of inoculum.

Methods
We monitored sanitized sites with a systematic sampling procedure using small, tubed Port-Orford-cedar seedlings as baits. Baits were planted in ten transects along a 0.4 to 0.8 kilometer segment of road at each site. Ten bait trees were used in each transect. Transects were located where introduction or movement of inoculum was likely (adjacent to existing dead Port-Orford-cedars or stumps, at stream crossings, in swampy areas, and on pullouts) and also at random points along the road. Bait seedlings were removed from their tubes and planted perpendicular to the road and on both sides, beginning at the road edge and then

periodically along the transect line across the sanitation treatment buffer and into the adjacent stand beyond. They were also planted in the roadside ditches above and below the intersections with each transect. At stream crossings where water was present, bait seedlings were left in their tubes and secured in the channels with metal stakes. The locations of all baits were mapped so that each transect could be resampled in the same fashion in subsequent years. Throughout the process, we took precautions to avoid contamination such as scrubbing boots and planting tools in chlorinated water before planting each new seedling. Bait seedlings were left in streams for two weeks, then retrieved and incubated in their tubes for four additional weeks. Planted baits were left on the site for six weeks. After six weeks, all baits were examined for evidence of infection by *P. lateralis*.

We have monitored 13 sanitized sites, one that was sanitized but was not infested and 12 that were infested and sanitized. At each site, once transects were originally installed, we have repeated the procedure with baits in the same locations for each subsequent year and have done so at approximately the same time of year as was used for the original installation. Sites have been followed for up to eight years since sanitation treatment.

Results and Discussion
There has been a consistent overall decrease in the numbers of infected bait trees over time since treatment in all sanitized infested sites evaluated (Fig. 1). Prior to treatment (year zero) an average of 20 percent of bait trees became infected on all sites. Percentages of infected trees began to drop in the first three years after treatment, and significant decreases were seen in the fourth year and thereafter. Percentages of bait trees infected from four to eight years after treatment have been low indeed. Our results suggest that sanitation treatments are worth doing even though inoculum decreases resulting from such treatments are not immediate. Sanitation treatments should be used in combination with other techniques that also reduce *P. lateralis* spread and establishment in an overall root disease management strategy for Port-Orford-cedar.

Within transects in our evaluation, the locations of infected bait seedlings has varied somewhat from year to year in the various sites monitored. This probably reflects the highly variable weather conditions during spring in Southwest Oregon. Soil moisture and temperature and the amount and temperature of water in streams and ditches have fluctuated from year to year and undoubtedly influenced activity of *P. lateralis*. In general in the years after a sanitation treatment, we have found the greatest numbers of infected baits in the roadside ditches, especially the ones on the upper sides of the roads. This suggests that the ditches function as traps for infested water. It means that design and maintenance of the ditches is an important component of managing roads to limit the spread of *P. lateralis*. Few if any infected baits were found near the outer edges of the sanitized areas.
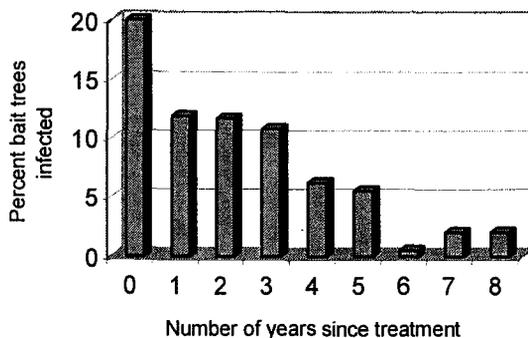

Fig. 1. Average percentage of Port-Orford-cedar bait trees infected in sample areas where roadside sanitation treatments were done.

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Phytophthora disease of alders in Bavaria: extent of damage, mode of spread, and management strategies

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Abstract. During a 2-year study it was shown that a new lethal *Phytophthora* root and collar rot of common (*Alnus glutinosa*) and grey alder (*A. incana*), which was first identified in 1993 in the UK, is widespread along most river systems in Bavaria. Symptoms include abnormally small and often yellowish leaves, a sparse canopy, tarry or rusty spots on the outer bark of the stem base with tongue shaped necroses of the inner bark. Once introduced to a river system, the alder *Phytophthora* spreads downstream infecting the collar or bare roots of riparian alders via lenticels and adventitious roots. The disease is also present in young plantations on former agricultural land without contact with any watercourses indicating disease transmission via infected nursery stocks. Baiting tests revealed the presence of the pathogen in rootstocks of alders from three out of four commercial nurseries, but not in rootstocks from four nurseries of the Bavarian State Forestry. In a new project, measures will be developed to prevent further disease spread, and facilitate the sanitation of diseased alder stands. As a first result, a code of good practice for the production of alders was negotiated with the Bavarian nursery owners. Furthermore, coppice trials with diseased alders growing under flooded and non-flooded conditions, and a screening for resistant alders in the field were started. Finally, a disease leaflet was produced, and sent to all local forestry, river and environmental authorities, to provide a detailed survey of the disease distribution. Preliminary results are presented and their implications discussed.

Introduction

The genus *Alnus* is represented in Europe by 4 species, most of them characterized by the ability to rapidly colonise abandoned or bare land and tolerate high groundwater tables and periodic flooding. A common feature of the genus is the ability to ameliorate soil due to the root symbiosis with actinomycetes of the genus *Franckia* which are able to fix atmospheric nitrogen. *Alnus glutinosa* (common alder) is the most widespread species occurring all across Europe at altitudes ranging from sea level up to 1800 msl. It is mainly adapted to wet and clay soils, colonising riverbanks (*Fraxino-alnetum*) or swamp areas (*Alnetum glutinosae*). It has high conservation value, and widely used in reforestation activities aiming to consolidate riverbank and slope stability, and produce valuable timber. *Alnus incana* (grey alder) has a Central Eastern distribution in Europe, being present in the south mainly in mountain areas. Being a pioneer species that tolerates both dry conditions and flooding it is extremely important for improving stability of slopes and riverbanks (*Alnetum incanae*). *A. viridis* (green alder) is a common shrub species in the Alps colonising steep clayey slopes and the banks of mountain streams. Finally, *A. cordata* (Italian alder) is a species endemic in Southern Italy growing in non-riparian pure and mixed forests in the Apennine mountains and in plantations across Italy.

In 1993 a previously unknown lethal disease of common alder (*Alnus glutinosa*) was recorded in southern Britain mainly along riverbanks, but also in orchard shelterbelts and woodland plantations (Gibbs 1995). In the following years the disease was also found on *A. incana* and *A. cordata*, and in other European countries, in particular Germany, France, Sweden, Netherlands, Belgium, Austria, Hungary, Estonia and Italy (Hartmann 1995; Cech 1997; Schmidt *et al.* 1998; Werres 1998; Gibbs *et al.* 1999; Jung *et al.* 2000b Streito and Gibbs 2000; Szabó *et al.* 2000; Santini *et al.* 2001; Werres *et al.* 2001; Gibbs *et al.* 2003). Affected trees show symptoms typical of root and collar rot by *Phytophthora*: abnormally small, sparse and often yellowish foliage, a dieback of the crown, early and often excessive fructification with unusual small cones, tongue shaped necroses of the inner bark and the cambium which extend from the stem base up to 3m, and usually are marked externally by the presence of tarry or rusty spots coming from exudations. It was shown by Brasier and colleagues that the causal organism is an interspecific hybrid involving

Phytophthora cambivora and an unknown *Phytophthora* species close to *P. fragariae* (Brasier *et al.* 1995; 1999; Brasier 2003;). The alder *Phytophthora* is morphologically similar to *P. cambivora*, but can be distinguished from the latter by its homothallism, high level of zygotic abortion, the occurrence of distorted oogonial shapes, and lower cardinal temperatures for growth. Beside the widespread standard form of the pathogen several variants are known which differ in their chromosome numbers, oogonial and antheridial morphology, cultural stability and colony morphology. Interestingly, viability of oospores is significantly different between the variants, but germination was never observed (Delcan and Brasier 2001). In pathogenicity tests the standard form and the Dutch variant of the alder *Phytophthora* came out as highly aggressive pathogens of common alder whereas the Swedish, German, and UK variants, and *P. cambivora* were only weakly aggressive (Brasier and Kirk 2001).

Alder plantations were extensively established in Bavaria since the 1980's due to (1) stabilisation of steep slopes in the Alps with degraded stands, (2) a new silvicultural concept of the State Forestry on wet forest sites as a consequence of the storm damages in January 1990, and (3) first afforestations of former agricultural land. Results of a one year study indicated that the *Phytophthora* disease of alders is widespread in Bavaria in both riparian ecosystems and forest plantations (Jung *et al.* 2000b). Therefore, the project was prolongated for another two years in order to assess the distribution of the disease in detail, to investigate modes of disease spread, and to develop a management concept for the disease. Preliminary results of the ongoing project are presented below, and their implications are discussed.

Methods
Disease survey
In order to assess the distribution of the disease in riparian stands and forest plantations a leaflet illustrating the disease symptoms was sent to local and national forestry, river and environmental authorities with the request to communicate suspected disease records. In addition, complementary to this survey, courses were offered to practitioners to facilitate the identification of disease symptoms in the field. Disease records were verified by isolations of the pathogen from necrotic alder bark.

A small-scale survey of the disease was conducted during wintertime in alder fields of 4 commercial private nurseries and 4 nurseries of the Bavarian State Forestry. In each nursery 10-20 alder plants were selected at random, and harvested together with adhering soil material. In a greenhouse the alder plants were potted in plastic buckets, flooded for 1 week and baited with oak leaflets (see below). Four weeks later after a second flooding and baiting procedure the root systems were harvested, washed and examined for root necrosis.

Isolation procedures
An orange-brown colour and a mottled appearance of the inner bark shows that the pathogen is still viable, whereas a uniform dark brown colour indictaes for an old inactive necrosis. Bark samples including the cambium from the upper c. 20 cm of active necroses were taken, put into distilled water, and transported to the lab. During summertime the jars with the flooded bark pieces were transported in cool boxes. The water was replaced 4 times daily over a period of 2-5 days in order to remove excess polyphenols, and then small pieces (c.8 x 3 x 3 mm) were cut from all parts and depths of the necrosis, blotted on filter paper, and plated onto selective PARPNH agar (Jung *et al.* 1996, 2000a) (c. 150 pieces per tree in 7-8 petridishes). In some cases, in particular with putatively inactive necroses, remaining pieces of the necrotic tissue were shredded, flooded with distilled water, and baited with oak leaflets according to the isolation protocol from soil (see below). The water was replaced daily in order to remove excess polyphenols and decrease bacterial populations.

Isolations from soil samples were carried out using 2–7-day-old leaflets of *Quercus robur* seedlings as baits floated over flooded soil (Jung 1998, Jung *et al.* 1996, 2000a, 2002). Infected brownish leaflets which normally appeared after 3-7 days were plotted dry, cut into pieces and plated onto selective PARPNH-agar. Petridishes were incubated at 20°C in the dark, and examined under the stereomicroscope for outgrowth of *Phytophthora* cultures which took 2-7 days in the case of plated bark pieces, and 24-48 hours in the case of plated leaf segments.

Phytophthora population in the rhizosphere of diseased alders in the field
Since the oospores of the standard form and all known variants of the alder *Phytophthora* failed to germinate *in vitro* (Delcan and Brasier 2001), it seems likely that

the pathogen has problems to establish and survive in soil under natural conditions. In order to verify this hypothesis isolations were carried out in three riparian stands from soil and necrotic bark of the same alder trees.

Coppice experiments

Preliminary results of coppice experiments performed on riparian sites in the UK indicate that a high percentage of the regrowth is still healthy four years after coppicing. However, these results may not be transferable to forest plantations where the inoculum comes from the nursery, and the primary infection court is the root system rather than the root collar. Therefore, in late winter 2001 coppice experiments were initiated in Bavarian plantations on two non-flooded sites with different water supply and one periodically flooded site with absence of the disease upstream. In each stand disease parameters such as necrosis length, proportion of stem girth with bark necrosis, vitality of the crown (1 = vital, 0 = non-vital, indicated by high transparency, stunted growth or even dieback), and fructification were recorded for at least 150 *A. glutinosa* trees before they were coppiced. In June and August 2001 weed concurrence was reduced mechanically. The number and vitality of the shoots per stool was recorded for the first time in July, and will be monitored over several years. Non-parametric Spearman Correlation between vitality of the crowns or sprouting rate after coppicing and proportion of necrotic stem girth was determined.

Results and Discussion

The detailed survey along rivers and streams in Bavaria was finished in November 2001. Disease records came mostly from river and forest authorities, and could be verified in most cases by isolations of *Phytophthora* from necrotic alder bark (Table 1). The results show that the disease is present in riparian stands of *A. glutinosa* and *A. incana* growing along at least 50 % of the river systems (Fig. 1). The disease is most likely even more widespread because neither absence nor presence of the disease was recorded from many rivers and streams. Along some rivers where disease records go back to the mid 1980's, disease incidences exceed 50%. In 25 rivers and streams which were investigated in detail the source of inoculum could be traced back to infested plantations established within the last 15 years on the river banks or on forest sites which drain into the rivers via ditches. In one river high disease incidences were found in natural alders growing downstream and on the banks of the raceways of a big commercial fish farm with international trade. The absence of any diseased alders upstream suggests introduction of the alder *Phytophthora* with basin water coming from another fish farm contaminated by infested river water. Once introduced to a river system the pathogen most likely spreads downstream with zoospores and infected bark debris. However, in some cases diseased oldgrowth was also found upstream of infested plantations indicating disease spread via vectors such as fishes or water birds.

Disease incidences were remarkably high in alders growing in permanent contact to the river water and in areas with a long retention time of river water after flooding such as flood plains and oxbows. There was a tendency to higher disease incidences in common alder stands growing along lowland rivers as compared to grey alder stands growing along alpine rivers. This might probably be due to longer durations of floodings, slower flowing rates, and higher water temperatures of lowland rivers rather than different susceptibilities of common and grey alder. This is supported by the observation that in mixed stands both alder species are suffering equally. High disease incidences were also found in alder stands growing in the reed zones of lakes with infested tributaries.

First results of the plantation survey of the State Forestry indicate that the disease might be present in about 50% of the alder plantations established within the last 15 years on non-flooded forest sites. A typical example may be the forest district 'Rotter Forst' near Rosenheim where about 40 alder plantations were established in the 1990's on wet gley soils. Although the alder plants came from various nurseries typical disease symptoms were found in all plantations, and several variants of the alder *Phytophthora* could be recovered from all 10 stands investigated in detail. On many of these sites free water is standing above ground after periods of heavy rain or snowmelt, and as a consequence the pathogen spreads from infected nursery plants to old growth and natural regeneration of alder growing in these stands and along drainage ditches.

Also on non-flooded sites, the rate of disease progression and the disease incidence are depend on the water supply of the site. This was exemplarily shown in two plantations which were established in 1995 with the same nursery material on a moderately moist site at
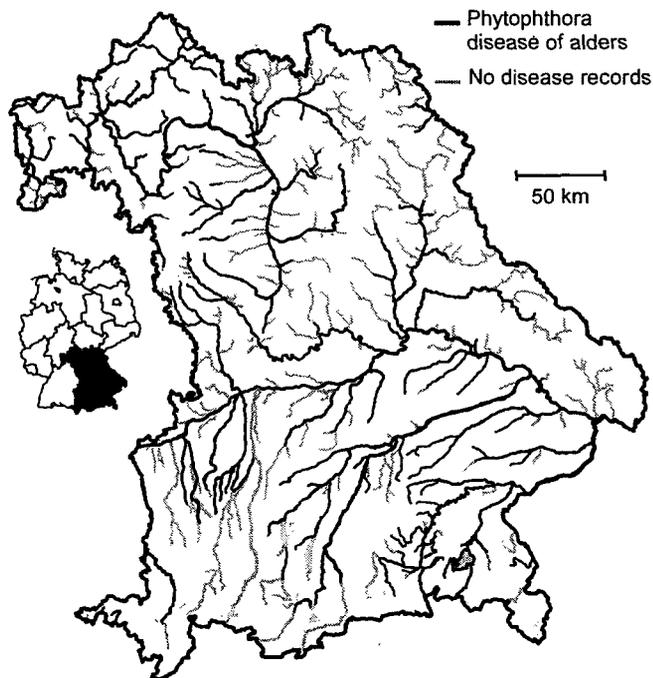
Fig. 1. Distribution of *Phytophthora* root and collar rot of alders along main rivers and streams in Bavaria; small map showing the location of Bavaria within Germany.

Table 1. Isolation results from necrotic bark of alders in the field

	No. of stands	No. of rivers and streams	No. of stands (rivers) with[A]			
			ALD	CIT	GON	PSEU
Riparian stands	106	95	97 (88)	5 (5)	1 (1)	-
Forest plantations	27	-	20	-	-	1
Total	133	39	117	5	1	1

[A] ALD = alder *Phytophthora*, CIT = *P. citricola*, GON = *P. gonapodyides*, PSEU = *Phytophthora pseudosyringae* sp. nov. (Jung *et al.* 2003).

Table 2. Effect of site conditions on disease incidence of *Alnus glutinosa* trees growing in plantations infested with the alder *Phytophthora*, and sprouting rate 4 months after coppicing

Site conditions	No. of alders	Diseased (dead) alders with collar rot symptoms	Spearman correlation r_S between proportion of necrotic stem girth and vitality	Sprouting rate after coppicing	Spearman correlation r_S between sprouting rate and	
					proportion of necrotic stem girth	vitality of the crown
Non-flooded sites						
moderately moist site on the top of a hill[A]	150	31.3 % (6.7 %)	-0.2759 (p = 0.0010)	72.9 %	-0.5248 (p < 0.0001)	0.1849 (p = 0.0305)
wet site at the base of a hill[A]	257	52.1 % (23.7 %)	-0.5744 (p < 0.0001)	30.6 %	-0.4463 (p < 0.0001)	0.2769 (p < 0.0001)
Riparian site[B]	699[C]	26.5 % (34.8 %)	-0.6553 (p < 0.0001)	28.3 %	-0.4179 (p < 0.0001)	0.1655 (p = 0.0371)

[A]Plantation established in 1995 with planting stock from nursery 2 (Table 5).
[B]Plantation established in 1988 with planting stock from nursery 4 (Table 5).
[C]Only 159 alders were coppiced.

229

the top of a hill and on a wet site with a periodically high watertable at the foot of the hill. Six years after planting, the frequency of diseased and dead alders was much higher at the wet site. Moreover, a strong and highly significant correlation between the proportion of necrotic stem girth and the vitality of the crowns was found on the wet site which in this regard was comparable to a riparian plantation, whereas on the dryer site this correlation was weaker and less significant (Table 2).

In infested plantations on moderately moist sites without high watertables the alder *Phytophthora* generally takes 5-10 years to reach the collar via the bark of the relatively tolerant suberized roots, whereas on wet or flooded sites this process takes less than 3-5 years; once in the collar the alder *Phytophthora* spreads rapidly, and within one year necroses often reach up to 1 m height and more than 50% of stem girth. Infected trees often show typical crown symptoms even without collar necrosis, and in these cases they may serve as early indicators for root infections; but one has to be aware that a high proportion of alders with more than 50% necrotic stem girth may have healthy looking crowns (Table 3).

The alder *Phytophthora* was isolated from necrotic bark throughout the year with isolation frequencies of more than 50%. About 300 isolates were recovered from 97 riparian stands growing along 88 rivers and streams, and from 20 out of 27 plantations on non-flooded sites (74.1%) (Table 1). *P. citricola* was recovered from 5 riparian stands. Most isolates of the alder *Phytophthora* belonged to the aggressive standard form, but the Swedish and the German variant, and several previously unknown, unique types were also found (Brasier, Kirk & Jung unpublished). In some cases two variants could be isolated from the same necrosis. Interestingly, the Swedish and the German variant were usually isolated from laterally restricted and sometimes stripe canker like necroses. This inability to girdle the tree under field conditions is congruent with the low aggressivity of these variants in bark inoculation tests (Brasier and Kirk 2001).

In three riparian stands isolations were made from necrotic bark and rhizosphere soil sampled from the same alder trees. While the isolation tests from necrotic bark always yielded the standard form of the alder *Phytophthora*, the baiting tests with the soil samples showed a converse picture. *P.*

citricola was isolated from all soil samples in two stands, whereas the alder *Phytophthora* was recovered from one alder (Table 4). This result supports the hypothesis that due to the inability of the oospores to germinate (Delcan and Brasier 2001) the alder *Phytophthora* has problems to establish and survive in natural soil. Therefore, it is supposed that the pathogen has to spread from infected bark via zoospores to another alder before all hyphae are lysed. Considering this short life cycle and the close specificity of the alder *Phytophthora* to the genus *Alnus* (Brasier and Kirk 2001) management concepts and control strategies which aim at starving this pathogen may be promising.

The results of the disease surveys in both riparian and non-flooded alder stands strongly supported the idea that dissemination of infested planting stock might be a major way of long distance spread of the disease. This was supported by the results of a small-scale nursery survey during which the alder *Phytophthora* was baited from alder plants of three out of four commercial private nurseries, but not from alder plants of 4 nurseries belonging to the State Forestry (Table 5). Most likely, the reason for this striking difference is that State Forestry nurseries raise their own planting stock from areas with potentially higher infestation levels, and grow them for a second year in their own beds. Moreover, at least two of the three infested nurseries may have introduced the alder *Phytophthora* with irrigation water taken from rivers with diseased alder stands upstream.

All the infested alder plants looked healthy and had no visible necroses on their roots when coming from the nurseries. However, two weeks after the second flooding necrotic fine roots and bark necroses on suberized roots were found on many plants, and the alder *Phytophthora* could be isolated easily. This result points out the inadequacy of visible controls and the necessity of a molecular based detection protocol which has to be developed in the future.

Various other *Phytophthora* species were also baited from the flooded root stocks, among them *P. cambivora* known as one of the parents of the hybrid and some isolates which according to ITS sequence analysis might be backcross products between *P. cambivora* and the alder *Phytophthora* (Jung *et al.* unpublished results). From one alder plant the

230

Table 3. Effect of the proportion of necrotic stem girth on the vitality and the sprouting rate after coppicing of 15-yr-old *Alnus glutinosa* trees growing in a riparian plantation infested with the alder *Phytophthora*

Proportion of necrotic stem girth (%)	No. of alders	Proportion of vital alders (%)	Sprouting rate (%)
100	6	0	0
76-99	44	7,3	12,2
51-75	33	44,8	13,8
26-50	21	60,9	23,8
11-25	16	71,4	33,3
0-10	10	80	60
0	29	86,2	62,1
Total	159	49.1	28.3

Table 4. Isolation results from necrotic bark and rhizosphere soil of alders in 3 riparian stands

River	No. of trees	No. of trees with [A]					
		ALD bark	soil	CIT bark	soil	CAC bark	soil
Glonn[B]	3	3	1	-	-	-	-
Große Vils[B]	5	5	-	-	5	-	-
Abens[C]	5	5	-	-	5	-	3

[A] ALD = alder *Phytophthora*, CIT = *P. citricola*, CAC = *P. cactorum*.
[B] Natural stand.
[C] 15-yr-old plantation

alder *Phytophthora, P. cambivora* and 3 other Phytophthoras were baited. The occurrence of multiple *Phytophthora* species in the rhizosphere of a single nursery plant enhances the probability of evolution and emergence of new *Phytophthora* pathogens, and new tree diseases, as it might have happened in the case of the hybrid alder Phytophthoras (Brasier and Jung this volume).

A further dissemination of inoculum with infested nursery stock would *a priori* undermine any management concept for the disease, and put an end to the silvicultural concept of pure common alder stands on wet forest sites. Consequently, no alders will be planted by the forest and river authorities which are not produced according to a code of good practice for the growth of alders which was recently arranged between most of the southern German nursery owners and the Bavarian State Forestry. The participating nurseries agree (1) to grow their alders on land where no alders were grown for at least five years, (2) to raise their own alder plants from seeds or to buy in alders for sale only from nurseries that grow their alders according to this code of good practice, (3) not to use river or surface water for irrigation, (4) not to grow

any other plants on the same fields in order to avoid passive introduction of the alder *Phytophthora* with non-host plants, (5) to avoid the introduction of the pathogen from infested fields via soil particles.

For the control of *Phytophthora* diseases in the field the application of systemic fungicides especially potassium phosphonate can be a successfull measure. This was shown in particular for root rot diseases caused by *P. cinnamomi* in eucalypts and many understorey species in Western Australia (Jarrah dieback; Hardy 2000), and in *Quercus suber* and *Q. ilex* in Iberia (Fernandez-Escobar *et al.* 1999). However, due to economic reasons and legislative restrictions this is not an option for controlling the *Phytophthora* disease of alders. Results of coppice experiments with riparian alder stands in the UK indicate that coppicing of infected trees or shoots could be an effective control measure. In these experiments most of the coppiced stools showed regrowth of which a high proportion was still healthy four years after coppicing (Gibbs 2003). However, the results of our coppice experiments in one riparian and two non-flooded plantations are less promising. Only in the stand on the moist site with the lowest disease incidence (ca.

38%) the sprouting rate was high enough (79%) for the formation of a new stand with a close canopy, whereas on the wet site and in the riparian plantation with high disease incidences and mortality rates, the sprouting rates were insufficient in terms of forest production (Tables 2 and 3), and markedly lower than in the UK experiments. The difference between the Bavarian and the UK experiments was most likely due to different infection processes. In natural riparian alders such as in the UK experiments infections mainly occur via the adventitious roots and the big lenticells at the root collar. Because the pathogen then primarily grows upwards the root system remains for some time functional allowing a good supply of the stools with carbohydrates, nutrients and water after coppicing. In contrast, in all 3 Bavarian plantations the roots of the alders had already been infected in the nurseries resulting in a high proportion of root damage before the appearance of first necroses at the collar. As a consequence, the supply of many of the coppiced stools with carbohydrates, nutrients and water was probably insufficient resulting in lower sprouting rates. Interestingly, the sprouting rate in all 3 forest plantations showed a highly significant and strong negative correlation to the proportion of necrotic stem girth whereas the correlation with the vitality of the crowns was weaker and less significant (Table 2). This apparent inconsistency was most likely due to an unknown proportion of healthy looking alders which were infected in the root system but not yet at the collar.

Another incalculable risk for the coppicing of diseased forest plantations is the possibility of reinfections of the collar region via infected roots. This is indicated by observations in another plantation on a non-flooded site where single shoots of some stools were infected 2 years after coppicing. From the Bavarian and the UK experiments it can be concluded that coppicing may be a good control measure for riparian naturally infected alders in terms of sustainable stabilisation of river banks, especially if the coppicing starts at the headwaters and tributaries. However, in forest plantations long-term data are required on disease progression in coppiced and non-coppiced stands on different sites before recommendations can be made. It has to be mentioned here that also in France and Belgium coppice experiments are in progress which in some years will deliver valuable results (Gibbs 2003).

The compliance with the new growth conditions for alder plants in nurseries may be sufficient to allow the sustainable production of alder timber on non-flooded forest sites in the future. However, on riparian sites an infection even of non-infested nursery plants by zoospores in the river water can not be excluded. Coppicing of infected trees and shoots may be a short-time control measure, but on a long-term scale a number of resistant clones is needed in order to sustain riparian alder stands. Two field experiments in the UK have disproved the idea of resistance of *A. glutinosa* against the alder *Phytophthora* at provenance level (Gibbs 2003). However, the observation that mature common alders with healthy crowns and overhealed inactive bark necroses at the stem base and alders without any infections are present in riparian stands with high disease incidences and long disease histories suggests the possibility of natural selection of resistant alder genotypes. Therefore, a selection breeding program was recently started in Bavaria using cuttings of such survivors for vegetative propagation and testing for resistance to the alder *Phytophthora*. After controlled crosses of resistant clones the heritability of resistance or tolerance against the alder *Phytophthora* will be tested with the F1 and F2 generation. The feasibility of such a selection breeding program was recently demonstrated for *Eucalyptus marginata* and *P. cinnamomi* (Stukely *et al.* this volume), and *Chamaecyparis lawsoniana* and *P. lateralis* (Bower *et al.* 2000, Hansen *et al.* 2000, Sniezko and Hansen 2000 and this volume).

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The contribution of research in the Forest Department of Western Australia and the Department of Conservation and Land Management to the fight against *Phytophthora* species in native vegetation of south-western Australia over the last two decades

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Abstract. Following a brief history of research on *Phytophthora cinnamomi* in Western Australia before 1980, the results of research on *Phytophthora* species since the 1980s by the Department of Conservation and Land Management and the Forests Department of Western Australia are described in relation to management outcomes. Because of the complexities of the pathogen-host-environment interactions affecting disease expression, control measures cannot be adequately developed and tested within a few years. Priority must be given to long-term research to ensure thorough testing of recommendations to management.

Introduction

Diseases caused by *Phytophthora* species are a major threat to the diversity, ecology and conservation of many plant communities of south-western Australia (Shearer 1990, 1994; Shearer and Smith 2000). Development of effective management strategies requires knowledge of the biology of the *Phytophthora* species in the Mediterranean environment experienced by native communities of south-western Australia, and the consequences of environmental change resulting from roading, logging, fire, recreation pressure, insect pests and climate. Plant disease research in the Department of Conservation and Land Management, incorporating the Forests Department of Western Australia, aims to predict the occurrence and development of *Phytophthora* species under different site, climatic and management conditions, and develop methods of control. This paper describes the history, structure and accomplishments of research on *Phytophthora* species by the Department of Conservation and Land Management, incorporating the Forests Department of Western Australia, since the 1980s.

Brief History of Research pre-1980

The establishment of a research station at Dwellingup in 1948 marked the beginning of organised formal forest research in Western Australia (Forests Department of Western Australia 1969). The investigations of Hamilton, Harding, Hatch, Wallace and Waring on the cause of unexplained deaths of *Eucalyptus marginata* during the period from the late 1940s to the mid-1950s are described in detail by Podger (1968). The first association between *P. cinnamomi* and death

of the *E. marginata* overstorey and understorey vegetation in forests of south-western Australia was established in the mid-1960s (Podger *et al.* 1965). This breakthrough stimulated extensive State and Commonwealth Government funding for investigation of the distribution of *P. cinnamomi* and environmental factors affecting the host-pathogen interaction.

The Forests Department of Western Australia quarantined northern *E. marginata* forest with little disease in high salt hazard areas in the mid-1970s in order to map disease distribution and give time to develop hygiene and other control measures. The accuracy of detecting and mapping disease distribution from black and white aerial photographs in the mid-1960s (Batini 1973), was greatly improved by the development of 70 mm shadowless colour aerial photography by the late 1970s (Bradshaw and Chandler 1978; Muir 1984). Greatest research emphasis was given to monitoring soil temperature and moisture to identify sites and seasons when conditions were most favourable for survival and sporulation of the pathogen and infection (Christensen 1975; Shea 1975; Schuster 1978). The effect of manipulation of understorey composition on the soil environment and inoculum levels of *P. cinnamomi* was determined (Shea *et al.* 1978). Symptoms were mainly used to monitor changes of the pathogen with time (Shea and Dillon 1980).

New and existing research was also funded from 1979 to 1987 by a Foundation for Jarrah Dieback research with contributions from the aluminium and timber industries.

Pathogen-Host-Environment Interactions	3-year period
	79-81 82-84 85-87 88-90 91-93 94-96 97-99 00-01

Pathogen
Population Dynamics
Detection
Phytophthora species
Eradication
Host
Pine resistance
Jarrah resistance
Host response
Host susceptibility
Chemotherapy-Phosphite
Environment
Understorey manipulation
Jarrah site characteristics
Site susceptibility
Disease
Impact

Fig. 1. Timelines of major research projects grouped under pathogen, host, environment and disease.

PATHOGEN-HOST-ENVIRONMENT INTERACTIONS	MANAGEMENT OUTCOMES	PATHOGEN-HOST-ENVIRONMENT INTERACTIONS
PATHOGEN		ENVIRONMENT
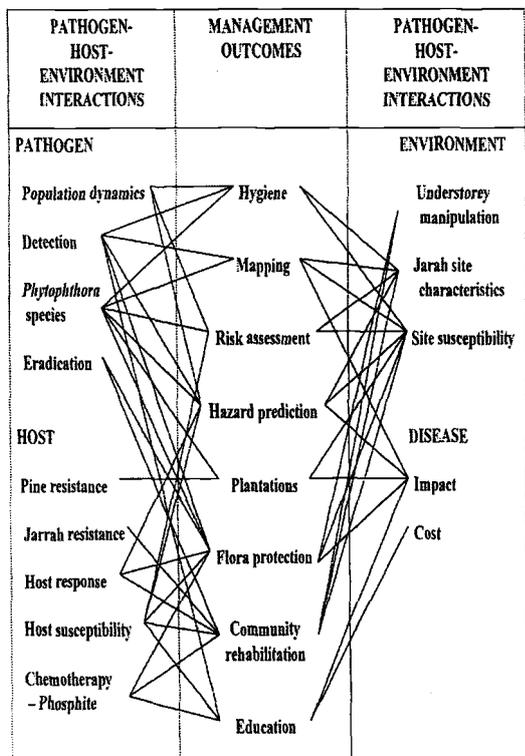
Fig. 2. Illustrating the complexity of the interactions between management outcomes and major research projects grouped under pathogen, host, environment and disease. Research project results will affect more than one management outcome and feedback occurs between outcomes and projects.

Contribution of Research to Management post-1980

Figure 1 shows the timelines for the various research projects grouped under those mainly involved with the pathogen, host, environment or disease. How the results from the projects contribute to management outcomes is shown in Figure 2. The interaction between research and management is complex as a research project must deal with a multiplicity of interactions resulting from extremes of scale between the pathogen and host and the diversity of microclimates affecting the host-pathogen interaction. The results from one project may affect more than one management outcome (Fig. 2).

Pathogen related projects

Greatest research emphasis in the 1980s was quantification of the effects of site and season on population levels of *P. cinnamomi* in surface soil (Fig. 1) associated with improvement of selective agars and baiting techniques (Shea *et al.* 1980; Shearer and Shea 1987; Shearer *et al.* 1989; Morgan 1991). This research showed that viable inoculum occurred in surface soil of infested, water-gaining low-land areas throughout the year. This was due to extended periods of moist conditions favourable for survival and maximum coincidence of warm, moist conditions suitable for sporulation. In contrast, inoculum levels in upland surface soils showed strong seasonal fluctuations with maximum levels in winter and least in summer when low soil water contents reduced survival and prevented sporulation of the pathogen. Occasional late spring or summer rains allowed inoculum to survive well into summer. Detailed description of inoculum fluctuations in the *E. marginata* forest are given in Shearer and Tippett (1989) and Shearer and Smith (2000). The importance of infected hosts with large woody roots which act as a buffered environment for survival of the pathogen was determined. Knowledge gained aided the development of hygiene prescriptions and the use of indicator plants in the interpretation of aerial photographs (Fig. 2).

Since the 1980s the division has maintained the Vegetation Health Service (Fig. 1) which tests at least 1000 soils samples each year for mapping and hygiene operations (Fig. 2). Details of the functions of the service are given by D'Souza *et al.* (2001).

Initially most of the research concentrated on *P. cinnamomi*, but by the mid 1980s there was a greater appreciation of the

236

importance of *Phytophthora* species other than *P. cinnamomi* as a threat to the diversity of south-western flora (Fig. 1). The behavior of 8 species of *Phytophthora* was compared in inoculated stems of *Banksia grandis* and eucalypts (Shearer *et al.* 1988). Hill (1990) showed the distribution and importance of *Phytophthora* species in the Northern Sandplains. Bunny and Shearer (1995) and Bellgard *et al.* (2001) determined the impact, epidemiology and variability of *P. citricola* and *P. megasperma* in native communities of south-western Australia and tested options for control. The results contributed to public education and hygiene prescriptions (Fig. 2).

Methods of chemical control to supplement hygiene were being tested by the late 1980s (Figs. 1 and 2). Various disinfectants were evaluated for use in hygiene prescriptions (Noske and Shearer 1985). Hill *et al.* (1995) compared a combination of herbicide and fungicide treatments for eradication of *P. cinnamomi* from infested *Banksia* woodland on deep leached sand. The use of chemicals to control spot infections suffered from the high cost of the chemical treatment. In addition the longevity and occurrence of the pathogen in the soil precluded any attempt at rapid eradication by chemicals.

Host related projects

Death of *Pinus radiata* in plantations following *P. cinnamomi* infection in the early-1980s led to screening for resistance to the pathogen (Fig. 1). Lines of *P. radiata* showing high levels of resistance to *P. cinnamomi* were identified and selected for use in the pine planting programme (Butcher *et al.* 1984).

Research up to the 1980s mainly concentrated on the behaviour and biology of *P. cinnamomi* in the soil environment with little attention being given to the host-pathogen interactions. Infection of *E. marginata* was thought to be mainly through fine feeder roots until invasion of large woody roots was observed in the early 1980s (Dell and Wallace 1981; Shearer *et al.* 1981). This discovery lead to greater emphasis being placed on host pathogen interactions (Fig. 1).

Resistant lines of *E. marginata* were selected in the glasshouse seedling tests and confirmed in the forest environment (Stukely and Crane 1994). Continued research of selected lines for use in rehabilitation strategies (Fig. 2) is a strong cooperative effort between ALCOA Australia, the Department of

Conservation and Land Management and Edith Cowan and Murdoch Universities.

Lesion development and resistance mechanisms in *E. marginata* roots were described (Tippett *et al.* 1983, 1985; Tippett and Hill 1984) and the effect of temperature and moisture on the host pathogen interaction determined (Davison and Tay 1987; Shearer *et al.* 1987a; Tippett *et al.* 1987). A greater understanding of lesion development in *E. marginata* led to studies on the relationships between infection and growth (Davison and Tay 1988; Crombie and Bunny 1994), root loss (Somerford *et al.* 1987) and the ecophysiology of *E. marginata* (Crombie *et al.* 1987; Crombie and Tippett 1990; Bunny *et al.* 1995).

Throughout the 1980s most of the research effort concentrated on the susceptibility of *E. marginata* and a few other species to *P. cinnamomi*. The susceptibility of the forest understorey and shrublands and woodlands throughout south-western Australia were largely ignored until the late 1980s (Fig. 1). During the 1990s susceptibility of species to *P. cinnamomi* have been assessed in the Northern Sandplains (Hill 1990), on the South Coast (Wills 1993), in *E. marginata* forest (Shearer and Dillon 1995) and *Banksia* woodland (Shearer and Dillon 1996b). Assessment of the susceptibility of rare and threatened flora to *P. cinnamomi* is a current research priority. Such information is required for accurate disease assessment and prioritisation of taxa and communities at risk from infection (Fig. 2).

Phosphite was tested as a control option in native communities in the late 1980s (Shearer and Tippett 1989; Shearer and Fairman 1991) (Fig. 1) following demonstration of the effectiveness of the chemical in the control of *P. cinnamomi* in agriculture. Lesion development of *P. cinnamomi* was inhibited for at least 4 years following injection of 50–100 g/L of phosphite into the trunks of *Banksia attenuata* and *B. grandis* (Shearer and Fairman 1979a). Foliar application using a backpack sprayer at the rate of 5 g/L reduced *Banksia* mortality for about 2 years in highly susceptible communities (Shearer and Fairman 1979b). Phosphite application induced strong host defence reactions against the pathogen (Smith *et al.* 1997). By the mid 1990s prescriptions had been developed to apply the chemical to susceptible communities by aircraft (Komorek *et al.* 1997) and are now in practice for the protection of threatened flora against *P.*

cinnamomi infection (Barrett 2001; Smith 2001). Continued research on phosphite is a strong cooperative effort between ALCOA Australia, CSIRO, the Department of Conservation and Land Management and Murdoch University.

Environment related projects

From the late 1970s to early 1980s, modification of the soil environment by replacement of a susceptible *B. grandis* understorey by *Acacia* species (Fig. 1) offered a potential management option for reducing disease development (Shea *et al.* 1978; Shea and Shearer 1980; Cary 1982). The concept has not been applied to forest management because of conservation issues, and the difficulty of controlling the high intensity fires needed to achieve significant reduction in *B. grandis* density.

Until the early 1980s, upland *E. marginata* forest sites were considered free draining. This supposition changed when *P. cinnamomi* was found at depth within the profile of upland sites, often just above a horizon that impeded vertical percolation of water (Shea *et al.* 1983). Emphasis was then given to determination of the relationships between soil profile characteristics, subsurface lateral movement of water and behaviour of *P. cinnamomi* at depth in the soil (Kinal 1986; Kinal *et al.* 1993). For the first time, disease behaviour in upland areas could be related to site characteristics that influence pathogen sporulation, survival and dispersal, and the infection of *E. marginata*. The determination of key site indicators that could be used to predict disease hazard (Fig. 2) was a high priority (Shearer *et al.* 1987*b*).

Site susceptibility research in the early 1980s and late 1990s (Fig. 1) studied the effect of soil type on the biology of *P. cinnamomi* and host infection (Sochacki 1982; Shearer and Crane 2001) in order to improve the precision of risk rating systems for managers (Fig. 2).

Disease related projects

It was not until the mid-1980s that priorities shifted within the programme to quantify the impact of *Phytophthora* species in woodlands and shrublands other than forest (Fig. 1) (Hill 1990; Wills 1993; Hill *et al.* 1994; Bunny and Shearer 1995; Shearer and Dillon 1996*a*; Bellgard *et al.* 2001). Shearer (1990) estimated that disease caused by *P. cinnamomi* cost Western Australian Government departments and Industries at least \$3.4 million in 1989. There is an obligation to continue monitoring changes in plant communities caused by *Phytophthora* species to help predict future progress of disease and prioritise areas in need of protection.

Prognosis

Most of the significant advances in the understanding and management of *Phytophthora* species in native flora of south-western Australia has come from long-term government funded research in the Forests Department of Western Australia and the Department of Conservation and Land Management. However future advances will depend on addressing the current minimum commitment of resources to *Phytophthora* research (Fig. 3).
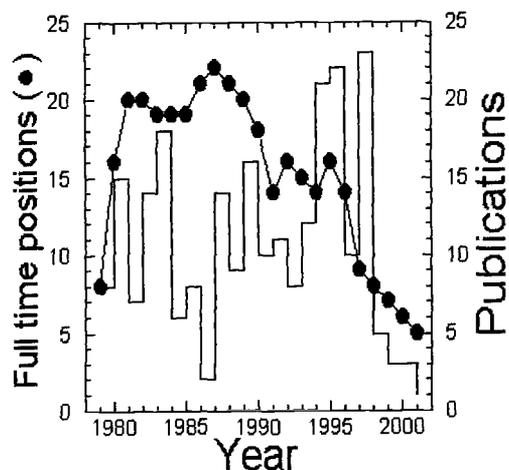
Fig. 3. Full time positions (•) in *Phytophthora* research in the Department of Conservation and Land Management and output of publications (bars) during the period 1980 to 2001.

Research on the biology of *Phytophthora* species, host reaction and the use of phosphite as a control measure is continuing as a strong cooperative effort between ALCOA Australia, CSIRO, the Department of Conservation and Land Management and Murdoch University, but under short-term funding. Because of the complexities of the pathogen-host-environment interactions (Fig. 2), factors affecting disease expression and control measures cannot be adequately tested within a few years. Priority must be given to long-term research to ensure thorough testing of recommendations to management.

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Abstracts and Poster Papers
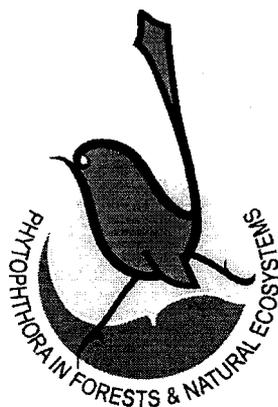
Development of disease caused by *Phytophthora cinnamomi* in mature *Xanthorrhoea australis*

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Abstract. Xanthorrhoea australis (Austral Grass-tree) is a species identified to be a good indicator of disease caused by *Phytophthora cinnamomi* in native forests and heathlands within Victoria. The plant is under serious threat from *P. cinnamomi* invasion and shows rapid death upon first infection from the disease. This research shows that the proportion of disease within the plant is relative to the disease symptoms or decline stage (DS) shown by the plant in the form of chlorosis to leaves. Plants showing severe disease symptoms had 67% and 86% of roots infected, while those with less severe disease symptoms had 40% of roots infected. One dead plant and all healthy plants had no infected roots. Isolation of the pathogen from roots showed a large variation in distribution of the pathogen within the roots of each plant. Microscopy showed that the pathogen is situated through xylem and metaxylem within the roots. Massive lesions were located in plants with severe disease symptoms but isolation from these lesions proved difficult

Phosphite controls *Phytophthora cinnamomi* at Anglesea and Wilson's Promontory National Park, Victoria

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Abstract. The use of phosphite at concentrations of 2 and 4g a.i./L proved to be successful in controlling the spread of *Phytophthora cinnamomi* within different vegetation communities at Anglesea (Alcoa lease area) and Tidal River (Wilson's Promontory National Park). At nine sites, phosphite was sprayed to runoff with a Solo® 422 motorised backpack sprayer with 2L/25m^2 quadrat. Using aerial and ground photography, quadrats were monitored over a two-year period to determine changes in species abundance due to the pathogen. There was a significant difference (p<0.05) between the proportions of healthy *Xanthorrhoea australis* in phosphite sprayed quadrats (2 and 4g a.i./L with 0.5% surfactant) compared to those sprayed with a control (water and 0.5% surfactant, water only). Vegetation in quadrats where *P. cinnamomi* was present was protected by phosphite for 2 years (4g a.i./L) and 12 months (2g a.i./L). However, vegetation in quadrats where the pathogen was present died where phosphite was not sprayed. Phosphite provided protection for *X. australis*, *Isopogon ceratophyllus* and *Monotoca scoparia* (3 species highly susceptible to *P. cinnamomi*). From the results of this research we recommend the use of phosphite in susceptible vegetation communities with 4g a.i./L phosphite to assist with already existing management strategies for the protection of Victorian heathlands and forests from *P. cinnamomi*.

The dilution of phosphite in rapidly growing plants and how soil and plant phosphate levels interact with phosphite and its ability to induce host-resistant responses when challenged by *Phytophthora cinnamomi*

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Abstract. The soil borne plant pathogen *Phytophthora cinnamomi* has irreversibly altered the make-up and diversity of the plant communities found in Australia. Recently, the fungicide phosphite has been used to effectively reduce the impact of this pathogen in natural plant communities. However, little is known (a) about how rapidly phosphite is diluted in the tissues of rapidly growing plants and (b) how soil and plant phosphate levels interact with phosphite and its ability to induce host-resistant responses when challenged by *P.cinnamomi*. This study examines the effects of phosphite dilution in different size classes of *Banksia grandis* and *B. hookeriana*. It also examines the effects of different soil phosphate levels on *in planta* phosphite and phosphate status in *B.hookeriana,* and its subsequent control of *P.cinnamomi,* and in the glasshouse and *Eucalyptus marginata* forest of Western Australia.

Rare flora threatened by *Phytophthora cinnamomi* in the Albany area, Western Australia

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Abstract. The plant pathogen *Phytophthora cinnamomi* is the key threatening process for 20 out of 70 threatened plant taxa in the Albany District with some or all populations of these species currently infested by the pathogen. Many of these species are naturally rare with highly localised distributions and small population sizes which is a feature of many south-western plants. Ten of these taxa are currently ranked 'critically endangered' using IUCN conservation categories, that is these are species facing an extremely high probability of extinction in the immediate future. Several poorly known 'Priority' taxa may also be threatened by *P. cinnamomi* but cannot be categorised as 'threatened' until adequate survey is carried out. Plant taxa threatened by *P. cinnamomi* are managed through the implementation of recovery plans and management programs for threatened flora. Recovery actions include regular monitoring, management of access to threatened flora populations, phosphite application and monitoring, seed collection or collection of other material for propagation by CALM's Threatened Flora Seed Centre, plant propagation by seed or cuttings to re-establish populations of threatened flora in the wild, research into the biology and ecology of threatened taxa, further survey to locate new populations, fire management and community education.

Alnus cordata mortality in Italy by a new *Phytophthora* species

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Abstract. Alnus cordata Loisel. is largely utilized in central Italy for reforestation of badly drained and wet soils, and for agro-forestry purposes. Recently wilting and mortality of *A. cordata* individuals were observed in a nursery on 1-2 years old seedlings in Northern Tuscany (4) and in a 6 years old plantation in Umbria. Among the different woody species present the latter site (alder, cherry and walnut), only *A. cordata* trees showed symptoms resembling those of Ink disease. They were characterized by sparse yellowish-brown foliage with abnormally small leaves and dark stained necrosis of the bark at collar level (1). In the nursery death of tap root and lateral roots and dying of seedlings were also observed. Tissue isolation from infected parts of the plants yielded a *Phytophthora cambivora*-like species in culture. The morpho-physiological features of the alder isolates from the nursery resembled those of the Swedish variant of the Alder *Phytophthora* (2, 3). Two-year-old alder seedlings (1.3 cm diameter, height 70 cm) grown in pots were wound-inoculated in the trunk with a *Phytophthora* isolate from the nursery. Six weeks later symptoms identical to those described above were observed and from diseased bark tissue yielded fungal colonies resembling those described above.

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ALNUS CORDATA MORTALITY IN ITALY BY A NEW PHYTOPHTHORA SPECIES

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Alnus cordata Loisel. is largely utilized in central Italy for reforestation of badly drained and wet soils, and for agro-forestry purposes. Recently wilting and mortality of *A. cordata* individuals were observed in a nursery on 1-2 years old seedlings in Northern Tuscany (Ref 4) and in a 6 years old plantation in Umbria Among the different woody species present the latter site (alder, cherry and walnut), only *A cordata* trees showed symptoms resembling those of Ink disease They were characterized by sparse yellowish-brown foliage with abnormally small leaves and dark stained necrosis of the bark at collar level (Ref. 1). In the nursery death of tap root and lateral roots and dying of seedlings were also observed.
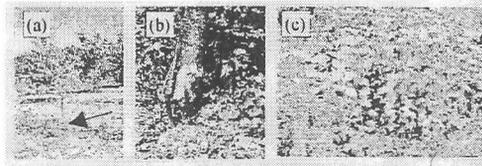
Alnus cordata trees (a, b) and seedlings (c) infected by alder *Phytophthora*.
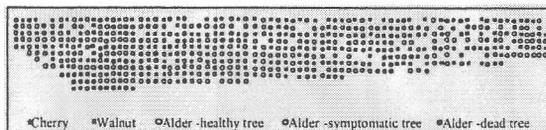
Plantation site map showing the distribution of the symptomatic trees

Material and Methods

Isolation and identification Tissue isolation from infected parts of the plants was attempted on material coming from the nursery and the plantation. After survey in a mixed plantation with Alder, Cherry and Walnut in Umbria, a site map showing the distribution of the symptomatic trees was prepared.

Pathogenicity tests. Two-year-old Alder seedlings (1,3 cm diameter, height 70 cm) growing in pots, were wound-inoculated at the collar level with a *Phytophthora* strain (CBS 109280) isolated from the nursery.

Results

Isolation and identification. *Phytophthora cambivora-like* colonies were obtained from the margin of the lesions of infected Alder plants. The fungus was identified as Alder *Phytophthora* and the isolate kept in collection (CBS 109280). Differently from *P. cambivora* the isolates were homothallic, with two-celled amphigynous antheridia and ovoid, non-papillate sporangia. The morpho-physiological features of the Alder isolates from the nursery resembled those of the Swedish variant of the Alder *Phytophthora* (Ref. 2, 3).

In the plantation the disease resulted extremely selective to Alder (no infection was found on Cherry and Walnut) and uniformly distributed (see map).

Pathogenicity tests. Six weeks after inoculations on Alder seedlings, symptoms identical to those described above were observed and isolations from infected bark tissue yielded fungal colonies resembling those of the original organism.
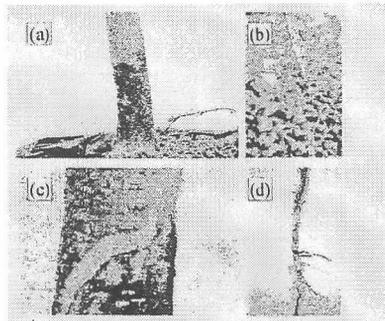
Phytophthora lesions at collar level on young trees (a, c) and seedlings (b, d).
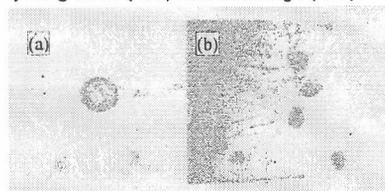
Smooth-walled oogonium with a two-celled amphigynous antheridium (a) and ovoid, non-papillate sporangia (b) of the alder *Phytophthora* nursery isolate

Discussion

Alder *Phytophthora* has been described for the first time in England in 1995 (Ref.2) and later also in northern and central Europe, particularly on *A. glutinosa*. This is the first time that this organism is also reported for the Mediterranean region. In this case the main host species was *A.cordata*, a more termophylous species. After these founding in central Italy, it seems that this recently reported fungus may cause disease on Alder also in the drier and warmer climates of southern Europe. Due to the large diffusion of *A cordata* both in natural and artificial stands, especially in the southern part of Italy, great attention must be devoted to this new pathogen. In particular, nurseries must be kept under control because they could be the source of inoculum of the disease in Italy.

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247

Variation, distribution and pathogenicity of the hybrid alder *Phytophthoras*

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Abstract. Results of further studies on comparative variation in the standard and variant heteroploid *Phytopthora* hybrids now spreading on alder in Europe, and on the putative parent species of the hybrids, will be presented. This will include data on differences in the stability of their asexual propagules; oospore viability; pathogenicity to alder bark; host specificity; molecular profiles and geographic distribution. The issue of the taxonomic status of the hybrids, and the issue of the potential threat to alders on other continents, will be discussed.

Phytophthora species infecting typical plants of the Mediterranean region

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Abstract. Phytophthora species can be spread worldwide through nursery stocks or propagation material of cultivated plant species. In recent years, typical plants of the Mediterranean flora have been used increasingly for reforestation of natural reserves or coastal areas, for ornamental purposes in park and gardens and to form edges lining cultivated fields or motorways. A survey was made of nurseries in Italy to identify the species of *Phytophthora* causing root rot of pot-grown forestry and ornamental plants typical of the Mediterranean flora.

Phytophthora isolates were obtained from either roots or soil, using both selective culture media and plant baiting, and were identified on the basis of morphological and cultural features, the electrophoretic pattern of mycelial proteins (total proteins and isozymes), the polymorphism of DNA sequences amplified by RAPD-PCR as well as the PCR amplification of all or part of the internal transcribed spacers (ITS) of rDNA with restriction digests (ITS-RFLP) of the resultant products combined with limited DNA sequencing.

P. nicotianae Breda de Haan was the most frequently recovered species. It was isolated from many hosts, including hawthorn, laurel, *Cystus* spp., strawberry-tree, lavender, lentisk, myrtle, rosemary, and English ivy. Both mating types (A1 and A2) of this species were found. Other species, such as *P. cactorum* (Lebert & Cohn) Schröeter, *P. cryptogea* Pethybr. & Laff. (A1 and A2), *P. drechsleri* Tucker (A1), *P. capsici* Leonian (A1 and A2), *P. palmivora* (E. Butler) E. Butler (A1), *P. gonapodyides* (Petersen) Buisman and *P. citrophthora* (R. E. Sm & E. H. Sm.) Leonian were isolated less frequently. *P. cinnamomi* Rands (A2), a polyphagous species, reported sporadically in Italy in last years and potentially very dangerous for ornamental and forestry plants, was isolated from potted myrtle, Port Orford cedar and chestnut plants. Most isolates recovered from chestnut were identified as *P. cambivora* (Petri) Buisman, a well-known pathogen of this tree species and widespread in forests and plantations of chestnut in Italy. A number of new or rare host-pathogen combinations were found, including *P. palmivora* on olive and *Pittosporum, Phytophthora* sp. "O-group" on *Prunus* and queen palm, *P. drechsleri* on rosemary, *P. capsici* on silverbush and *P. gonapodyides* on *Prunus*. The morphological features of the *P. capsici* isolate from silverbush were very similar to those of *P. tropicalis sensu* Aragaki and Uchida.

248

Strategic review of *Phytophthora cinnamomi* in parks and reserves in Victoria, Australia

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Abstract. *Phytophthora cinnamomi* continues to have a significant impact on flora and fauna in Victoria. Parks Victoria aims to quantify the effects of threatening processes and this project was initiated to review the impact of disease across the park estate. It addresses several of the objectives of the draft National Threat Abatement Plan for *P. cinnamomi* including promoting recovery of endangered or vulnerable species and ecological communities, preventing invasion into new areas, and improving knowledge of the disease process. Specific objectives were to determine the current and potential distribution of the pathogen and distributions of susceptible species and communities. Also examined were environmental and anthropogenic factors that may limit or spread *P. cinnamomi*. A literature review and geospatial analyses have determined the historical, current and predicted extent of *P. cinnamomi* across Victoria. The analyses identify and map potentially susceptible vegetation associations and individual species. Risk of infestation is predicted through analyses of environmental factors, vectors, and pathogen distribution. A GIS layer documents the distribution and potential impact of *P. cinnamomi* on native communities. Modelling the potential for disease spread and the level of risk by geographic locality enables management options and priorities to be identified throughout the park estate.

Long term survival of *Phytophthora cinnamomi* in mature *Banksia grandis* trees in remnant jarrah forest

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Abstract. Effective management of *Phytophthora cinnamomi* requires knowledge of its ability to survive adverse conditions in soil and plant tissue. We have assessed the long-term survival of *P. cinnamomi* in *Banksia grandis* trees over 18 months in jarrah forest in the Southwest of Western Australia. Thirty-six *B. grandis* trees were killed by underbark inoculation with *P. cinnamomi* 10cm below ground level. To assess distribution and survival of the pathogen, 4 dead trees were harvested at the time of death and a further 4 at each of 12 and 18 months after death. A further 9 standing dead trees were sampled bi-monthly for 18 months by removing 1 cm diameter cores 10cm and 40cm above and below the soil line. *P. cinnamomi* colonisation of standing dead trees declined over time. The pathogen was isolated from 54% of sample cores 2 months after death, and only 2.4% after 12 months. In the early months after death, there was a higher percentage of recovery of the fungus from cores from above, rather than below ground tissue (eg. after 2 months 61.1% of samples above and 46.4% from below the soil line were colonised), while approximately 12 months later the values were 0.3% of colonised samples above and 4.3% from below the soil.

Aspects of the interaction between *Xanthorrhoea australis* and *Phytophthora cinnamomi* in Victoria, Australia

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Abstract. Xanthorrhoea australis, a monocotyledon indigenous to Australia, forms an important component of the eucalypt woodland and heathland ecosystems of southern Victoria. The species is highly susceptible to infection by *Phytophthora cinnamomi*, a destructive soil-borne pathogen of many native Australian species. This project investigated the morphological and genetic variation within and between populations of *P. cinnamomi* in Victoria. A large number of isolates from the Anglesea heathlands were analysed using characteristics such as the morphology of sporangia and oogonia, pathogenicity and growth rates. Victorian isolates were also compared with isolates from Western Australia. Recently, the systemic fungicide potassium phosphonate (phosphite) has been used to control disease caused by *P. cinnamomi* in native ecosystems. The study also examined characteristics of the interaction between *X. australis* and *P. cinnamomi* and the effect of phosphite on the host defence response. Cell suspension cultures of *X. australis* were developed to investigate the mechanisms by which phosphite induces host resistance in the host-pathogen interaction. The effect of phosphite on the host response to pathogen attack was also examined using histological techniques and biochemical analyses. Defence responses were enhanced in tissues treated with phosphite, including elevated production of phenolic compounds, callose and lignin.

Histological analysis of the effect of phosphonate on the interaction between *Phytophthora cinnamomi* and *Xanthorrhoea australis*

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Abstract. Plants respond to pathogen attack by producing chemical and physical barriers. Where the plant's defence response is sufficient, invasion by the pathogen is successfully prevented and a resistant reaction occurs. Susceptible plants are unable to mount a defence response of sufficient speed or magnitude to overcome the pathogen. The systemic fungicide, potassium phosphonate (phosphite), successfully controls disease caused by Oomycetes. It is believed to have two modes of action: at higher concentrations phosphite directly inhibits pathogen growth, while at lower concentrations it acts indirectly to alter pathogen metabolism and stimulates cellular defence mechanisms. *Xanthorrhoea australis*, an important component of the native vegetation communities of southern Victoria, is highly susceptible to *Phytophthora cinnamomi*. The mechanisms by which phosphite induces host resistance in the interaction between *X. australis* and *P. cinnamomi* were investigated using light and transmission electron microscopy. Defence responses including lignin, callose and phenolic production were found to be enhanced in tissues treated with phosphonate. Cells in untreated tissues displayed greater disruption of tissue structure, nuclear degeneration and shrinkage of the cytoplasm and cell membrane upon inoculation compared with phosphite treated seedlings. Thickening of the cell wall, particularly in vascular tissues, was also observed following phosphite treatment.

Will *Phytophthora cinnamomi* become resistant to phosphite with its increasing use?

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Abstract. Phosphite is increasingly being used as a means of control for dieback caused by *Phytophthora cinnamomi*. We wish to study the likelihood of *P. cinnamomi* evolving resistance to phosphite, given the clonal populations of the fungus present in Western Australia. We have collected isolates of *P. cinnamomi* from areas where phosphite has been used intensively for up to 15 years (avocado orchards) as well as areas of less frequent use and no use of phosphite. Our testing involved stem inoculating a clonally propagated host (*Leucadendron* sp.) that was treated with one of three levels of phosphite (0%, 0.25% and 0.5%). We measured the extent of colonisation by each *P. cinnamomi* isolate after eight days of incubation in a controlled temperature plant growth cabinet. Preliminary results suggest that less aggressive isolates are not present in populations obtained from areas where phosphite has been used. Also, the few isolates that colonise the phosphite treated host to a large extent, all come from areas of phosphite use. Research is continuing to replicate these results and investigate their significance to the control of *P. cinnamomi* using phosphite.

Continent-wide clonal lineages of *Phytophthora cinnamomi* show frequent mitotic recombination

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Abstract. Genetic studies of *Phytophthora cinnamomi* using isozymes have revealed low levels of diversity suggesting, though not proving, clonality in a large proportion of worldwide populations (1). In Australia, only three isozyme types (representing both mating types) are found with no evidence for sexual recombination (2). Using microsatellite markers, we have shown that these isozyme types are clonal lineages of *P. cinnamomi* and that these same lineages are found elsewhere in the world (3). Our study used 647 isolates from three intensively and hierarchically sampled *P. cinnamomi* disease fronts located in south-west Australia. In addition 133 isolates from an Australia-wide culture collection and 27 isolates from elsewhere in the world were analysed with four microsatellite markers. One disease front contained all three clonal lineages within close proximity in soil and plant tissue but no sexual recombinant isolates were found, even with very intensive sampling. However, within these clonal lineages we frequently found evidence for mitotic recombination (mitotic crossing over). This mechanism for producing genetic variation may explain phenotypic variation known to occur within the identified clonal lineages.

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Biological control of *Phytophthora cinnamomi*: the potential of five Western Australian native *Acacia* species to protect *Banksia grandis*

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Abstract. Mortality of *Eucalyptus marginata* seedlings following infection by *Phytophthora cinnamomi* was lower when planted with *Acacia pulchella* than when planted with *Banksia grandis* (1). The protective effects of native legumes other than *A. pulchella* against *P. cinnamomi* have not been determined. Field and glasshouse inoculation trials were set up to investigate the protective potential of five Western Australian native *Acacia* species for *B. grandis*. In the field only *A. pulchella* protected *B. grandis* against *P. cinnamomi* infection. Mortality of *B. grandis* planted with *A. pulchella* was as low as uninoculated *B. grandis* planted alone. Mortality of *B. grandis* planted with *A. urophylla*, *A. extensa*, *A. latericola* or *A. drummondii* was high and similar to inoculated *B. grandis* planted alone. In the glasshouse none of the *Acacia* species definitively protected *B. grandis*. Mean mortality due to infection by *P. cinnamomi* of *B. grandis* planted with *A. pulchella* or *A. latericola* was less than the control. However the remaining *B. grandis* seedlings died following infection by an unidentified fungus, hence protection could not be concluded.

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Biological control of *Phytophthora cinnamomi*: the potential of Western Australian native legume species to reduce inoculum levels in soil

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Abstract. Sporulation by *Phytophthora cinnamomi* is significantly suppressed in forest sites dominated by *Acacia pulchella* compared to forest sites dominated by species of Proteaceae (1). In this investigation an inoculation trial was conducted to determine the effect of 14 other Western Australian native legumes on population levels of *P. cinnamomi* in the soil compared to *Banksia grandis*. Direct plating of soil onto *Phytophthora* selective agar was used to quantify inoculum levels. *A. alata*, *A. extensa A. latericola*, *A. pulchella*, *A. stenoptera*, *Kennedia coccinea* and *K. prostrata* showed low mortality and decreased inoculum of *P. cinnamomi* in soil compared to *B. grandis*. *A. drummondii*, *A. urophylla* and *Viminaria juncea* also showed low mortality but had no effect on inoculum of *P. cinnamomi*. *Bossiaea aquifolium*, *Daviesia decurrens*, *Hovea chorizemifolia*, *Labichea punctata*, *Mirbelia dilatata* and *B. grandis* showed high mortality due to *P. cinnamomi* infection. Of these species population levels were only quantified from *B. grandis* pots for comparison.

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The Vegetation Health Service: a resource for researchers and managers of *Phytophthora* disease

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Abstract. The mapping of disease caused by *Phytophthora cinnamomi* in Western Australian native forest is a major component of the Department of Conservation and Land Management's (CALM's) management responsibilities prior to logging and mining activities. The Vegetation Health Service (VHS) within the CALMScience Division is currently responsible for accurate detection and identification of *Phytophthora* species for disease mapping. Since 1984 the VHS has managed a comprehensive database of every sample assessed for *Phytophthora*. To date there are approximately 21,000 records from areas ranging from Eneabba north of Perth to Albany south east of Perth. Records include 6,759 positive detections of *P. cinnamomi* and 1,430 of other species of *Phytophthora* including the first report of *P. boehmeriae* in Western Australia. A culture collection of *Phytophthora* species is maintained by the VHS with currently 830 cultures of *Phytophthora*, 377 of which are *P. cinnamomi*. Other species include *P. cactorum, P. citricola, P. cryptogea, P. drechsleri, P. megasperma* and *P. nicotianae*. The database and culture collection are maintained as a resource for internal and external groups involved in *Phytophthora* research and management.

Developing Christmas trees resistant to Phytophthora root rot

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Objective:
To combat the threat of phytophthora root rot to North Carolina's Christmas tree industry by developing resistant planting stock of Fraser fir (*Abies fraseri* [Pursh] Poir.) and other *Abies* species.

North Carolina's Christmas Tree Industry

Fraser fir is one of the premier Christmas tree species in the United States. In North Carolina, this species accounts for 98% of the $100+ million annual revenue from Christmas tree sales. Over 80% of the 6-7 million Fraser fir Christmas trees produced in North Carolina each year are sold wholesale and shipped nationwide.

Phytophthora Root Rot

Phytophthora root rot, primarily caused by *Phytophthora cinnamomi* Rands., results in an estimated $1.3 million in direct losses and an additional $4.5 million in lost potential revenue to North Carolina's Christmas tree industry annually. The incidence of phytophthora root rot averaged 9% over Fraser fir sites sampled in a recent survey. An unknown number of additional infested sites have been abandoned for Christmas tree production. While control in nursery beds is feasible with metalaxyl, proper site selection is the only practical preventative measure for the field, but often, is not effective.

Fraser Fir

The development of resistant Fraser fir planting stock could provide North Carolina Christmas tree growers an effective, low-cost option to combat losses from phytophthora root rot.

Greenhouse inoculation trials have confirmed conclusions from experiences in highly infested Christmas tree plantations that Fraser fir is extremely susceptible to *P. cinnamomi*. Overall, mortality of Fraser fir seedlings in one greenhouse inoculation trial was 90% after 122 days but varied significantly among geographic seed sources (Figure 1).

In another greenhouse inoculation trial, mortality of 100 Fraser fir open-pollinated families from a N.C. Division of Forest Resources seed orchard ranged from 91 to 100% with 42 families exhibiting 100% mortality (Figure 2). The inoculated families were largely from Roan Mountain. In a follow-up study, seedlings are currently being grown to inoculate 15-20 open-pollinated families from each of the six major sources of Fraser fir.

Putatively resistant Fraser fir seedlings surviving greenhouse inoculation trials are presently being vegetatively propagated via rooted cuttings. Additionally, healthy Fraser fir seedlings selected from heavily infested Christmas tree plantations are being propagated. Unfortunately, vegetative propagation of Fraser fir is problematic due to 1) very slow growth rates, 2) low frequency of shoot production and 3) plagiotropic growth of cuttings rooted from lateral shoots.

Any putative resistant Fraser fir seedlings that are successfully cloned will be further tested for resistance to an array of *P. cinnamomi* isolates in additional greenhouse inoculation trials as well as established in field trials on highly infested sites.
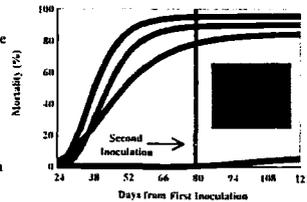
Figure 1. Mortality curves for three geographic sources of Fraser fir inoculated in the greenhouse with *P cinnamomi* (combined over five inoculum isolates).
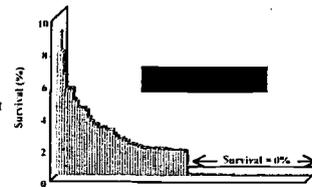

Figure 2. Survival of 100 open-pollinated families of Fraser fir (Roan Mountain source) 16 weeks after the first of two inoculations in the greenhouse with *P. cinnamomi*.

Alternative Fir Species

Alternative fir species are also being investigated as potential sources of phytophthora rot root resistance because 1) the frequency of resistance in Fraser fir is low or nonexistent and 2) propagation difficulties will impede deploying any resistance identified in Fraser fir.

In two independent greenhouse inoculation trials of selected *Abies* species at Washington State University (Figure 3, left) and at North Carolina State University (Figure 3, right). momi or Japanese fir (*A. firma* Sieb. & Zucc.) and Turkish fir (*A. bournmuelleriana* Mattf.) proved to be very resistant to *P. cinnamomi*. Seedlings from 35 species and 44 taxa of *Abies* are being cultured for a more comprehensive greenhouse resistance screening trial.

Field tests have been established to further evaluate the phytophthora root rot resistance of alternative fir species as well as their growth, adaptability and Christmas tree quality. Of the two most resistant species identified to date, Turkish fir is used as a Christmas tree species in Europe while momi fir is not considered to be of Christmas tree quality due to its coarse foliage.

Alternative fir species may also be used as resistant rootstock for grafting Fraser fir planting stock. A grafting study has demonstrated that interspecific grafting within the *Abies* genus is feasible (Figures 4 and 5). Field trials have been and will be established to evaluate the field resistance, growth and Christmas tree quality of these grafts.
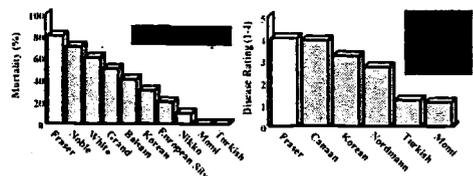

Figure 3. Mortality (left) and disease rating (right) of greenhouse seedlings of selected *Abies* species inoculated with *P. cinnamomi* in two independent studies.

Rootstock Species	Survival (%)
Canaan	93
Fraser	100
Korean	95
Momi	83
Nordmann	99
Turkish	97

Figure 4. Survival of Fraser fir scions one year after grafting onto rootstock of selected *Abies* species.

Figure 5. Fraser fir scion (above line) grafted onto momi rootstock (below line).

Genetic variation in *Phytophthora cinnamomi* isolated from Fraser fir in western North Carolina

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Abstract. Fraser fir (*Abies fraseri* [Pursh] Poir.) Christmas tree production is an economically important industry to the mountainous western region of North Carolina generating over $US 100 million in annual sales. Root rot disease caused by *Phytophthora cinnamomi* Rands. limits or prevents Fraser fir production on many sites of this region. Genomic DNA was extracted from 34 single zoospore cultures of *P. cinnamomi* and 1 culture of *P. dreschsleri* Tucker isolated from Fraser fir Christmas trees from 5 different counties. DNA fingerprints of these isolates were developed by amplified fragment length polymorphism (AFLP) technique using five primer pair combinations (*EcoR* I-AC with *Mse* I-AG, -CG, -GG-, -CT and -CA). Genetic similarity estimates and cluster analyses were used to group individual *P. cinnamomi* isolates and sub-populations

The use of mulches as a method of controlling *Phytophthora cinnamomi* in avocados

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Abstract. Phytophthora cinnamomi is a major pathogen of avocado trees grown commercially in New Zealand. It attacks the feeder roots that lie near the surface in the topsoil and litter layers. Soil microorganisms may have an important role in controlling *P. cinnamomi* (1). The application in avocado orchards of different mulches and composted materials has been reported to be disease suppressive by contributing to a proliferation and presence of controlling microorganisms with appropriate biological activity, although the mechanisms by which this occurs are not well understood. A study is being conducted into the effects of various organic mulches on the incidence of Phytophthora root rot on six-year-old plantings of Hass avocados in an orchard under certified organic management in the Bay of Plenty, New Zealand. Four different mulches have been applied and compared to a control without mulch on a total of 91 trees. Changes to various parameters of tree health are being monitored. In addition to assessing tree responses, an understanding of possible mechanisms of disease suppression is being sought with using molecular and biochemical methods as well as traditional culturing techniques.

1. You MP, Sivasithamparam K (1994) Hydrolysis of FDA in an avocado plantation suppressive to *P. cinnamomi* and its relationship with certain biotic and abiotic factors. *Soil Biology and Biochemistry* **26**, 1355-1361.

A new *Phytophthora* infects several plant species and causes extensive mortality of three tree species in coastal woodlands in California

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Abstract. Since 1994, epidemic level mortality of *Lithocarpus densiflorus, Quercus agrifolia,* and *Q. kelloggii* has been reported in a 300 km long stretch within the coastal region of central California. A causal agent was unknown until June 2000, when we isolated a *Phytophthora* sp. from cankers on diseased trees. The morphology and the ITS DNA sequence of the isolated pathogen match a newly described species from ornamental rhododendrons in Europe. Cankers are usually initiated at the basal part of the tree, but do not enlarge below the soil line. On the other hand, the pathogen can be frequently isolated from aerial cankers up to 20 m from the soil line. The pathogenicity of the new *Phytophthora* was confirmed through inoculation experiments on seedlings, saplings, and mature trees of *Q. agrifolia* and *L. densiflorus.* In addition to *Quercus* and *Lithocarpus* spp., the pathogen has also been found infecting ornamental *Rhododendron* spp., native huckleberry (*Vaccinium ovatum*), California bay laurel (*Umbellularia californica*) and Pacific madrone (*Arbutus menziesii*). On these additional hosts, the pathogen appears to cause a foliar blight often leading to a twig and branch dieback. While these hosts may not necessarily succumb to the disease, they are an important source of inoculum.

The decline of Australian mammals: Implications for ecosystem function in *Phytophthora* affected communities

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Abstract. Phytophthora cinnamomi has major effects on floristics and structure in native sclerophyll vegetation in Australia and recent studies have shown that *P. cinnamomi* can also alter the diversity and abundance of small mammals within affected areas. Overseas research has found that vertebrate fauna can influence a number of key ecosystem processes. This paper reviews some of the evidence for similar effects in Australia and examines the implications of a decline in vertebrate fauna for ecosystem function in *Phytophthora* affected communities.

The effects of digging on ecosystem function is demonstrated by studies of the Woylie (*Bettongia penicillata*) in Australia. Woylies forage for the underground fruiting bodies of ectomycorrhizal fungi and create up to 110 diggings per night. At this rate individual Woylies can disturb in excess of 5.5 tonnes of soil annually. Experiments using simulated woylie diggings show that they reduce soil water repellency, affect the availability of nutrients and alter the particle size distribution of the soil. These studies also show that a decline in their population results in a loss of this digging activity and suggests any large scale disturbance that alters the guild of fauna within an ecosystem may alter functional processes as well.

In experiments currently being initiated, the changes in functional processes due to altered fauna guilds will be examined in *Phytophthora* affected communities in southern Australia. It will provide a detailed examination of soil disturbance (biopedturbation) by a suite of digging species as they forage for the fruiting bodies of underground fungi (mycophagy). These fungi (ectomycorrhizae) play a vital role in the supply of nutrients to plants. However, there is little information on the impact of *Phytophthora* on the production of fruiting bodies, or the effects on vertebrate foraging and soil disturbance. Nectar resources and vertebrate pollination will also be measured.

Sudden oak death in Oregon

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COOP Aerial Survey and Ground Check

Sudden Oak Death (SOD), caused by the pathogen *Phytophthora ramorum*, was first discovered in the United States in California. There the disease is responsible for the death of thousands of trees, including tan oaks (*Lithocarpus densiflorus*) and coastal live oaks (*Quercus agrifolia*). Southwest Oregon is home to many of *P. ramorum's* host plants. In an effort to evaluate if SOD was present in Oregon. the Oregon Department of Forestry and USDA Forest Service did an aerial survey. Eighteen potential sites were spotted from the air. These sites were further pinpointed with a helicopter survey. The following week a ground check was done by a team of forest pathologists from the Oregon Department of Forestry, the Oregon Department of Agricultural, the Forest Service and Oregon State University. Four additional clumps of dead tanoak were located at that time. Plant and soil samples were taken.

Survey Findings

Confirmed SOD sites in Oregon are all within an area of about 10 square miles, close to Brookings on the South Coast (Fig.1). The Oregon infestation is about 350 km north of the nearest confirmed site in the California epidemic area. SOD sites in Oregon have been confirmed by symptoms, isolation and morphology, and PCR. A non-specific *Phytophthora* ELISA test was also used. At two additional sites there is apparently another *Phytophthora* species present (Table 1).

Oregon Confirmed Hosts and their Symptoms

To date only 3 hosts have been confirmed in Oregon forests. The most common host is *Lithocarpus densiflorus* where SOD causes bleeding cankers and eventually death (Fig.2).
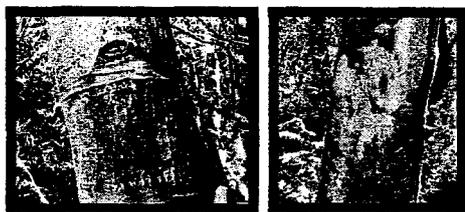
Figure 2. Tan oak bleeding canker on left and stem lesion on right.

Rhododendron macrophyllum and *Vaccinium ovatum*, common understory plants in the tanoak forest, both display tip dieback and stem lesions (fig.3).

Figure 2. Rhododendron tip dieback on left and stem lesion on right.

Table 2. Isolation success for *Phytophthora ramorum* at SOD sites in August 2001.

Sample Type	Success/ Plant or Soil Sampled	Success
Lithocarpus		
bark	11/22	50%
leaves and shoots	2/7	29%
Rhododendron	5/8	62%
Vaccinium	3/6	50%
Soil	4/16	25%

Quarantine

When the presence of *P. ramorum* is confirmed at a site. the center and buffer zone are flagged off. The Oregon Department of Agriculture has enacted a quarantine for these centers that encompasses its square mile section. The quarantine forbids the transportation of tanoak, black oak, coast live oak, evergreen huckleberry, Oregon myrtle, rhododendron, and madrone. This includes all plant material and associated soils.

Eradication of the Sudden Oak Death pathogen. *Phytophthora ramorum,* in infected sites in Southwestern Oregon

- All host plants for *P. ramorum* with in the infection center will be cut down and bucked as needed.

- Host plants within a 50-100 foot buffer zone around the active infection centers will also be cut down.

- All host material from within the infection center and buffer zone will be piled and burned.

- There will also be a broadcast burn throughout the center and buffer zone to incinerate fallen leaves and duff.
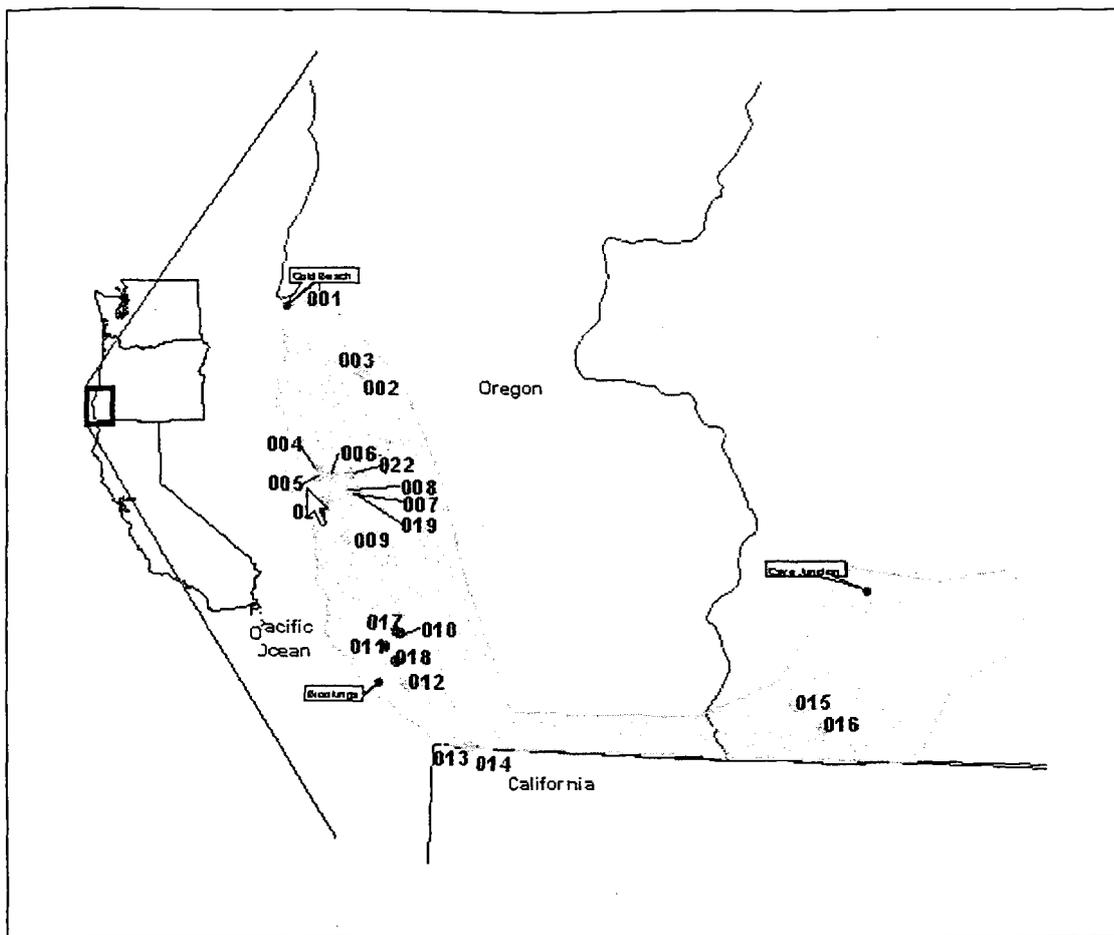
Figure 1. Shaded area represents the aerial survey. Suspected sites are numbered, with the presence of P. ramorum confirmed at sites 010, 011, 017 and 018.

Table 1. Survey findings.

Sites ID'd by Survey	Survey Findings	P. ramorum results		Phytophthora ELISA
		Culture	Tissue PCR	
001	Oceanspray			
002	Armillaria and SOD symptoms	-	-	
003	Landslide with down tanoak			
004	Armillaria	-		
005	Armillaria and SOD symptoms	-	-	
006	Armillaria and SOD symptoms	-	-	
007	Tanoak with callused growth	-	-	
008	Armillaria and SOD symptoms	-	-	+
009	Powerline – brush cutting			
010	SOD symptoms	+	+	
011	SOD symptoms	+	+	+
012	Declining alder			
013	SOD symptoms	-	-	
014	SOD symptoms	-	-	+
015	Herbicide treatment			
016	Herbicide treatment			
017	SOD symptoms	+		
018	SOD symptoms	+	+	+
019	Armillaria and SOD symptoms	-	-	
020	SOD symptoms	-	-	
021	Armillaria and SOD symptoms	-	-	
022	Single dead tanoak	-	-	

258

Phytophthora in Australasia and the way forward in disease management

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Abstract. Unlike the USA and Europe, Australasia has not in the last two years faced any unexpected developments in new *Phytophthora* diseases in forests and natural ecosystems. Consequently, we will focus on the way forward for disease management and examine the challenges that will need to be faced over the next few years to control the impact and rate of spread of this disease.

Measuring resistance in jarrah, *Eucalyptus marginata*, to *Phytophthora cinnamomi*: What factors change disease expression?

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Abstract. The interaction between *Eucalyptus marginata* (jarrah), the dominant and important timber species in jarrah forests, and *Phytophthora cinnamomi*, is not a co-evolved one. Jarrah appears to have a wide range of variability in resistance to *P. cinnamomi* in the forest. Jarrah clonal lines resistant (RR) and susceptible (SS) to the pathogen have been produced (1).

Our glasshouse mortality trial showed that the capacity of 73 isolates to cause disease ranged from killing all plants (59 days) to plants being symptomless (182 days) (2). Comparison of branch and root inoculations in situ confirmed that branches are a valid option for testing resistance of young jarrah (3). No jarrah clonal line maintained its resistance level in a series of experiments using different inoculation methods, different environmental conditions and when challenged by individuals from a large range of *P. cinnamomi* isolates (2-4). Even the most promising RR line had replicates that became diseased with time in various treatments.

To develop robust resistance, further screening work may be required using more isolates varying in their capacity to cause disease and a broader range of environmental conditions that favour the pathogen, particularly at 25-30ºC (4). Jarrah trees are affected by many environmental conditions during their life cycle (500-1000 years). Consequently, clonal lines that survive such rigorous screening may be durably resistant and survive in disease impacted sites.

1. McComb *et al.* (1990). Selection and propagation of jarrah for dieback resistance- -A progress report. *Combined Proceedings of the International Plant Propagators' Society* **40**, 86-90
2. Hüberli *et al.* (2001). Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. *Mycological Research,*. **105**, 1053-1064.
3. Hüberli *et al.* Evaluation of resistance to *Phytophthora cinnamomi* in seed-grown trees and clonal lines of Eucalyptus marginata inoculated in lateral branches and roots. *Plant Pathology.* **51**, 435-442.
4. Hüberli *et al.* (2002). Temperature and inoculation method influence disease phenotypes and mortality of *Eucalyptus marginata* clonal lines inoculated with *Phytophthora cinnamomi. Australasian Plant Pathology.* **31**, 107-118.

Summer rainfall and the development of disease caused by *Phytophthora cinnamomi* in droughted *Eucalyptus marginata* plants

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Abstract. Summer rainfall seldom occurs in the south-west of Western Australia. Flora of the region has evolved under a Mediterranean climate having hot dry summers with rare thunder storms, and cool wet winters. The consequence of high summer rainfall is significant because the resulting conditions can favour an outbreak of the introduced soil pathogen, *Phytophthora cinnamomi*, to which many plant species in the south-west forests are susceptible.

A glasshouse experiment compared the response of drought stressed and non-stressed *Eucalyptus marginata* (jarrah) plants when inoculated with *P. cinnamomi* after a simulated summer rainfall event. Clonal plants, resistant or susceptible to *P. cinnamomi*, were tested. The moisture content of the container substrate for inoculated and non-inoculated plants was either kept at container capacity or at a pre-determined level just above that of the wilting point of each plant for 3 weeks. Sudden restoration to container capacity simulated summer rainfall and plants were inoculated immediately. Higher proportions of non-stressed clonal plants, both resistant and susceptible, became infected and were more extensively colonized by the pathogen than plants subjected to drought. Results supported our hypothesis : tissue of drought affected *E. marginata* plants is less susceptible to infection by *P. cinnamomi* than tissue of plants which have recently experienced no water deficit.

Phytophthora spp. associated with *Eucalyptus smithii* dieback in South Africa

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Abstract. *Eucalyptus smithii* is cold tolerant and thus ideal for commercial propagation in high altitude areas of South Africa. It grows fast and has superior pulping properties compared to other commercially grown eucalypts. *Phytophthora* dieback, however, limits the afforestation potential of this species. In this study, a survey was conducted in newly established *E. smithii* stands in the KwaZulu/Natal province of South Africa to identify and map the occurrence of *Phytophthora* spp. Soil and plant samples were collected from diseased trees and their Global Positioning System (GPS) co-ordinates recorded. *Phytophthora* spp. recovered from the soil and diseased plant material were identified using morphological characteristics and sequence data from the ITS region of the rDNA operon. Three *Phytophthora* spp., namely *P. cinnamomi*, *P. citricola* and *P. nicotianae* were recovered from soil and diseased plant material. Our results suggest that *P. nicotianae* is the most common species associated with *E. smithii* death. This is in contrast the previous view that *P. cinnamoni* is the dominant pathogen in this environment.

Ink disease distribution on sweet chestnut in France, Italy and Greece and *Phytophthora* species associated

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Abstract. Ink disease of sweet chestnut (*Castanea sativa*) represents a serious threat to the survival of chestnut groves in several European countries, including France and Italy. In these two countries, the disease is present throughout the range of the host in different stands types, including orchards, coppices and naturalised forests. Recent surveys carried out in representative chestnut areas in France and Italy confirmed a widespread presence of the disease with few exceptions. *Phytophthora cinnamomi* and *Phytophthora cambivora* were the species most frequently isolated from soils in infested groves in France and in Italy respectively. These two species are also considered the most aggressive to chestnut. However, other *Phytophthora* species have been identified from soil in chestnut stands. *P. citricola* and *P. cactorum* were occasionally recovered in France and Italy but their presence is not constantly associated with symptomatic trees. More species have been identified, among which *P. gonapodyides* (mainly associated with seasonal streams crossing chestnut groves) and *P. syringae*. Of particular interest is the detection of *P. cryptogea* previously reported as associated with severe wilting of chestnuts in South Australia.

Ink disease distribution on sweet chestnut in France, Italy and Greece and *Phytophthora* species associated.

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On sweet chestnut (*Castanea sativa* Mill.), *Phytophthora cinnamomi* and *P. cambivora* cause rapid tree death by infecting and invading the root system and by producing extensive lesions in the collar. This disease was designated as ink disease because of the inky fluid which occasionally exudes from the lesions. In Europe, it has been reported since 1838 (in Portugal). Currently, it is present in most chestnut areas in forest plantations, coppices and orchards. In southern Europe, ink disease has increased significantly in last decades (Vettraino *et al.* 2000).

In favour of the European Union project **CASCADE** (EU Environment Project, No. EVK2-CT-1999-00006) dealing with the conservation of chestnut in Europe, a common study was carried out in the main chestnut areas in France, Italy, Greece, Spain and United Kingdom in order to document the distribution of *Phytophthora* species and the intraspecific variability of resistance of chestnut to this disease in these countries. We report here preliminary data on ink disease distribution in three of these countries.

Root infection of a seedling and bark lesion caused by *P. cinnamomi*.

Countries where CASCADE survey was carried out (in yellow)

Material and methods

In each country, **6 to 8 chestnut sites** were visited, and in each site 2 or 3 chestnut plots were studied. They could be old chestnut groves, chestnut coppices or naturalised chestnut areas. Health status of **26 chestnut trees** per plot was assessed with a five level score.

Soil sampling was performed (4 subsamples per tree) for 10 trees per plot, on healthy or declining trees, in spring and autumn. **Isolation from soil** of *Phytophthora* species is achieved by biological baiting (using chestnut leaf disks and PARBHy selective medium, Robin *et al.* 1998). When bark lesions were present, isolation was attempted from bark.

Identification of isolates was performed using morphological and molecular markers.

Chestnut mortality caused by *P. cinnamomi*.

Results

Ink distribution and Phytophthora species identified :

France

P. citricola
P. cryptogea

Britany

Dordogne

Aveyron
Cévennes

P. cambivora

Pyrénées

P. cambivora

P. cambivora

P. citricola

P. cambivora

P. citricola
P.cactorum

Italy

Var

Corsica

P. cactorum

P. cambivora
P. citricola
P.cactorum
P.syringae
P.cryptogea
P.gonapodyides

P. cambivora

P. citricola

P. cambivora

P. cambivora

P. cambivora

P. cambivora

Greece

- Ink disease reported before CASCADE survey
  (P. cambivora isolated in Italy, *P. cambivora* and *P. cinnamomi* in France)
- during CASCADE survey:
- *P. cinnamomi* or *P. cambivora* isolated
- *P. cinnamomi* and *P. cambivora* not isolated, but ink disease observed
- No symptoms of ink disease

During CASCADE surveys ink disease symptoms were observed in all areas investigated in Italy and Greece, except in Sicily. However, Phytophthora species have not been isolated everywhere. Drought which occurred last years, especially in Greece, may have exacerbated dieback symptoms and decreased soil inoculum pressure.

In France, studied chestnut trees were healthy in Britany, the Cévennes and in Corsica, but ink disease has already been reported in these areas.

In France, both *P. cinnamomi* and *P. cambivora* were isolated. We never found both species in the same plot. However we know they can coexist at the region level. For example, in the Pyrénées we detected *P. cambivora* in the studied plots but *P. cinnamomi* has been isolated several times in this area on oaks or chestnuts.

In Italy and Greece, *P. cambivora* was the most frequent pathogenic Phytophthora species detected in soil or bark tissues. Before this study *P. cinnamomi* had not been isolated in Italian chestnut plots (Vettraino *et al.* 2000).

P. cinnamomi and *P. cambivora* was found in all type of chestnut plots: forest coppices, orchards or abandoned chestnut groves. However, presence of these species seemed to be linked to human activities: plant introduction from nurseries, irrigation or sylvicultural works...

References:

Robin *et al.* 1998, Ann Sci. For. 55:869–883; Vettraino *et al.* 2000, Plant Pathology 49:1-8.

Second International IUFRO meeting on Phytophthora in Forests and Natural Ecosystems.

The holistic approach to *Phytophthora* management on Kangaroo Island, South Australia

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Abstract. Kangaroo Island is a unique natural environment with approximately 47% of its native vegetation still intact. The Island's habitats support the largest number of endemic species of any region in SA and many species of state and national conservation significance. The Island's isolation has saved it from the impact of foxes and rabbits, but unfortunately, not the introduction of *Phytophthora*. Loss of native vegetation and animal habitat through *Phytophthora* infestation is a major threatening process that has had devastating impacts on Kangaroo Island's biodiversity.

Kangaroo Island is at the forefront of *Phytophthora* management in South Australia. The Island's isolation, distinct boundary, and presence of only one port for vehicle access, has enabled a very holistic and unique approach to *Phytophthora* management.

While our management approach focuses on maintaining hygienic practices, we are broadening our efforts to understanding and actively managing outbreaks, particularly in regards to the protection of threatened species. To do this we are undertaking the following activities:

*mapping the distribution and extent of *Phytophthora*;
*developing a set of ecological criteria and monitoring sites to predict *Phytophthora* distribution, rate of spread and potential for infection;
*developing a strategy for prioritising areas in need of protection given the proximity and degree of threat from *Phytophthora*;
*determining the impact of *Phytophthora* on nationally threatened species, and developing Management Plans for and initiating threat abatement strategies; and
*determining the effectiveness of Phosphite as a means of preventing *Phytophthora* infection in SA.

With this integrated approach, we hope to better manage *Phytophthora* on Kangaroo Island, and maintain the region as an area of high conservation significance.

Occurrence of the alder (*Alnus glutinosa* L.) decline in Sweden and affinities of the causal *Phytophthora* pathogen as assessed by isozyme analysis

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Abstract. In 1996, a *Phytophthora* sp. was isolated from bark lesions on the stem bases of diseased alder trees on the banks of the river Säveån in Gothenburg. The isolated fungus was pathogenic to alder seedlings in pot tests and in its morphology and cultural characteristics, it resembled the alder *Phytophthora* originally isolated from the UK. Although clearly similar to *P. cambivora*, the Swedish alder isolates could be distinguished from this species on the basis of their homothallism, disrupted gametogenesis, growing with only small amounts of aerial mycelium on agar, and a lower maximum growth temperature than *P. cambivora*. In isozyme studies, all but one of six Swedish isolates formed a distinct group with similarities to *P. cambivora* and *P. fragariae* var. *fragariae*. The exception was closely related to a British alder isolate, and both these isolates were attacking alder trees in a more aggressive way. This more aggressive Swedish alder *Phytophthora* was isolated from a dying alder on the shore of the lake Stensjön, Mölndal, not associated with the water of Säveån.

1. Brasier CM, Rose J, Gibbs JN (1995) An unusual *Phytophthora* associated with widespread alder mortality in Britain. *Plant Pathology* **44**, 999-1007.

2. Olsson CHB (1999) Occurence of the alder (*Alnus glutinosa* L.) decline in Sweden and affinities of the causal *Phytophthora* pathogen as assessed by isozyme analysis. In: Agraria **161**, Acta Universitatis Agriculturae Sueciae, 'Diagnosis of root-infecting *Phytophthora* spp'.

Results of 10 years of investigations on ink disease caused by *Phytophthora cinnamomi* on *Quercus rubra* and *Q. robur*: Etiology, breeding for resistance, and hazard mapping

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Abstract. Phytophthora cinnamomi Rands is the causal agent of ink disease on *Quercus rubra* and *Q. robur*. Using controlled root inoculations, we showed that root infection in both species is low and the principal symptom of ink disease is cortical and bleeding cankers which develop on main roots, collar and then trunks. Our aim during the last ten years was to understand and predict the disease development oak trunks. Techniques of stem analysis and dendrochronolgy were adapted to study the spatial and temporal evolution of lesions in trunks of naturally infected trees. Intraspecific variablity of susceptibility was studied using a provenance and progeny tests of *Q. rubra* which was introduced in France at the beginning of the 19th century. Estimates of heritability of resistance were obtained. A model was developed in order to relate winter temperatures (sum of negative degree days) that are likely to be a limiting factor of disease development in trunks to the survival of the fungus at the cambium level. Disease hazard, related to the frequency of predicted survival over the last 30 years, was mapped for France. Present disease distribution in France only covers a part of high hazard zones.

Method of strategic conservation planning for species and communities highly susceptible to *P. cinnamomi* in Tasmania

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Abstract: The method employed to establish a representative suite of management areas for the conservation of plant communities that are severely degraded by *P. cinnamomi* in Tasmania is described. Target plant communities are identified by screening communities that occur within the environmental domain of *P. cinnamomi* and contain elements known to be highly susceptible to *P. cinnamomi* in the field. Mapped vegetation assemblages are used in planning where knowledge of the distribution of individual plant communities is insufficient for spatial analysis. Potential *P. cinnamomi* management areas are identified by spatial analysis of *P. cinnamomi* distribution, community/ vegetation assemblage distribution, natural boundaries for pathogen spread, land use and access. Field surveys are undertaken in these areas to update information on plant communities, disease status and management issues. For each community/ vegetation assemblage the overall disease status is determined and optimum disease-free management units are identified against a suite of risk and manageability criteria. Where disease free management areas are not available, the most feasible management area is chosen. Selection of management areas is reduced by favouring areas that capture a range of target communities. For each management area management recommendations and actions are to be prepared.

Time course studies of the effect of temperature and stimulation of soil at different depths on sporangium production by *Phytophthora cinnamoni*

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Abstract. Time course studies of the effect of temperature on sporangium production of *Phytophthora cinnamomi* have not been reported. Little is understood of the soil's capacity to stimulate sporulation at different depths from the surface. Sporangium production was counted on sterilised *Banksia grandis* discs colonised by mycelium of *P. cinnamomi* and incubated in soil extracts on a temperature plate between 10-30 °C. Soil was collected from two sites; only surface soil at one site and soil from the surface and 20, 60 and 90 cm depth from the other site. Extracts were incubated at the different temperatures for 2, 4, 6, 8 and 12 days. Time and temperature were combined in the one equation using the procedure of Pegelow *et al.* (1977). The predicted response surfaces show that numbers of sporangia increase rapidly as temperatures rise from 16-28 °C and incubation periods increase from 2-12 days. There was a more rapid response of sporangium production to temperature in extracts from surface soils than soils collected at depth.

1. Pegelow EJ, Taylor BB, Horrocks RD, Buxton DR, Marx, DB Wanjura DF (1977). The gompertz function as a model for cotton hypocotyl elongation. *Agronomy Journal* **69**, 875-878.

Time course studies of the effect of temperature and stimulation of soil at different depths on sporangium production by *Phytophthora cinnamomi*

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Introduction

- Time-course studies of the effect of temperature on sporangium production of *Phytophthora cinnamomi* have not been reported.
- Little understood is the soil's capacity to stimulate sporulation of *P. cinnamomi* at different depths from the surface.

Materials and Methods

- Soil was collected from 2 sites in the jarrah forest: surface soil at one site and soil from the surface and at 60 and 90 cm depth at the second site.
- Sporangium production was counted on sterilised *Banksia grandis* discs colonised with *P. cinnamomi* and incubated in soil extract on a temperature plate between 10-30 °C. Extracts were incubated at the different temperatures for 2, 4, 6, 8 and 12 days.
- For each soil or depth, sporangium numbers were regressed against time. The regression coefficients of the time-sporangium equation were then regressed against temperature.

Results

- Soils from 60 and 90 cm below the soil surface were less fertile than surface soil (Table 1).

Table. 1. Nutrient status and pH of soils collected from 2 sites in the jarrah forest.

Character	Soil 1	Soil 2		
	Surface	Surface	60 cm	90 cm
Total nitrogen (%)	0.07	0.07	0.02	0.03
Extractable Potassium ($\mu g\,g^{-1}$)	37.8	12.5	7.9	8.5
Extractable Phosphorus ($\mu g\,g^{-1}$)	5.6	9.9	2.5	2.9
Organic carbon (%)	2.6	2.0	0.3	0.2
pH	5.0	4.5	4.4	4.5

- Greatest number of sporangia formed at 26-30 °C and none formed at 10 °C (Fig. 1).
- A significantly greater number of sporangia formed in extract from surface soil than in extract from the less fertile soil collected at 60 and 90 cm depth (Fig. 1).

Surface soil   Soil at 90 cm
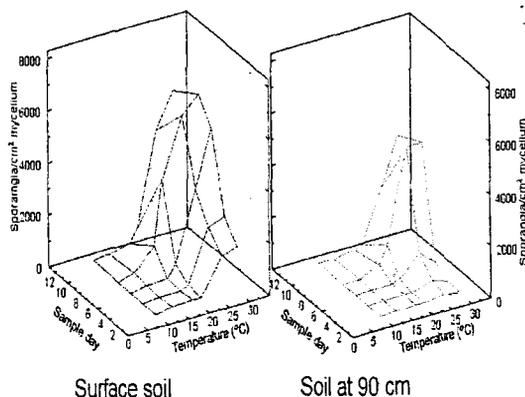
Fig. 1. Observed response surfaces of the effect of temperature and sample day on sporangia production of *Phytophthora cinnamomi* in soil extracts from surface soil and 90 cm below the soil surface at site 2.

- A cubic equation through the origin best described changes in sporangia with assessment time and the resulting regression coefficients and temperature.
- The regression models were combined as illustrated for Site 2 surface soil ($R^2_{adj} = 0.97$):
$$N = (-0.1073TEMP^2 + 0.0128TEMP^3)DAY^2 + (0.0055TEMP^2 - 0.0008TEMP^3)DAY^3$$
- There was a very satisfactory fit between pooled observed and predicted values (Fig. 2) of the form:
$$PREDICTED = 0.98\ OBSERVED\ (R^2_{adj} = 0.91, n = 138)$$
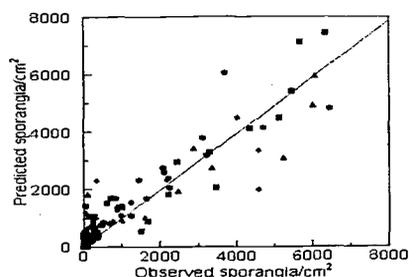
Fig. 2. Plot of observed and predicted sporangia of *Phytophthora cinnamomi* in extracts from site 1 surface soil (●); site 2 surface soil (■) and soil at 60 cm (▲) and 90 cm (◆) below the soil surface.

Surface soil   Soil at 90 cm
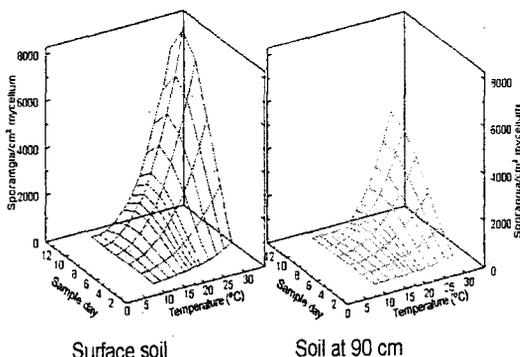
Fig. 3. Predicted response surfaces of the effect of temperature and sample day on sporangia production of *Phytophthora cinnamomi* in soil extracts from surface soil and 90 cm below the soil surface at site 2.

- The slope of the predicted temperature-sporangia response surface increases with increased incubation time (Fig. 3).
- There is a more rapid response of number of sporangia to temperature for extracts from surface soil than soils collected at depth.
- Few sporangia formed at temperatures ≤ 14 °C for surface soil and ≤ 16 °C for soil at depth.

Conclusions

- Incubation time and soil depth significantly affected the size and shape of the temperature-sporangium response surface.
- Quantification of the factors affecting *P. cinnamomi* development must be a research priority for accurate prediction of disease development.

266

The influence of soil from a topographic gradient in the Fitzgerald River National Park on mortality of *Banksia baxteri* following infection by *Phytophthora cinnamoni*

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Abstract. The Fitzgerald River National Park is an international biome in which *Phytophthora cinnamomi* is destroying *Banksia baxteri-Lambertia* scrub-heath in a disease centre over 6 km long in the middle of the park. Current assessment of the vulnerability of healthy areas to infestation by *P. cinnamomi* depends on estimating the probable susceptibility of component plants within the vegetation associations, without knowledge of the potential for the pathogen to develop within the major soil types of the park. Intact soil cores were removed from 5 major soil associations of the park (a relatively fertile loam from the floor of the gorges to the more infertile sandy soils of the plains and uplands) and placed in free draining pots. Cores were also taken from a red loam from incised drainage systems and a gravel and sandy soil from the nearby Ravensthorpe Range for comparison. The cores were planted with seedlings of *B. baxteri* and maintained in a shadehouse. Following establishment of the plants, the cores were inoculated in summer with an isolate of *P. cinnamomi* from the only disease centre in the park. The rate of mortality was greatest in the infertile sandy soils and the soils of the Ravensthorpe Range and lowest in the red loam. The rate of mortality for the Perkin Loam from the gorge floor was intermediate between that of the sands and red loam. While the results of pot experiments have limitations in predicting disease development and must be used with caution, the results do identify the soil types most conducive for disease development.

The influence of soil from a topographic gradient in the Fitzgerald River National Park on mortality of *Banksia baxteri* following infection by *Phytophthora cinnamomi*

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Introduction

- The Fitzgerald River National Park (FRNP) is an international biome in which *Phytophthora cinnamomi* is destroying *Banksia baxteri-Lambertia* scrub-heath in a disease centre over 6-km-long in the centre of the park.
- Current assessment of the vulnerability of healthy areas to infestation by *P. cinnamomi* depends on estimating the probable susceptibility of component plants within vegetation associations, without knowledge of the potential for the pathogen to develop within the major soil types of the park.

Materials and Methods

- Intact soil cores were removed from 5 major soil associations of the park (Table 1) and placed in 15 cm diameter free-draining pots.
- Cores were also taken from a Red Loam in the FRNP and a sandy and gravel soil from the nearby Ravensthorpe Range for comparison (Table 1).

Table. 1. Soils from the Fitzgerald River National Park and Ravensthorpe Range and associated geomorphic units and textures.

Soil	Geomorphic Unit	Area of Park covered by unit (%)	Texture
Red loam (RL)	River valley	1	Sandy loam
Perkins loam (PL)	Floor of spongolite gorges	10	Silty loam
Qualinup Sand - Shallow phase (QSS)	Marine plain	44	Sandy
Qualinup Sand - Deep phase (QSD)			Loamy sand
Nyerilup Sand - Dry phase (NSD)	Upland	31	Silty sand
Nyerilup Sand - Gravelly phase (NSG)			Loamy sand
Ravensthorpe Sand (RS)	Plain	.	Loamy sand
Ravensthorpe Gravel (RG)	Upland slope	.	Loamy sand gravel

- The cores were planted with seedlings of *B. baxteri* and maintained in a shadehouse. Following establishment of the seedlings, the pots were inoculated in summer with an isolate of *P. cinnamomi* from the only disease centre in the park.

Results

- The Red and Perkin loams had higher nitrogen, phosphorus, potassium and organic carbon than the more leached sands of the ancient marine plain and upland of the FRNP and the soils of the Ravensthorpe Range (Fig. 1).
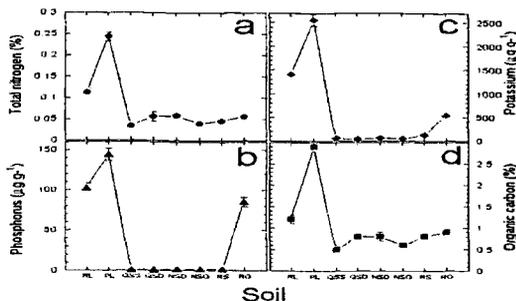
Fig. 1. Total nitrogen (a), phosphorus (b), potassium (c) and organic carbon (d) for the soils from the Fitzgerald River Natioal Park (RL, PL, QSS, QSD, NSD, NSG) and Ravensthorpe Range (RS, RG). Meaning of soil abbreviations given in Table 1.
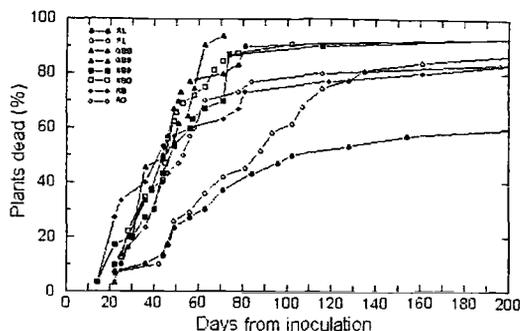
Fig. 2. Mortality of *Banksia baxteri* growing in 8 soils inoculated with *Phytophthora cinnamomi* in pots in a shadehouse. Fitzgerald River National Park soils: Red Loam (RL), Perkins Loam (PL), Qualinup Sand shallow (QSS) and deep phase (QSD), Nyerilup Sand dry (NSD) and gravel phase (NSG). Ravensthorpe Range soils: loamy sand (RS) and loamy sandy gravel (RG).

- Mortality was least in the relative fertile Red and Perkins loam, and greatest in the infertile sands and gravel (Fig. 2).
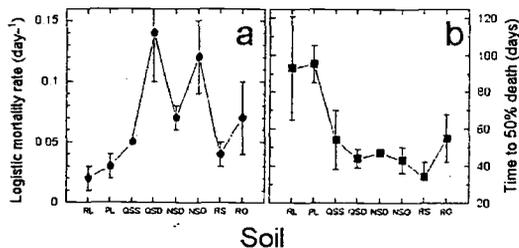
Fig. 3. Effect of soil type on the logistic mortality rate (a) and the time to 50% death (b) calculated from the mortality curves of *Banksia baxteri* infected with *Phytophthora cinnamomi* (see Fig. 2). Fitzgerald River National Park soils: Red Loam (RL), Perkins Loam (PL), Qualinup Sand shallow (QSS) and deep phase (QSD), Nyerilup Sand dry (NSD) and gravel phase (NSG). Ravensthorpe Range soils: loamy sand (RS) and loamy sandy gravel (RG).

- The rate of mortality of *B. baxteri* was least in the fertile loams compared to the infertile sands (Fig. 3). Mortality was delayed in the fertile loams with time to 50% death greater in the loams compared to the infertile sands (Fig. 3).
- Rate and delay in mortality were not significantly correlated with soil characteristics.

Conclusions

- The rate of mortality of *B. baxteri* infected with *P. cinnamomi* was reduced and mortality delayed in the relatively fertile loams compared to infertile sandy soils.
- While the results of pot trials have limitations and must be used with caution, the results do identify the soil types most conducive for disease development.
- Elucidation of the mechanisms of how soil type influences pathogen development, the infection process and host response must be a research priority for accurate prediction of disease development.

Foliar application of phosphite delays and reduces the rate of mortality of three *Banksia* species in communities infested with *Phytophthora cinnamomi*

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Abstract. Our aim in this study was to determine the efficacy of foliar application of phosphite in controlling mortality of three *Banksia* species in *P. cinnamomi* disease centres. Plots, 5 x 5 m, along *P. cinnamomi* disease fronts in *Banksia brownii* Baxter ex R. Brown, *B. baxteri* R. Brown and *B. coccinea* R. Brown communities were sprayed with 2.5, 5 and 10 g L^{-1} phosphite and surfactant (0.2% Pulse) using a backpack sprayer. Controls were only sprayed with surfactant. Treatments were replicated four times in a randomised block design. Mortality was monitored in the plots for 4 year in *B. brownii*, by which time the controls reached extinction, and 6 year in *B. baxteri* and *B. coccinea*. For both delay and rate of mortality, differences between phosphite concentration and *Banksia* species were highly significantly (P < 0.01), but the interaction of phosphite and *Banksia* species was not significant. The non-significant interaction suggest a similar action of phosphite occurred in all three communities. In the sprayed plots, 10 g L^{-1} phosphite reduced mortality the greatest, 2.5 g L^{-1} the least with, 5 g L^{-1} being intermediate between the two. One application of phosphite reduced mortality for up to 2.5 year, after which the plots were resprayed. Unacceptable phytotoxic reactions, such as growth retardation and leaf burning, occurred at concentrations > 10 g L^{-1}.

Phosphite inhibits lesion development of *Phytophthora cinnamomi* for at least four years following trunk injection of *Banksia* species and *Eucalyptus marginata*

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Abstract. We sought to determine the duration of effectiveness of phosphite in limiting lesion development of *P. cinnamomi*, following trunk injection of *Banksia* species and *Eucalyptus marginata* Donn ex Smith. Sufficient numbers of *B. grandis* and *E. marginata* were trunk injected with 50, 100 and 200 g L^{-1} phosphite in 1988 to give estimates of the duration of effectiveness of phosphite over time. For each species, groups of 10 trees were inoculated with *P. cinnamomi* in mid-summer approximately every two years, with a different group being inoculated each time. Control trees nearby were not injected with phosphite. Lesion size was determined 6-weeks after inoculation. At each inoculation time, more groups of ten trees were injected with phosphite to give a range of periods after injection. Along two disease fronts, trees of *B. attenuata* R. Brown close to one another and of similar size were paired and one tree at random injected with 100 g L^{-1} phosphite at the rate of 1 ml/cm trunk circumference and the other tree not treated. There was 41 pairs of trees at one site and 13 at the other. Mortality was monitored during a 8-year-period. Increase in *P. cinnamomi* lesion length with time after injection was greatest for trees injected with 50 g L^{-1} phosphite, least for those injected with 200 g L^{-1} and intermediate for trees injected with 100 g L^{-1} phosphite. All three concentrations of phosphite effectively controlled lesion extension for at least 4 years after injection. Trends in tangential spread of lesions were similar to those described for linear extension. Injection of 200 g L^{-1} phosphite caused tissue necrosis at the site of injection and inhibited canopy growth. Mortality of trees injected with 100 g L^{-1} phosphite was significantly less than trees receiving no phosphite in both disease fronts. One injection protected trees for at least 4 years, after which mortality of injected trees increased. This period of effectiveness was similar to that obtained from controlled inoculation. The results suggest that the period of effectiveness of phosphite against *P. cinnamomi* in the low phosphate environment of native communities in south-western Australia is considerably greater than that found in the high phosphate, frequently harvested horticultural situation.

Phosphite reduces the rate of spread of *Phytophthora cinnamoni* in *Banksia* woodland, even after fire

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Within the Department of Conservation and Land Management, phosphite application is an important and effective management tool for the protection of native plant species from infection and death by *Phytophthora cinnamomi*. In 1993, as part of ongoing testing of application strategies, a trial was commenced to test the effect of application of phosphite as a spray of understorey and injection of overstorey species, on the rate of spread of *Phytophthora cinnamomi* along a disease front in *Banksia* woodland. There were 5 treatments: no phosphite, with all the 4 remaining treatments having the overstorey injected with 50 g phosphite/l and the understorey sprayed either once or twice with 2 or 5 g phosphite/l with a backpack sprayer. The treatments were applied in 10 x 15 metre plots along an active disease front in a randomised block design with four replicates. The plots were positioned in healthy vegetation with one edge of the long axis aligned along the disease front. Six months after phosphite application, movement of the disease front was greater in non treated plots than in sprayed and injected plots. The *Banksia* woodland was burnt a year after phosphite application. By 3 years after burning the understorey vegetation had re-established and a disease front was evident. When the disease front was replotted 3 and 4 years after burning and 4 and 5 years after phosphite application, disease extension was least in phosphite treated plots and greatest in the non treated plots. There appears to be some residual effect of phosphite application after fire.

Phosphite reduces disease extension and the rate of extension of *Phytophthora cinnamomi* in *Banksia* woodland, even after fire

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Introduction

- *Phytophthora cinnamomi* Rands causes reduced floristic diversity and a conspicuous decline in biomass (Fig. 1) and is a major threat to the conservation of the *Banksia* woodland of the Swan Coastal Plain, Western Australia.
- We tested the effect of phosphite application as a spray to understorey and injection of overstorey on extension of a *P.cinnamomi* disease front in *Banksia* woodland. Burning of the trial site by the owner gave an unplanned effect of fire on phosphite treatment.
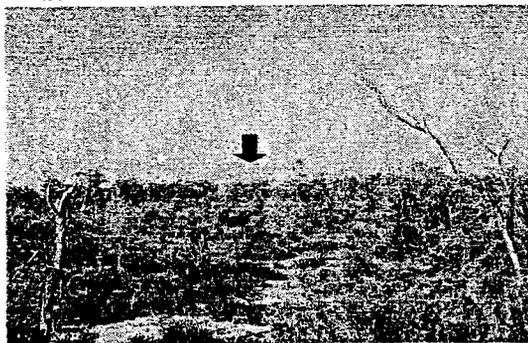
Fig. 1. High impact of *Phytophthora cinnamomi* in *Banksia* woodland. The arrow indicates the recently dead disease front between the infested area and healthy woodland along which plots were positioned. Most susceptible hosts have been killed in the diseased area.

Materials and Methods

- The five phosphite treatments were: no phosphite (treatment 1); all trees injected with 50 g L⁻¹ phosphite and the understorey sprayed with 2 g L⁻¹ phosphite once or twice (treatments 2 & 3); or all trees injected with 50 g L⁻¹ phosphite and the understorey sprayed with 5 g L⁻¹ phosphite once or twice (treatments 4 & 5). Treatments were replicated 4 times in a randomised block design.
- Assessment 1 of the disease front position occurred 0.5 yr after the first spray, assessment 2 occurred 4.3 yr after the first spray and 3.2 yr after the fire and assessment 3, 5.3 yr after the first spray and 4.1 yr after the fire.

Results

Disease front extension

- Phosphite treatments 2-5 significantly reduced disease extension by an average of 0.9 ± 0.1 m, 4.0 ± 0.2 m and 4.1 ± 0.2 m at assessments 1, 2 and 3 respectively, compared to the non phosphite treatment 1 (Fig. 2).
- There were no consistent differences in disease front extension between phosphite treatments 2-5.
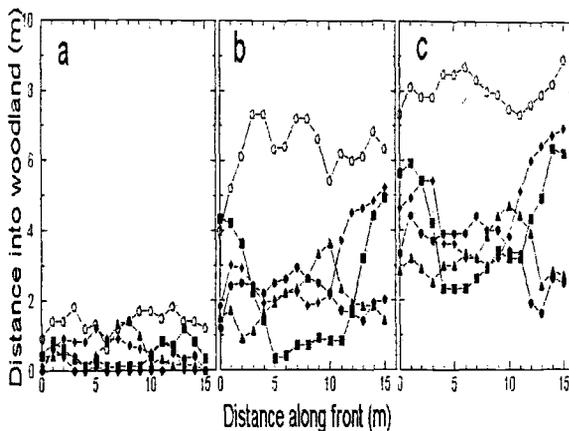
Fig. 2. Mean extension of a *Phytophthora cinnamomi* disease front in *Banksia* woodland not treated with phosphite (O) or all trees injected with 50 g L⁻¹ phosphite and the understorey sprayed with either 2 g L⁻¹ phosphite once (●) or twice (■) or 5 g L⁻¹ phosphite once (▲) or twice (◆) , a) 0.5 yr after spray and before the fire, b) 4.3 after spray and 3.2 yr after the fire, and c) 5.3 yr after spray and 4.1 yr after the fire.

Rate of extension

- Rates of extension 1 and 2 (calculated between assessments 2 and 1 and 3 and 2, respectively) were reduced by a half to a third by phosphite treatment (Table 1).
- As for disease front extension there were no consistent differences in rate between phosphite treatments 2-5.

Table 1. Mean rate of extension of *Phytophthora cinnamomi* front in *Banksia* woodland not treated with phosphite or given four phosphite applications.

Phosphite Treatment	Rate (m yr⁻¹)		
	Rate 1	Rate 2	Rate 3
1. None	1.3 ± 0.1	1.4 ± 0.1	1.8 ± 0.2
2. Injection + 1 spray 2 g L⁻¹	0.5 ± 0.1	0.7 ± 0.1	1.3 ± 0.2
3. Injection + 2 spray 2 g L⁻¹	0.4 ± 0.1	0.8 ± 0.1	2.0 ± 0.2
4. Injection + 1 spray 5 g L⁻¹	0.4 ± 0.04	0.6 ± 0.05	1.4 ± 0.2
5. Injection + 2 spray 5 g L⁻¹	0.6 ± 0.1	0.9 ± 0.1	1.9 ± 0.2

Conclusions

- Phosphite application significantly reduced disease extension in *Banksia* woodland infected with *P. cinnamomi*, even after fire.
- Disease extension in *Banksia* woodland is probably mainly through growth of the pathogen through major woody roots.
- Residual action of phosphite would not be expected to last in understorey vegetation destroyed by fire.
- The residual action of phosphite probably persisted after the fire in the woody roots of injected overstorey trees.
- This study demonstrates that injection of overstorey must accompany spray of foliage to ensure long lasting protection by phosphite.

271

Identification of pathogenicity genes in *Phytophthora nicotianae*

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Abstract. Zoospores are the main infective agent for most plant pathogenic oomycetes, including
Phytophthora. The focus of our research is identification of zoospore-specific genes that may be crucial
for pathogenicity of *Phytophthora.* In order to achieve this, a high quality cDNA library was made
from mRNA isolated from zoospores of *Phytophthora nicotianae.* This species was chosen because it
produces abundant zoospores and because of its wide host range which includes scientific model plants
such as tobacco. The library was plated on duplicate grids that were subsequently used to differentially
screen with cDNA probes originating from either mycelium or zoospore mRNA. Candidate zoospore
specific genes, that were identified in this way were then passed through another round of screening
and then partially sequenced from their 5' end. Comparison with other sequences in Genebank revealed
homologies with known genes involved in a number of metabolic pathways as well as a number of
unannotated sequences identified in the *Phytophthora* genome project. Of particular interest were
clones that had multiple hits solely in the *P. sojae* zoospore-derived library. These clones will be
characterised further. Future experiments will involve identifying the function of these genes through
transformation experiments. Continuation of this research will identify important pathogenicity factors
that could be used as targets in novel control measures for *Phytophthora* diseases.

The impact of Phytophthora disease on riparian populations of common alder
(*Alnus glutinosa*) along the Moselle River (North-eastern France)

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1. INTRODUCTION

Alder Phytophthora caused significant damages in north-eastern France in the last years. A survey was established in August 2000 along the Moselle river, to obtain informations on the disease. Its severity was investigated and discussed in relation to several environmental and tree parameters

2. METHODS

The survey concerned the Moselle river from its sources to the luxembourg border (along 350km). A plot was established each 5 km when it was possible (easy access and alders present). 15 alder trees (maiden or coppice stool) were studied by plot. The 3 larger stems were studied in case of coppice stool. For each stem the following information was collected : maiden or coppice stool, diameter of the trunk, distance from water, distance from neighbour tree, presence of tarry spots, severity of crown symptoms (evaluated with a notation scale, table 1).

The incidence of the disease was expressed by the percentage of trees with tarry spots or crown symptoms (notation scale). It was examined in relation to the various factors of the alder population (distance from water, diameter etc.) Data were analysed with the software SAS
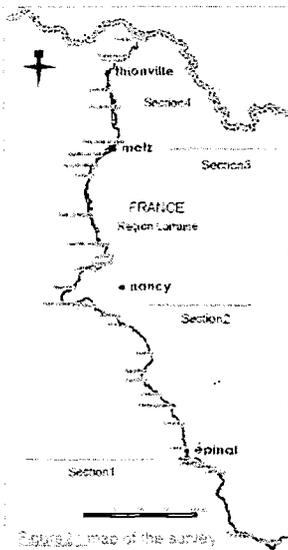
Figure 1 : left healthy tree (note 0) right typical crown symptoms (note 2)

Scale	Symptoms
0	Healthy
1	50% defoliation
2	50% defoliation
3	Dead

Table 1 : Symptoms notation scale

Figure 2 : map of the survey

3. RESULTS AND DISCUSSION
3.1. Incidence of diseased alders

	No alders	Alders with crown symptoms % dead (note 2 and 3) (in %)	Alders with tarry spots %
From Sources to Epinal	251	9	6
From epinal to Messein	197	21	34
From Maron to Moulin les Metz	214	11	16
From Ay sur M. to Apach	103	16	16
Total	765	13	17

Overall, nearly 13% of the trees were classified as diseased or dead. 17% showed tarry spots. These damages may be considered hight compared with those reported in UK by J. Gibbs, 1999. We considered 4 sections of the river. Before Dinozé, alder trees form a curtain continuous. The Number of diseased trees is relatively low. From Epinal to Messein alder trees are sparse but frequently diseased. From Maron to Moulin, alders are more abundant and the disease less frequent. After Ay, the banks are artificial and trees very rare. Percentage of decaying alders is hight whereas the number of tarry spots is limited. It is not evident that most of these trees are affected by the Phytophthora.

4. CONCLUSION

3.2. Effect of distance from water

The effect of distance from water on incidence of the disease shown by Gibbs. 1999 is confirmed, and hightly significant, (likelihood chisquare = 8.10; Pr = 0.004)

3.3. Aggregation pattern of the disease

3.3.1 At the intra-coppice level

Canker present on another stool of the coppice / Maximum crown rating given on the other stool of the coppice
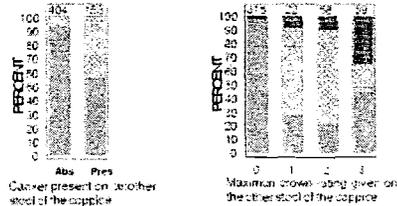
Canker on the stool / Stool crown rating

Figure 3 / Figure 4

The status of single coppice stool was related with the general status of the coppice as far as canker presence and crown rating are concerned. When another stool of the coppice showed canker presence or crown symptoms, the chance of the stool to show canker and/or deteriorated crown was greatly enhanced (fig. 3-4).

3.3.2 At the plot level
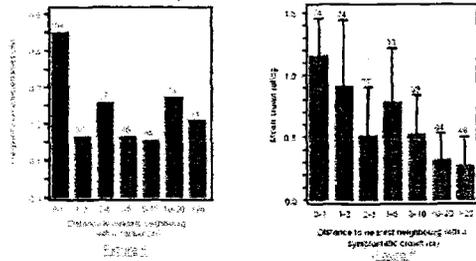
Figure 5 / Figure 6

Both figure 5 and 6 shows that probability for a tree to be affected is higher if a diseased tree is within few meter from it. This is highly significant (likelihood chisquare = 21.3, p = 0.0001 for tarry spot and Fisher = 32.2, p = 0.0001 for crown symptoms).

### 3.4. Correlation between crown symptoms and tarry spots
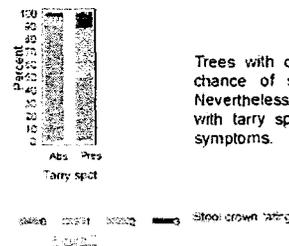
Trees with cankers have much higher chance of showing crown symptoms Nevertheless, about 50% of the stems with tarry spots do not express crown symptoms.

Stool crown rating

Figure 7

3.6. Disease incidence in relation to diameter of the stem

Stem diameter showed no significant relationship with disease.

In this study, several parameters relating to the trees or their position were investigated. Results shows that the didease is very frequent on the Moselle river and concentrate within one meter from the river bank. The disease shows aggregation pattern suggesting that parasite population dynamic in the soil is important in the disease development. Other factors such as water pollution. management of river banks etc. will be studied later.

Phytophthoras in Oregon Forests

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Abstract. Ten or more species of *Phytophthora* are present in Oregon forests; only *P. lateralis* is associated with dramatic disease. Sampling of forest soils and streams, as well as "minor" diseases of several forest plants, continues to produce new reports. *P. cinnamomi* has been recovered for the first time from Oregon forest soils, not always associated with observed disease. *P. cambivora* causes a canker disease of *Castanopsis* in forests, and may be associated with a basal canker of Douglas-fir. *P. citricola* has been recovered from soil and symptomatic *Gaultheria* leaves. Other intriguing finds include *P. ilicis* "like", *P. fragariae* "like", and *Halophytophthora* "like" isolates from soil, without observed disease on surrounding vegetation. Several members of the *P. megasperma, P. gonapodyides* clade have been recovered. We monitored strong seasonal fluctuations in populations of *P. gonapodyides* in stream water. *Phytophthora* is widespread and locally abundant in Oregon forests. Most of its impacts remain unknown. While sampling to determine the full extent and diversity of *Phytophthora* in Oregon forests continues, attention is increasingly focused on behavior of these organisms in the ecosystems where they are found.

Introduction

At least 13 species of *Phytophthora* are present in Oregon forests (table 1). Three species cause dramatic mortality on host trees: *P. ramorum* causing Sudden Oak Death on *Lithocarpus* and several associated understory plants in SW Oregon; *P. lateralis* killing *Chamaecyparis lawsoniana* and associated *Taxus brevifolia* in forests and horticultural plantings; and *P. cambivora* causing a new disease of chinquapin (*Castinopsis*) in the mountainous forests of south central Oregon.

Several species are recovered from soil or water in forest areas but have not been associated with any disease in the forest. Collectively, they are widespread and locally abundant, at least beneath *Quercus, Acer,* and *Alnus* riparian stands where sampling has been concentrated to date.

Phytophthora species are recovered from soil by baiting with Port-Orford-cedar and rhododendron foliage baits, from water by filtration and plating the filters on selective medium or by baiting from leaf litter collected from the stream, and from symptomatic plants by direct plating on selective medium. We use CARP (cornmeal, ampicillin, rifampicin, pimaricin), often with hymexazol and benomyl added.

Results

Phytophthora ramorum

P. ramorum was recently discovered at 7 sites in SW Oregon , about 350 km north of the nearest known California infestation. It is killing *Lithocarpus* and *Rhododendron.* An eradication effort is underway.

Phytophthora cambivora

P. cambivora is isolated from lethal basal cankers and associated soil on chinquapin (*Castanopsis*) in south central Oregon. This is a newly recognized disease, and is causing serious damage in the epidemic area. Elsewhere *P. cambivora* is recovered from soil around seemingly healthy hardwood trees, and from soil around Douglas-fir with basal cankers. It is also an important pathogen of trees, especially chestnut, in horticultural situations and nurseries.

Phytophthora cinnamomi

P. cinnamomi is widespread and damaging on many hosts in irrigated horticultural situations in Oregon. This is the first report of the pathogen in forest soils in the state, and 2 of these 3 locations are disturbed sites with recent introduction likely. The third location, however, was from beneath wild *Rhododendron, Vaccinium, and Arctostaphylos* in an undisturbed coastal forest. There were no symptoms noted on the associated plants. Members of the plant family Ericaceae are generally susceptible to *P. cinnamomi* and are important in many forest plant communities

Phytophthora gonapodyides

P. gonapodyides is widespread and abundant in Oregon. It is present in every forest stream sampled to date, even in extreme headwaters locations in remote, undisturbed areas. It is also the species most commonly isolated from

soil, especially in riparian areas. It is not associated with any disease symptoms in the forest.

the native forest in SW Oregon. Intensive programs to limit further spread in the forest through sanitation and local quarantine, and to breed resistant trees are in operation.

Phytophthora lateralis

P. lateralis was introduced to Oregon about 1920. It kills *Chamaecyparis lawsoniana* in horticultural situations, and in
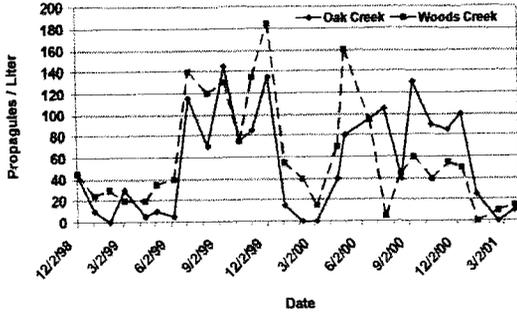

Fig. 1. *Phytophthora* propagules.

Table 1. Phytophthoras isolated from Oregon forests.

Phytophthora species	Water	Soil	Nursery
lateralis	X	X	X
ramorum		X	
cinnamomi		X	X
cambivora		X	X
citricola	X	X	
cactorum		X	X
gonapodyides	X	X	X
"chlamydospores"	X	X	
megasperma	X	X	X
"*ilicis*"	X		
"*fragariae*"	X		
cryptogea	X		X
Halophytophthora	X		

Table 2. Isolation success.

Substrate	Successful isolations/ samples	Number of species recovered
Soil	37/127 (29%)	10
Water	95/98 (97%)	8
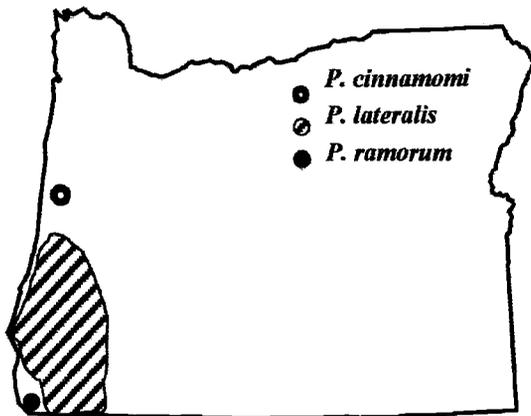
Fig. 2. Map of Oregon showing isolation sites.

Interaction of sudden oak death *Phytophthora* with associated organisms

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Abstract. Sudden Oak Death is a new disease that has killed large numbers of oaks (*Quercus agrifolia, Quercus kelloggii, Quercus parvula var. shrevei*) and tanoaks (*Lithocarpus densiflorus*) in some of central California's coastal counties. The name Sudden Oak Death is used because of the rapid colour change of leaves from green to brown. In June 2000 an unknown species of *Phytophthora* sp. was discovered as the underlying cause of Sudden Oak Death, which was later identified as a new species, *Phytophthora ramorum*. A tree may be infected with *P. ramorum* for a number of months or years before exhibiting this sudden change in foliage. After the infection begins, attacks by oak bark beetles, *Pseudopityophthorus pubipennis*, and oak ambrosia beetles, *Monarthrum scutellare*, and the growth of *Hypoxylon thouarsianum* also occur, which might contribute to the hastening of the tree's death.

The role of *Phytophthoras* in tree yellowing and death in Texas

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Abstract. The major symptom of *Phytophthora* spp. infection was a generalized slight yellowing of leaves, persisting for months or years. Later the colour change accelerated, the leaves become orange, and then turned brown and remained attached. Two to four weeks later the tree died. Since 1934, symptoms of foliage discoloration ("yellowing"), retarded growth, and premature death of post oak, live oak, winged elm, and hackberry have been reported in central Texas. These symptoms are distinctly different from those caused by the oak wilt (*Ceratocystis fagacearum*) and oak decline (*Cephalosporium*) fungi. Of the 34 trees sampled, 24 exhibited "yellowing" decline symptoms. All of the symptomatic trees yielded *Phytophthora* in fruit culture and 83% of these yielded *Phytophthora* upon culture on media. 30 percent of the symptomless trees yielded cultures of *Phytophthora*. *P. cinnamomi* was observed in many of the isolates. Symptomatic trees were prevalent in places with frequent standing water, or water flows across the roots where there was exposure to slightly saline or alkali well water. Gas leaks and cattle trampling also increased susceptibility. Soil injections in the root zone with ethazol and metalaxyl provided symptom remission (greener leaves). French drains to carry saline water past low sites also resulted in symptom remission. *Phytophthora* played an important role in tree "yellowing" and subsequent death in Texas. These symptoms could be minimized with control.

THE ROLE OF PHYTOPHTHORAS IN TREE YELLOWING AND DEATH IN TEXAS

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DEFINITION - What is tree "yellowing" - The major symptom associated with *Phytophthora* spp. infection was a generalized slight yellowing of leaves, persisting for several months to several years. At some point the rate of color change accelerated, the leaves became orange, and then turned brown and remained attached. Two to four weeks later the tree died.
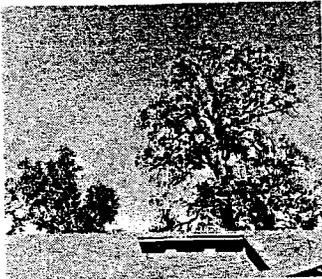
Symptomatic oak (on right) with yellowing symptoms of decline. This will be followed by rapid death of the tree. Trees on the left are healthy.

INTRODUCTION - The work described here was done before scientists had a full appreciation or understanding of the important role of species of *Phytophthora* in declines of urban and forest trees. With the development of selective media and accurate identification methods has come the recognition that *Phytophthora* spp. have been implicated in tree declines in wide geographic regions (Ann. Sci. For. 53:347-58; Bol. Sanidad Vegetal, Plagas 22:491-9; Plant Dis. 61:66-70; Plant Dis. 78:369-74; Plant Dis. 84:394-8) in addition to the long and well-known, destructive jarrah tree decline in Australia (Phytopathology 62:972-81).

Since 1934, symptoms of foliage discoloration ("yellowing"), retarded growth, and premature death of urban and woodlot trees of post oak, live oak, winged elm, hackberry, and other broadleaved species have been reported in central Texas. These symptoms are distinctly different from those caused by the oak wilt (*Ceratocystis fagacearum*) and oak decline (*Cephalosporum*) pathogens. This research identified biological and site factors contributing to this disease syndrome and determined the efficacy of commercially applied chemical and physical control measures.

MATERIALS AND METHODS - The research was designed to test efficacy of commercial remediation efforts applied to 34 trees in 9 areas within 3 counties in central Texas. Small roots, and adjacent soil samples collected from trees with "yellowing" symptoms, and nearby symptomless trees, were baited with fresh Kiefer pears or Granny Smith apples, and resulting cultures were plated onto media to confirm presence of *Phytophthora* spp. Soil was injected with metalaxyl and ethazol in the root zones of symptomatic trees, along with applications of sulfur, ammonium, magnesium, and zinc sulfates. The local physical environment was studied to detect potentially stressful growth conditions and removal of fill soil and installation of French drains was done. The irrigation cycles were also modified to increase soil aeration.

RESULTS - Of the 34 trees sampled, 24 exhibited "yellowing" decline symptoms. All of the symptomatic trees yielded *Phytophthora* in fruit culture and 83% of these yielded *Phytophthora* upon culture on media. Thirty percent of the symptomless trees also yielded cultures of *Phytophthora*. Cultures were not identified to species but it was observed that *P. cinnamomi* was present in many of the isolations.

Ecological Considerations. Symptomatic trees were prevalent in places with frequent standing water, frequent water flow across the roots, and particularly where there was exposure to slightly saline and alkaline water such as local well water. Trees with earth fill over the root zone were more susceptible. Other factors favoring decline were trash on the soil, roots under patio decks and concrete driveways, and construction disturbances. House footings created dams so that trees on the uphill side of the house had symptoms where puddles formed. Underground natural gas leaks as well as cattle trampling also increased susceptibility.

Physical modification of the site during construction or landscaping.   Soil compaction.

Low areas with frequent standing water or with exposure to saline or alkaline water.

Physical Modification of the Environment. Physical site modification often resulted in symptom remission. Installation of shallow surface ditches to drain puddles, or plastic drainpipes to carry saline water past low sites were conducive to symptom remission. To be most effective, these control measures were usually applied in an integrated fashion. Direct chemical controls were seldom applied without indirect controls and at least some site modifications.

Indirect Chemical Control. Nitrogen fertilization improved leaf color and possibly tree health by improving plant growth. All chemicals improved coloration when applied separately, adding them together produced a more intense greening effect. These indirect chemical treatments were temporary measures to keep the tree alive and improve growth during the period while physical modifications were being made. Soils were often alkaline from the sodium in the irrigation water, with pH as high as 9.5 being fairly common. Sulfur, and various sulfates, were routinely used to acidify.

ISOLATION RESULTS FOR *PHYTOPHTHORAS*

Symptomatic trees

Media	Tree species	Quantity	Isolation % Fruit	
	Ulmus alata	2	100	75
	Celtis occidentalis	1	100	50
	Quercus falcata	1	100	25
	Q. nigra	6	100	78
	Q. stellata	8	100	33
	Q. virginiana	6	100	25
	Total	24		

Symptomless trees

	U. alata	1	100	0
	Q. marylandica	1	100	0
	Q. nigra	2	100	33
	Q. stellata	2	100	17
	Q. virginiana	4	100	0
	Total	10		

Direct Chemical Control. Soil injections in the root zone with ethazol provided symptom remission, visible as a return to greener leaf coloration, and were long lasting where there was less than 15 cm of soil fill. Soil injections with metalaxyl produced symptom remission in a higher percentage of cases but the effects seemed to be shorter lived than with ethazol.

Declined oak, 60 days after chemical treatment. Note dark, green foliage.

CONCLUSIONS - This research shows that species of *Phytophthora* played an important role in tree "yellowing" and subsequent decline in Texas. The research also showed that these symptoms could be minimized with an integrated approach to control.

277

Phytophthora in South Australia: a quantum shift in awareness

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Abstract. Mainland South Australia has seen a quantum shift in its level of *Phytophthora* awareness over the past 18 months. A *Phytophthora* Project Officer is leading a statewide campaign that is generating a ground swell of interest in, and action against, *Phytophthora*. The campaign has centred on education and training, with a multitude of workshops and presentations undertaken, a quarterly newsletter distributed and information articles, posters and brochures produced. Distribution mapping and sampling has also occurred and *Phytophthora* Management Plans and hygiene strategies have been developed for key groups including Local Councils, Friends of Parks, Transport SA and forestry industries. A diversity of other activities have been undertaken including assisting the Country Fire Service to incorporate *Phytophthora* management into its fire fighting operations, Local Councils have taken on responsibility for threat mitigation and community education and a set of Standard Operating Procedures has been developed for National Parks and Wildlife SA for implementation across the state. Maintaining communication links between these groups, and encouraging a collaborative approach to *Phytophthora* management will be the key to the success of this program in South Australia.

New alarm of *Phytophthora* root rot of walnut in Italy

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Abstract. Root rot caused by *Phytophthora* spp. has been recently recorded in several plantation of *Juglans regia* in Northern and Central Italy. Isolation trials from soil and infected roots confirmed the presence of *Phytophthora* spp. in the collected samples. Symptoms of the disease are very similar to those of Ink disease of chestnut. Morphological and molecular identification evidenced the presence of 5 species. Among them, *P. cinnamomi*, whose presence in Italy has been probably confused with *P. cambivora* in the past; *P. cambivora, P. cactorum, P. citricola,* and *P. cryptogea.* This is the first record of the latter 2 species on walnut in Italy. Pathogenicity tests carried out on 2 years seedlings through soil infestation, confirmed *P. cinnamomi* as the most aggressive species on *J. regia.*

Seasonal vitality of *Phytophthora cambivora* in soil and chestnut tissues

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Abstract. Phytophthora cambivora is the most frequent species causing Ink disease in Italy. The biological characteristic of this organism and the disequilibrium of mating types evidenced in the Italian population, limit its ability to survive in adverse condition as resting structure, both in soil and host tissues. Experiences carried put over a 3 year period by sampling soil samples and host infected tissues monthly, found two peaks of vitality of *Phytophthora cambivora* during the year. The pathogen can easily be isolated from soil at soil temperature between 15 to 20 °C following abundant precipitation. Such conditions correspond at Italian latitudes with early spring and fall. Vitality in host tissues is independent to precipitation and is

associated with presence of sap flow in the stem, average air temperatures generally close to 20 °C and relatively high values of vapour pressure deficit (VPD), corresponding in Italy to the late spring and early fall.

Variability in resistance to *Phytophthora cambivora* of *Castanea sativa* wild population and selected cultivars in Italy and Spain

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Abstract. The behaviour of 4 wild population of *Castanea sativa* from Sicily (Southern Italy), Pedmont (Northern Italy), A Coruña (Northern Spain), Málaga (Southern Spain) to *Phytophthora cambivora* was tested on 4 month old half-sib progenies through soil infestation. A number of qualitative and quantitative parameters were assessed at the beginning, during and after the experiment, including growth in height, number of leaves, dry weight, length of the stem and root necroses, crow condition and mortality. Results showed a wide variability in the response to the pathogen challenge. Some progenies showed a resistance to *Phytophthora cambivora* similar to that of the resistant hybrid Marsol used as control. An additional experiment was carried out through stem inoculation on 87 cultivars of *Castanea* spp., including French hybrids. In this case, a relatively wide variability in susceptibility was assessed among the Italian cultivars, some of which showed comparable behaviour with the French hybrids.

The distribution and impact of *Phytophthora cinnamomi* in Royal National Park, New South Wales

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Abstract. Phytophthora cinnamomi was recorded within Royal National Park (New South Wales) more than 30 years ago. However, the distribution of the pathogen within the Park and its impact on vegetation has yet to be established. It is known that *Telopea speciosissima* (the floral emblem of New South Wales) is susceptible to *P cinnamomi*, both in the glasshouse and in cultivation, yet knowledge of the susceptibility of this species in native vegetation is sparse. A range of populations of *T. speciosissima* and *Xanthorrhoea resinifera* (a species known to be in decline within the Park) were tested using *Phytophthora*-selective medium to assess disease presence. No *P. cinnamomi* was detected in any of the five *T. speciosissima* sites sampled but it was present within seven of nine *X. resinifera* sites. It is likely that *P. cinnamomi* is contributing to the decline of *X. resinifera* within the Park, but it is not yet possible to assess the potential susceptibility of *T. speciosissima* in the field, so we are using cuttings of this species in glasshouse susceptibility trials. Currently we are examining whether soils in the Park are conducive to the spread of *P. cinnamomi*, by measuring sporangial production from soil leachate and chemical composition of soils.

Introduction

Royal National Park is an important reserve for the characteristic sandstone flora and fauna located on the southern edge of Sydney. *Phytophthora cinnamomi* was first recorded in the Park in 1974 and, until recently, the potential for impact on vegetation was regarded as minimal.

Populations of *Xanthorrhoea resinifera* have been monitored in the Park since 1988. Over this period mortality has exceeded recruitment (David Keith, NPWS, *pers. comm.*). In 1999, *P. cinnamomi* was isolated from dying *X. resinifera*.

The waratah, *Telopea speciosissima*, is the floral emblem of New South Wales. Protecting this species is of cultural and biological importance. *Telopea speciosissima* is susceptible to infection by *P. cinnamomi* in cultivation, but the impact of the pathogen in native vegetation is unknown.

This study sought to 1) determine if *P. cinnamomi* is present on sites containing *T. speciosissima* or *X. resinifera* in Royal National Park, 2) determine the susceptibility of populations of *T. speciosissima* in Royal National Park to infection by *P. cinnamomi* and 3) examine potential differences in infested and uninfested sites.

Materials and Methods

Distribution of the pathogen

Soil samples taken from five sites containing *T. speciosissima* and nine sites containing *X. resinifera* were baited with *Phytophthora*-selective medium in a 1:4 soil/water slurry to detect the presence of the pathogen. *Phytophthora cinnamomi* was not detected in any of the *T. speciosissima* sites, but it was present in seven of the nine *X. resinifera* sites. Even in these sites, the percentage of samples testing positive was low, ranging from 5% to 35%. This indicates that the population density of the pathogen is likely to be quite low and the distribution patchy.

Susceptibility of *T. speciosissima*

Ten cuttings obtained from each *T. speciosissima* study site were inoculated in a glasshouse trial, with an isolate of *P. cinnamomi* obtained in the soil sampling (above). Pots containing *Banksia* species known to be susceptible (*B. grandis, B. brownii, B. attenuata*) or resistant (*B. integrifolia*) to infection (McCredie *et al.* 1985) were inoculated at the same time.

Within 6 weeks following inoculation, all of the susceptible *Banksia* species had died, and *P.*

cinnamomi was recovered from 69% of the root systems. By the end of the 10-week trial, 60% of the *B. integrifolia* plants had died, but only 20% of the plants yielded *P. cinnamomi* from the root systems. All of the *T. speciosissima* cuttings displayed symptoms of infection. Death occurred in 43% of plants by 10 weeks, and 74% of plants yielded *P. cinnamomi* from the root systems.

Site characteristics

Sporangial production by *P. cinnamomi* was measured in soil leachates from each of the sites. No significant difference (t=1.454, d.f.=54, P=0.1518) was found between the number of sporangia produced in leachates from infested and uninfested soils

Environmental and soil chemistry variables were measured at each site. Environmental variables measured were slope, aspect, canopy height, canopy cover, leaf litter depth, vegetation structure, and dominant vegetation species. Soil chemistry variables included exchangeable calcium, exchangeable magnesium, nitrate nitrogen, ammonium nitrogen, available phosphorus, organic carbon, and pH. All infested sites occurred in heathland vegetation. Infested sites had significantly lower pH (pH 5.1±0.09 cf. 5.7±0.08) (t=4.4, d.f.=12, P=<0.005). Differences in other variables between infested and uninfested vegetation were not significant.

Discussion

Phytophthora cinnamomi is widespread in heathland vegetation in Royal National Park, and has the potential to be contributing to decline in *X. resinifera* populations.

The similarity in the rate of infection of *T. speciosissima* cuttings from the Park to *Banksia* species known to be susceptible in the field, and the expectation that more *T. speciosissima* plants would have died had the trial been run for longer, indicate that *T. speciosissima* is likely to be susceptible to *P. cinnamomi* in the field at Royal National Park.

The slightly lower pH of infested sites is likely to be a reflection of the heathland habitat of *X. resinifera* compared to the woodland habitat of *T. speciosissima*. The heath sites of *X. resinifera* are, in general, more poorly drained than the woodland habitats of *T. speciosissima* in the Park. *Phytophthora cinnamomi* relies on moisture for sporulation, dispersal and infection (Shearer and Tippett 1989), so the greater availability of water in the heathland sites could be a determining factor in the distribution of *P. cinnamomi* throughout Royal National Park.

No environmental factor examined in this study is expected to inhibit *P. cinnamomi* if it were to colonise *T. speciosissima* sites. Management procedures for the protection of *T. speciosissima* and other significant or threatened species in the Park are therefore essential. The patchy distribution of the pathogen indicates the use of fungicides on these species in infested populations may be a viable management option.

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Impact of *Phytophthora cinnamomi* on mammals in southern Australia

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Abstract. The plant pathogen *Phytophthora cinnamomi* (cinnamon fungus) has a major effect on vegetation floristics and structure in sclerophyll vegetation in Australia. Effects include loss of plant species, decline in vegetation cover, increases in bare ground and the abundance of resistant plant species. These changes would be predicted to have effects on faunal communities inhabiting infected habitats. Analyses in heathlands and woodlands of south-eastern Australia have identified *P. cinnamomi* infection as being associated with low species richness, and low abundance of small mammals. Studies of *Antechinus stuartii* (Brown Antechinus) in woodlands found that there were lower capture rates in infected areas, and habitat utilisation was altered. The major contributing factor was alterations to vegetation structure, rather than food availability. In heathlands, species such as *Rattus lutreolus* (Swamp Rat), *Rattus fuscipes* (Bush Rat), *Antechinus agilis* (Agile Antechinus) and *Sminthopsis leucopus* (White-footed Dunnart) were found to be less abundant in diseased areas, or utilised them less frequently. An analysis of mammals that occur in Victoria found that for twenty-two species, five of which are rare or endangered, more than 20% of their range coincides with the reported distribution of *P. cinnamomi.*

Distribution of *Phytophthora* species in forest soils of upstate South Carolina, USA

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Abstract. The Jocassee Gorges tract in northwest South Carolina, USA recently has been designated by state and federal agencies as a major conservation and land preservation site. The tract contains an abundance of native plants that are largely unique to the area. Species of *Phytophthora* pose a threat to natural areas because, if present or introduced, they may attack and devastate susceptible plant species. A baiting bioassay (with camellia disks, shore juniper and hemlock needles as baits) was used to assay fresh and air-dried composite soil samples for *Phytopthora* species. *P. heveae* only was detected with hemlock needles whereas *P. cinnamomi* was detected primarily with camellia leaf disks and shore juniper needles. *P. heveae* and *P. cinnamomi* were recovered from 23% and 41%, respectively, of the samples collected. These species of *Phytophthora* are a potential threat to plant biodiversity in this region.

Distribution of *Phytophthora* Species in Forest Soils of the Jocassee Gorges Natural Area in South Carolina, USA

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INTRODUCTION

The Jocassee Gorges natural area (Fig 1), located in Pickens and Oconee Counties of South Carolina, covers approximately 17,600 ha. The area was logged exploitively in the early 1900s, during which time numerous roads and access trails were constructed. Roads, trails, equipment, natural waterways, and foot and vehicle traffic can introduce and spread *Phytophthora* species; therefore, the area is at risk for potential infestation. *Phytophthora* species are known to attack thousands of plant species worldwide (3).

Figure 1 Location map of research area.

OBJECTIVE

To determine the occurrence and distribution of *Phytophthora* species in the forest soils of the Jocassee Gorges Natural Area

MATERIALS AND METHODS

SITE LOCATION. Twenty-two 0.1-acre (0 04 ha) randomly located plots (Figs. 2, 3, and 4) were established using 7.5 minute quadrangle maps. Global positioning system (GPS) data were collected to record the location of each plot (Fig. 4) and to provide information to create a geographical information system (GIS). The GIS included site descriptions, soil analyses, and data on detection of *Phytophthora* species.
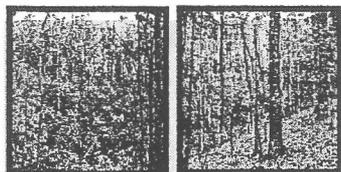
Figure 2. Center of plot 1   Figure 3. Center of plot 18

SAMPLE COLLECTION. From each plot, 15-20 soil cores (2x20 cm) were collected and pooled to make a composite sample. A baiting bioassay (4) was used to recover *Phytophthora* species. Soil samples were mixed and screened through a 2-mm sieve; root pieces were chopped and incorporated into samples. For each sample, 100-ml aliquots of soil were placed into each of 12 475-ml plastic containers. Three bait types were used: leaf disks of camellia (*Camellia japonica* cv. 'Governor Mouton'), and whole needles of both shore juniper (*Juniperus conferta* cv. 'Blue Pacific') and eastern hemlock (*Tsuga canadensis*). The soil aliquots were flooded with 200 ml of distilled water and five baits of each type were floated in each container. Baited soils were incubated in the dark at 25°C. Baits were removed at 24 and 72-h, blotted on paper towels, and placed on plates of PARPH/V8, a medium selective for *Phytophthora* species (4). Isolation plates were incubated at 25°C for 7 days and examined periodically for *Phytophthora* species. Isolates were subcultured to fresh PARPH/V8 and then to V8A and amended, clarified V8A media for identification (4).

Figure 4a *Phytophthora* species recovered during fall collection

Figure 4b *Phytophthora* species recovered during spring collection.

RESULTS AND DISCUSSION

During both the fall and spring collections, *P heveae* (Fig 5) was recovered from five (23%) of the plots (Figs. 4a,4b). *P. heveae* was first identified in 1929 from rubber trees in Malaysia and since then has been found only infrequently in other locations around the world (3), including soils in old-growth hardwood and hemlock forests in Tennessee and North Carolina (2) and on container-grown rhododendrons in North Carolina (1). Interestingly, *P. heveae* was detected only by hemlock needles

Figure 5 Funnel-shaped oogonia of *P heveae*.

During the fall collection, *P. cinnamomi* was recovered from nine (41%) of the plots (Fig. 4a). During the spring collection, it was recovered from 11 plots (50%) (Fig. 4b) This fungus is a serious pathogen of many plant species native to this area (3), including *Pinus*, *Quercus*, *Rhododendron*, and *Vaccinium*. The fungus causes littleleaf disease of *Pinus echinata* in the piedmont, upper coastal plain, and contiguous physiographic regions from Virginia to Mississippi. *P cinnamomi* was detected primarily by camellia leaf disks and juniper needles.

CONCLUSIONS

Phytophthora species are present within the Jocassee Gorges Natural Area and, therefore, are a potential threat to the native vegetation.

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The susceptibility of nance, *Byrsonima crassifolia*, to *Phytophthora cinnamomi*

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Abstract. Prior to 1987, the root pathogen *Phytophthora cinnamomi* Rands was introduced accidentally into a pristine oak forest in the state of Colima, Mexico. Within the affected area most of the oaks were killed. Nance, (*Byrsonima crassifolia* (L.) HBK.), is an understory shrub that disappeared soon after the oak mortality began. To determine susceptibility of *B. crassifolia* to *P. cinnamomi*, 84 Nance seedlings were inoculated at the root collar or lower stem with mycelium from one of two isolates of *P. cinnamomi* (MX-12 from Colima, Mexico or AF-027 from South Carolina). After three months, lesions formed on 100% of the inoculated seedlings; there was no difference in lesion length between isolates. Mean lesion lengths above and below the inoculation point were 95 and 78 mm, respectively; *P. cinnamomi* was reisolated from 43.5% of the lesions. It appears that *B. crassifolia* is susceptible to *P. cinnamomi*, which may explain the disappearance of this understory plant during the early part of the oak mortality epidemic.

The Susceptibility of *Byrsonima crassifolia* to *Phytophthora cinnamomi*

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INTRODUCTION

The residents of a village in the state of Colima, Mexico observed a rapid mortality of trees in the oak forest surrounding this village (Fig. 1). Ook mortality was caused by the root pathogen *Phytophthora cinnamomi* Rands which was accidentally introduced into the forest prior to 1987 (4). The residents also observed that the understory species *Byrsonima crassifolia* (L.) HBK., which is known locally as Nance, also began dying shortly after oaks began dying. As of 2001, the shrub has re-established itself to some extent in older portions of the mortality area, but these individuals are now dying (Fig. 2) and their root crowns exhibit necrotic phloem lesions (Fig. 3).

Nance is a slow-growing, tree-like shrub which may attain a height of 15 m. It is native to southern Mexico and is abundant throughout the Pacific side of Central America. The shrub is an important watershed species in oak forests because it thrives on droughty and rocky soils. The area with affected plants has now spread to over 600 ha. Consequently, there is considerable concern for the future of this important understory species, particularly if it is susceptible to

Figure 1. Location of oak mortality area in Mexico.

OBJECTIVE

To determine the susceptibility of Nance

to *Phytophthora cinnamomi*

Figure 3. Nance shrub showing phloem lesions on root crown.

MATERIALS AND METHODS

SEEDLING PRODUCTION. Seeds were collected in Colima, Mexico in 1998 and brought back to Clemson University. They were planted in soilless container mix in cone-tainers and germinated in o greenhouse. Seedlings then were moved into a growth room and maintained with a 14-h photoperiod.

ISOLATES. Two isolates of *P. cinnamomi* were used: isolate MX-12, which was isolated from soil in the oak mortality area in Mexico, and isolate SC-AF027, which was isolated from field soil at an ornamental crop nursery in South Carolina. Isolates were maintained and grown on acidified potato-dextrose agar (aPDA).

SEEDLING INOCULATION. 84 seedlings were selected for inoculation with *P. cinnamomi* or sterile disks of aPDA (controls). Plants were inoculated at one of two places: on the lower stem ~ 4 cm above the soil line or at the root collar ~ 1 cm above the soil line. Plants were wounded with a sterile razor blade by making a 3- to 4-mm-deep cut through the bark into the outer xylem tissue. Agar plugs (3-mm diameter) from 7-day-old cultures were inserted into wounds beneath the tissue flaps. Wounds were wrapped with Parafilm and aluminum foil to prevent desiccation. Seedlings were placed in the growth room and observed for symptom development.

After inoculation, three different watering treatments were randomly assigned to the seedlings: 28 seedlings received each treatment. Plants either were: watered normally (control)--i.e., watered once every three days: watered lightly--i.e., once every 10 days; or watered heavily--i.e., placed in plastic saucers and watered four times a week.

After three months, individual seedlings were examined for lesion development. The periderm was carefully removed from around the inoculation site, and the length of the resulting necrotic lesion was measured. Tissue sections from lesions were placed on selective medium (3) for re-isolation.

Figure 4. Necrotic lesion on xylem / phloem interface on stem of Nance seedling.

EXCISED TWIG ASSAY. Only 84 Nance seedlings were available for testing and all of the seedlings had been used in the previous test. Therefore, after the stem assay, all seedlings inoculated on the root-collar were used for an excised twig bioassay. Stem segments (55 mm long) were cut from seedlings, surface disinfested, and inoculated following the procedure of Jeffers *et al.* (1). Each twig segment was placed into a 50-ml centrifuge tube containing 10 ml of half-strength cornmeal agar amended with 5 ppm pimaricin. Tubes were infested with one of the two isolates, sealed in plastic bags, and incubated for 10 days at 25°C. Lesion lengths were measured and pathogens were re-isolated as above.

STATISTICAL ANALYSIS. All data were analyzed using ANOVA and Duncan's multiple range test (2).

RESULTS

SEEDLING INOCULATION. Inoculation of Nance seedlings with *P. cinnamomi* produced no foliage symptoms during the 3-month duration of this experiment. However, necrotic lesions developed around wounds on all plants inoculated with *P. cinnamomi* (Fig. 4). There was no difference in lesion length between plants inoculated at the collar or on the stem so data were combined. Lesion length (for all treatments combined) was similar for the two isolates (Fig. 5). There was a significant interaction (P=0.022) between isolate and watering treatment in a 2-way ANOVA so individual treatments were compared (Table 1). There was a difference (P=0.037) in lesion length between plants normally watered and inoculated with isolate AF-027 and the other treatments. *P. cinnamomi* was re-isolated from 43% of the seedlings.

EXCISED TWIG ASSAY. Due to contamination on both media and twig segments, only 30% of the twigs inoculated developed lesions that could be assessed. There was no significant difference (P=0.083) between the isolates in lesion length. Mean lesion length was 7.3 mm for isolate MX-12 and 12.8 mm for isolate AF-027; lesions did not develop on non-inoculated control twigs. *P. cinnamomi* was re-isolated from 77% of the twigs.
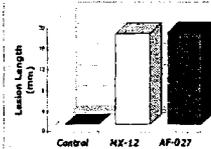

Figure 5. Total length of lesions produced by two isolates of *P. cinnamomi* on Nance seedlings.

Table 1. Effect of watering treatment on the length of lesions (mm) produced by two isolates of *P. cinnamomi*.

| Isolate	Watering Treatment		
	Normal	Light	Heavy
MX-12	13.8b	13.9b	15.8b
AF-027	27.6a	12.2b	15.7b
Control	0a	0a	0a

CONCLUSIONS

Nance appears to be susceptible to *P. cinnamomi*. This may explain the rapid disappearance of this shrub species within the oak mortality area and suggests that care should be taken to avoid introduction of *P. cinnamomi* into other similar forests.

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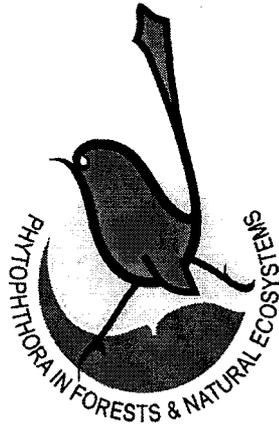
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290

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Author index