IUFRO Working Party 7.02.09
Proceedings from the First International
Meeting on Phytophthoras in Forest and
Wildland Ecosystems

Phytophthora Diseases of Forest Trees

Editors
Everett M. Hansen and Wendy Sutton

Sponsored by the
Department of Forest Science, Oregon State University
and the
USDA Forest Service, Southwest Oregon Forest Insect and Disease Technical Center

Grants Pass, Oregon USA
August 30 – September 3, 1999

Forest Research Laboratory, Oregon State University
Corvallis, Oregon 97331

ISBN 0-87437-002-7

CONTENTS

Posters, Abstracts and Field Trip

IUFRO Working Party 7.02.09 Phytophthora Diseases of Forest Trees

SUMMARY OF THE MEETING

The inaugural meeting of Working Group 7.02.09, "*Phytophthora* Diseases of Forest Trees," under the auspices of the International Union of Forestry Research Organizations, brought together 44 scientists from 10 countries in Grants Pass, Oregon. The convening committee of Dr. Clive Brasier, Forestry Authority, Great Britain, Dr. Everett Hansen, Oregon State University, and Dr. Ken Old, CSIRO, Australia, had noted that despite active research and management programs on *Phytophthora* diseases in forest ecosystems on three continents, there has been no forum for scientists to gather and share information. These Proceedings document the breadth and depth of the dialog that was established in Grants Pass during this First International meeting on Phytophthoras in Forest and Wildland Ecosystems.

The meeting was sponsored by the Department of Forest Science, Oregon State University and the USDA Forest Service, Southwest Oregon Forest Insect and Disease Technical Center. Wendy Sutton managed the many details of the meeting and Ellen Goheen provided local arrangements. Their collaboration assured a smooth, relaxed meeting in comfortable surroundings, with ample opportunity for social as well as professional exchange. Don Goheen, Katy Marshall, and Pete Angwin, with the assistance of their colleagues in the Forest Service, organized and led a field trip focused on the challenges of managing Port-Orford-cedar, *Chamaecyparis lawsoniana*, under the threat of the exotic pathogen *Phytophthora lateralis*. Together with a pre-meeting general forest pathology field trip, an afternoon in the redwoods of northern California, and an evening jet boat trip on the Rogue River, participants were exposed to a diverse array of forest ecosystems and forest management objectives.

The meeting began with three summary papers, presenting a broad view of the history and current understanding of the ecology of *Phytophthora* species and the effects they have on forest and wildland ecosystems in Australia, Europe, and the Americas. *Phytophthora cinnamomi* continues to demand attention in Australia, as a threat to ecological as well as economic values. This pathogen, with its enormous host range spanning many genera of rare and endemic plants, has been named one of 5 "Key Threatening Processes" endangering ecosystems and ecological processes on the continent. A more diverse array of pathogenic species affect European trees. *Phytophthora cinnamomi* is present and causing new concerns in cork oak in the Iberian Peninsula, as well as its more familiar role as cause of root diseases of chestnut and red oak. In addition, newly described species threaten alders in northern Europe, and are implicated in oak decline in Germany and surrounding countries. Little is known about *Phytophthora* species in American forests, especially in the tropics, but old records suggest that *P. cinnamomi* devastated chestnut forests in the southern United States long before it attracted attention in other parts of the world and in the SE United States as cause of littleleaf disease. Today, the multi-faceted research and disease management program aimed at *P. lateralis* in Oregon and northern California attracts the most attention.

The opening session addressed the ecology, epidemiology, and impacts of *Phytophthora* species in forests through a series of papers, poster presentations, and lively discussion. The evidence implicating several species of *Phytophthora* in European oak decline, in a new disease of alder, and in cork oak decline was presented. There were also descriptions of previously poorly understood species with saprophytic or perhaps opportunistic behaviors in European forest soils. Hypotheses to explain the recovery of susceptible vegetation many years after the passage of a *P. cinnamomi* killing front in Australian forests were presented. Several presentations described new diagnostic tools for *P. cinnamomi* and *P. lateralis* based on molecular markers, and improved baiting techniques for recovery of *Phytophthora* species.

The second session addressed *Phytophthora* biology and genetics. The hybrid nature of the alder *Phytophthora* was described and the implications of this newly recognized route to speciation were explored. Other presentations focused on genetic variation in populations of *P. lateralis, P. quercina,* and *P. cinnamomi*, with discussion of implications for epidemic origin and disease management. These themes were revisited in the Business Meeting and resulted in a resolution from the Working Group.

The session on disease management focused on *P. lateralis* in western North America and *P. cinnamomi* in western Australia. The determination and multifaceted efforts of a private mining company and their cooperators in University and government agencies in Australia to reverse the impacts of *Phytophthora cinnamomi* in Jarrah forests were especially impressive. The program to halt the spread of *P. lateralis* on Federally managed forest lands and restore Port-Orford-cedar through resistance breeding in Oregon and California provided interesting parallels and contrasts to the Australian situation. The field trip allowed meeting participants to see *P. lateralis* and root rot up close, and appreciate the challenges of stopping further spread. Visitors were asked to wash their boots before moving from infested to healthy stands. The importance of human vectors was demonstrated later when Don Goheen reported that *P. lateralis* was isolated from the wash water of the group!

The meeting concluded with a business session. It was decided that the next meeting will be in two years (August 2001) in western Australia. Giles Hardy will assume leadership of the Working Group, with Everett Hansen, Clive Brasier, and Ken Old continuing to serve as an advisory executive committee. Giles will be joined by colleagues from CSIRO, ALCOA, CALM, and other Australian Universities in organizing the meeting. Several recommendations and resolutions, described in the Minutes of the meeting (see below), were presented, discussed, and voted upon. These resolutions have subsequently been forwarded to IUFRO and to officials in the United States Forest Service. Members are encouraged to distribute the resolutions as useful to reduce the risks that *Phytophthora* species pose to the world's forests.

Everett M. Hansen
Professor and
Chair, IUFRO Working Party 7.02.09

MINUTES OF FIRST BUSINESS MEETING GRANTS PASS OREGON, SEPTEMBER 3 1999

IUFRO Working Party 7.02.09 Phytophthora Diseases of Forest Trees

Present, 40 members of the working party plus Chairman and deputies

Agenda item 1
Proceedings of first working party meeting
Everett Hansen outlined the proposals for publication of the proceedings by Oregon State University press:

- Papers from all contributions (including posters) will be published
- The full versions of each paper should be sent to Everett as Email attachments in MS Word.
- There will be minimal editing so authors (especially non-native English speakers) should arrange for a colleague, fluent in English, to check the text for language use and grammar.
- Format will be fixed up where needed.
- Text of offered papers and posters should be limited to 4500 words plus no more than 1 page of tables or figures.
- Proceedings will be bound and sent to all participants.
- Depending on costs the print run will provide for extra copies which will be made available to libraries and an ISBN number will be obtained.
- Deadline for text will be October 31, publication of papers received after that date will not be guaranteed.
- Abstracts may be published in the Phytophthora Newsletter and put on the IUFRO Web page.

Item 2
Report to IUFRO by Chairman
Everett Hansen agreed to send a short report of the meeting to the IUFRO Secretariat.

Item 3
Recommendations to IUFRO from Working Party 7.02.09
Ken Old proposed three draft recommendations based on issues raised and discussed during the workshop:

Recommendation 1
Issue
There is a need to assess the capacity of "wild' *Phytophthora* spp., newly emerging species (including hybrids) and species with currently limited geographical ranges (e.g. *P. lateralis*), to cause disease in forests and other vegetation in countries where they are not known to occur.

Recommendation
Germplasm should be made available between countries for pathogenicity testing of a broad range of related and non-related host genera and species against *Phytophthora* species which infect forest trees and native vegetation in their known range. Diagnostic methods need to be improved and communicated widely so that the distribution and impacts of *Phytophthora* spp in forests and wildland ecosystems can be determined.

Recommendation 2
Issue
Global warming and associated climate change patterns are likely to favor *Phytophthora*-induced diseases.

Recommendation
Robust data is needed on the distribution and impacts of *Phytophthora* spp on forests and other vegetation worldwide and on the climatic requirements of the pathogens. Such information should then be used to model the potential effects of climate change on *Phytophthora* epidemics in forests and wildlands on a global basis.

Recommendation 3
Issue
There is circumstantial evidence that, despite internationally agreed protocols, *Phytophthora* epidemics and possibly the formation of novel hybrids between *Phytophthora* spp. have been initiated by movement of diseased planting stock between and within countries.

Recommendation
To reduce these risks, and in view of the limited knowledge of the consequences of genotype variation on disease, the issue of phytosanitary certificates for planting stock should be based on more comprehensive and effective diagnostic testing. In addition, concepts of "area freedom" should be applied with great caution to *Phytophthora* diseases.

The above draft recommendations were discussed and met with the support of the meeting. Improved versions will be submitted to IUFRO with the report of the meeting.

Item 4
Communication out of session
Clive Brasier led a discussion of ways in which to maintain communication within the working party.
The following suggestions were made;

- Email addresses of all participants and most other members of the working party will be maintained and will be available.
- A request was made that areas of interest of members should be appended to Email addresses.
- There was little enthusiasm for a separate Web site for the working party as much can be achieved with an Email corresponding group.
- Forpath. An existing Website is available for open letter correspondence.
- The organizer or working party No 2 can be contacted on issues to be included on the program.

Item 5
Next meeting
There was general support for the next meeting to be held in September or October 2001 in Perth , Australia, with a possibility of an extension of the program to Melbourne for field trips to sites of *Phytophthora* damage to native vegetation within reach of the city.
Giles Hardy of Murdoch University WA offered to be the principal organizer of the meeting.
This offer was accepted.

Item 6
Vote of thanks to the organizers of the Grants Pass workshop.
At the end of the morning's activities, Thomas Jung gave a vote of thanks on behalf of all participants to Everett Hansen and his team (especially Wendy Sutton and Ellen Goheen) for organizing and running what has been an extremely successful workshop, scientifically and socially. Thanks are also due to USDA Forest Service staff who led or participated in the field day.

Ken Old

9/9/99

THE ROLE OF *PHYTOPHTHORA* PATHOGENS IN FORESTS AND SEMI-NATURAL COMMUNITIES IN EUROPE AND AFRICA

Clive M. Brasier

Forestry Commission Research Agency, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK

Dedicated to W.C.T. (Bill) Young and R.G. (Bob) Strouts of the FC Pathology Advisory Service, who recognised in the 1960s a need for greatly increased research into *Phytophthora* diseases of trees.

Europe before 1990

Until the beginning of the 1990s, economic and environmental concern about Phytophthoras on forest trees in Europe was focused mainly on root and collar rot, or 'ink disease', of native European chestnut, *Castanea sativa.* Between 1900 and 1950, *C. sativa* suffered heavy mortality in the main chestnut growing areas of southern Europe, especially Italy, France and Iberia, due to the spread of *Phytophthora cambivora* and *P. cinnamomi* (Peace, 1962; Moreau & Moreau, 1952). This epidemic paralleled a destructive epidemic on native chestnuts in the south eastern USA, also caused by the spread of *P. cinnamomi* (Crandall, Gravatt & Ryan, 1945; Hansen, this volume). In northern Spain, c. 75% of the chestnuts were killed (Urquijo Landaluze, 1936; and Artaza, 1949) while in Portugal the death of several million chestnuts was reported (Del Canizo, 1942). Both *P. cinnamomi* and *P. cambivora* are considered introduced to Europe. *P. cinnamomi* was probably introduced in the late 18th century from a centre of origin in the Papua New Guinea-Celebes region; (see Fig. 3). Possibly, *P. cambivora* was introduced at around the same time.

There is confusion in the European literature of the mid 1900s over the identity of these two species (e.g. Moreau & Moreau, 1952; Urquijo Landaluze, 1947). However Grente (1952) found no appreciable difference in aggressiveness between them, *C. sativa* being highly susceptible to both. Multiple epidemic waves probably occurred across Europe as a result of their separate patterns of introduction and spread (Brasier, 1993). Day (1938) concluded that, at its most severe, the disease was consistently associated with high soil moisture content. Certainly *P. cambivora* appears to require heavier, more moisture-holding soils. *P. cinnamomi* may also be very active on seasonally dry soils. Today, the two pathogens remain a problem in the main commercial chestnut growing regions; and continue to be responsible for death of *C. sativa* across many other parts of Europe, from southern Britain (where the disease partly restricts the area available for chestnut coppice cultivation; Peace, 1962) to Greece. Programmes of selective breeding and genetic manipulation of chestnut for resistance are in progress in several countries (e.g. Robin, Capron & Capdevielle, this volume).

Another higher profile *Phytophthora* disease is *Phytophthora* stem canker of American red oak, *Quercus rubra*, in south western France. This is caused by *P. cinnamomi* (Moreau & Moreau, 1952). Although 'ink disease' is often used as an informal name for this disease, this is in fact a misnomer (Peace, 1962). Both host and parasite are introduced, the oak being used in the cooperage industry. The pathogen produces multiple necroses of the phloem and cambium around the trunk of the tree. Microclimate (especially frost) and topography have a significant impact on disease distribution (Marcais, Dupuis & Desprez-Loustau, 1996a; Levy, this volume). Since the initial inoculum probably comes from the soil, the pathogen is, by implication, likely to be active on *Q. rubra* roots. Pathogenicity tests show that fine roots of *Q. rubra* are susceptible; but the secondary root tissues are less susceptible than are those of the collar and stem (Marcais *et al*., 1996b).

Somewhat outside the main scope of this paper, but of economic and epidemiological importance, is a damaging epidemic of *P. cinnamomi* root disease on conifers and woody ornamentals that occurred in nurseries and gardens across Europe during the 1960s and 70s. Particularly affected were rhododendrons, azaleas, heathers (*Erica* spp.) and horticultural varieties of *Chamaecyparis lawsoniana* (Port Orford Cedar or Lawson Cypress) (e.g. Smith, 1988). The epidemic probably reflected the spread of *P. cinnamomi* within the nursery trade and from there out into the landscape. By the 1980s, the epidemic was largely under control in north western Europe due to management procedures such as containerisation of planting stock; while fungistatic chemicals have been widely used to suppress symptoms. Nonetheless, losses of susceptible ornamentals in gardens and shelterbelts, including small to large *C. lawsoniana*, remains a frequent occurrence across southern Britain, the disease often appearing some years after initial planting out from a nursery.

Other records of* Phytophthoras *on trees in Europe

The spread of *P. cinnamomi* in horticulture, together with routine disease enquiries indicating that *Phytophthora* diseases of trees were more widespread than previously considered, led the UK Forestry Commission disease advisory service to initiate routine isolation from suspected cases of *Phytophthora* damage in forests and in urban situations (cf. Strouts, 1981). Between 1968 and 1990, these investigations yielded many new records of host-*Phytophthora* associations. Some of these records are summarised in Table 1; native European hosts are shown in bold type, non-native hosts in normal type. It should be noted that only for about 30% of these associations have Koch's postulates been carried out. Also, some of these associations were already known from the pre-1960s literature. Thus, in addition to problems with chestnut and ornamentals described above, it was also known that *P. syringae* and *P. cambivora* caused root and collar rot of European beech (Day, 1938, 1939); that *P. cactorum* attacked beech seedlings in the forest and in nurseries (Liese, 1926); and that *Taxus* was very susceptible to *P. cinnamomi* (Buddenhagen, 1955).

Notable among the new records were the susceptibility of *Aesculus hippocastanum* to collar rot and aerial stem cankers caused by *P. megasperma*, *P. cactorum* and *P. citricola* (Brasier & Strouts, 1976); the susceptibility of *Tilia* spp. to collar rots caused by *P. citricola* and *P. megasperma*; and the susceptibility of *Acer* (especially *A. pennsylvannicum*) to *P. cambivora*, *P. citricola* and *P. megasperma*. Other unusual records included the occurrence of *P. hibernalis* on foliage of *C. lawsoniana* (Brasier & Strouts, 1978); the occurrence in Ireland of *P. eruigena* sp. nov. on the same host (Clancy & Kavanagh, 1977); and the first European record of *P. ilicis* on leaves and young shoots of ornamental holly (Strouts, Rose & Reffold, 1989).

Many of the records involving hardwood trees reflected locally severe episodes of root and collar rot, e.g. collar rots of *Tilia*, *Acer*, *Platanus*, *Sorbus* and *Populus*. Such episodes were often associated with heavy clay soils, soil compaction around stem bases, or high nitrogen inputs due to undergrazing by cattle. *P. megasperma* and *P. citricola* are especially associated with wet or waterlogged soils.

Many of these hardwood records are also from roadsides, parks, pastures or gardens where the trees tended to be subject to a variety of stresses in addition to climate.

The records in Table 1 largely represent cases of overt or acute disease reported to the advisory service, rather than instances of mild disease. Nor do they encompass many potential European hosts of *Phytophthora* that occur only outside Britain or north-west Europe.

Nonetheless, they indicated that a wide variety of tree species were potentially susceptible to Phytophthoras, given appropriate conditions for infection and disease development. It is likely, therefore, that a great deal of chronic and even severe Phytophthora root damage on trees in Europe remains unnoticed or uninvestigated because the symptoms are only 'moderate'; because the damage is mainly below ground level and therefore unseen; or because they are attributed to other causes such as *Armillaria* root rot, or to general decline phenomena.

It is also notable from Table 1 that certain tree genera, such as *Aesculus* and *Chamaecyparis* are susceptible to a wide range of *Phytophthora* species. Indeed, *C. lawsoniana* in particular is attacked at its roots, collar and foliage and appears to be something approaching a 'universal suscept'. Likewise, certain Phytophthoras such as *P. cinnamomi, P. citricola* and *P. megasperma* have rather wide host ranges; while others such as *P. ilicis* are apparently very host specific.

TABLE 1. *Phytophthora*-host associations in Europe pre-1990 (compare also Table 2).

	***Phytophthora*[1]**																	
***Host*[+]**	*P. cactorum*	*P. cambivora*	*P. cinnamomi*	*P. citricola*	*P. cryptogea*	*P. drechsleri*	***'P. ereugena'*[2]**	*P. hibernalis*	*P. ilicis*	*P. lateralis*	*P. megasperma*	*P. syringae*	***P. sp 'O group'***	*P. gonapodyides*	***'P. alni' sp. nov***	***P. quercina sp. nov***	***P. spp. nov***	***P. italica sp. nov***
Acer spp.	○	○		○							○							
Aesculus hippocastanum	○	○		○							○							
Castanea sativa	○	●	●															
Chamaecyparis lawsoniana		○	●	○	○		○	○			○							
Crataegus sp.			○								○			○				
Eucalyptus spp.			○															
Fagus sylvatica	○	○		○								○						
Ilex aquifolium									○[3]					○				
Larix sp.			○															
Malus spp.	●											○						
Nothofagus spp.			○	○	○						○							
Olea europea											○							
Platanus sp.		○		○														
Populus sp.											○	○						
Prunus sp.	○			○										○				
Quercus rubra			●[4]															
Salix spp.				○									○	○				
Sorbus sp.	○	○									○							
Taxus baccata			○	○	○													
Thuja plicata				○	○													
Tilia spp.				○							○							
Ulmus sp.											○							

Based mainly on records of UK Forestry Commission disease advisory services (R.G. Strouts and colleagues) 1970-1990; also including well known, published records for Europe pre-1970.

[+]Bold type, native European host; normal type, non-native host. 1=New taxa shown in bold. 2=Clancy & Kavanagh (1977). NB Formal description incomplete. 3= Pathogens so far confined to the UK. 4=Disease so far confined to France.

○, Occasional record; ●, economically or environmentally important disease, or potentially so.

Other records include *P. cinnamomi* on *Abies*; *P. citricola* on *Sambucus, Liquidambar, Pseudotsuga menzeisii, Cupressocyparis* and *Abies; P. megasperma* on *Sambucus* etc.

TABLE 2. New *Phytophthora*-host associations recorded in Europe since 1990 (compare also Table 1).

	Phytophthora[1]															
Host[+]	*P. cactorum*	*P. cambivora*	*P. cinnamomi*	*P. citricola*	*P. cryptogea*	*P. drechsleri*	*P. ilicis*	*P. lateralis*	*P. megasperma*	*P. syringae*	***P. sp. 'O group'***	*P. gonapodyides*	***'P. alni' sp. Nov***[2]	***P. quercina sp. nov***	***P. spp. nov. various***	***P. italica sp. nov***
Alnus spp.													●			
Arbutus sp.			○													
Chamaecyparis lawsoniana								○[3]								
Myrtus communis																○[5]
Olea europaea									●		●[4]					
Quercus ilex			●													
Quercus robur	●	●		●						●		●		●	●	
Quercus spp.*	●	●		●						●		●		●	●	
Quercus suber			●			○										

[+] Bold type, native European host; normal type, non-native host.
1=New taxa shown in bold. 2=Alder Phytophthora: not yet formally designated; 3= Pathogen so far confined to France; 4= Disease so far confined to Spain; 5=Pathogen so far confined to Italy (Ref).
○, Occasional record; ●, economically or environmentally important disease, or potentially so.
*****, includes *Q. petraea, Q. ceris, Q. pubescens, Q. frainetto* and other *Quercus* spp. See for example Jung *et al.*, (1999) and Hansen & Delatour (1999)

Europe since 1990

Between 1990 and 2000, the profile of Phytophthoras in forests and natural ecosystems in Europe has risen appreciatively as a result of three prominent 'new developments' (Table 2). These will now be summarised.

P. cinnamomi associated mortality of evergreen oaks in the Mediterranean region

In 1991 a widespread mortality of native cork oak, *Q. suber*, and holm oak, *Q. ilex* (*Q. rotundifolia*) in central and south western Spain was reported by Spanish government authorities. Symptoms ranged from sudden death, usually in spring or autumn, to decline over several years. Concern was expressed in Spain about the possible arrival of North American oak wilt, *Ceratocystis fagacearum*. Until this time, this and similar declines in Portugal and Italy had been ascribed to factors such as drought and attacks by insects, *Hypoxylon mediterraneum* and *Armillaria*.

When representative sites were investigated in May 1991, affected trees were found to have substantial loss of fine feeder roots, while some had extensive lesions on major roots. Dead and dying trees were often in groups and associated with valleys, seasonally lying water, depressions and disturbances such as ploughing. Maquis species such as *Cistus* and *Lavendula* were also affected. *P. cinnamomi* was isolated from root necroses on oaks at a number of sites (Brasier, 1991, 1992); and further investigations confirmed that *P. cinnamomi* was commonly associated with roots of declining oaks at decline foci across southern Spain and Portugal (Brasier, Robredo & Ferraz, 1993; Tuset *et al.*, 1996). A decline hypothesis was presented, proposing a variety of interactive decline factors. These included the spread of *P. cinnamomi* from chestnuts onto oaks; locally explosive build-up of *P. cinnamomi* inoculum in the soil; recurrent drought stress and unseasonal heavy rains since the early 1980s; changes in landuse; depleted mycorrhizae; and secondary attacks by other fungi and insects (Brasier, 1993, 1996).

In a further study using apple baits to sample fine feeder roots of oaks in Alentjo, central Portugal, in February-June 1994, E. Sanchez (personal communication) obtained *P. cinnamomi* from eight of thirteen *Q. suber* trees tested at different decline sites; but no *P. cinnamomi* from nine *Q. suber* tested at various 'healthy' sites. Similarly, three of five *Q. ilex* trees from decline sites yielded *P. cinnamomi*; but only one of seven *Q. ilex* trees from 'healthy' sites did so. Overall, therefore, c.62.5% of trees from decline sites but only 4.5% of trees from healthy sites yielded *P. cinnamomi*. All the soils were very dry at the time of sampling. In another study, Gallego, De Algaba & Fernando-Escobar (1999) planted *Q. ilex* seedlings in soils collected from oak decline sites in Extremadura, Spain. A majority of the oak seedlings died, and *P. cinnamomi* was consistently isolated from their roots. *P. cinnamomi* has also been isolated from a range of maquis understory species in Portugal (A.C. Moreira, personal communication) and from oak decline sites in France (Robin *et al*., 1998) and Italy (N. Luisi, personal communication).

In experimental inoculations, *P. cinnamomi* was shown to be pathogenic to bark of standing *Q. suber* and *Q. ilex* trees in the field in Spain (Cobos *et al*., 1993; Tuset *et al*., 1996). When roots of potted seedlings of *Q. ilex* and *Q. suber* were exposed to a zoospore inoculum of *P. cinnamomi*, those of *Q. ilex* were rather more susceptible than those of *Q. suber*, while the latter showed increased susceptibility with flooding (Moreira *et al*., this volume; Gallego *et al*., 1999; Robin *et al*., 1998). To date, there are no reports of how these oak species respond if subjected to drought stress following inoculation. Roots of maquis understory shrubs, including *Cistus* and *Lavendula*, however, have also been shown to be susceptible to zoospore inoculum of *P. cinnamomi* in pot inoculations (A.C. Moreira, personal communication).

In fungicide trials, mist spraying of abaxial leaf surfaces of heavily declining *Q. suber* trees in Portugal with phosphonate-containing fungicide (Aliette ®; at 80 g/tree) is reported to have led to their recovery within 6 months compared with similarly declining unsprayed control trees (Rhone Poulenc Agro, Lisbon; Commercial leaflet, 1995). In addition, in Spain, *Q. suber* trees given single direct trunk injections of potassium phosphonate or another fungicide showed a significant improvement in vegetative growth within two

years and some recovery by the third year (Fernandez-Escobar *et al*., 1999).

Elicitin proteins ('cinnamomins') produced by *P. cinnamomi* have recently been purified and shown to induce a hypersensitive response in young *Q. suber* seedlings, indicating that they may have a role in pathogenesis. The genes encoding the elicitins have also been sequenced. These sequences may provide a PCR-based method for diagnosing the pathogen in roots of oaks and other tree species (Coelho *et al*., 1997 and this volume, Duclos *et al*., 1998). With regard to selection of resistant *Q. suber* material for future planting, an investigation of DNA markers linked to *P. cinnamomi* resistance is in progress; and *Q. suber* plantlets suitable for genetic transformation had been regenerated from callus tissue. In addition, three polypeptides produced in roots or leaves of *Q. suber* following infection with *P. cinnamomi* are under investigation as potential resistance markers (A.C. Coelho and A. Cravador, personal communication).

On the whole, roots of oaks are probably considerably more resistant to *P. cinnamomi* than those of their close relatives, the chestnuts. (cf. Crandall *et al*., 1945; Marcais *et al*., 1996b). Equally, however, it is clear that some oaks such as *Q. ilex* are potentially very susceptible to *P. cinnamomi*; that *P. cinnamomi* is closely associated with decline sites of *Q. suber*, *Q. ilex* and other oak species in the Mediterranean region; and that *P. cinnamomi* can be frequently isolated from their roots. It is likely, therefore, that this pathogen has a significant role, whether primary or interacting with other factors, in the current mortality and decline of evergreen oaks in the region.

Phytophthoras and deciduous oak decline in central and western Europe

Episodes of recurrent, severe decline of native deciduous oaks, in particular *Q. robur* and *Q. petraea*, have occurred across central Europe from Britain to the Caucasus since c.1900. Like evergreen oak mortality in the Mediterranean, this has been ascribed previously to various biotic and abiotic factors (Delatour, 1983; Luisi, Lerario & Vannini, 1993); Dreyer & Aussenac, 1996). The demonstration that *P. cinnamomi* was associated with the Mediterranean oak declines led inevitably to a proposal that *P. cinnamomi* or other Phytophthoras, might be involved in the oak declines across central Europe also (Brasier, 1993).

A research programme initiated in Germany, subsequently extended to several other European countries, has resulted in many new and interesting observations. Sample surveys from roots of declining oaks led to the demonstration that a number of Phytophthoras could be isolated from necroses on feeder and secondary roots (Blashke, 1994; Blashke & Jung, 1996; Jung, Blashke & Neumann, 1996; T. Jung, *et al.*, this volume). These included well-known species such as *P. cambivora*, *P. citricola*, *P. syringae* and *P. gonapodyides*; together with several new species, including *P. quercina* sp. nov., which appears to be relatively specific to *Quercus* (Jung *et al*., 1999). In addition, at least three other previously unknown taxa have recently been found associated with oak roots in Poland, France and Germany (Hansen & Delatour, 1999; and T. Jung, personal communication). It now appears that *P. cambivora*, *P. quercina*, *P. citricola*, *P. gonapodyides* and *P. syringae* are associated with roots of deciduous oaks across much of Europe, from the UK to Hungary.

In Germany, a positive correlation has been demonstrated in *Q. robur* between crown dieback level and loss of feeder roots. It has also been demonstrated that there is a 2–4 fold increase in the risk of oak decline when *Phytophthora* is present in the soil (T. Jung, this volume). In France, it has been observed that the presence of Phytophthoras in the soil is not consistently associated with the crown condition of oaks, the Phytophthoras also being present on 'healthy' oak sites (Hansen & Delatour, 1999).

In pathogenicity tests on roots of potted *Q. robur* seedlings involving zoospore inoculum of a range of Phytophthoras, *P. quercina*, *P. cambivora*, *P. cactorum* and *P. citricola* caused the most root rot among the species tested (Jung *et al*., 1999). Root infection by *P. quercina* and *P. cambivora* also led to foliar symptoms. Overall, *P. quercina* was considered the most aggressive pathogen in these tests (though the incubation temperature of *c.* 20° used would probably not favour *P. cambivora*). In a similar test (C. Delatour, personal communication, and Delatour this volume), *P. cinnamomi* (a species not tested by Jung *et al*., 1999) caused more damage to roots of potted *Q. robur* than *P. quercina*, *P. citricola* and a *Phytophthora* from oak in France (*Phytophthora* "species 6", Hansen & Delatour, 1999). *P. quercina* produces elicitin proteins, called 'Quercinins' that cause necrosis of leaves of excised oak shoots; similar elicitins are produced by *P. gonapodyides* and *P. citricola*. The possibility that they act as translocatable 'toxins', inducing foliar symptoms in affected oak trees, is under investigation (Heiser *et al*., 1999; Osswald *et al*., this volume).

It has recently become clear, therefore, that central European deciduous oaks are host to a remarkably wide range of root-rotting *Phytophthora* species (though probably not often to *P. cinnamomi* because of climatic constraints, see below). Their association with so many different Phytophthoras, some with apparently restricted and some with wide distributions, makes elucidating the role of Phytophthoras in these declines especially difficult. Some may be co-evolved endemic Phytophthoras, that, under unstressed or normal conditions, live in a reasonable balance with the host. If the host is stressed, however, they could well become a critical factor in its survival. They could also predispose oaks to attack by other organisms such as *Armillaria*, *Collybia* and *Agrilus* spp.. Other Phytophthoras may be introduced species able to cause more acute damage.

Again, different Phytophthoras may have different ecological strategies. Some species, such as *P. quercina*, may be fine-root 'nibblers' with a further potential to produce toxins that reduce crown vigour. Others, such as *P. cambivora*, might tend to infect the larger roots and collar, and be able to inflict substantial bark injury. In addition to such possibilities, much has yet to be learned about the influence of host resistance, site factors and climate change on Phytophthora root rots of oak.

The new hybrid alder phytophthoras

In 1993, a serious new *Phytophthora* disease of the common riparian Alder, *A. glutinosa*, was diagnosed in Britain (R.G. Strouts, unpublished). The disease was soon shown to be widespread, locally severe, and spreading, along UK river systems. It also occurs in some horticultural shelterbelts and woodland plantings of alder, including *A. cordata* and *A. incana* (Gibbs, 1995). The symptoms are generally those of a classic collar rot, with bark lesions sometimes spreading up the trunk to a height of *c.* 0.5-1.5 m. The current rate of loss of alder along UK rivers is estimated, from annual surveys, to be *c.* 2% per annum (Gibbs, Lipscombe & Peace, 1999).

Surveys have shown the disease to be present in other parts of Europe. In the Netherlands, it occurs among alders in the meers or swampy wetlands (C. van Dyck and H. De Gruyter, personal communication). In France, it is causing serious damage to alder on the major rivers of the north-east, and is also present elsewhere (Anon, 1997; J. C. Streito & J. N. Gibbs, this volume). The disease is locally severe in parts of northern and southern Germany and in Austria (Hartman, 1995; S. Werres, T. Jung & T. Chezch, personal communications). It also occurs locally in western Sweden (Olsson, 1999). Given this wide distribution, the disease is probably present in some neighbouring countries also. However, despite careful investigation, it has not so far been found in Norway, Denmark and Finland, nor in eastern Sweden. Nevertheless, this new disease appears to represent a serious threat to natural and planted alder stands in Europe and to the stability of riparian ecosystems (Gibbs, *et al.*, 1999). It may also represent a threat to alders outside Europe, such as those of North America.

Phytophthora has not previously been reported to be a pathogen of alder. An initial study of the causal *Phytophthora* showed that it superficially resembled *P. cambivora*, but differed from it in many

unusual features. These included different cardinal temperatures for growth, self-fertility rather than heterothallism and an unusually high level of zygotic abortion. On this evidence, it was suggested that the *Phytophthora* might be a species hybrid involving *P. cambivora* as one of the parents (Brasier, Rose & Gibbs, 1995).

When the species hybrid hypothesis was investigated, it was demonstrated that the alder *Phytophthora* comprised a range of heteroploid species hybrids (Brasier, Cooke & Duncan, 1999). A common, 'standard' hybrid occurs across much of Europe, from Scotland and Sweden to Austria and southeast France. This type is near-tetraploid and is apparently unable to complete meiosis beyond metaphase I. Standard isolates also have dimorphic ITS sites (duplicate DNA bases) in their rDNA genes, representing the DNA signatures of more than one species. These observations are consistent with their being allopolyploid hybrids between *P. cambivora* and another *Phytophthora* close to *P. fragariae*.

Phenotypically and genetically distinct alder phytophthoras, collectively termed 'variants,' also occur naturally in parts of Sweden, UK, Germany and the Netherlands. These have unique combinations of colony morphologies, gametangial morphologies (including either smooth-walled or ornamented oogonia and either amphigynous or paragynous antheridia), and temperature-growth relationships. Some are highly unstable in culture. The chromosome numbers of variants tend to be intermediate between diploid and tetraploid, and some of the 'lower chromosome number' variants are able to complete meiosis. Their ITS profiles are also different from the standard type, tending to be more similar to those of *P. cambivora* or those of *P. fragariae*. The variants may be genetic breakdown products of the standard near-tetraploid hybrid, or backcross products (Brasier *et al.,* 1999).

Pathogenicity tests on freshly cut logs show the standard hybrid to be a highly aggressive pathogen of alder bark, whereas the two putative parent species, *P. cambivora* and *P. fragariae*, are not. In addition, the standard hybrid is non-pathogenic to bark of a range of other hardwood and conifer hosts, indicating that it is relatively host specific. The different variant types, on the other hand, range from being weakly to highly aggressive to alder bark, consistent to their widely differing genotypes(C. M. Brasier & S. Kirk, unpublished). In another test, young alder seedlings inoculated with zoospores of the Dutch variant exhibited massive root rot compared with the controls. A *P. citricola* isolate, obtained from the water of an alder meer in the Netherlands, also caused substantial root rot of alder seedlings (C. van Dyck, personal communication).

As a whole, the alder phytophthoras appear to be a swarm of relatively recent species hybrids that are still in the process of evolution. This process includes continuing recombination and homogenisation of their ITS arrays (Brasier *et al.,* 1999). During the hybridisation process, the new alder phytophthoras may also have acquired the ability to attack a new host that the parent species could not attack (Brasier, *et al.,* 1999). Together with the likelihood that the new alder Phytophthoras have arisen via hybridisation between two introduced Phytophthoras, this raises important quarantine issues (Brasier, 2000). It also raises critical taxonomic issues. While it is intended that the new alder Phytophthoras will be described as a 'new species' (probably as '*P. alni*'; see Table 1), the existence of a range of unique hybrid genotypes and phenotypes means that it will require unusually careful definition (Brasier *et al.,* 1999).

Other new records in Europe

In addition to the above developments, a new species of *Phytophthora, P. italica*, has been described on young *Myrtus communis* in nurseries (Cacciola, Magnano di San Leo & Belisario 1996; *P. italica* was previously assigned to *P. iranica* by Belisario, Magnano di San Leo & Cacciola, 1993); *Phytophthora* sp. 'O-group' (Brasier, Hamm & Hansen, 1993; also to be described as a new species) has been found, along with *P. megasperma*, to be a causal agent of root and collar rot of olive trees in southern Spain (E. Sanchez & C. M. Brasier, unpublished); and *P. lateralis* has been found on seedling *Chamaecyparis lawsoniana* at a nursery in France, probably as a result of its introduction from North America (C. Delatour and E.M. Hansen, personal communication). (Table 2).

***Phytophthora* in forests and semi-natural communities of Africa**

In South Africa, *P. cinnamomi* was once suggested to be an endemic pathogen (von Broemsen & Kruger, 1985). However, molecular fingerprinting studies have shown that the South African population has very limited genetic variability, and the pathogen is now considered to be introduced, as in Europe (Linde *et al*., 1997; Linde, 1999). *P. cinnamomi* is causing significant damage to the famous Gondwana-origin fejnbos (mediterranean heath) vegetation of the southern Cape area. The native Proteaceae are especially susceptible. It has also been isolated from native tree species in Transvaal, to the north-east (Von Broembsen & Kruger, 1985). In the Transvaal, *P. cinnamomi* is a serious pathogen of established plantation *Eucalyptus* spp., especially *E. fastigata* and *E. fraxinoides*. As a result, the use of these high elevation species in commercial forestry is greatly restricted (Wingfield & Knox-Davies, 1980). In field-based pathogenicity tests, *P. cinnamomi* shows a significant range of variation in its pathogenicity to *E. smithii* (Linde *et al*., 1998). Breeding and selection programmes for resistance in these eucalypts are in progress. In addition to the above, *P. nicotianae* causes a root and collar rot of introduced black wattle, *Acacia mearnsii*, in South Africa (Zeijlemaker, 1971); while various other Phytophthoras can be isolated from root tips of native South African trees (Jolanda Roux, personal communication).

The situation regarding Phytophthoras on trees in other parts of Africa is very uncertain. A serious decline of cork oak, comparable to that in Iberia, has been reported in North Africa, from Morocco to Tunisia. To the authors' knowledge, its association with *P. cinnamomi* has not been investigated. Computer models predict considerable activity for *P. cinnamomi* in parts of east, central and west Africa (see below). However, the situation in much of the African continent is greatly in need of investigation; particularly with regard to native forests. This could well harbour many interesting local or introduced phytophthoras.

Influence of global warming on *Phytophthora* activity in Europe, Africa and worldwide

The question whether *P. cinnamomi* might be as active a pathogen on oak in central as in southern Europe led Brasier & Scott (1994) to model the distribution and activity of *P. cinnamomi* in Europe (i) under current conditions and (ii) under conditions of global warming. The model utilized as its variables the known distribution of the pathogen in south-western Australia; known climatic constraints on the pathogen's survival, such as its sensitivity to frost: and the pathogen's reported optimal temperatures for growth rate and pathogenicity of c. 25-30°C. Details of the model are given in Brasier & Scott (1994).

Activity predicted for *P. cinnamomi* under current climatic conditions in Europe (Fig. 1) represented a good fit to the known activity of the pathogen (IMI distribution map, Anon., 1984). Significantly, it indicated that the pathogen was not likely to be active in those continental areas of Europe subject to intense winter cold. Indeed, Marcais *et al.* (1996; and see also this volume) have also demonstrated that the distribution of *P. cinnamomi* on red oak in south-western France is constrained by frost.

Given the currently predicted moderate climatic warming of ca. +1.5°C (mean annual temperature) for the year 2050 and the more severe warming of c. +3°C for the year 2100, the model suggests a significant increase in the activity of *P. cinnamomi* in maritime and coastal regions of Europe (Fig. 2). Its activity will probably increase in central Europe also, but winter cold will still continue to be a significant constraint. It is important to caution here that an increase in *P. cinamomi* activity would also depend upon availability of susceptible hosts; and on other ecological constraints, such as soil

pH, competition for the host resource from other pathogens and the natural biological control activity of microbial antagonists. It should also be noted that general stress effects imposed by global warming could render some currently resistant hosts more susceptible to *P. cinnamomi*, and other Phytophthoras (Brasier & Scott, 1994),

FIGURES 1-5. Activity of *Phytophthora cinnamomi* as predicted by computer models. In Figs. 1-4, activity level is indicated by the relative size of the dot. In Fig. 5: open circles, reduction in activity predicted; closed circles, increase in activity predicted.

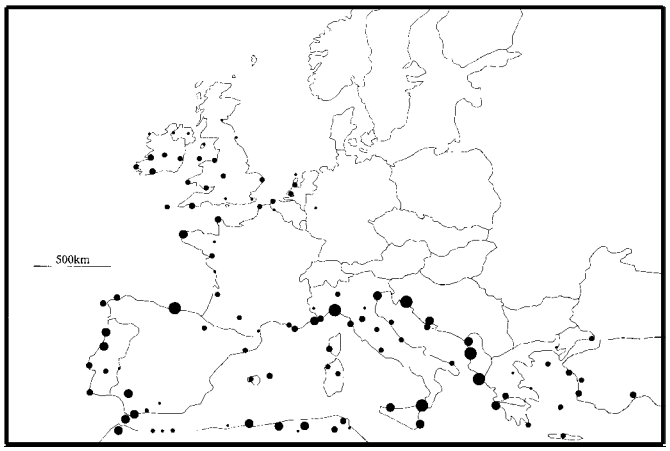


FIGURE 1. Activity in Europe under current climatic conditions.

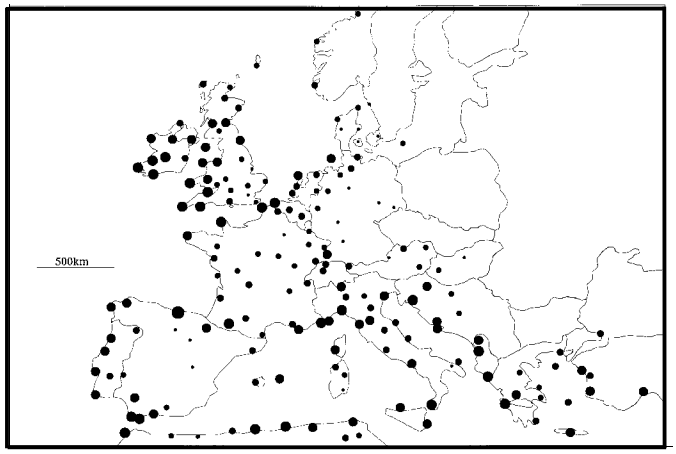


FIGURE 2. Activity in Europe assuming an increase of +3°C in mean annual temperatures - a current climate change prediction for around 2100.

Based on this model, further predictions have been produced for *P. cinnamomi* activity in Africa and in the rest of world (J. Scott & C. M. Brasier, previously unpublished). The predicted activity of *P. cinnamomi* world-wide under 'current' climatic conditions (Fig. 3) is again a good fit to the known distribution of *P. cinnamomi* (IMI distribution map; Anon., 1984). Note the high levels of activity predicted for *P. cinnamomi* in the highlands of Papua New Guinea, southeastern China and Taiwan, consistent with the view that *P. cinnamomi* is native to PNG and possibly also to China and Taiwan.

An increase in mean annual temperature of +1.5°C (not shown) and +3°C (Fig. 4) is predicted to lead to a general reduction in activity of *P. cinnamomi* in equatorial west Africa; but an increase in its activity in east Africa, in central southern Africa and along the north African coast. Indeed, a similar trend is predicted worldwide (Fig. 4): a general reduction in activity in *P. cinnamomi* across the equatorial zone; accompanied by an increase in its activity in temperate and hot temperate zones, especially in some maritime regions. The details must again be treated with some caution in view of other influential factors mentioned above; and in view of the simplicity of the model. It is interesting to note, however, that a projected mean increase of +3°C (Fig. 4) appears to make little difference to the activity of *P. cinnamomi* in PNG or in south-east China. Fig. 5 summarises the contrast in predicted outcomes between the 'current' and the +3°C models worldwide. Open circles represent a predicted reduction and closed circles an increase in *P. cinnamomi* activity given a mean +3 °C warming. It more clearly illustrates the shift in activity from the equator to the temperate zones.

Climate change is likely to influence the behaviour not only of *P. cinnamomi* but of many other *Phytophthora* tree pathogens. Indeed, Jung *et al.* (1996) have suggested that increasingly milder winters (and also increased nitrogen deposition) of recent decades may have influenced the role of Phytophthoras in deciduous oak decline in Europe by enhancing the size and activity of *Phytophthora* populations in the soil. The result may have been more frequent infection of non-mycorrhizal oak roots.

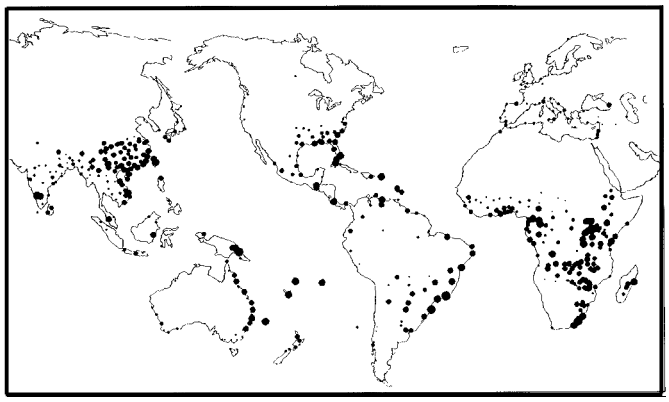


FIGURE 3. Activity worldwide under current climatic conditions.

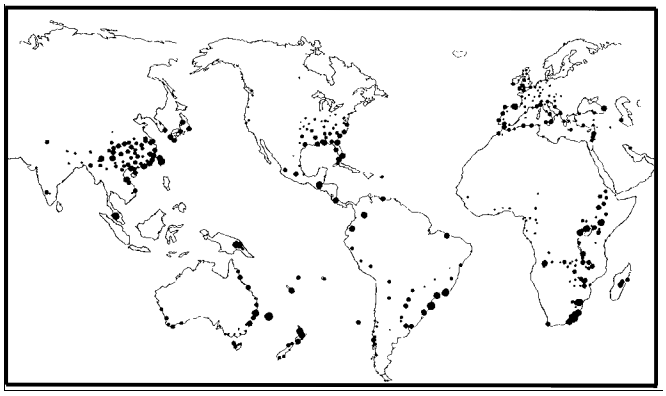


FIGURE 4. Activity worldwide assuming a 3°C increase in mean annual temperatures.

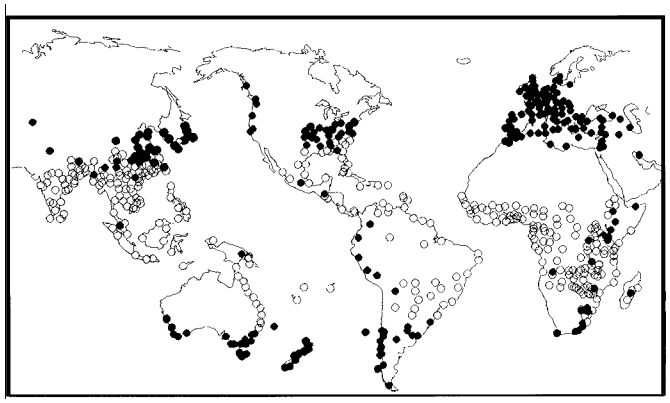


FIGURE 5. Contrast between data in Figs. 3 and 4. Figs. 1 and 2, redrawn from Brasier & Scott (1994). Figs. 3-5, J.K. Scott (CSIRO Division of Entomology, Perth, Western Australia) & C. M. Brasier, previously unpublished.

Concluding comments

Prior to 1990, a solid foundation of knowledge about Phytophthora diseases of trees in Europe was laid down through much careful observational and descriptive work. Since 1990, our understanding has accelerated through an emphasis on experimental investigation and on the investigation of Phytophthoras in forests and other semi-natural communities. In consequence of the latter, a remarkable new range of Phytophthora taxa have been recorded. Which of these taxa are endemic and which are introduced is yet to be determined. However, it is clear from developments in Iberia that aggressive, introduced pathogens such as *P. cinnamomi* continue to play a major role; and this and other developments including human disturbance factors, such as climate change, changes in land use and nitrogen deposition may be accentuating the activity of both introduced and endemic Phytophthoras. It has also become apparent since 1990 that accelerated evolution of 'tree Phytophthoras' may be occurring, as a result of genetic exchange between endemic and introduced (or introduced and introduced) species.

The application of molecular methods has led to many new insights into the population and evolutionary biology of Phytophthoras on trees. They now promise further insights into their ecology, detection and host-pathogen interactions. Hopefully the new technology can be used to elucidate the genetical and physiological basis of aggressiveness and host specificity. This in turn may provide information for use in resistance breeding. It could also enhance our meagre understanding of *Phytophthora* ecological strategies.

In general, the rise in the profile of Phytophthoras in trees in Europe since 1990 has been accompanied by good progress in scientific understanding, but it is clear that there is little room for complacency.

ACKNOWLEDGEMENT

I am grateful to many colleagues for permission to use their unpublished information.

LITERATURE CITED

Anon. (1984). *Phytophthora cinnamomi*. *Distribution maps of plant diseases no. 302*. Edition 5. CAB International, Wallingford (GB).

Anon. (1997). Departement de la santé des forêts, 1997: La santé des forêts (France) en 1996. *Les Cahiers du DSF*, 1-1997, 87 pp.

Artaza, J.E.Y. (1949). El castaño en España. Instituto Forestal de Investigaciones y Experiencas. 303 pp.

Belisario, A., Magnano di San Lio, G., Pane, A. & Cacciola, S.O. (1993). *Phytophthora iranica*, a new root pathogen of myrtle in Italy. *Plant Disease* **77**, 1050-1055.

Blaschke, H. (1994). Decline symptoms on roots of *Quercus robur*. *European Journal of Forest Pathology* **24**, 386-398.

Blaschke, H. & Jung, T. (1996). Symptome und Nachweis eines Phytophthora-Befalls an Eichen. *Mitteilungen aus der Biologischen bundesanstalt für Land- und Forstwirtschaft* **318**, 61-78. Berlin-Dahlem (in German).

Brasier, C.M. (1991). Survey of widespread oak mortality in Spain, 13-17 May 1991, conducted on behalf of ICONA and the Direccion General de la Samdad de la Produccion Agraria, Madrid. Report on isolation of *Phytophthora cinnamomi* (6 pp).

Brasier, C.M. (1992). Oak mortality in Iberia. *Nature* **360**, 539.

Brasier, C.M. (1993). *Phytophthora cinnamomi* as a contributory factor in European oak declines. In: *Recent advances in studies on oak decline*, eds N. Luisi, P. Lerario and A. Vannini, 49-57. Universita degli Studi, Bari.

Brasier, C.M. (1996). *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Annales des Sciences Forestière* **53**, 347-358.

Brasier, C.M. (2000). Rapid evolution of introduced plant pathogens via interspecific hybridization. *Bioscience*, in press.

Brasier, C.M., Cooke, D. and Duncan, J.M. (1999). Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences* USA **96**, 5878-5883.

Brasier, C.M., Hamm, P.B. & Hansen, E.M. (1993). Cultural characteristics, protein patterns and unusual mating behaviour of *Phytophthora gonapodyides* isolates from Britain and North America. *Mycological Research* **97**, 1287-1298.

Brasier, C.M., Robredo, F. and Ferraz, J.F.P. (1993). Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology* **42**, 140-145.

Brasier, C.M., Rose, J. and Gibbs, J.N. (1995). An unusual *Phytophthora* associated with widespread alder mortality in Britain. *Plant Pathology* **44**, 999-1007.

Brasier, C.M. and Scott, J. (1994). European oak declines and global warming: a theoretical assessment with special reference to the activity of *Phytophthora cinnamomi*. *EPPO Bulletin* **24**, 221-232.

Brasier, C.M. & Strouts, R.G. (1976). New records of *Phytophthora* on trees in Britain. I. Phytophthora root rot and bleeding canker of horse chestnut (*Aesculus hippocastanum* L.). *European Journal of Forest Pathology* **6**, 129-136.

Brasier, C.M. & Strouts, R.G. (1978). *Phytophthora hibernalis* recorded in Britain. *Plant Pathology* **27**, 99-100.

Buddenhagen, I.W. (1955). *Phytophthora cinnamomi* (Rands) bij *Chamaecyparis lawsoniana* (Parl.) en *Taxus baccata* (L.) in Nederland. *Tijdschrift Planten Ziekt* **61**, 17-18.

Cacciola, S.O., Magnano di San Lio, G. & Belisario, A. (1996). *Phytophthora italica* sp. nov. on myrtle. *Phytopathologia Mediterannea* **35**, 177-190.

Clancy, K.J. & Kavanagh, J.A. (1977). The isolation and pathogenicity of a new *Phytophthora* species. University College Dublin Fac. Gen. Agric. Res. Rep. 1976-1977: 27-28.

Cobos, J.M., Montoya, R., Tuset, J.J. (1992). New damages of the *Quercus* woodlands in Spain. In: Proc. Int. Congr. Recent Advances in Studies on Oak Decline, Brindisi, Italy. pp 163-169.

Coelho, A.C., Cravador, A., Bollen, A., Ferraz, J.F.P., Moreira, A.C., Fauconnier, A. & Godfroid, E. (1997). Highly specific and sensitive non-radioactive molecular identification of *Phytophthora cinnamomi*. *Mycological Research* **101**, 1499-1507.

Crandall, B.S., Gravatt, G.F., Ryan, M.M. (1945). Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. *Phytopathology* **3**, 162-180.

Day, W.R. (1938). Root rot of sweet chestnut and beech caused by species of *Phytophthora*. I. Cause and symptoms of disease: its relation to soil conditions. *Forestry* **12**, 101-106.

Day, W.R. (1939). Root rot of sweet chestnut and beech caused by species of *Phytophthora*. II. Inoculation experiments and methods of control. *Forestry* **13**, 46-58.

Delatour, C. (1983). Les dépérissements de chênes en Europe. *Biologie et Forêt* **35**, 265-282.

Del Canizo, J. (1942). Spain. A new method of controlling ink disease of Spanish chestnut. *International Bulletin of Plant Protection* **15**, 2M-3M.

Dreyer, E. and Aussenac, G. (eds) (1996). Ecology and physiology of oaks in a changing environment. *Annales des Sciences Forestières* **53**, 161-800.

Duclos, J., Fauconnier, A., Coelho, A.C., Bollen, A., Cravador, A. and Godfroid, E. Identification of an elicitin gene cluster in *Phytophthora cinnamomi. DNA Sequence* **9**, 231-237.

Fernandez-Escobar, R., Gallego, F.J., Benlloch, M., Membrillo, J., Infante, J. & Perez de Algaba, A. (1999). Treatment of oak decline using pressurized injection capsules of antifungal materials. *European Journal of Forest Pathology* **29**, 29-38.

Gallego, F.J., Perez de Algaba, A. & Fernandez-Escobar, R. (1999). Etiology of oak decline in Spain. *European Journal of Forest Pathology* **29**, 17-27.

Gibbs, J.N. (1995). *Phytophthora* root disease of alder in Britain. *EPPO Bulletin* **25**, 661-664.

Gibbs, J.N., Lipscombe, M.A. and Peace, A.J. (1999). The impact of Phytophthora disease on riparian populations of common alder (*Alnus glutinosa*) in southern Britain. *European Journal of Forest Pathology* **29**, 39-50.

Grente, J. (1952). Le *Phytophthora cinnamomi* parasite du châtaignier en France. Comptes Rendus de l'Academie de Science Paris **234**, 2226-8.

Hansen, E.M. & Delatour, C. (1999). *Phytophthora* species in oak forests of north-east France. *Annales des Sciences Forestiere* **56**, 539-547.

Hartmann, G. (1995). Wurzelhalsfäule der Schwarzerle (*Alnus glutinosa*) - eine bisher unbekannte Pilzkrankheit durch *Phytophthora cambivora*. *Forst und Holz* **18**, (25 September, 1995), 3 pp.

Heiser, I., Fromm, J., Geifing, M., Koehl, J., Jung, T. & Osswald, W. (1999). Investigations on the action of *Phytophthora quercina*, *P. citricola* and *P. gonapodyides* toxins on tobacco plants. *Plant Physiology and Biochemistry* **37**, 73-81.

Jung, T., Blaschke, H. and Neumann, P. (1996). Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* **26**, 253-272.

Liese, J. (1926). Achet auf Buchenkeimlingspilz! *Forstarchiv* **2**, 217-218.

Linde, C. (1999). Population structure of *Phytophthora cinnamomi* in South Africa. PhD thesis, University of Orange Free State, Bloemfantem.

Linde, C., Drenth, A., Kemp, G.H.J., Wingfield, M.J. & Von Broemsen, S.L. (1997). Population structure of *Phytophthora cinnamomi* in South Africa. *Phytopathology* **87**, 822-827.

Linde, C., Kemp, G.H.J. & Wingfield, M.J. (1999). Variation in pathogenicity among South African isolates of *Phytophthora cinnamomi*. *European Journal of Plant Pathology* **103**, 231-239.

Luisi, N., Lerario, P. and Vannini, A. (eds) (1993). *Recent advances in studies on oak decline*. University of Bari, Italy.

Marcais, B., Dupuis, F. & Desprez-Loustau, M.L. (1996a). Modelling the influence of winter frosts on the development of the stem canker of red oak, caused by *Phytophthora cinnamomi*. *Annales des Sciences Forestieres* **53**, 369-382.

Marcais, B., Dupuis, F. & Desprez-Loustau, M.L. (1996b). Susceptibility of the *Quercus rubra* root system to *Phytophthora cinnamomi*; comparison with chestnut and other oak species. *European Journal of Forest Pathology* **26**, 133-143.

Moreau, C., Moreau, M. (1952). Etude mycologique de la maladie de l'encre du chêne. *Revue de Pathologie Végétale et d'Entomologie Agricole de France* **31**, 201-231.

Olsson, C.H.B. (1999). Occurrence of the alder (*Alnus glutinosa* L.) decline in Sweden and affinities of the causal *Phytophthora* pathogen as assessed by isozyme analysis. In *Diagnosis of root-infecting Phytophthora* spp. PhD thesis, Swedish University of Agricultural Sciences, Uppsala.

Peace, T.R. (1962). *Pathology of trees and shrubs*. Oxford University Press.

Robin, C., Desprez-Loustau, M.L., Capron, G. & Delatour, C. (1998). First record of *Phytophthora cinnamomi* on oak and holm oaks in France and evidence of pathogenicity. *Ann. Sci. For.* **55**, 869-883.

Smith, P. (1988). *Phytophthora cinnamomi*. In *European Handbook of Plant Diseases I* (Smith, I.M., Dunez, J., Phillips, D.H. Lelliot, R.A. and Archer, S.A. eds.) pp 213–215. Blackwell Scientific Publications, Oxford.

Strouts, R.G. (1981). *Phytophthora diseases of trees and shrubs.* Arboricultural Leaflet 8. HMSO, London.

Strouts, R.G., Rose, D.R. & Reffold, T.C. (1989). Advisory services. Wales and southern England. In *Report on Forest Research 1988*, pp 42-43. HMSO, London.

Tuset, J.J., Hinarejos, C., Mira, J.L., Cobos, J.M. (1996). *Phytophthora cinnamomi*, una causa de la "Seca" de los *Quercus* mediterráneos. In: Actas del VIII Congreso de la Sociedad Española de Fitopatología, Córdoba. p.31.

Urquijo Landaluze, P. (1936). Hacia la solución del problema del Castaño. *La Coruña, Papeleria & Imprenta Lambardero*. 38 pp.

Urquijo Landaluze, P. (1947). Revision taxonomica de los hongos productores de la enfermedad del Castaño Llamada de la "tinta". *Bol. Pat. Veg. Ent. agric. Madrid* **16**, 253-70.

Von Broemsen, S.L. & Kruger, F.J. (1985). *Phytophthora cinnamomi* associated with mortality of native vegetation in South Africa. *Plant Disease* **69**, 715-717.

Wingfield, M.J. & Knox-Davies, P.S. (1980). Observations on diseases of pine and eucalyptus plantations in South Africa. *Phytophylactica* **12**, 57-60.

Zeijlemaker, F.C.J. (1971). Black butt disease of black wattle caused by *P. nocotianae* var. *parasitica*. *Phytopathology* **61**, 144-145.

PHYTOPHTHORA IN FORESTS AND NATIVE VEGETATION IN AUSTRALASIA AND EASTERN ASIA

Ken Old and Mark Dudzinski

CSIRO Forestry and Forest Products, PO Box E4008 Kingston, Canberra, ACT 2604, Australia

SUMMARY

Information is summarised on *Phytophthora* species associated with forest trees and native vegetation in Australasia (including Papua New Guinea (PNG)), South East and Eastern Asia. Only a few species appear to offer significant threats, namely *P. cinnamomi, P. citricola, P. cryptogea, P. drechsleri, P. megasperma* and *P. nicotianae*, with *P. cinnamomi* being by far the most severe. Genetic variability in populations of A1 and A2 mating types of *P. cinnamomi* differs markedly. The A2 mating type in Australia and PNG shows little variation, only two isoenzyme genotypes being found in more than 400 isolates tested. This is consistent with the low variability detected world wide in A2 isolates. In contrast, 8 separate genotypes were found in ten PNG A1 isolates indicating that the two mating types may not share centres of origin. Evidence from isoenzymes and DNA markers suggests that *P. cinnamomi* does not undergo sexual exchange of markers in field populations. Impacts on softwood and hardwood plantations have been relatively small, though locally significant where climatic and edaphic conditions are conducive and susceptible species are planted. Damage to native ecosystems in several regions of southern Australia is very severe and *Phytophthora cinnamomi* has been recognised by the Federal Government as one of five 'Key Threatening Processes' endangering species and ecological processes. Summaries are given of approaches to managing *Phytophthora* diseases in native vegetation in Australia.

INTRODUCTION

In this overview we do not propose to review in detail the huge amount of work carried out on this topic in Australia, New Zealand, Papua New Guinea (PNG), South East Asia, and Eastern Asia (in this paper, referred to collectively as the "region"), primarily in Australia. There have been at least 11 thorough reviews at the State, national, or regional level over the last 30 years (Newhook and Podger1972; Podger 1972; Old 1979; Weste and Marks 1987; Shearer and Tippett 1989; Davison and Shearer 1989; Newhook 1989; Marks and Smith 1991; Weste 1994; Irwin *et al*. 1995; Erwin and Ribeiro 1996). Instead we will summarise information that has become available over the last 25-30 years on the spectrum of *Phytophthora* species associated with native vegetation in Australasia and Eastern Asia, introduce some aspects of the population structure of some of these pathogens and provide a brief account of the impacts of *P. cinnamomi* on native vegetation in Australia and approaches to management and mitigation of disease impacts.

Phytophthora spp. associated with native vegetation and forest plantations.

Table 1 lists *Phytophthora* species associated with native vegetation communities and forest plantations in the region. The information available is heavily weighted toward Australia where a whole generation of forest and native vegetation pathologists has been drawn into the search for ways to mitigate damage to plant communities inflicted by *P. cinnamomi.*

With the exception of New Zealand (Newhook 1970; Podger andNewhook 1971) and PNG (Arentz 1986; Arentz and Simpson 1986), information on forest trees and native vegetation for other parts of the region, e.g. South East Asia, China, Taiwan and Japan, is fragmentary. Despite Sumatra having been the location of the original type isolate of *P. cinnamomi* (Rands 1922), there are very few records of *Phytophthora* in native plant communities in South East Asia. It may be significant that two recent reports, from northern Sumatra (Anggraeni and Suharti 1997) and from West Java, (Intari *et al.* 1995) cite unidentified *Phytophthora* spp. as causing root damage on *Eucalyptus urophylla* and *Dalbergia latifolia* respectively. The cryptic nature of root disease especially on forest trees and an absence of specialised taxonomic support in forest pathology may contribute to this lack of recent records.

This shortage of information is in contrast to the many records of *Phytophthora* disease in Indonesia and Malaysia on a wide variety of plantation crops such as rubber (Tan 1979), cocoa (Kueh 1985), cloves (Lee 1974), pepper, vanilla, and nutmeg (Kobayashi and Oniki 1993). In a monograph prepared by Kobayashi and Guzman (1988) on tree diseases in the Philippines, *Phytophthora* spp. are not listed as agents of significant disease. With the exception of a number of records from China, of *Phytophthora* spp. on *Cedrus deodora, Robinia pseudoacacia*, and in forest soils, *Phytophthora* records for forest species in the south-east and eastern parts of Asia (Table 1) are mostly from nurseries as ubiquitous damping-off and root-rot pathogens and are not further discussed in this paper.

Note, in addition to the above records, Arentz isolated several other *Phytophthora* species from soil under rainforest in PNG. These included *P. katsurae* Ko and Chang, *P. megasperma* var. *sojae* Hilderbrand, and *P. palmivora* (Butler) Butler. There was no indication that these fungi were associated with disease. There is also a small number of recent unpublished records of *P. cactorum* and *P. gonapodyides* in native vegetation landscapes in Australia. An undetermined *Phytophthora* sp. has been associated with dieback in *Eucalyptus tetrodonta* at Nhulunbuy in the Northern Territory, Australia (Dept. of CALM 1999).

Several descriptions have been published of marine *Phytophthora* species associated with mangrove ecosystems in eastern Australia (Pegg and Alcorn 1982; Gerrettson-Cornell and Simpson 1984), but these species have since been assigned to *Halophytophthora* (Ho and Jong 1990). Weste *et al*. (1982) also attributed *P. nicotianae* to dieback of mangroves in Queensland.

Table 1 indicates that forests and native vegetation ecosystems in the region harbour a large number of *Phytophthora* species. Podger (1979) listed ten species recorded in Australia from nurseries, plantations and native forest, but Weste considered that only eight different taxa of *Phytophthora* were associated with dieback of native vegetation in Western Australia (Weste 1994). Davison and Shearer (1989) indicated that 11 species of *Phytophthora* were known from Queensland rainforest, but only *P. cinnamomi* and *P. heveae* were widespread. Carstairs and Stukely (1996) examined 433 field isolates of *Phytophthora*, collected in Western Australia, and compared their morphology and isoenzyme profiles with standard isolates. The isolates fell into six taxonomic groups and use of isozymes was found to improve the speed and accuracy of diagnosis for species other than *P. cinnamomi.* The total number of records of *Phytophthora* spp. in forest and native vegetation ecosystems in Australasia, eastern and south-east Asia is unlikely to represent the actual number of *Phytophthora* spp. present and is also confounded by variation in taxonomical combination and nomenclature.

Despite likely differences in their environmental and host adaptation, only a few of the species present in the region appear to offer significant threats to forest plantations and native vegetation namely: *P. cinnamomi, P. cryptogea, P. drechsleri, P. megasperma, P. citricola* and *P. nicotianae.*

Variation in field populations of *Phytophthora* spp.

In the context of forests and wildlands, the main focus of *Phytophthora* population genetics in the region has been on *P. cinnamomi*, with some work on variation within the *P. cryptogea/drechsleri* complex. In addition, the report by Carstairs and Stukely (1996) casts some light on variation in isozyme markers used to assist identification of field isolates of *Phytophthora*. Whereas isolates of *P. cinnamomi* and the *P. nicotianae* complex matched known standards with a high degree of fidelity, matches of the *P. megasperma*, *P. drechsleri/cryptogea*, and *P. citricola* complexes were very variable. The relationship of variability in isozyme characteristics to disease impacts in the field, for these latter groups, is unknown.

TABLE 1. *Phytophthora* spp. associated with forests and natural vegetation in Australia, New Zealand, Papua New Guinea, South East and Eastern Asia with selected records.

***Phytophthora* sp.**	Location	Vegetation type	References
P. cinnamomi Rands	Australia	Pine plantation	Gerrettson-Cornell 1977; Bumbieris 1981; Chevis and Stukely 1982
		Hardwood plantation	I.W. Smith, Department of Natural Resources and Environment, Victoria, Australia (pers. comm.)
		Native forest	Podger 1972; Podger and Brown 1989; Shearer and Tippett 1989
		Heathland	Pegg and Alcorn 1972; Weste 1974; Hill *et al*. 1994; Weste 1994; Shearer and Dillon 1996
P. cinnamomi	New Zealand	Pine plantation	Newhook 1970; Newhook and Podger 1972
		Hardwood plantation	Hill 1979
		Native vegetation	Podger and Newhook 1971; Gadgil 1974
P. cinnamomi	Papua New Guinea	Pine plantation	Arentz and Simpson 1986
		Native vegetation	Shaw *et al*. 1972; Arentz 1983; Arentz 1986; Arentz and Simpson 1986; Ash 1988
P. cinnamomi	Indonesia	*Cinnamomum burmannii* (native species grown for spice)	Rands 1922
P. cinnamomi	China	Variety of wild plants and crops	Zheng and Lu 1989
		Forest plantations	Ho *et al*. 1983, 1984; Ho 1996
P. cinnamomi	Japan	Ornamental rhododendron	Hagiwara *et al*. 1980
P. cinnamomi	Taiwan	Hardwood plantation	Chang 1993
P. drechsleri Tucker	Australia	Pine plantation	Heather and Pratt 1975; Bumbieris 1976
		Native forest	Shepherd and Pratt 1973
P. cryptogea Pethybr. & Laff.	Australia	Pine plantation	Cannon 1978
		Hardwood plantation	Gerrettson-Cornell 1978a
P. cryptogea	Papua New Guinea	Pine plantation	Arentz 1986
		Hardwood plantation	Arentz 1986
		Native vegetation	Arentz 1986
P. citricola Sawada	Australia	Pine plantation	Chevis and Stukely 1982
		Native forest	Gerrettson-Cornell 1983; Shearer *et al*. 1987; Shearer 1990; Bunny *et al*. 1994
P. nicotianae Breda de Haan	Australia	Native forest	Davison and Tay 1983
		Mangroves	Weste *et al*. 1982
P. nicotianae	Papua New Guinea	Pine plantation	Arentz 1986
		Hardwood plantation	Arentz 1986
		Native forest	Arentz 1986
P. megasperma Drechsl.	Australia	Pine plantation	Chevis and Stukely 1982
		Native forest	CSIRO (unpubl. records)
		Heathland	Carstairs and Newcombe 1997
P. heveae Thomson	Australia	Hardwood plantation	Gerrettson-Cornell 1976
P. heveae	New Zealand	Native vegetation	Gadgil 1974
P. heveae	Papua New Guinea	Hardwood plantation	Arentz 1986
		Native vegetation	Arentz 1986
P. cambivora (Petri) Buisman	Australia	Hardwood plantation	Gerrettson-Cornell 1978b
P. cambivora	Papua New Guinea	Native vegetation	Ash 1988
P. gonapodyide Petersen (Buisman)	Australia	Native vegetation	Dept. of CALM 1999
P. boehmeriae Sawada	Australia	Pine plantation	Oxenham and Winks 1963; Gerrettson-Cornell 1976
		Hardwood plantation	Gerrettson-Cornell 1976
		Native vegetation	D'Souza *et al*. 1997

Prior to 1984, work on variability in those species of *Phytophthora* associated with disease in forests and native vegetation was restricted to mating type, morphological and physiological properties (Chee and Newhook 1965; Shepherd and Pratt 1974; Shepherd *et al*. 1974; Ann and Ko 1985), and more commonly to studies of pathogenicity. There was much speculation regarding the origin of *P. cinnamomi* although the theory espoused by Shepherd (1975) that the fungus was an ancient immigrant to Australia, and epidemic disease was primarily the outcome of site disturbance, has received little support. Isoenzyme markers which are relatively unequivocal in interpretation and not subject to large environmental influences for their expression were seen by Old *et al.* (1984) as providing a powerful new tool for investigating the population structure of *P. cinnamomi* in Australia and PNG. Variation was assessed initially in 183 isolates from that region (later extended to more than 400 isolates). Low levels of variation (total of 4 genotypes) were found in both A1 and A2 mating types isolated within Australia. The A2 isolates tested from PNG were identical to those from Australia but of the eight A1 isolates from this source, seven distinct genotypes were found. This provided evidence, since confirmed by Dobrowolski *et al*. (1999) using DNA

markers, that the population of *P. cinnamomi* in Australia is of very restricted lineage. Considering the relative rarity of the A1 mating type worldwide, with notable exceptions such as Taiwan (Ko *et al*. 1978) and some areas of South Africa (Broembsen 1984; Linde *et al*. 1997), this high level of variation in the A1 population in PNG suggests that the centre of origin of the A1 mating type of the organism may lie in the PNG/Indonesian archipelago. This hypothesis is further developed by Arentz and Simpson (1986), and Zentmyer (1988).

The apparent lack of interaction of the two mating types was further noted by Old *et al.* (1988) who showed that populations of A1 and A2 coexisting within a native forest in south-eastern Australia showed no evidence of recombination at 6 variable loci. Intensive studies of field populations at 4 locations in the south-west of Western Australia by Dobrowolski *et al*. (1999) using DNA markers has confirmed the absence of sexual reproduction in the field. Although distinct lineages of two A2 and one A1 populations were present, variability which was attributable to asexual mechanisms could be detected within these strains. Similar conclusions were reached by Chang *et al*. (1996) for *P. cinnamomi* in Taiwan.

In addition to examining the distribution of mating types of *P. cinnamomi* in Australia (Shepherd and Cunningham 1978) two distinct ecotypes of *P. drechsleri* were designated by Shepherd and Pratt (1973). Isolates from northern Australia generally differed in their upper temperature limit for growth (36-37°C) from isolates that originated from southern Australia (33-36°C). However, more recent knowledge suggests that the *P. cryptogea*/*drechsleri* complex appears to consist of several distinct species (Erwin and Ribeiro 1996).

A further potential aspect of variability in *P. cinnamomi* has been addressed using the large collection of Australian and PNG isolates of the oomycete maintained at CSIRO, Canberra. Research over the last three decades has established the widespread occurrence of double-stranded RNA elements in fungal thalli of plant pathogenic fungi including *Phytophthora infestans* (Newhouse *et al*. 1992) and *Pythium irregulare* (Gillings *et al*. 1993). Consequences for phenotypic variability vary from undetectable to major, including modification of the pathogenic capacity of the infected strain (Nuss and Koltin 1990).

A total of 160 isolates of *P. cinnamomi* representing a broad range of geographic and host origins and the isozyme genotypes and mating types present in Australia were examined to determine the presence of dsRNA. A further 20 overseas isolates, including 17 from PNG, two from Japan and one from the USA were also examined. Isolates of *P. irregulare* were used for comparison and a number of dsRNA species have been cloned from this species. However, despite numerous attempts, virus particles were not detected in isolates of *P. cinnamomi* (A. Davidson, Department of Microbiology, Monash University, Victoria, Australia, pers. comm.).

Pathogenic variation in *Phytophthora cinnamomi*

Zentmyer (1980) reviewed available information on *P. cinnamomi* and the diseases it causes, presenting a picture of a pathogen with an extremely wide host range across many taxa, especially perennial plants, shrubs and trees. The extreme manifestation of host range and vegetation impacts is found in southern Australia (Weste 1994), especially in the megadiverse native heath and shrubland communities of the south-west of Western Australia. For example Wills (1993) suggested that as many as 2000 of the 9000 species found in that region may be susceptible to *P. cinnamomi*, and Weste and Taylor (1971) considered that 50%-70% of the woody perennial understorey species of the Brisbane Ranges in Victoria are susceptible. Podger and Brown (1989) studied the impacts of *P. cinnamomi* on temperate rainforest communities in Tasmania and concluded that 30% of the 142 species present were highly susceptible and less than 5% resistant. However, evidence of intra-specific resistance within susceptible eucalypts has been recorded in Western Australia and Victoria.

Compared to the wealth of information on the range of species susceptible to the pathogen, there are very few studies of variation in pathogenicity of *P. cinnamomi.* The availability of the large CSIRO collection of isolates of known mating type, isozyme genotype, geographical and host origin has been used by Podger (1989) and by Dudzinski *et al.* (1993) to assess pathogenic variation in *P. cinnamomi.* Podger compared the pathogenicity of fourteen isolates on transplants of Tasmanian temperate heathland species. He found that all were pathogenic, with no evidence of strong differences in pathogenicity between isolates grouped according to isoenzyme characteristics, geographic origin of isolates or host source. Podger's conclusions with respect to the groupings of isolates were borne out by Dudzinski *et al*. (1993), who inoculated *E. marginata* plantlets, produced by tissue culture, under controlled environment conditions, but significant differences between individual isolates with respect to pathogenicity were clearly demonstrated. The pathogenicity of 42 isolates was tested in six successive trials. When isolates were rated according to days needed to induce first visible symptoms and to kill plantlets, consistent differences in pathogenicity between isolates were shown. These data were confirmed using two further clones of the host and by stem-inoculating seedlings of five additional susceptible eucalypt species.

Impacts on native vegetation and plantations

As noted above, the species present in the region which appear to offer significant threats to forest plantations and native vegetation are *P. cinnamomi, P. megasperma* in some areas of coastal vegetation of Western Australia (Bellgard *et al.* 1994), and *P. citricola* in rehabilitated mine sites in the jarrah (*Eucalyptus marginata*) forest (Bunny *et al.* 1994). *Phytophthora cinnamomi* is by far the species most damaging to indigenous forests and also causes root rot of exotic *Pinus* spp. in plantations established on sites conducive to disease. In Australia and New Zealand *P. cinnamomi* has been associated with shelterbelt mortalities in mature *P. radiata* (Newhook 1970; Batini and Podger 1968) and sometimes in young plantations on wet sites before canopy closure (Chevis and Stukely 1982). A range of other *Phytophthora* spp. has also been occasionally associated with deaths of pines, also usually in water-gaining sites, e.g. *P. drechsleri* and *P. cryptogea* (Davison and Bumbieris 1973). *Phytophthora heveae* was associated with damage in the native kauri pine, *Agathis australis*, in New Zealand (Gadgil 1974). An undetermined *Phytophthora* sp. has recently been associated with foliar disease in advanced plantations of *Eucalyptus saligna* and *E. botryoides* trees in the North Island of New Zealand (Dick 1997).

There are many examples in the region where *Phytophthora* spp., including *P. cinnamomi*, can be readily isolated from forests and other vegetation that show little evidence of significant disease (Pratt *et al*. 1973; Bunny *et al.* (1994) (*P. citricola*)). In other instances tree dieback and/or understorey symptoms appear to be coincidental with presence of *Phytophthora* spp., with attempts to establish any causal relationship proving inconclusive in some eucalypt forests (Blowes *et al*. 1982; Gerrettson-Cornell 1973) and in *Nothofagus* forest in PNG (Arentz and Simpson 1986).

Successive reviews have summarised the available information on impacts of *P. cinnamomi* on forests and native vegetation in Australia, New Zealand and PNG. The published proceedings of the *Symposium on Plant Diseases in Ecosystems: Threats and impacts in south-western Australia* (Withers *et al*. 1994) provide the most recent comprehensive assembly of information on severe impacts of disease on one of the world's ecologically most diverse areas, from the point of view of land management and ecosystem conservation. *Phytophthora* impacts and management constitute a major part of the Volume. Papers by Shearer; Wills and Keighery; Crombie and Bunny; Keighery, *et al.* (all 1994) are of particular relevance to this workshop, with a paper by Wilson *et al*. addressing the issue of impacts of plant disease on faunal communities at the catastrophic scale current in the south-west of Western Australia.

Shearer has used a comprehensive database on disease incidence assembled by the Western Australian Department of Conservation and Land Management to show the distribution of *P. cinnamomi* in that State. This covers a crescent shape including the area west of the

600 mm annual rainfall isohyet from Eneabba north of Perth (Lat. 31.5°S) (Hill *et al*. 1994) including the coastal plain (Shearer and Dillon 1996) and the range of low hills south-east of Perth, which are covered by jarrah forest to Walpole, in the most southerly part of Western Australia (Lat. 35°S). The eastern distribution of the pathogen in the State then continues many hundreds of kilometres, to the east of Esperance. Impacts of the pathogen are minor to severe throughout this region, depending partly on edaphic factors and vegetation composition. There are several significant National Parks through this area including the Stirling Range National Park; the impacts of *P. cinnamomi* on the vegetation of the Park were studied by Dudzinski (data in Wills 1993) and Wills (1993). The majority of species from the Proteaceae, Epacridaceae and Papilionaceae were found to be highly susceptible to the pathogen. Myrtaceae on the other hand were found generally to show less susceptibility. Losses in these families, which include many species (e.g. *Banksia* spp.) of great ecological significance including a role as food for nectarivorous birds and mammals, could accelerate the rate of ecosystem collapse.

Weste (1994) has provided the most recent summary of the situation for other parts of Australia where impacts are significant, including certain areas of South Australia, Victoria and Tasmania. She has also provided detailed accounts of vegetation changes over more than two decades in National Parks in Victoria affected by *P. cinnamomi* (Weste and Ashton 1994; Weste and Kennedy 1997). Whereas among the large number of susceptible plant taxa it appears that six rare shrub species in the Brisbane Ranges are vulnerable and may become endangered (Peters and Weste 1997), there is also evidence of regeneration of some susceptible species even in the presence of the pathogen (Weste *et al*. 1999). The impact of loss of susceptible species on small mammal populations has also been recorded for the Brisbane Ranges (Newell and Wilson 1993).

In Tasmania, Podger and Brown (1989) showed that disturbed rainforest at elevations below 900 m is at risk from invasion by *P. cinnamomi.* About one third of the species which make up these cool-temperate rainforest communities are highly susceptible to infection. The lack of disease in undisturbed forest appears to be related to soil temperature. Outbreaks of disease in regenerating rainforest after fire appear to be due to increased soil temperatures with removal of the forest canopy. *Phytophthora cinnamomi*, already present as cryptic infestations or possibly introduced during fire suppression activities, becomes invasive and incites disease. Podger *et al.* (1990) carried out a comprehensive survey of Tasmania for the presence of the pathogen and generated climatic profiles for more than 1400 sites. The analysis suggested that damage by *P. cinnamomi* is unlikely on sites in Tasmania where annual mean temperature does not exceed 7.5°C and annual mean rainfall is <600 mm. They were able to identify twelve substantial areas of native vegetation in climates suited to infection by the pathogen. Such information is of critical value in planning forest and land management to limit spread of *P. cinnamomi* into uninfested areas where vulnerable vegetation exists.

Impacts of *P. cinnamomi* have also been significant in restricted areas of rainforest in Queensland near Ingham and Mackay in Australia's tropical north. Brown (1976) reported some patch deaths where *P. cinnamomi* could readily be isolated from dead plants and from soil samples taken within patches. Several areas of tree death could not be related to road-making or logging activities. These appeared to be associated with wallows used by feral pigs. Re-colonisation of the sites by species less susceptible to *Phytophthora* appears to have occurred over the last two decades and impact is not significant. The cessation of tropical rainforest logging in Australia is likely to reduce opportunities for the pathogen (which is widely distributed in most east coast forests) to cause disease. There is, however, some concern that population pressure on forests outside of reserves, through soil disturbance associated with development, will be associated with local impacts by soil pathogens.

In terms of loss of forest timber production from native forests the only areas where *P. cinnamomi* is considered to be significant are the south-west of Western Australia (jarrah forest) and coastal forests of eastern Victoria (Davison and Shearer 1989). In Victoria many of the severely affected production forests have had a change of land use and are now within areas zoned as National Parks.

Management of *Phytophthora* diseases

Management of soil borne diseases of even the most intensively managed crop is a major challenge. For pathogens of forests and native vegetation the task is at the least daunting; nevertheless control or management to reduce impacts of disease is the raison d'être of forest pathologists. Approaches to management are influenced by the value of the crop and the extent of the resource to be protected, and its intensity of management. When native vegetation is considered, especially from a conservation standpoint, non-commodity values become paramount with attendant difficulties in integration of decision-making within an economics-driven society.

Phytophthora diseases of woody hosts impact across the whole value spectrum from nursery operations, amenity plantings and orchard trees, commercial plantations, native forests managed for timber, and natural ecosystems managed for conservation and preservation of amenity and public good values. Attempts to develop and apply management to forests and native ecosystems have been focused in southern Australia, especially south-west Western Australia. Efforts in other regions of Australia have been modelled on the Western Australian experiences with variation based on local circumstances.

The three land management systems that will be touched on here are native forests managed for multiple use (timber and non-wood values), rehabilitation of grossly disturbed native ecosystems (associated with mineral mining) and management of National Parks and other reserves.

Native forests

The need to manage *Phytophthora* disease of forest trees and understorey in the jarrah forest of Western Australia followed the demonstration in 1964 of an association between *P. cinnamomi* and a progressive dieback of *Eucalyptus marginata* (Podger *et al*. 1965). Studies of the etiology of the disease were later summarised by Podger (1972). The next two decades saw an unprecedented level of research activity, aimed at elucidating the dynamics of the disease in the forest environment, on which attempts to ameliorate the impacts of the disease could be based. Investigations were carried out by a succession of researchers from the Department of Conservation and Land Management, Western Australia and staff and graduate students of institutions of higher education in the Perth region. The outcomes of this work were summarised and integrated by Shearer and Tippett (1989). Management has been based on extensive surveys of the whole jarrah forest using aerial photography and photo-interpretation to map the distribution of disease.

As part of the effort to map the distribution of the pathogen in Western Australia, in the mid-1970s areas of the northern jarrah forest with little disease were quarantined. This had the dual benefits of increasing mapping accuracy by allowing time for existing recent infections to become symptomatic in a suite of highly susceptible understorey species (especially *Banksia* spp.) and reducing vehicular transfer of the pathogen into disease-free areas (Shea 1979). During the early 1980s a site hazard rating system was developed based on disease expression, landform, geology, soils, vegetation site history, and vegetation composition and structure (Shearer and Tippett 1989). Healthy areas of forest are rated for disease hazard and managers are able to systematically predict the effect of disturbance on hazard level and what the consequences will be of introduction of *P. cinnamomi* into any particular location.

The hazard rating is used to assist in the application of hygiene measures with regard to access to particular areas of forest, especially by vehicles. These measures include washing down of vehicles when moving from areas of high infestation to low; harvesting operation planning e.g. confining coupes within a single catchment; and

restriction of movement of vehicles and/or implementation of road closures during wet weather to reduce movement of infested soil.

Other approaches to control include the local and strategic use of phosphonate (Komorek *et al*. 1995; Peters and Weste 1997) athough this has found more application in the protection of rare and endangered species in woody heathland communities, and in nurseries.

The above approach to managing forests affected by *Phytophthora* has been used on a much reduced scale, and with variations to suit local conditions, in Central and East Gippsland, Victoria. Many of the eucalypt species in that region, particularly the "ash group" species, are highly susceptible to disease caused by *P. cinnamomi* (Podger and Batini 1971) but the climatic conditions in the upland forests are not suited to epidemics and significant disease impacts have been limited to the coastal forests (Marks and Smith 1991). The silvertop ash/stringy bark coastal forests are dominated by eucalypt species in the informal sub-genus *Monocalyptus* and are moderately to highly susceptible to disease caused by *P. cinnamomi.* Selective logging in these forests since European settlement appears to have resulted in a change in species composition towards susceptible species. Although the rainfall is not strongly seasonal there are occasional combinations of very wet summers followed by dry autumn seasons. Tregonning and Fagg (1984) attributed a marked increase in dieback severity in East Gippsland to such events occurring during 1971-72.

Although *P. cinnamomi* is ubiquitous throughout the coastal forests, vigorously growing regrowth stands, even those dominated by the susceptible species *Eucalyptus sieberi*, are healthy. Sites previously showing a high level of dieback have been successfully regenerated with indigenous species including mixtures of disease susceptible and tolerant species (Fagg 1987). The dense stocking of these sites, despite high early mortality, appears to have resulted in environmental conditions less conducive to disease and twenty-four years after the first eucalypt seeding trials were initiated all indigenous eucalypt species have been successfully established.

Native vegetation communities

Appreciation of the level of impacts of *P. cinnamomi* on native vegetation other than production forests was low until the early 1980s when pioneering work was carried out in several National Parks in Western Australia (Wills 1993) and by Podger in Tasmania (Podger and Brown 1989). Since that time the enormity of the problem has been defined and major efforts are being made to mitigate further damage and to put in place appropriate management practices. For Western Australia, summaries are provided by Shearer (1994), Wills and Keighery (1994), and Gillen and Napier (1994) on various aspects of the impacts of disease on native plant ecosystems and attempts to find management solutions.

Measures include the integration of disease management with more broadly based landscape management, including the development of a GIS-based decision support system, management of access by vehicles and foot traffic for recreation purposes, and strategic use of aerially applied phosphonate fungicide to protect rare and endangered species. In addition a major program of *ex situ* conservation has been mounted.

In Tasmania impacts of *P. cinnamomi* on native vegetation are more cryptic, but locally severe, with local populations of both common and rare and endangered species being threatened. Communities affected include heaths, dry sclerophyll forests, moorlands and disturbed rainforests below 600 m elevation. Management areas have been selected and designed for the purpose of safeguarding 44 species that appear on state and national lists of rare and endangered species (Barker *et al.* 1996).

Management of *Phytophthora* in areas affected by mining operations

Particular features of disease epidemiology and management of *Phytophthora* diseases in Western Australia relate to the consequences of former mineral exploration and mining operations for the spread of *Phytophthora* spp. in the jarrah forest. Having recognized the potential of these operations to exacerbate the impacts of disease, preventative and remedial measures have significantly impacted on the mining industry. Two major mining companies incorporate *Phytophthora* management into their operations. The companies are Alcoa World-Wide-Alumina Australia Limited (which mines bauxite deposits of the northern jarrah forest) and RGC Mineral Sands Limited which mines and processes mineral sands on the coastal plain about 300 km north of Perth to produce ilmenite, rutile and zircon (Colquhoun and Petersen 1994).

Major costs associated with *Phytophthora* management programs, for these two operations, were estimated by the above authors to be in excess of A$1m and $300,000 respectively. These costs included construction of vehicle cleaning facilities (A$300,000 each), dieback interpretation and mapping (A$200,000 and A$20,000 annually), site vegetation mapping and hazard prediction, and support of research carried out in Universities and other institutes of higher education (A$201,000 and A$10,000 annually).

A further cost to the mining industry is incurred during mine-site rehabilitation, which involves the regeneration of the site with indigenous vegetation (including *Phytophthora*-susceptible species). In 1994 it was estimated that Alcoa rehabilitates in the order of 450 ha of forest each year (Colquhoun and Peterson 1994). Many of the operations carried out during stripping of overburden prior to mining and during rehabilitation include dieback control measures. For example, site rehabilitation aims to create soil conditions unfavourable for the activity of *Phytophthora* by avoiding water-gaining micro-sites. Although *P. cinnamomi* has been isolated from dead jarrah trees and soils in trials established in rehabilitated sites during 1986 and later, survival of jarrah in these areas up to the late 1990s has been high. Species richness has also been maintained at a level which meets management objectives (Colquhoun and Petersen 1994).

Future

Phytophthora-related diseases are currently the most damaging influence on the health and future conservation of native vegetation in Australia, although the impact varies from minor to severe, depending on climate, edaphic factors, vegetation composition and land management. This review has not found evidence of damage on a comparable scale in other parts of the region although the cryptic nature of root disease on vegetation, which is concurrently affected by more obvious agencies e.g. clearing, soil and site degradation, and wildfire, often escapes detection.

The unique extent of the threat of *Phytophthora* spp. to Australia's biodiversity is recognized by the *Commonwealth Endangered Species Protection Act* of 1992 as one of five "Key Threatening Processes" endangering species and ecosystems, on a par with feral pests such as foxes and rabbits. The Act carries with it an obligation by the Federal agency, Environment Australia, to develop and implement a "Threat Abatement Plan" and in July 1999 a draft plan was released for public comment. Other State and Federal initiatives in Australia include the Review of *Phytophthora* Diseases in Australia (Cahill 1994); and the Report on Dieback in Western Australia (Podger *et al.* 1996).

These initiatives have created a policy environment that should assist in maintaining the level of R&D effort on these pathogens. State governments responsible for forest and land management in southern Australia have supported research on these diseases since the 1970s although, with the possible exception of Western Australia, activity has waned over the last decade. The unprecedented level of resources afforded to mitigation of impacts of the diseases on native ecosystems by mining companies in Western Australia has also been a lynchpin of practical measures to reduce impacts in highly disturbed sites.

It seems likely that for production forestry in Australia enough is known regarding the biology of *P. cinnamomi* and the epidemiology and etiology of disease to successfully manage forests in the presence of the pathogen with acceptable levels of disease impacts. Other

Phytophthora species do not appear to have impacts on the scale of *P. cinnamomi*, and measures to control the major threat should render effects of lesser pathogens to be of little consequence. The outlook, however, especially in Western Australia and parts of Victoria, for susceptible understorey species in forests and for the highly diverse woody shrub communities of coastal and hinterland regions is far from promising. Vast changes have already occurred in species diversity and abundance, following incursions of *P. cinnamomi*, which are likely to be irreversible. The damage inflicted by the pathogen has gone hand in hand with changes in land use, e.g. partial clearing, construction of roads and access tracks, greatly increased recreational use and in some areas commercial harvesting of wild flowers. Some of these activities can be curtailed, but the widespread changes in vegetation patterns in the landscape are probably irreversible.

On the macroscale in some parts of Australia, global warming may render vulnerable to damage areas that are currently little affected by *Phytophthora*, through increasing soil temperature and changing patterns of precipitation (Chakraborty *et al*. 1998). Depending on modes of reproduction of susceptible species after perturbation, e.g. seed regeneration versus vegetative recovery, natural selection may partly offset the impacts of *Phytophthora* on vegetation communities. For example in East Gippsland there is some evidence that the successful regeneration of forest sites by the strongly seeding *E. sieberi* may partly be assisted by natural selection for *P. cinnamomi*-tolerant individuals. In the Brisbane Ranges, also in Victoria, Weste and Kennedy (1997) have chronicled some re-colonisation of former dieback sites by *Xanthorrhoea australis* and other susceptible species. Whether this is disease tolerance or escape is yet to be ascertained.

The outlook for native plant communities in other areas of high disease hazard is, however, less than promising. Podger, reported in James (1994), considered the invasion by *P. cinnamomi* essentially unstoppable so that it will ultimately affect all the vulnerable plant populations on the Australian continent. Some of the work summarised above has shown that reduction in the spread of disease across a wide range of land tenures and uses can undoubtedly be achieved if resources are made available. In the time scale relevant to conservation of native ecosystems, however, these measures will delay invasion rather than prevent it.

Hardy *et al.* (1994) reviewed the control options available for *Phytophthora* and other pathogens impacting on native plant communities in south-west Australia and presented a more encouraging picture in the light of rapidly developing molecular technologies. Most of these strategies at their present stage of development present options for only selective application and the potential for broad-scale use across the hundreds of thousands of hectares in Western Australia affected by *Phytophthora* disease remains small.

REFERENCES

Anggraeni, I. and Suharti, M. (1997). Identification of some pathogens as causal agents of root rot disease on forest plants. *Buletin Penelitian Hutan* 610, 17-35.

Ann, P.J. and Ko, W.H. (1985). Variants of *Phytophthora cinnamomi* extend the known limits of the species. *Mycologia* 77, 946-950.

Arentz, F. (1983). *Nothofagus* dieback on Mt Giluwe, Papua New Guinea. *Pacific Science* 37, 453-458.

Arentz, F. (1986). A key to *Phytophthora* species found in Papua New Guinea with notes on their distribution and morphology. *Papua New Guinea Journal of Agriculture, Forestry and Fisheries* 34, 9-18.

Arentz, F. and Simpson, J.A. (1986). Distribution of *Phytophthora cinnamomi* in Papua New Guinea and notes on its origin. *Transactions of the British Mycological Society* 87, 289-295.

Ash, J. (1988). *Nothofagus* (Fagaceae) forest on Mt Giluwe, New Guinea. *New Zealand Journal of Botany* 26, 245-258.

Barker, P.C.J., Wardlaw, T.J. and Brown, M.J. (1996). Selection and design of *Phytophthora* management areas for the conservation of threatened flora in Tasmania. *Biological Conservation* 76, 187-193.

Batini, F. and Podger, F.D. (1968). Shelterbelt mortalities on the Swan Coastal Plain. *Australian Forest Research* 3(4), 39-45.

Bellgard, S.E., Shearer, B.L., Crane, C.E. and Smith, B.J. (1994). Morphological variability exhibited by *Phytophthora megasperma* retrieved from diseased areas of Western Australian bushland. In: R.T. Wills and W.A. Cowling (Eds). Handbook of the Symposium on Plant Diseases in Ecosystems: threats and impacts in south-western Australia; 16 April 1994; Perth, Western Australia. p 20.

Blowes, W.M., Heather, W.A., Malajczuk, N. and Shea, S.R. (1982). The distribution of *Phytophthora cinnamomi* Rands at two sites in southern Western Australia and at Durras in south-eastern New South Wales. *Australian Journal of Botany* 30, 139-145.

Broembsen, S. von. (1984). Occurrence of *Phytophthora cinnamomi* indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. *Phytophylactica* 16, 221-225.

Brown, B.N. (1976). *Phytophthora cinnamomi* associated with patch death in tropical rain forests in Queensland. *Australian Plant Pathology Society Newsletter* 5, 1-4.

Bumbieris, M. (1976). The role of *Phytophthora cryptogea* and waterlogging in a decline of *Pinus radiata*. *Australian Journal of Botany* 24, 703-709.

Bumbieris, M. (1981). Association of *Phytophthora cinnamomi* with *Pinus radiata* in South Australia. *Australian Journal of Botany* 29, 11-15.

Bunny, F.J., Hardy, G.E.St.J., Shearer, B.L. and Colquhoun, I.J. (1994). Distribution of *Phytophthora citricola* on soil surface and at depth in the northern jarrah forest. In: R.T. Wills and W.A. Cowling (Eds). Handbook of the Symposium on Plant Diseases in Ecosystems: threats and impacts in south-western Australia; 16 April 1994; Perth, Western Australia. p 21.

Cahill, D. (1994). Review of *Phytophthora* diseases in Australia. Canberra, Australia. RIRDC Research Paper Series No. 93/4, Rural Industries Research and Development Corporation, Canberra.

Cannon, M. (1978). Brief note on pine diseases in Weipa area, north Queensland. Meeting of IUFRO Working Groups S 2.06.12 and 2.07.07 on pests and diseases of tropical pines, 3-14 September 1978, Bogota, Colombia.

Carstairs, S.A. and Newcombe, L.E. (1997). The control and management of *Phytophthora megasperma* in the native plant communities of Western Australia. In 'Control of *Phytophthora* and *Diplodina* canker in Western Australia'. Final report to Environment Australia, Department of Conservation and Land Management, Perth. pp 98-148.

Carstairs, S.A. and Stukely, M.J.C. (1996). Rapid identification of species of *Phytophthora*. Minerals and Energy Research of Western Australia Report No. 175, Perth.

Chakraborty, S., Murray, G.M., Magarey, P.A., Yonow, T., O'Brien, R.G., Croft, B.J., Barbetti, M.J., Sivasithamparam, K., Old, K.M., Dudzinski, M.J. and others. (1998). Potential impact of climate change on plant diseases of economic significance to Australia. *Australasian Plant Pathology* 27, 15-35.

Chang, T.T. (1993). Decline of two *Cinnamomum* species associated with *Phytophthora cinnamomi* in Taiwan. *Plant Pathology Bulletin* 2, 1-6.

Chang, T.T., Yang, W.W. and Wang, W.Y. (1996). Use of random amplified polymorphic DNA markers for the detection of genetic variation in *Phytophthora cinnamomi* in Taiwan. *Botanical Bulletin of Academica Sinica* 37, 165-171.

Chee, K.H. and Newhook, F.J. (1965). Variability in *Phytophthora cinnamomi* Rands. *New Zealand Journal of Agricultural Research* 8, 96-103.

Chevis, H.W. and Stukely, M.J.C. (1982). Mortalities of young established radiata pine associated with *Phytophthora* spp. in the Donnybrook Sunkland plantations in Western Australia. *Australian Forestry* 45, 193-200.

Colquhoun, I.J. and Petersen, A.E. (1994). The impact of plant disease on mining. *Journal of the Royal Society of Western Australia* 77, 151-158.

Crombie, D.S. and Bunny, F.J. (1994). Disease and forest production in Western Australia with particular reference to the effects of *Phytophthora cinnamomi*. *Journal of the Royal Society of Western Australia* 77, 145-149.

Davison, E.M. and Bumbieris, M. (1973). *Phytophthora* and *Pythium* spp. from pine plantations in South Australia. *Australian Journal of Biological Sciences* 26, 163-169.

Davison, E.M. and Shearer, B.L. (1989). *Phytophthora* spp. in indigenous forests in Australia. *New Zealand Journal of Forestry Science* 19, 277-289.

Davison, E.M. and Tay, C.S. (1983). Twig, branch, and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* 67, 1285-1287.

Department of Conservation and Land Management, Western Australia (1999). A National overview of *Phytophthora cinnamomi* in Australia: supplementary information to accompany the draft national Threat Abatement Plan. Report prepared for Environment Australia. 95 pp.

Dick, M. (1997). Foliage death of *Eucalyptus saligna*: request for soil samples. Forest Health News 66, 2.

Dobrowolski, M.P., Tommerup, I.C., Shearer, B.L. and O'Brien, P.A. (1999). Mitotic recombination in continent-wide clonal lineages of *Phytophthora cinnamomi*. (Submitted to *Nature*).

D'Souza, N.K., Webster, J.L. and Tay, F.C.S. (1997). *Phytophthora boehmeriae* isolated for the first time in Western Australia. *Australasian Plant Pathology* 26, 204.

Dudzinski, M.J., Old, K.M. and Gibbs, R.J. (1993). Pathogenic variability in Australian isolates of *Phytophthora cinnamomi*. *Australian Journal of Botany* 41, 721-732.

Erwin, D.C. and Ribeiro, O.K. (1996). *Phytophthora* diseases worldwide. APS Press, St Paul, MN, 562 pp.

Fagg, P.C. (1987). Establishment and early growth of indigenous eucalypts sown on forest sites infested with *Phytophthora cinnamomi* in East Gippsland, Australia. *Forest Ecology and Management* 20, 53-78.

Gadgil, P.D. (1974). *Phytophthora heveae*, a pathogen of kauri. *New Zealand Journal of Forestry Science* 4, 59-63.

Gerrettson-Cornell, L. (1973). The recovery of *Phytophthora cinnamomi* Rands from Ourimbah State Forest (Wyong, Newcastle district, New South Wales, Australia) and its pathogenicity to eucalypt seedlings under controlled conditions. *Phyton, Argentina* 31, 111-116.

Gerrettson-Cornell, L. (1976). *Phytophthora heveae* and *P. boehmeriae* in a *Eucalyptus pilularis* plantation in New South Wales. *Australian Plant Pathology Society Newsletter* 5, 8-9.

Gerrettson-Cornell L. (1977). Results of a survey on *Phytophthora cinnamomi* Rands in the *Pinus radiata* plantations at Sunny Corner State Forest (Bathurst District, New South Wales) in 1972-1974. *Phyton, Argentina* 35, 159-162.

Gerrettson-Cornell, L. (1978a). *Phytophthora* spp. in a plantation of *Eucalyptus paniculata* Sm. in New South Wales. *Phyton, Argentina* 36, 69-70.

Gerrettson-Cornell, L. (1978b). Notes on the morphology of an isolate of *Phytophthora cambivora* (Petri) Buis. from Australia. *Phyton, Argentina* 36, 85-89.

Gerrettson-Cornell, L. (1983). Note on the morphology of *Phytophthora citricola* Sawada from Australia. *Nova Hedwigia* 38, 187-195.

Gerrettson-Cornell, L., and Simpson, J.A. (1984). Three new marine *Phytophthora* species from New South Wales. *Mycotaxon* 19, 453-470.

Gillen, K. and Napier, A. (1994). Management of access. *Journal of the Royal Society of Western Australia* 77, 163-168.

Gillings, M.R., Tesoriero, L.A. and Gunn, L.V. (1993). Detection of double-stranded RNA and virus-like particles in Australian isolates of *Pythium irregulare*. *Plant Pathology* 42, 6-15.

Hagiwara, H., Koizumi, S., Takeuchi, S. and Masago, H. (1980). Rhododendron root rot caused by *Phytophthora cinnamomi* Rands in Japan. *Annals of the Phytopathological Society of Japan* 46, 442-447.

Hardy, G.E.St.J., O'Brien, P.A. and Shearer, B.L. (1994). Control options of plant pathogens in native plant communities in south-western Australia. *Journal of the Royal Society of Western Australia* 77, 169-177.

Heather, W.A. and Pratt, B.H. (1975). Association of *Phytophthora drechsleri* Tucker with death of *Pinus radiata* D. Don in southern New South Wales. *Australian Journal of Botany* 23, 285-288.

Hill, C.F. (1979). New plant disease records in New Zealand. *New Zealand Journal of Agricultural Research* 22, 641-645.

Hill, T.C.J., Tippett, J.T. and Shearer, B.L. (1994). Invasion of Bassendean Dune Banksia woodland by *Phytophthora cinnamomi*. *Australian Journal of Botany* 42, 725-738.

Ho, H.H. (1996). The genus *Phytophthora* in mainland China. *Mycotaxon* 58, 93-118.

Ho, H.H. and Jong, S.C. (1990). *Halophytophthora* gen. nov., a new member of the family Pythiaceae. *Mycotaxon* 36, 377-382.

Ho, H.H., Lu, J. and Gong, L. (1984). *Phytophthora* spp. associated with the decline of *Cedrus deodara* in Nanjing, China. *Mycopathologia* 85, 59-64.

Ho, H.H., Zhuang, W.Y., Liang, Z.R. and Yu, Y.N. (1983). *Phytophthora cinnamomi* on black locust (*Robinia pseudoacacia*) in Jiangsu Province of China. *Mycologia* 75, 881-886.

Intari, S.E., Santoso, E. and Sophie, M.M. (1995). An experiment to control termites and rot root diseases attacking the *Dalbergia latifolia* plantation in the District Wanayasa, Purwakarta, West Java. *Duta Rimba* 20, 36-40.

Irwin, J.A.G., Cahill, D.M. and Drenth, A. (1995). *Phytophthora* in Australia. *Australian Journal of Agricultural Research* 46, 1311-1337.

James, S.H. (1994). Plant diseases in ecosystems: threats and impacts in south-western Australia. Symposium summary. *Journal of the Royal Society of Western Australia* 77, 99-100.

Keighery, G.J., Coates, D.J. and Gibson, N. (1994). Future ecosystems - ecological balance (ecological impact of disease causing fungi in south-western Australia). *Journal of the Royal Society of Western Australia* 77, 181-184.

Ko, W.H., Chang, M.S. and Su, H.J. (1978). Isolates of *Phytophthora cinnamomi* from Taiwan as evidence for an Asian origin of the species. *Transactions of the British Mycological Society* 71, 496-499.

Kobayashi, T. and Guzman, E.D. de. (1988). Monograph of tree diseases in the Philippines with taxonomical notes on their associated microorganisms. *Bulletin of the Forestry and Forest Products Research Institute* No. 351, Ibaraki, Japan, 200 pp.

Kobayashi, T. and Oniki, M. (1993). Diagnostic Manual for Industrial Crop Diseases in Indonesia. Japan International Cooperation Agency and Research Institute for Spice and Medicinal Crops, Indonesia. 107 pp.

Komorek, B., Shearer, B., Smith, B. and Fairman, R. (1995). The control of *Phytophthora* in native plant communities. Annual report to the Endangered Species Unit, Australian Nature Conservation Agency, Department of Conservation and Land Management, Como, Western Australia.

Kueh, T.K. (1985). Cocoa diseases. Annual Report of the Research Branch, Department of Agriculture, for the year 1983, Ministry Agriculture and Community Development, Sarawak, p 72-73.

Lee, B.S. (1974). *Phytophthora cinnamomi*: a new pathogen on cloves in Peninsular Malaysia. *MARDI Research Bulletin* 2, 26-30.

Linde, C., Drenth, A., Kemp, G.H.J., Wingfield, M.J. and Broembsen, S.L. von. (1997). Population structure of *Phytophthora cinnamomi* in South Africa. *Phytopathology* 87, 822-827.

Marks, G.C. and Smith, I.W. (1991). The cinnamon fungus in Victorian forests: history, distribution, management and control. No. 31. Department of Conservation and Environment, East Melbourne.

Newell, G.R. and Wilson, B.A. (1993). The relationship between the Cinnamon Fungus (*Phytophthora cinnamomi*) and the abundance of

Antechinus stuartii (Dasyuridae: Marsupiala) in the Brisbane Ranges, Victoria. *Wildlife Research* 20, 251-259.

Newhook, F.J. (1970). *Phytophthora cinnamomi* in New Zealand. In: T.A. Toussoun, R.V. Bega, and P.E. Nelson, Eds. *Root Diseases and Soil-borne Pathogens*, University of Calif. Press, Berkeley, CA. pp 173-176.

Newhook, F.J. (1989). Keynote address: Indigenous forest health in the South Pacific - a plant pathologist's view. *New Zealand Journal of Forestry Science* 19, 231-242.

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

Newhouse, J.R., Tooley, P.W., Smith, O.P. and Fishel, R.A. (1992). Characterization of double-stranded RNA in isolates of *Phytophthora infestans* from Mexico, the Netherlands, and Peru. *Phytopathology* 82, 164-169.

Nuss, D.L. and Koltin, Y. (1990). Significance of dsRNA genetic elements in plant pathogenic fungi. *Annual Review of Phytopathology* 28, 37-58.

Old, K.M. (Ed.) (1979). *Phytophthora* and forest management in Australia. Report of a Conference held 18-20 October 1978, Canberra. CSIRO, Melbourne, 114 pp.

Old, K.M., Dudzinski, M.J. and Bell, J.C. (1988). Isozyme variability in field populations of *Phytophthora cinnamomi* in Australia. *Australian Journal of Botany* 36, 355-360.

Old, K.M., Moran, G.F. and Bell, J.C. (1984). Isozyme variability among isolates of *Phytophthora cinnamomi* from Australia and Papua New Guinea. *Canadian Journal of Botany* 62, 2016-2022.

Oxenham, B.L. and Winks, B.L. (1963). *Phytophthora* root rot of *Pinus* in Queensland. *Queensland Journal of Agricultural Science* 20, 355-366.

Pegg, K.G. and Alcorn, J.L. (1972). *Phytophthora cinnamomi* in indigenous flora in southern Queensland. *Search* 3, 257.

Pegg, K.G. and, J.L. (1982). *Phytophthora operculata* sp. nov., a new marine fungus. *Mycotaxon* 16, 99-102.

Peters, D. and Weste, G. (1997). The impact of *Phytophthora cinnamomi* on six rare native tree and shrub species in the Brisbane Ranges, Victoria. *Australian Journal of Botany* 45, 975-995.

Podger, F.D. (1972). *Phytophthora cinnamomi*, a cause of lethal disease in indigenous plant communities in Western Australia. *Phytopathology* 62, 972-981.

Podger, F.D. (1979). Biology of the pathogens and its relevance to forest management. In: K.M. Old (Ed.) (1979). pp 14-27.

Podger, F.D. (1989). Comparative pathogenicity of fourteen Australian isolates of *Phytophthora cinnamomi* determined on transplants of Tasmanian temperate heathland. *Australian Journal of Botany* 37, 491-500.

Podger, F.D. and Batini, F. (1971). Susceptibility to *Phytophthora cinnamomi* root-rot of thirty six species of *Eucalyptus*. *Australian Forest Research* 5(3), 9-20.

Podger, F.D. and Brown, M.J. (1989). Vegetation damage caused by *Phytophthora cinnamomi* on disturbed sites in temperate rainforest in western Tasmania. *Australian Journal of Botany* 37, 443-480.

Podger, F.D., Doepel, R.F. and Zentmyer, G.A. (1965). Association of *Phytophthora cinnamomi* with a disease of *Eucalyptus marginata* forest in Western Australia. *Plant Disease Reporter* 49, 943-947.

Podger, F.D., James, S.H. and Mulcahy, M.J. (1996). Review of Dieback in Western Australia. Vol 1 - Report and Recommendations. Report to the Western Australian Minister for the Environment, Western Australia.

Podger, F.D., Mummery, D.C., Palzer, C.R. and Brown, M.J. (1990). Bioclimatic analysis of the distribution of damage to native plants in Tasmania by *Phytophthora cinnamomi*. *Australian Journal of Ecology* 15, 281-289.

Podger, F.D. and Newhook, F.J. (1971). *Phytophthora cinnamomi* in indigenous plant communities in New Zealand. *New Zealand Journal of Botany* 9, 625-638.

Pratt, B.H., Heather, W.A. and Shepherd, C.J. (1973). Recovery of *Phytophthora cinnamomi* from native vegetation in a remote area of New South Wales. *Transactions of the British Mycological Society* 60, 197-204.

Rands, R.D. (1922). Streepkanker van Kaneel, veroorzaakt door *Phytophthora cinnamomi* n. sp. *Mededelingen Instituut voor Plantenziekten* 54, 41pp.

Shaw, D.E., Cartledge, E.G. and Stamps, D.J. (1972). First records of *Phytophthora cinnamomi* in Papua New Guinea. *Papua New Guinea Agricultural Journal* 23, 46-48.

Shea, S.R. (1979). Forest Management and *Phytophthora cinnamomi* in Australia. In: K.M. Old, (Ed.) (1979), pp 73-100.

Shearer, B. (1990). Dieback of native plant communities caused by *Phytophthora* species - a major factor affecting land use in south-western Australia. *Land and Water Research News*, 15-26.

Shearer, B.L. (1994). The major plant pathogens occurring in native ecosystems of south-western Australia. *Journal of the Royal Society of Western Australia* 77, 113-112.

Shearer, B.L. and Dillon, M. (1996). Susceptibility of plant species in *Banksia* woodlands on the Swan Coastal Plain, Western Australia, to infection by *Phytophthora cinnamomi*. *Australian Journal of Botany* 44, 433-445.

Shearer, B.L., Michaelsen, B.J. and Warren, H.J. (1987). Comparative behaviour of *Phytophthora* species in the secondary phloem of stems and excised roots of *Banksia grandis* and *Eucalyptus marginata*. *Australian Journal of Botany* 35, 103-110.

Shearer, B.L. and Tippett, J.T. (1989). Jarrah dieback: the dynamics and management of *Phytophthora cinnamomi* in the jarrah (*Eucalyptus marginata*) forest of south-western Australia. Research Bulletin No. 3., Department of Conservation and Land Management, Western Australia.

Shepherd, C.J. (1975). *Phytophthora cinnamomi* - an ancient immigrant to Australia. *Search* 6, 484-490.

Shepherd, C.J. and Cunningham, R.B. (1978). Mating behaviour of Australian isolates of *Phytophthora* species. II. *Phytophthora cinnamomi*. *Australian Journal of Botany* 26, 139-151.

Shepherd, C.J. and Pratt, B.H. (1973). Separation of two ecotypes of *Phytophthora drechsleri* Tucker occurring in Australian native forests. *Australian Journal of Biological Sciences* 26, 1095-1107.

Shepherd, C.J. and Pratt, B.H. (1974). Temperature/growth relations and genetic diversity of A2 mating-type isolates of *Phytophthora cinnamomi* in Australia. *Australian Journal of Botany* 22, 231-249.

Shepherd, C.J., Pratt, B.H. and Taylor, P.A. (1974). Comparative morphology and behaviour of A1 and A2 isolates of *Phytophthora cinnamomi*. *Australian Journal of Botany* 22, 461-470.

Tan, A.M. (1979). *Phytophthora* diseases of rubber in Peninsular Malaysia. *Planters' Bulletin of the Rubber Research Institute of **Malaysia*, 11-19.**

Tregonning, K.C. and Fagg, P.C. (1984). Seasonal rainfall and *Eucalyptus* dieback epidemics associated with *Phytophthora cinnamomi* in Gippsland, Victoria. *Australian Forest Research* 14, 219-234.

Weste, G. (1974). *Phytophthora cinnamomi* - the cause of severe disease in certain native communities in Victoria. *Australian Journal of Botany* 22, 1-8.

Weste, G. (1975). *Phytophthora drechsleri* and *Eucalyptus viminalis*. ***Australian Plant Pathology Society Newsletter* 4, 38.**

Weste, G. (1994). Impact of *Phytophthora* species on native vegetation of Australia and Papua New Guinea. *Australasian Plant Pathology* 23, 190-209.

Weste, G. and Ashton, D.H. (1994). Regeneration and survival of indigenous dry sclerophyll species in the Brisbane Ranges, Victoria, after a *Phytophthora cinnamomi* epidemic. *Australian Journal of Botany* 42, 239-253.

Weste, G., Cahill, D. and Stamps, D.J. (1982). Mangrove dieback in North Queensland, Australia. *Transactions of the British Mycological Society* 79, 165-167.

Weste, G. and Kennedy, J. (1997). Regeneration of susceptible native species following a decline of *Phytophthora cinnamomi* over a period of 20 years on defined plots in the Grampians, Western Victoria. *Australian Journal of Botany* 45, 167-190.

Weste, G. and Marks, G.C. (1987). The biology of *Phytophthora cinnamomi* in Australasian forests. *Annual Review of Phytopathology* 25, 207-229.

Weste, G. and Taylor, P. (1971). The invasion of native forest by *Phytophthora cinnamomi*: I. Brisbane Ranges, Victoria. *Australian Journal of Botany* 19, 281-294.

Weste, G., Walchhuetter, T. and Walshe, T. (1999). Regeneration of *Xanthorrhoea australis* following epidemic disease due to *Phytophthora cinnamomi* in the Brisbane Ranges, Victoria. *Australasian Plant Pathology* 28, 162-169.

Wills, R.T. (1993). The ecological impact of *Phytophthora cinnamomi* in the Stirling Range National Park, Western Australia. *Australian Journal of Ecology* 18, 145-159.

Wills, R.T. and Keighery, G.J. (1994). Ecological impacts of plant disease on plant communities. *Journal of the Royal Society of Western Australia* 77, 127-131.

Wilson, B.A., Newell, G., Laidlaw, W.S. and Friend, G. (1994). Impact of plant diseases on faunal communities. *Journal of the Royal Society of Western Australia* 77, 139-143.

Withers, P.C., Cowling, W.A. and Wills, R.T. (Eds) (1994). Plant diseases in ecosystems: threats and impacts in south-western Australia. Proceedings of a Symposium of the Royal Society of Western Australia and The Ecological Society of Australia; 16 April 1994; Murdoch University, Perth. *Journal of the Royal Society of Western Australia* 77, 97-186.

Zentmyer, G.A. (1988). Origin and distribution of four species of *Phytophthora*. *Transactions of the British Mycological Society* 91, 367-378.

Zentmyer G.A. (1980). *Phytophthora cinnamomi* and the diseases it causes. American Phytopathological Society Monograph No. 10, St. Paul MN, 96 pp.

Zheng, X.B. and Lu, J.Y. (1989). Studies on *Phytophthora* species in Fujian, Zhejiang, Jiangsu provinces and Shanghai, China. *Acta Mycologica Sinica* 8, 161-168.

PHYTOPHTHORA IN THE AMERICAS

Everett M. Hansen

Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR USA

Phytophthora began in the Americas - at least the scientific world's first awareness of this genus of "plant killers" began - when an inconspicuous wild American *Phytophthora* on wild and native cultivated species of *Solanum* stormed European shores in 1845. The potato late blight pathogen set the standard for destruction by an exotic pathogen. 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*, official mascot of this meeting, exemplifies the dangers. The omnivorous, and seemingly ubiquitous *Phytophthora cinnamomi* provides dramatic counterpoint through several examples. There are other species of *Phytophthora* in the forests, but we know very little about either their pathology or their ecology. In contrast to growing attention in other parts of the world, there are few active research programs in North or South America focusing on forest Phytophthoras.

EXOTIC PATHOGENS

Phytophthora lateralis. Port–Orford-cedar, *Chamaecyparis lawsoniana*, 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. It is the largest species of its genus, and its wood is probably the most valuable of any conifer in the world. POC tolerates high concentrations of heavy metals in the ultramafic soils scattered throughout the region. In the southern part of its range, POC is usually found primarily along streams and areas with year-round seepage. It often grows within the active stream channel. In the north, POC commonly grows mixed with other conifers, in upland as well as riparian areas (Zobel and Hawk 1979).

Phytophthora lateralis was introduced for sure. It colonizes trees rapidly, advancing upward from infection points on fine roots until it reaches the main stem. The demarcation between infected and healthy tissue is clearly marked as the fungus advances up the stem. While we know approximately when and where the epidemic began, there is only supposition about the origin of the fungus. In 1923, John Boyce, author of the first and still the best American textbook on Forest Pathology, was working for the Forest Service in Portland Oregon. He received a letter from an ornamental nursery near Seattle Washington, describing a serious "fungus disease" of POC in the nursery and in landscape plantings in western Washington state. He visited the nursery and observed that "any disease which is so virulent as this one appears to be is potentially dangerous" (Zobel et al. 1985). He noted that the disease was also present in Portland, Oregon, and went on to describe the international trade between Europe and the United States in horticultural varieties of POC. More than 200 named cultivars have been selected and propagated, mostly in Europe. In 1923, the Seattle nursery in question was rooting cuttings from stock plants of several cultivars imported from France. There was no implication that the disease originated in Europe, and indeed we are confident that it did not, but here is another documentation of a system of unregulated international plant movements in the horticultural trade that was undoubtedly responsible at some point for the introduction.

There have been reports of *P. lateralis* from other parts of the world, but only one has been confirmed. It is reported as a pathogen of kiwi in New Zealand, rhododendron and other ornamentals in North Carolina (Abad et al. 1988), and *Chamaecyparis* in Germany. The first two reports cannot be confirmed because no cultures survive. The German report proved to be a misidentification, and the others probably are as well (Hansen et al. 1999). The fungus was recently in the nursery industry in France (Hansen et al 1999), probably imported from the United States or Canada on horticultural stock. As with other *Phytophthora* species, identification of *P. lateralis* is not easy. Oospores are seldom formed in culture and the sporangia lack distinguishing features, except they are dehiscent under some cultural conditions, and this isn't noted in the literature (Tucker and Milbrath 1942). It comes down to the characteristic lateral chlamydospores. With practice *P. lateralis* is readily distinguished from other chlamydospore-forming species such as *P. gonapodyides*.

Taxus brevifolia, Pacific yew, is also susceptible (DeNitto and Kliejunas 1991), but is naturally infected only when growing together with POC along streams in the forest (Murray and Hansen 1997). A limited field survey found yew killed by *Phytophthora* only where it was growing in close association with dead and dying POC. In a streamside survey, 46% of the cedar was dead, compared to 10% of the yew. The moderate susceptibility of Pacific yew does highlght the possibility of other unknown hosts, perhaps elsewhere in the world. Other *Chamaecyparis* species are all much more tolerant of infection than *C. lawsoniana*, although *C. obtusa* from Japan is sometimes killed in artificial inoculaton and in the landscape when growing mixed with POC (Torgeson et al. 1954). *C. nootkatensis*, from western North America, is regularly infected but seldom killed (Torgeson et al. 1954, Hansen and McWilliams, unpublished data), even after direct inoculation. Eastern Asia is the evolutionary center for the genus, and the tolerance of the Asian species supports this region as origin for the pathogen.

It was 30 years before the fungus was introduced to POC in its native range. By the early 1950s *P. lateralis* was killing POC 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. In a recent survey, four stretches of long-infested forest road were mapped. Cedars were still present along the roads in all areas, but mortality continues and inoculum from these roadside trees has spread further along the roads as well as downslope. The situation along streams is especially critical. Essentially all POC growing with their roots in contact with normal winter high water flows are killed within a few years of introduction of the pathogen to the stream. In a 1993 survey of 3 infested streams, mortality of larger POC growing within 3 m of the streams ranged from 65% to 92% (Hansen et al. 1999).

Today the rate of disease increase has slowed dramatically, 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, sanitation, silviculture including targeted planting and spacing of POC, and genetic resistance.

DISEASE MANAGEMENT

Most of the effort to date has been directed at road management. Year-round road closures provide the greatest protection against the spread of the disease. Wet season closures, enforced by gates, are used in many areas in order to preserve motorized access. In places official vehicles and maintenance and harvesting equipment are

washed before passing between infested and uninfested areas. Sanitation reduces the probability of spread and intensification by reducing inoculum loads along roads. The most vulnerable cedar trees growing adjacent to roads are cut.

Heritable resistance to *P. lateralis* was first demonstrated in 1989 (Hansen et al. 1989). Richard Sniezko (USDA Forest Service) calculated family mean resistance heritabilities of 0.21 and 0.91 for stem and root resistance tests, respectively. The family correlation between tests was low, suggesting the possibility of independently inherited resistance mechanisms. The earliest replicated outplanting test of trees selected for resistance to *P. lateralis* is now 10 years old. Survival of resistant families ranges from 25% to 80%, while mortality is near 100% for susceptible families. In a continuing program to screen, propagate, and breed resistant trees from the forest, candidate trees are challenged using a stem lesion test on excized branches. Trees that score well are vegetatively propagated, and will be retested before entering a breeding program to provide planting stock adapted for the broad range of forest conditions where POC grows.

***Phytophthora cinnamomi*.** About the same time as the export of *P. infestans* to Europe, or maybe earlier, *P. cinnamomi* arrived in North America, unannounced, and spread silently but with lethal effect across the SE United States. Nothing is known of the landing, although it seems fair to assume that fascination with plants from foreign lands was somehow to blame. Any one of a thousand plantation owners across the South in their pre-Civil War aristocratic glory might have imported the lethal cargo; travel and trade within the region assured its further spread. Regardless of where or when it began, 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 range of these species. 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). This was 75 years before the introduction of the Chestnut blight fungus, *Cryphonectria parasitica*, into the northern part of the tree's range, and about the same time that Ink Disease became a concern in Europe on *Castanea sativa*. Chestnut was already largely gone from the southern Appalachian foothills before chestnut blight reached that region.

Phytophthora was first associated with dying chestnut in 1932, and *P. cinnamomi* was convincingly shown to be the the cause in 1945. Root lesions, with necrotic phloem sometimes extending above the root collar and inky black exudations, were described on both naturally infected and inoculated trees. It is perhaps significant that northern red oak (*Quercus borealis*) was reported dying where it grew in association with chestnut, although *Phytophthora* was apparantly never isolated from the oaks. The pathogen was isolated at that time from 20 additional tree species in nurseries from Pennsylvania and Delaware to Louisiana (Crandall et al. 1945).

Phytophthora cinnamomi continues to attract attention in forestry in the SE United States, today primarily as the cause of Phytophthora root rot of Frasier fir- a Christmas tree disease (Kuhlman and Hendrix 1963). The pathogen is apparantly 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 (Benson and Grand 1999). One infected seedling per hectare may trigger an epidemic in downslope areas following heavy rains.

When *P. cinnamomi* isn't enough. On some hosts, such as *Castanea, P. cinnamomi* can aggressively colonize the root system, like *P. lateralis* does on POC. In other (most?) cases, it is confined to the fine roots and the role of the pathogen is less easily demonstrated. Littleleaf disease exemplifies this situation in North America.

Littleleaf disease of shortleaf pine (*Pinus echinata*) first attracted attention in the 1930s (Hepting et al. 1945). Shortleaf pine has a broad geographic range across the southern United States. It became an increasingly abundant species after about 1900 as the tree colonized abandoned agricultural lands in the Piedmont and Coastal Plain of the SE United States. An early map of local disease incidence indicates that symptomatic trees were first evident adjacent to roads and forest tracks (Campbell and Copeland 1954). Littleleaf disease was most destructive in stands older than 20 years on severely eroded soils in this region (Tainter 1997). At its height, the disease was present across about one third of the tree's range, and forced changes in management on about 2 million hectares of forest land (Campbell and Copeland 1954). 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, addressing decay fungi, nematodes, viruses, nutrient deficiencies, and bark beetles led in 1948 to the isolation of *P. cinnamomi* from symptomatic shortleaf pine trees (Campbell 1948). Even with a pathogen in hand, however, many puzzles, and skeptics, remained. For one thing, *P. cinnamomi* was seemingly everywhere across the south, in soil beneath healthy as well as diseased stands (Campbell 1949 and 1951, Campbell et al. 1963). It took careful, quantitative surveys to show that populations were greater beneath diseased trees than beneath nearby healthy trees in a stand, and greater in littleleaf stands than in adjacent healthy stands (Table 1).

Table 1. Occurrence of *P. cinnamomi* in soil samples collected beneath healthy and diseased trees in healthy and diseased stands of shortleaf pine in Georgia and South Carolina (Campbell 1949).

TREE CONDITION	STAND CONDITION	SAMPLES WITH P. CINNAMOMI (N= 480)
Healthy	Healthy	4.6 %
Healthy	Littleleaf	14.6 %
Littleleaf	Littleleaf	41.7%

Second, while seedling inoculations confirmed the pathogenicity of *P. cinnamomi*, symptoms were less severe on inoculated older trees. Only nonmycorrhizal, nonsuberized root tips were killed, and trees readily regenerated new rootlets. Third, nitrogen fertilization led to tree recovery, and the close field correlation with severely eroded, nutrient depleted sites was very strong. But experiments with nutrition alone did not replicate the disease syndrome either, and gradually it was accepted that *P. cinnamomi* plays a central role in littleleaf disease. 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*. To a plant pathologist appreciative of Phytophthora diseases, littleleaf provides a classic example of the interactions highlighted in the disease triangle with an emphasis on the soil environment as it impacts both pathogen and host (Zak 1961). A by-product of the littleleaf research program was the demonstration by Marx (1973) of the important role that ectomycorhizae can play in protecting healthy trees from fine root pathogens.

What limits *P. cinnamomi*?

With *Phytophthora cinnamomi* the important question might be "Why aren't there more examples of forest destruction?" It has been introduced to most forested areas of the western hemisphere, especially on planting stock from nurseries, but with few and mostly localized exceptions, it has not become established outside of the southeastern U.S. In the Pacific Northwest, it is almost ubiquitous in the ornamental nursery industry. From the nursery centers it has been

carried throughout the region by homeowners, and regularly kills susceptible landscape plants. It is present, although seldom causing significant damage, in the forest tree nurseries. But despite the widespread occurance of susceptible genera in the forest, including *Rhododendron, Taxus, Chamaecyparis,* and even *Pseudotsuga*, the fungus has never been recovered from plants in the forest (but see Middleton and Baxter 1955). In one of the first studies of its kind, Drs. Lewis Roth and George Kuhlman (1966) showed why *P. cinnamomi* has not become a forest pathogen in this region of Mediterranian climate. In short, it is too cold in the winter when it is wet, and too dry in the summer for this pathogen. Mortality resulted from soil infestation only when soil temperatures exceeded 16 Celsius. It is not a problem of pathogen short-term survival but of pathogenic activity (Table 2).

Table 2. Effect of temperature and moisture of infested field soils on cumulative survival of *P. cinnamomi* and mortality of Douglas-fir seedlings after 27 months (Roth and Kuhlman 1966).

TREATMENT	P. CINNAMOMI RECOVERY	DOUGLAS-FIR MORTALITY
Unmodified environment	40 %	5 %
Summer water	42%	27 %
Winter heat	39%	72 %
Both heat and water	49%	88 %

Port-Orford-cedar is also susceptible, and in an early survey of POC mortality in landscape and nursery plantings, *P. cinnamomi* was isolated from about 30% of the dead trees (Torgeson et al. 1954). *P. lateralis* killed the rest. Thirtyfive years later, in a similar survey, *P. cinnamomi* was associated with less than 2% of the POC mortality (Hansen and Hamm 1996). When POC was being widely transplanted from the ornamental nurseries, both *Phytophthora* species were being distributed with it. After the collapse of the POC ornamental trade caused by *P. lateralis*, the cedar hedgerows continued to die from *P. lateralis*, but *P. cinnamomi* gradually disappeared.

It is much less clear why there are so few reports of *P. cinnamomi* from tropical forest soils in the Americas. The pathogen is reported from plantation crops, especially *Persea* and *Cinchona*, in several countries. Certainly lack of systematic sampling must be a factor, but George Zentmeyer (1988) went looking on several occassions, and came back with very limited success. Over 400 samples were collected from native species of genera known to be susceptible, especially *Persea* and *Cinchona*, throughout tropical America, with most samples from wet soils in areas remote from cultivation. The only recoveries of *P. cinnamomi* came from disturbed areas adjacent to cultivated land.

PHYTOPHTHORA IN ALASKA, HAWAII, AND ARGENTINA

Forest decline, in the mysterious sense of Waldsterben, is a largely discredited topic in North America. But there are legitimate decline type diseases, of complex etiology (Manion 1992). *Phytophthora* has figured in at least four of these: Alaska cedar decline; Mal des cipres; Ohia decline; and and the previously described littleleaf disease. Alaska cedar decline began in the late 1800s on the islands of SE Alaska (Hennon et al. 1990). It "looks" like it might be a Phytophthora disease. Mortality is centered around bogs or muskegs, and progresses slowly into surrounding stands on better drained soils. Alaska cedar trees of all ages are affected but other species are not. Symptoms include progressive phloem necrosis extending from roots up the stem, often marked by a distinct demarcation between necrotic and healthy inner bark. Indeed one initial hypothesis was that *P. lateralis* might be the causal agent. A substantial multifaceted research effort starting in 1980 has so far failed to establish an unambiguous etiology, but it has eliminated many possibilities, including *P. lateralis* (Hennon et al. 1990). That fungus is simply not present in these stands. There is a widespread *Phytophthora* species in the water and bog soil, but it is *P. gonapodyides* (initially misidentified as *P. drechsleri*, Hansen et al. 1988). It was never isolated from lesions or roots, however, and we never succeeded in inducing symptoms in pathogenicity tests. *P. gonapodyides* does not cause Alaska cedar decline, but it is a most interesting fungus (see below). Best current thinking on the decline invokes some triggering climatic event, such as a hard freeze in a winter without protective snow, that killed many bog trees outright and injured others. With loss of the protective canopy, new trees were exposed to future events, and mortality "spreads" in a continuing abiotic cascade of injury.

Mal des cipres pulls us "down under," to Pategonia in Argentina. *Austrocedrus chilensis*, cipres, forms pure, even-aged stands as well as mixed stands with *Nothofagus pumilo*, covering about 200,000 ha in the Andes and adjacent piedmont. About 50 years ago a distinctive pattern of slow mortality was first recorded, that came to be called Mal des cipres (Baccala et al. 1998). Again the pattern is suggestive of *Phytophthora* involvement. Mortality is aggregated and appears to be contagious. It is concentrated on poorly drained soils where water accumulates during winter rains. Several pathogens have been identified on dead trees, but they are either not regularly associated with the disease, or are found only after trees are dead. Recently, however, *Phytophthora pseudotsugae* was associated with the disease (Rajchenberg 1998). It was recovered from soil by baiting with *Citrus* leaves, and from fine roots by direct plating on selective media. *P. pseudotsugae* was isolated from both soil and roots in 6 of 33 declining stands of *Austrocedrus*, and from 2 or 3 of 23 adjacent healthy areas.

The authors are appropriately cautious in their interpretation, identification needs to be confirmed and pathogenicity tests completed, but the results are very interesting none the less. This is apparantly the first report of *P. pseudotsugae* outside of the Pacific Northwest, where we described it as a pathogen of conifer seedlings in forest tree nurseries (Hamm and Hansen 1983). A number of Northwest conifers have been tried in Argentina and ponderosa pine is widely grown. It seems possible that *P. pseudotsugae* was introduced to Argentina on conifer nursery stock imported from the Northwest. It is morphologically similar to *P. cactorum*, and phylogenetically it is very closely related to that species. It differs in having noncaducous sporangia and rather lax sporangiophores.

Now to Hawaii and Ohia decline. Here we have both undisputed pathogen and susceptible host (Kleijunas and Ko 1973), and it still doesn't add up to a completely satisfying Phytophthora disease. *P. cinnamomi* was presumably introduced to the Hawaiian Archipelago, perhaps with the first colonizing Pacific Islanders (Zentmeyer 1988). It is now widespread, in healthy as well as diseased areas, spread by wild pigs as well as human activities (Table 3) (Kleijunas et al. 1977). Ohia grows in many forest situations, on a variety of soils and in association with several other forest trees. It is particularly important as an early seral colonizer of lava flows from the island's several active volcanos. These are the stands that have been devastated by mortality, especially on the "pahoehoe" type of lava. Ohia on the better drained a'a lava flows is generally healthy. Stands on recent lava flows tend to be even aged, depending on the age of the flow. A puzzling factor is that in many decline stands, understory ohia, or trees regenerating after death of the overstory, do not die (Hodges et al. 1986). This is a disease of mature trees. In one possible scenario, drainage in the dense pahoehoe 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*. As the stress intensifies, the trees become susceptible to secondary agents, including a wood boring beetle.

A competing ecological theory of Ohia decline is labeled "synchronous cohort senescence," suggesting that entire cohorts or generations of trees are established together, grow old together, and die together, naturally, in response to some abiotic stress agent exacerbated by intertree competition (Hodges et al. 1986). In this view, *P. cinnamomi* is only a secondary agent, invading only weakened and dead roots.

Table 3. Populations of *P. cinnamomi* in soil from three areas of ohia decline and adjacent healthy ohia forest in Hawaii (Kliejunas and Ko 1976).

LOCATION	HEALTH	PROPAGULES/g
1	Decline	2.0a
	Healthy	2.4a
2	Decline	0.9a
	Healthy	1.1a
3	Decline	3.8a
	Healthy	2.4b

WILD PHYTOPHTHORAS

We know that some *Phytophthora* species can cause great economic and ecological destruction when introduced to new forest ecosystems. But what does an endemic *Phytophthora* do in its land of origin? Could we even detect its presence? There is almost no systematic survey information available for most of the western hemisphere, but what exists suggests that Phytophthoras are widespread but not often abundant in many temperate forest ecosytems, in the absence of dramatic disease (Table 4). *P. gonapodyides* is the best documented. It seems to be ubiquitous in forest streams of the western United States, including very remote areas, never disturbed by European man, in Alaska and Oregon. We recover it abundantly in all seasons from forest streams near Corvallis. It is harder to make a claim for "undisturbed" in Europe, but *P. gonapodyides* is at least as widespread in water in the forest, including ephemeral puddles (Hansen and Delatour 1999). We also recover it from soil in riparian hardwood stands in Oregon (and in France). *P. gonapodyides* has a reputation as a weak pathogen, and is capable of a saprophytic existance, but when circumstances are right, at least in artificial inoculation, it can induce significant disease. What is this abundant organism doing in the forest? Evidently not inciting disease, in most places most of the time. Are there circumstances, however, combinations of climatic and biotic stress perhaps, in which it could play a more decisive role? *P. gonapodyides* is also interesting because of questions about its evolutionary history. It is sterile, at least under normal lab conditions (Brasier et al.1993), but it is closely related to the homothallic *P. megasperma* s.s. and an array of related isolates (Cooke et al. 1999). It is also commonly misidentified as *P. drechsleri*, *P. cryptogea*, or even *P. lateralis.*

Table 4. *Phytophthora* species (other than *P. cinnamomi* and *P. lateralis*) isolated from forest soils or from forest trees in the Western Hemisphere.

SPECIES	HABITAT	LOCATION
P. cactorum	Hardwood forest soil	Eastern and SE USA
P. cactorum	Hardwood forest soil	Oregon USA
P. cactorum	Austrocedrus roots and soil	Argentina
P. citricola	Hardwood forest soil, roots, streams	Oregon and SE USA
P. drechsleri	Pine forest soil	SE USA
P. gonapodyides	Streams and soil	Alaska, Oregon USA
P. hevea	Hardwood forest soil	SE USA
P. parasitica	Pine forest soil	SE USA
P. pseudotsugae	Austrocedrus roots and soil	Argentina

Phytophthora cactorum is another species reported with some regularity from forest soils, especially beneath hardwoods (Darmano et al. 1991, Campbell and Hendrix 1967, Jeffers and Aldwinkle 1988). In most cases there is no disease associated, but Toole (1951) isolated *P. cactorum* from basal bleeding cankers on maple trees in the eastern U.S. forests and Campbell (1965) associated it with cankers on red maple, especially in wet areas and after heavy rains. Rajchenberg (1998) recovered it occasionally beneath *Austrocedrus* in Argentina. *P. citricola* has been isolated form soil in the SE (Campbell 1965) and from cankers on alders in Oregon (Brasier and Hansen unpubl.). We also recover *P. citricola* from forest streams with some regularity. There are scattered reports *P. hevea* (Campbell and Gallegly 1965), and *P. parasitica* and *P. drechsleri* from forest soils in the south (Campbell 1965). And there are more; in our continuing work in Oregon, we regularly recover isolates that can not be readily assigned to any known species (Hansen and Hamm 1988).

There are also the very poorly understood species *P. quininea* from quinine trees (*Cinchona* species) in Peru (Crandall 1947), and *P. inflata* from elms in the midwestern United States (Caroselli and Tucker 1949). Are these forest species that strayed into civilization and were spotted? Why are there so few reports of *Phytophthora* species from tropical forests? Certainly there are pathogenic species in the plantations of rubber, avocado and *Cinchona*. Zentmeyer (1988) hypothesized from indirect evidence that *P. palmivora* originated in the American tropics with its hosts. Yet he failed to isolate it from forest plants or soil. We must conclude that we know almost nothing about the potentially very important occurance and plant interactions of *Phytophthora* species in forests, especially tropical forests.

It is perhaps relevant (and certainly fortunate) that most *Phytophthora* pathogens of agricultural crops, including forest trees grown in nurseries, cannot often survive in forest condtions. In Oregon and Washington nearly all forest regeneration is accomplished by planting seedlings raised in nurseries. Until about 1980, *Phytophthora* species were unrecognized limiting factors in nursery production. They are still present, but masked by better soil management and fungicide application (Hamm et al. 1981). At least 6 species are common (Pratt et al. 1976). To assess the risk to forestry, we planted *Phytophthora*-infected seedlings together in the same planting hole with healthy seedlings on several forest sites (Hansen et al. 1980). After two years, *Phytophthora* was isolated from 14% of the initially diseased seedlings that were still alive (5% recovery from dead seedlings), but was recovered from only 2 of 720 healthy trees planted in the same hole and we failed completely to isolate *Phytophthora* from healthy seedlings planted 0.6 meter downslope. The reasons for lack of *Phytophthora* spread were not elucidated, but clearly the nursery/agricultural environment is more conducive to *Phytophthora* survival and parasitism than forest soils. Candidate factors include year-round water and a relative lack of microbial competition in the nursery soils.

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; that *Phytophthora* species, including undescribed species, are widespread in forests; and that it takes more than the proximity of a dangerous pathogen and a susceptible host in the same spot to trigger disease. Most significantly, we must acknowledge how little we know about the interactions that regulate disease in the forest.

LITERATURE CITED

Abad, Z.G., T. Creswell, R.K. Jones, and H.D. Shew. 1994. Occurrence of *Phytophthora* species on various hosts in North Carolina. Plant Disease 78:830.

Baccala, N. B., P. H. Rosso, and M. Havrylenko. 1998. *Austrocedrus chilensis* mortality in the Nahuel Huapi National Park (Argentina). Forest Ecology and Management 109:261-269.

Benson, D. M. and L. F. Grand. 1999. Disease incidence of Phytophthora root rot of Frasier fir in North carolina. Phytopathology 89 No. 6 (Supplement): 6.

Brasier, C. M., P.B. Hamm, and E.M. Hansen. 1993 Cultural characters, protein patterns and unusual mating behaviour of *Phytophthora gonapodyides* isolates from Britain and North America. Mycol. Res. 97:1287-1298.

Campbell, W.A. 1948. *Phytophthora cinnamomi* associated with the roots of littleleaf-diseased shortleaf pine. Plant Disease Reporter 32:472.

Campbell, W.A. 1949. Relative abundance of *Phytophthora cinnamomi* in the root zones of healthy and littleleaf-diseased shortleaf pine. Phytopathology 39: 752-753.

Campbell, W.A. 1951. The occurrence of *Phytophthora cinnamomi* in the soil under pine stands in the southeast. Phytopathology 41:742-746.

Campbell, W.A. 1965. *Phytophtora* species isolated from forest soils of the southeast. Georgia Acadamy of Science. Bull. 23:89-90.

Campbell, W.A., and O.L. Copeland, Jr. 1954. Littlefleaf disease of shortleaf and loblolly pines. U.S.D.A. Dept. of Agriculture Circular no. 940. 41 pages.

Campbell, W.A and M. E. Gallegly. 1965. *Phytophthora heveae* from eastern Tennessee and western North Carolina. Plant Disease Reporter 49:233-234.

Campbell, W.A. and F.F. Hendrix, Jr. 1967. *Pythium* and *Phytophthora* species in forest soils in the southeastern United States. Plant Disease Reporter 51:929-932.

Campbell, W. A., G.V. Gooding, and F.A. Haasis. 1963. The occurrence of *Phytophthora cinnamomi* in Kentucky, North Carolina, Tennessee, and Virginia. Plant Disease Reporter 47:924-926.

Caroselli, N. E. and C. M. Tucker. 1949. Pit canker of elm. Phytopathology 39:481-488.

Cooke, D.E.L., A. Drenth, J.M. Duncan, G. Wagels, and C.M. Brasier. 1999. A molecular phylogeny of *Phytophthora* and related Oomycetes. Mycol. Res. (In Press).

Crandall,B.S. 1947. A new *Phytophthora* causing root and collar rot of *Cinchona* in Peru. Mycologia 39:218-223.

Crandall, B. S., G.F. Gravatt, and M. M. Ryan. 1945. Root disese of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. Phytopathology 35:162-180.

Darmono, T.W., M.L. Owen, and J. L. Parke. 1991. Isolation and pathogenicity of *Phytophthora cactorum* from forest and ginseng garden soils in Wisconsin. Plant Disease 75:610-612.

DeNitto, G.A., and J.T. Kliejunas. 1991. First report of *Phytophthora lateralis* on Pacific yew. Plant Disease 75:968.

E. M. Hansen, P. B. Hamm, A.J. Julis, and L. F. Roth. 1981. Isolation, incidence, and management of *Phytophthora* in forest tree nrseries in the Pacific Northwest. Plant Disease Reporter 63:607-611.

Hamm, P. B. and E. M. Hansen. 1983. *Phytophthora pseudotsugae*, a new species causing root rot of Douglas-fir. Canadian Journal of Botany 61:2626-2631.

Hansen, E.M. and C. Delatour. 1999. *Phytophthora gonapodyides* and other *Phytophthora* species in French oak forests. Annals of Forestry (In Press).

Hansen, E. M., D. J. Goheen, E. S. Jules, and B. Ullian. 1999. Managing Port-Orford-cedar and the introduced pathogen, *Phytophthora lateralis*. Plant Disease 84:4-14.

Hansen, E.M. and P.B. Hamm. 1988. *Phytophthora* species from remote forests of western North America. Phytopathology 78:1519.

Hansen, E.M. and P.B. Hamm. 1996. Survival of *Phytophthora lateralis* in infected roots of Port-Orford-cedar. Plant Disease 80:1075-1078.

Hansen, E.M., P. B. Hamm, and L.F. Roth. 1989.Testing Port-Orford-cedar for resistance to *Phytophthora*. Plant Disease 73:791-794.

Hansen, E.M., P. B. Hamm, C.G. Shaw III, and P. Hennon. 1988. *Phytophthora drechsleri* from remote areas of Southeast Alaska. Trans. British Mycological Society 91:379-384.

Hansen, E.M., L. F. Roth, P.B. Hamm, and A.J. Julis. 1980. Survival, spread, and pathogenicity of species of *Phytophthora* spp. on Douglas-fir trees planted on forest sites. Phytopathology 70:422-425.

Hansen, E.M., J.-C. Streito, and C. Delatour. 1999.First confirmation of *Phytophthora lateralis* in Europe. Plant Disease 83:587.

Hennon, P. E., C.G.Shaw III, and E. M.Hansen. 1990. Symptoms and fungal associations of declining *Chamaecyparis nootkatensis* in southeast Alaska. Plant Disease 74:267-273.

Hennon, P. E., C.G.Shaw III, and E. M.Hansen. 1990. Dating decline and mortality of *Chamaecyparis nootkatensis* in southeast Alaska. Forest Science 36:502-515.

Hodges, C. S., K. T. Adee, J. D. Stein, H. B. Wood, and R. D. Doty. 1986. Decline of Ohia (*Metrosideros polymorpha*) in Hawaii: a review. United States Department of Agriculture Forest Service General Technical Report PSW-86. 22p.

Hepting, G. H., T. S. Buchanan, and L. W. R. Jackson. 1945. Littleleaf disease of pine. U. S. Dept. Agr. Circ. 716. 15 p.

Jeffers, S.N., and H.S. Aldwinckle. 1988. Phytophthora crown rot of apple trees: Sources of *Phtophthora cactorum* and *Phytophthora cambivora* as primary inoculum. Phytopathology 78:328-335.

Kliejunas J. T. and W. H. Ko. 1973. Root rot of ohia (*Metrosideros collina subsp. polymorpha*) caused by *Phytophthora cinnamomi*. Plant Disease Reporter 57:383-384.

Kliejunas J. T. and W. H. Ko. 1976. Association of *Phytophthora cinnamomi* with ohia decline on the island of Hawaii. Phytophthology 66:116-121.

Kliejunas, J. T., R. F. Scharpf, and R. S. Smith, Jr. 1977. The occurrence of *Phytophthora cinnamomi* in Hawaii in relation to ohia forest site and edaphic factors. Plant Disease Reporter 61:290-293.

Kuhlman, E.G. and F.F. Hendrix Jr. 1963. Phytophthora root rot of Fraser fir. Plant Disease Reporter 47:552-553.

Middleton, J.T. and D.V. Baxter. 1955. The occurrence of *Phytophthora* and *Pythium* species on roots of native plants in northern California and southern Oregon. Phytopathology 45:694 (Abst.).

Manion, P. D. 1981. Tree Disease Concepts. Prentice Hall. 399 p.

Marx, D. H. 1973. Growth of ectomycorrhizal and nonmycorrhizal shortleaf pine seedlings in soil with *Phytophthora cinnamomi*. Phytopathology 63:18-23.

Murray, M. S. and E. M. Hansen. 1997. Susceptibility of Pacific yew to *Phytophthora lateralis*. Plant Disease 81:1400-1404.

Pratt, R.G., L.F. Roth, E. M. Hansen, and W.D. Ostrofsky. 1976. Identity and pathogenicity of species of *Phytophthora* causing root rot of Douglas-fir in the Pacific Northwest. Phytopathology 66:710-714.

Rajchenberg, M., C. Barroetavena, P.P. Cwielong, M. Rossini, D. Cabral, and A. Sivori. 1997. Fungal species associated with the decline of *Austrocedrus chilensis* in Patagonia, Argentina: preliminary results. Pp 235-244 in Root and Butt Rot of Forest Trees. Proceedings of the 9th International IUFRO Conference. Les Colloques (INRA Editions) No. 89. Paris. 459 p.

Roth, L. F. and E.G. Kuhlman. 1966. *Phytophthora cinnamomi*, an unlikely threat to Douglas-fir forestry. Forest Science 12:147-159.

Tainter, F. 1997. Littleleaf disease. pp 4-6 , In, E.M. Hansen and K. J. Lewis eds. Compendium of Conifer Diseases. APS Press. St Paul. 101p.

Toole, E.R. 1951. Bleeding canker of maple in North Carolina and Tennessee. Plant Disease Reporter 35:119.

Torgeson, D.C., R. A. Young, and J. A. Milbrath. 1954. Phytophthra root diseases of Lawson cypress and other ornamentals. Oregon State College, Agricultural Experiment Station, Station Bulletin 537. 18 p.

Tucker, C.M. and J. A. Milbrath. 1942. Root rot of *Chamaecyparis* caused by a species of *Phytophthora*. Mycologia 34: 94-103.

Zak, B. 1961. Littleleaf disease of shortleaf pine (*Pinus echinata* Mill.). Recent Advances in Botany. Pp1525-1528. Univ. Toronto Press.

Zentmyer, G.A. 1988. Origin and distribution of four species of *Phytophthora*. Trans. Br. Mycol. Soc. 91:367-378.

Zobel, D.B. and G.M. Hawk. 1980. The environment of *Chamaecyparis lawsoniana*. American Midland Naturalist 103:280-297.

Zobel, D.B., L. F. Roth, and G.M. Hawk . 1985. Ecology, Pathology, and Management of Port-Orford-Cedar (*Chamaecyparis lawsoniana*). U.S.D.A. Forest Service General Technical Report PNW-184. 161 p

INVOLVEMENT OF *PHYTOPHTHORA* SPECIES IN CENTRAL AND WESTERN EUROPEAN OAK DECLINE AND THE INFLUENCE OF SITE FACTORS AND NITROGEN INPUT ON THE DISEASE

T. Jung, H. Blaschke and W. Oßwald

Institute of Forest Botany, Forest Phytopathology, Am Hochanger 13, D-85354 Freising, Germany.

ABSTRACT

In 1996 and 1997 a survey was made in 35 oak stands on a range of different sites in Bavaria on the occurrence of Phytophthora species in the rhizosphere. The most widespread species were *Phytophthora quercina* sp. nov., *P. citricola* and *P. cambivora*. Seven other Phytophthora species were isolated infrequently. The results of an intense study concerning the health condition of the crowns and fine root systems of 217 oaks in these 35 stands indicate that there are at least two different complex diseases being referred to under the name 'oak decline'. In stands with a mean soil-pH ($CaCl_2$) > 3.5 and silty, loamy or clayey soil-texture *Phytophthora* spp. were commonly isolated from rhizosphere soil and an extremely significant correlation existed between the health condition of the crown and different root parameters, whereas no such correlation was found in stands without *Phytophthora* on sandy-loamy to sandy soils with a mean pH < 3.9. Considering their high aggressivity to oak *Phytophthora* species are most likely to be heavily involved in oak decline on neutral to moderately acidic sites. Anthropogenic nitrogen input as well as climatic changes are discussed as triggering factors.

INTRODUCTION

Oak decline is a serious and frequently recurring threat to European forestry. 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 & Liese, 1991; Luisi *et al.*, 1993), all symptoms indicative for water stress and poor nutrition. Mortality rates reach 2 to 5 trees per hectare and year (Hartmann *et al.*, 1989). An assessment of root systems of declining and healthy oaks in 33 stands of 5 European oak species all over Central Europe revealed a progressive destruction of the fine root system, dieback of longroots and necrotic lesions on suberized and nonsuberized roots. These symptoms had been present in both healthy and declining oaks, but the extent of damage was obviously higher in declining ones (Blaschke, 1994; Jung, 1998). Isolations from fine roots and rhizosphere soil samples showed the widespread occurrence of several *Phytophthora* species including *P. cactorum*, *P. citricola*, *P. cambivora*, *P. gonapodyides*, *P. undulata* and the new species *P. quercina* in 29 out of the 33 stands (Jung, 1996 & 1998; Jung *et al.*, 1996 & 1999). Up to now *Phytophthora* spp. have been isolated from soil samples of 68 out of 98 oak stands (69.4%) in 10 Central- and Western European countries. The most widespread species have been *P. quercina* (41 stands in 7 countries) and *P. citricola* (36 stands in 7 countries) (Jung *et al.* in prep.). Several soil infestation tests showed *P. quercina* and *P. cambivora* being the most aggressive species to root systems of young *Quercus robur* plants (Jung, 1998; Jung *et al.*, 1996&1999). In a soil infestation test with different *Phytophthora* species and a range of broadleaf species *P. quercina* proved to be host specific to the genus *Quercus* (Jung *et al.* in prep).

The aim of this project was to study in detail the influence of site factors, especially geological substrate, soil-pH and soil-texture on the distribution of *Phytophthora* species. Furthermore, the root status of healthy and declining oaks on a broad range of sites and possible correlations between crown transparency and fine and weak root specific parameters should be investigated.

MATERIALS AND METHODS

In 1997 and 1998 3–4 healthy (crown transparency ≤25%) and 3-4 declining oaks (crown transparency ≥35%) per stand were sampled in 35 oak stands on a broad range of typical oak forest sites (Tab.1) (in total 106 healthy and 111 declining oaks). In August 1997 crown transparency was assessed according to EC-UN/ECE (1994) and Anonymus (1996). The mean crown transparency of the declining oaks was 63.2% compared to 21.0% in the healthy oaks.

Sampling procedure, isolation method and root analysis

Root samples in each stand were taken at the first sampling date whereas soil for isolations was sampled 2-3 times. The sampling procedure was as follows: 3-5 soil-root-monoliths (20x30x30cm) were taken in opposite directions around the tree in a distance of 50-150cm from the stem base. Most fine and weak roots of oak in the monoliths were forwarded to root analysis. About 1 liter of rhizosphere soil of all monoliths was bulked and used for the isolation tests.

Isolations were carried out using 2–5 d-old leaflets of *Q. robur* seedlings as baits floated over flooded soil (Jung 1998; Jung *et al.*, 1996). Infected brownish leaflets were cut into pieces, plated onto selective PARPNH-agar (V8A amended with 10 µg ml^{-1} pimaricin, 200 µg ml^{-1} ampicillin, 10 µg ml^{-1} rifampicin, 25 µg ml^{-1} pentachloronitrobenzene (PCNB), 50 µg ml^{-1} nystatin, and 50 µg ml^{-1} hymexazol) and incubated at 20°C in the dark. After 24-48 h *Phytophthora* hyphae were transferred under the stereomicroscope onto V8A.

Root samples of each oak were analysed as follows: The percentage of root damage was estimated visually, and the number of living fine roots (∅ <2mm) per m mother root (=weak roots; ∅ 2-5mm) was counted. After drying at 65 °C fine roots were weighed and scanned. Using the computer program Delta-T Scan 2.04 (Delta-T Devices Ltd., Cambridge, UK) the total fine root length was measured and the number of root tips counted. Afterwards the specific fine root length (SFRL; = fine root length/fine root dry weight [cm/g]) and the specific root tip density (SRTD; = fine root tips/fine root dry weight [n/g]) could be calculated. After drying at 65 °C the length and dry weight of the mother roots was also measured. With these data the parameters fine root length/mother root length (FRL/MRL), fine root tips per m mother root and fine root length/dry weight mother root could be calculated.

Statistical analysis

The root samples of different stands have been taken at different dates and different weather conditions. Since the fine root system is underlying a strong seasonal and weather-induced turnover, the absolute values of the root parameters of healthy and declining trees can certainly be compared within one stand but comparisons between stands should be interpreted with caution. Therefore, the absolute values of all root parameters of each oak were transformed into season and weather corrected relative values. For this purpose for

each root parameter the mean value of the 3-4 healthy sample trees of a stand was considered as stand internal standard and set 100%. Then the single values of all healthy and declining sample trees of the stand were related to this standard. The statistical analysis was made with the absolute and the relative values. The data of all *Phytophthora* infested stands and the data of all *Phytophthora* free stands were combined, and the two collectives were analysed separately. The significance of the difference between the mean values of the healthy and the declining oaks was tested using the non-parametric Mann-Whitney test. Furthermore, using the non-parametric Spearman Correlation, it was tested whether there was a significant correlation between crown transparency and the different root parameters. The relationship between the presence of *P. quercina* and *Phytophthora* spp. in the rhizosphere and the risk of an oak to develop above-ground symptoms was tested with contingency tables. Furthermore, the relationship between crown status and presence/absence of *P. quercina* was analysed using a logistic regression. All statistical analyses except of the logistic regression (programme SPSS for Windows 6.1) were made using the programme InStat 3.01 for Windows 95/NT (GraphPad, San Diego, USA).

RESULTS

Distribution of *Phytophthora* species and correlation with crown status and site factors

Ten different *Phytophthora* species have been isolated from rhizosphere soil of 73 (out of 124) oaks in 19 (out of 35) stands. *P. quercina* was the species with the widest geographical distribution (18 stands in 5 growth regions) and the highest isolation frequency. *P. quercina* was recovered from 41 out of 65 declining (=63.1%) and from 14 out of 59 healthy oaks (=23.7%). *P. cambivora* was isolated from 9 and *P. citricola* and *P. undulata* from 7 stands, while the other species occurred only sporadically (Tab.3).

The statistical analysis of a contingency table revealed that an oak with *P. quercina* in its rhizosphere has an extremely significant 2.1 fold higher relative risk to develop crown symptoms than an oak without *P. quercina* (Tab.1). Considering all 10 *Phytophthora* species the relative risk increases to 2.8 (Tab.2). A logistic regression analysis revealed an extremely significant correlation between crown status and the occurrence of *P. quercina* in the rhizosphere ($r = 0.2870$; $p < 0.0001$).

TABLE 1. Contingency table: occurrence of *P. quercina*/crown status

Quercina	Declining oaks	Healthy oaks	Total
Q. present	41	14	55
Q. absent	24	45	69
Total	65	59	124

Relative risk = 2.143
95% - Confidence interval: 1.498-3.066
Fishers exact test: p<0.0001

TABLE 2: Contingency table: occurrence of *Phytophthora* spp./crown status.

Phytophthora	Declining oaks	Healthy oaks	Total
P. present	52	21	73
P. absent	13	38	51
Total	65	59	124

Relative risk = 2.795
95 % - Confidence interval: 1.709-4.568
Fishers exact test: p<0.0001

The distribution of *Phytophthora* species showed a clear relationship to the geological substrate and the texture and pH of the soil (Tab.3). *Phytophthora* spp. were isolated from soil samples on a broad range of geological substrates with silty, loamy or clayy soil-texture and a mean soil-pH ($CaCl_2$) between 3.5 and 7.0, whereas isolations failed on Triassic and Jurassic sandstones, Pleistocene gravels and chalk with mean soil-pH values ≤3.9 and mainly sandy or sandy-loamy soil-texture. The statistical analysis revealed that soils of *Phytophthora* infested and *Phytophthora* free stands differed significantly ($p<0.0001$) in their pH (4.16 ± 0.85 *versus* 3.56 ± 0.28) and their calcium content (28.35 ± 37.36 ppm *versus* 5.54 ± 4.61 ppm). Nitrate concentrations have been quite high in both stand collectives (35.60 ± 40.49 & 31.0 ± 25.7 ppm). Table 4 shows that *P. quercina* (3.53-7.0) has the highest plasticity to the soil-pH followed by *P. citricola* (4.49-6.63), whereas *P. syringae* (3.39-3.85; only 1 isolate at 6.02) and *P. undulata* (3.3-3.75) seem to be restricted to acidic soils.

TABLE 3: Occurrence of Phytophthora species in Bavarian oak stands on different geological substrates and soil types.

Geological substrate	No. of stands	Main soil types (FAO)	Texture[a]	pH-range[b]	No. of stands with occurrence of [c]									
					Que	Cac	Cam	Cit	Gon	Meg	Syr	Und	sp.2	sp.5
Triassic and Jurassic sandstones	13	Dystric & ferric cambisols, cambic & orthic podzols,	(sL) – lS – S	3.2-4.1										
Pleistocene gravels	1	Dystric cambisol	lgS – gS	3.4-3.5										
Pleistocene gravels	1	Calcaric cambisol	sL-uL	3.6-6.0	1			1			1		1	
Chalk	2	Dystric cambisols	ulS-S	3.5-3.7										
	1	Eutric cambisol	sL-uL	4.5-5.1	1	1								
Triassic and Jurassic claystones[d]	7	Vertic cambisols, vertic luvisols, gleysols, stagnic gleysols	ulC-lC	3.4-6.9	6		4	1	1		2	2	1	1
Triassic gypsum	1	Vertic cambisol, eutric cambisol	uL-cL sL-uL	3.5-4.1	1		1							
Triassic and Jurassic limestones[d]	4	Orthic rendzinas, chromo-calcic, (luvo-& gleyo-) chromic cambisols	ugL-uL	3.7-6.7	4	1	1	1						
Tertiary deposits[d]	1	Dystric cambisols	sgL	3.5-3.7	1			1	1			1		
Loess	3	Orthic, stagno-gleyic & gleyic luvisols, stagnic gleysols	suL, uL, cL	3.5-5.4	3		2	2	1		1	3		
Alluvial deposits	1	Calcaric fluvisol	suL, uL, cL	5.6-7.0	1		1	1		1		1		
Total	35				18	2	9	7	3	1	3	7	2	1

[a] =: g = gravelly; s = sandy, u = silty, l = loamy, c = clayy, S = sand, L = loam, C = clay.
[b] =: pH measured in $CaCl_2$.
[c] =: CAC=*Phytophthora cactorum*; CAM=*P. cambivora*; CIT=*P. citricola*; GON=*P. gonapodyides*; MEG=*P. megasperma*; QUE=*P. quercina*; SYR=*P. syringae*; UND=*P. undulata*; sp.2=*Phytophthora* species 2; sp.5=*Phytophthora* species 5.
[d] =: some stands in parts with layers of loess.

TABLE 4.: pH-range of oak forest soils with occurrence of different Phytophthora species

Phytophthora spp.	No. of isolates	pH - range ($CaCl_2$)
P. cactorum	2	4.49 – 6.71
P. cambivora	8	3.65 - 4.39 (1 isolate 6.95)
P. citricola	11	3.53 - 6.63
P. gonapodyides	4	3.62 – 6.02
P. megasperma	1	6.85
P. quercina	55	3.53 – 7.0
P. syringae	7	3.39 – 3.85 (1 isolate 6.02)
P. undulata	6	3.3 – 3.75
Phytophthora sp.2	2	6.02
Phytophthora sp.5	1	6.85

Health status of the root systems and correlation with the crown status

The results of the root analyses including statistical analysis are summarized in Table 5. Considering the absolute values of all root parameters except of root rot, it is obvious that both healthy and declining oaks in the 16 Phytophthora free stands on sandy to sandy-loamy sites with lower nutritional levels have higher values than those in the 19 Phytophthora infested stands on silty, loamy and clayy sites with higher nutritional levels. This is congruent with the results of Meyer (1987) for damaged and healthy spruce stands on limestone and sandstone, and with the results of Thomas & Hartmann (1998) for oak stands on sandy and clayey soils in Germany.

In the Phytophthora infested stands the differences between healthy and declining oaks for absolute values of the root parameters root rot, number of fine roots per m mother root, FRL/MRL, fine root tips per m mother root and FRL / dry weight mother roots have been extremely significant. The nonsignificance for the parameters SFRL and SRTD is not astonishing because their absolute values are heavily influenced by season and weather. In the Phytophthora free stands no or only weakly significant differences have been found between healthy and declining trees.

Considering the season and weather corrected relative values, the totally different situation of the two stand collectives is becoming even more obvious. The differences between healthy and declining oaks in the Phytophthora infested stands have been extremely significant for all parameters, whereas in the Phytophthora free stands the differences for nearly all root parameters were only weakly or not significant.

The nonparametric Spearman-correlation analysis revealed for the Phytophthora infested stands extremely significant correlations between crown transparency and the absolute values of five and the relative values of all root parameters (Tab.6).

On the other hand, in the Phytophthora free stands there was nearly no significant correlation between crown transparency and the absolute values of all root parameters, and even for the relative values significant correlations existed only for the parameters root rot, FRL/MRL and fine root tips per m mother root.

DISCUSSION

Oak decline is at least since the beginning of the 20th century a frequently recurring threat to European forestry (von Hey 1914; Falck 1918; Skoric 1929; Delatour 1983; Oleksyn und Przybyl 1987). Being a shorttime phenomenon of local or regional importance in the past, oak decline in its current phase has been going on since the beginning of the 1980s and is occurring all over Europe (Delatour, 1983; Hartmann *et al.*, 1989; Ragazzi *et al.*, 1989 & 1993; Siwecki & Liese, 1991; Luisi *et al.*, 1993). In 1998 29% of the oaks in Bavaria showed severe crown damage (crown transparency >25%; Anonymus, 1998). A lot of causal agents as frost, droughts, air pollution, sinking groundwater levels, silvicultural mistakes, leaf defoliators, bark beetles, *Ophiostoma* and *Ceratocystis* species, bacteria, MLO and viruses have been discussed, but all of them failed in explaining more than local or regional problems (Delatour, 1983; Nienhaus, 1987; Oleksyn & Przybyl, 1987; Hartmann *et al.*, 1989; Siwecki & Liese, 1991; Luisi *et al.*, 1993; Büttner & Führling,1993; Scorticchini *et al.* 1993; Ragazzi *et al.*, 1993; Ahrens & Seemüller, 1994; Schlag, 1995). In Spain and Portugal a strong association of *P. cinnamomi* interacting with prolonged drought periods and rapid mortality and decline of cork (*Q. suber*) and holm oaks (*Q. ilex*) which display similar disease symptoms as declining pedunculate (*Q. robur*) and sessile oaks (*Q. petraea*) in Central and Western Europe could be shown (Brasier, 1993; Brasier *et al.*, 1993a&b; Cobos *et al.*, 1993; Gallego *et al.*, 1999). In Southeastern France *P. cinnamomi* could also be isolated from cork and holm oak. Its pathogenicity towards both oak species was proven, and a possible involvement in the decline process supposed (Robin *et al.*, 1998). In Spain declining cork and holm oaks have been revitalized by trunk injections of potassium phosphonate, quinosol and carbendazim confirming that Iberian oak decline is a Phytophthora disease. Also in Central and Western Europe fine root destructions have been found in oaks (Näveke & Meyer, 1990; Eichhorn, 1992; Blaschke, 1994; Jung, 1998). In 1990 Galoux & Dutrecq isolated a *Phytophthora* species from fine roots of declining oaks in France, and from 1993-1996 a survey in Central Europe concerning the root status of healthy and declining oaks revealed the widespread occurrence of different *Phytophthora* species in the rhizosphere. Soil infestation tests proved *P. quercina* and *P. cambivora* to be the most aggressive species to fine roots of *Q. robur* and *Q. petraea* (Jung *et al.*, 1996; Jung, 1998; Jung *et al.* in prep). Schlag (1994) mentioned that oak decline is a complex of different diseases. This hypothesis could be confirmed by the results of this study.

It could be shown that *Phytophthora* species are widespread on a range of different geological substrates with silty, loamy or clayey soils with a mean pH ($CaCl_2$) between 3.5 and 7.0. *P. quercina* was by far the most frequently isolated species with an obviously higher isolation frequency from declining (63.1%) than from healthy oaks (23.7%). The statistical analysis revealed that an oak with *P. quercina* in its rhizosphere has a 2.1 fold higher relative risk to develop above-ground disease symptoms compared to an oak without *P. quercina*. In the Phytophthora infested stands highly significant correlations between crown transparency and all root parameters assessed existed. The transformation of the absolute values of the root parameters into relative values eliminated the influence of the seasonal and wheather-induced fine root turnover, thus proving to be a suitable method for comparing the fine root status of oaks from different stands. From soil samples of sites with sandy to sandy-loamy soil-texture and a mean soil-pH <3.9 no *Phytophthora* species was recovered. In these stands correlations between crown transparency and most root parameters have been not or only weakly significant. The soil-pH seems to be a limiting factor for the occurrence of *Phytophthora* species. In this study no *Phytophthora* could be isolated from soil samples with a pH ($CaCl_2$) <3.4 (= c.4.2 measured in water). This is congruent with the data of an *in vitro* sporangia test in soil leachate with a pH (H_2O) row from 3.5-7.0. All *Phytophthora* species tested failed in producing sporangia at pH values ≤4.0 and had an exponential increase in sporangia formation with increasing pH. Exceptions had been *P. cinnamomi* which produced sporangia even at 3.5 and *P. syringae* which had an optimum at 5.0 (Jung *et al.* in prep.). These results fit well the statement of Ribeiro (1983) who mentioned that sporangia may not form at pH values <4.0. This is probably caused by the inability of *Phytophthora* oospores to germinate at low pH values (Ribeiro, 1983). In a pH range from 3.4 to 3.9 *Phytophthora* spp. were isolated from silty, loamy and clayey soils but not from sandy to sandy-loamy soils. An explanation could be that in sandy soils even after heavy rains the period with free water is too short to allow sufficient production of sporangia and release of viable zoospores at these unsuitable pH values.

TABLE 5: Differences of root parameters between healthy and declining oaks and their significance (Mann-Whitney test).

	Root damage (%)		Fine roots / m mother root		SRTD		SFRL		FRL/MRL		FR tips / m mother root		FRL/dry weight mother roots	
19 Stands with Phytophthora (59 healthy and 65 declining oaks)														
Absolute values	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Mean	60.4	82.1	59.2	44.6	702.5	615.2	614.8	531.3	3.5	2.2	400.0	250.0	121.0	76.1
Standard deviation	19.1	13.5	24.8	19.8	320.9	368.2	265.0	279.3	2.3	1.7	260.0	200.0	66.8	52.2
Significance[a]	***		***		n.s.		n.s.		***		***		***	
Relative values	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Mean	100.0	144.9	99.9	78.4	100.0	85.9	100.0	85.8	99.9	64.6	100.0	65.1	100.0	64.1
Standard deviation	29.9	39.5	28.0	35.3	21.5	30.3	19.0	28.3	32.1	38.1	34.6	40.3	34.0	44.5
Significance[a]	***		***		***		***		***		***		***	
16 Stands without Phytophthora (47 healthy and 46 declining oaks)														
Absolute values	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Mean	62,5	68,6	62.8	57.3	923.3	968.8	814.7	863.4	5.2	3.9	540.0	410.0	174.1	144.6
Standard deviation	14,8	11,1	48.3	35.1	360.1	404.0	278.7	297.0	4.3	3.5	430.0	320.0	120.6	121.5
Significance[a]	*		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
Relative values	H	D	H	D	H	D	H	D	H	D	H	D	H	D
Mean	100,0	114,7	100.0	95.8	100.0	110.0	100.0	112.6	100.1	85.0	100.0	86.8	100.0	90.9
Standard deviation	16,5	29,5	30.3	30.0	23.3	40.7	24.6	38.7	40.2	65.0	41.2	86.8	38.6	70.0
Significance[a]	**		n.s.		n.s.		n.s.		*		*		*	

[a] =: n.s. = not significant

TABLE 6: Correlations between crown transparency and different root parameters and their significance

	Root damage (%)	Fine roots / m mother root	SRTD	SFRL	FRL/MRL	FR tips / m mother root	FRL/dry weight mother roots
19 stands with Phytophthora (59 healthy and 65 declining oaks)							
Absolute values							
Spearman Correlation rS	0.5691	- 0.3231	- 0.1462	- 0.1389	- 0.3154	- 0.3006	- 0.3949
Significance[a]	***	***	n.s.	n.s.	***	***	***
Relative values							
Spearman Correlation rS	0.5453	- 0.3265	- 0.3024	- 0.3238	- 0.4595	- 0.4442	-0.4545
Significance[a]	***	***	***	***	***	***	***
16 stands without Phytophthora (47 healthy and 46 declining oaks)							
Absolute values							
Spearman Correlation rS	0.2473	- 0.0080	0.0240	0.0129	- 0.07048	- 0.0575	-0.0570
Significance[a]	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Relative values							
Spearman Correlation rS	0.3265	- 0.0159	0.05060	0.0883	- 0.2750	- 0.2870	- 0.1976
Significance[a]	**	n.s.	n.s.	n.s.	**	**	n.s.

[a] =: n.s. = not significant

The isolation of *Phytophthora* species from soil samples of 68 out of 98 oak stands in Germany, France, England, Scotland, Luxembourgh, Poland, Switzerland, Italy, Slovenia and Hungary, and similar relationships between soil-pH and soil-texture and the occurrence of *Phytophthora* spp. (Jung *et al.* in prep.) indicate that the Bavarian results might have general validity for Central and Western Europe.

From our data we conclude that there are at least two different diseases being referred to under the name 'oak decline':

On sites with a mean soil-pH ($CaCl_2$) ≥3.5–7.0 and silty, loamy or clayey soil-texture decline of oaks is primarily a Phytophthora fine root disease with highly significant correlations between crown transparency and most root parameters, in which *P. quercina* plays the major role because of its widespread occurrence, the high plasticity concerning site conditions and soil-pH, and the host specifity and high aggressiveness to oak species. Normally, this fine root disease is causing a chronic dieback of the crown. However, in combination with high or periodically fluctuating water tables, summer droughts or heavy defoliations dramatic and rapid mortality in groups of trees can occur (Oosterbaan & Naabuurs, 1991; Malaisse *et al.*, 1993; Block *et al.*, 1995; Hartmann & Blank, 1998; Thomas & Hartmann, 1998; Jung *et al.* in prep.).

On sites with a mean soil-pH ($CaCl_2$) ≤3.9 and sandy to sandy-loamy soil-texture oak decline generally is a chronic disease without highly significant correlations between root and crown status, and with most members of the stand suffering equally. On these sites, *Phytophthora* species have not been isolated and, therefore, cannot be considered as causal agent of the decline. Also on these sites prolonged droughts can lead to rapid mortality because of the low water retention capacity of the sandy soils. In this case mortality is starting on or even restricted to the most shallow and sandy parts of the stand or the upper slope.

If the hypothesis is put forward that the onset of this decline-type disease (Manion and Lachance, 1992) is triggered by root-dieback, the question arises, which environmental changes in Central and Western Europe during the last decades have unbalanced the host-parasite relationship between the oaks and the naturally occurring soil-borne *Phytophthora* species. The following ideas are presented for discussion:

Forest soils in Central Europe are getting oversaturated with nitrogen (Kreutzer, 1991; Mohr, 1994; Thomas & Kiene, 1995). Excess nitrogen input into forest soils (Nihlgard, 1985; Thomas & Kiehne, 1995) leads to the observed reduction of mycorrhiza (Zare-Maivan, 1983; Jung & Blaschke unpublished data), which is known as effective mechanical and biochemical barrier against infection by *Phytophthora* species (Zak, 1964). In general, the effects of nitrogen on *Phytophthora* diseases vary with different host-pathogen combinations and with the nitrogen form applied (Huber & Watson, 1974; Schmitthenner & Canaday, 1983). It is known that crown rot of apple trees by *P. cactorum* (Utkhede & Smith, 1995), root rot of

citrus trees by *P. parasitica* (Klotz *et al.*, 1958) and littleleaf disease of pines in New Zealand (Newhook & Podger, 1972) are increased by nitrogen fertilization especially if the nitrogen/phosphate ratio becomes imbalanced, but there are also many examples of nitrogen decreasing disease severity (Schmitthenner & Canaday, 1983). In an *in vitro* sporangia test in soil leachate with nitrate concentrations of 2, 25, 50 and 100 ppm *P. quercina*, *P. citricola*, *P. cambivora* and 3 other *Phytophthora* species showed an exponential increase in the numbers of sporangia formed with increasing nitrate concentrations. The mean nitrate concentration of the soils of the Phytophthora infested oak stands in this study was 35.6 ± 40.5 ppm, indicating favourable conditions for sporangia formation in the field.

The frequent occurrence of mild-humid periods during wintertime and springtime of the last decades indicated by a rise in mean winter temperature of 0.03 K per year and a seasonal shift of precipitation from summer into wintertime (Rapp & Schönwiese, 1995) has favoured the infection of nonmycorrhizal roots by zoospores during the cool season thus triggering an increasing population of *Phytophthora* and a progressive destruction of root systems from year to year. This is confirmed by the fact, that the isolated *Phytophthora* species in this study are able to form sporangia and to release zoospores in soil leachate at temperatures between 2- 8°C (Jung, 1998). During the frequently occurring droughts in summertime the oaks are not sufficiently able to regenerate their fine root systems thus showing aboveground symptoms of waterstress and malnutrition. Brasier and Scott (1994) suggested that a further increase of the winter temperatures by global warming might favour spread of root rot of oaks caused by *P. cinnamomi* from Iberia to Central Europe.

ACKNOWLEDGEMENTS

We are grateful to the Allianz Stiftung zum Schutz der Umwelt and the Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten for the generous financial support and to all the Bavarian Foresters for their kind help. We also want to thank PhD student Jan Nechwatal and the technicians Johanna Lebherz and Renate Bernhard for their assistance in the laboratory routines.

REFERENCES

Ahrens, U.; Seemüller, **E**., 1994: Detection of mycoplasmalike organisms in declining oaks by polymerase chain reaction. Eur. J. For. Path. **24**, 55-63.

Anonymous, 1996: Waldzustandsbericht 1996, Bayerische Landesanstalt für Wald- und Forstwirtschaft (Ed.), pp. 54.

Anonymous, 1998: Waldzustandsbericht 1998, Bayerische Landesanstalt für Wald- und Forstwirtschaft (Ed.), pp. 60.

Blaschke, **H**., 1994: Decline symptoms on roots of *Quercus robur*. Eur. J. For. Path. **24**, 386-398.

Block, J.; Delb, H.; Hartmann, G.; Seemann, D. and Schröck, H.W., 1995: Schwere Folgeschäden nach Kahlfraß durch Schwammspinner im Bienwald. AFZ **38**, 1278-1281.

Brasier, C.M., 1993: *Phytophthora cinnamomi* as a contributory factor in european oak declines.In: Recent advances in studies on oak decline. Proc. Int. Cong., Selva di Fassano (Brindisi), Italy - Sep. 13-18, 1992. Ed. by LUISI, N.; LERARIO, P.; VANNINI, A. pp. 49-57.

Brasier, C.M.; Scott, J.K., 1994: European oak declines and global warming: a theoretical assessment with special reference to the activity of *Phytophthora cinnamomi*. Bulletin OEPP/EPPO Bulletin **24**, 221-234.

Brasier, C.M.; Robredo, F.; Ferraz, J.F.P., 1993a: Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. Plant Pathol. **42**, 140-145.

Brasier, C.M.; Moreira, A.C.; Ferraz, J.F.P.; Kirk, S., 1993b: High mortality of cork oak in Portugal associated with *Phytophthora cinnamomi*. In: Recent advances in studies on oak decline. Proc. Int. Cong., Selva di Fassano (Brindisi), Italy - Sep. 13-18, 1992. Ed. by Luisi, N.; Lerario, P.; Vannini, A. pp. 461-462.

Buttner, C.; Fuhrling, M., 1993: Beobachtungen zu virusbedingten Symptomen an erkrankten Stieleichen (*Quercus robur* L.) - eine Abgrenzung zu ähnlichen, nicht virusbedingten Krankheitsbildern. Nachrichtenbl. Deut. Pflanzenschutzd. **45**, 110-115.

Delatour, C., 1983: Les dépérissements de chênes en Europe. Revue forestière francaise **15**, 265-282.

EC-UN/ECE ,1994: Forest condition in Europe. Results of the 1993 survey. Convention on long-range transboundary air pollution. International co-operative programme on assessment and monitoring of air pollution effects on forests. EC-UN/ECE, Brussels, Geneva, pp.93.

Eichhorn, J., 1992: Wurzeluntersuchungen an sturmgeworfenen Bäumen in Hessen. Forst und Holz **47**, 555-559.

Falck, R., 1918: Eichenerkrankungen in der Oberforstdirektion Lödderitz und in Westfalen. Zeitschrift f. Forst- und Jagdwesen **50**, 123-132.

Fernandez-Escobar, R.; Gallego, F.J.; Benlloch, M.; Membrillo, J.; Infante, J.; Perez de Algaba, A., 1999: Treatment of oak decline using pressurized injection capsules of antifungal materials. Eur. J. For. Path. **29**, 29-38.

Gallego, F.J.; Perez de Algaba, A Fernandez-Escobar, R., 1999: Etiology of oak decline in Spain. Eur. J. For. **29**, 17-27.

Galoux, D.; Dutrecq, A., 1990: Le dépérissement du chêne. A propos des mortalités récentes des chênes pédonculé et sessile en Hesbaye, en Condroz et en Ardenne couvinoise. Forêt Wallone **7**, 3-8.

Hartmann, G.; Blank, R., 1998: Aktuelles Eichensterben in Niedersachsen – Ursachen und Gegenmaßnahmen. Forst und Holz **53**, 733-735.

Hartmann, G.; Blank, R.; Lewark, S., 1989: Eichensterben in Norddeutschland: Verbreitung, Schadbilder, mögliche Ursachen. Forst und Holz **44,** 475-487.

Hey, von, D., 1914: Das Absterben der Eichen in Westfalen. Zeitschrift f. Forst- und Jagdwesen **46**, 595-598.

Huber, D.M.; Watson, R.D., 1974: Nitrogen form and plant disease. Annu. Rev. Phytopathol. **12**, 139-165.

Jung, T., 1998: Die Phytophthora-Erkrankung der europäischen Eichenarten – wurzelzerstörende Pilze als Ursache des Eichensterbens. LINCOM EUROPE, München, pp.143.

Jung, T.; Blaschke, H.; Neumann, P., 1996: Isolation, identification and pathogenicity of Phytophthora species from declining oak stands. Eur. J. For. Path. **26**, 253-272.

Jung, T.; Cooke, D.E.L.; Blaschke, H.; Duncan, J.M.; Oβwald, W., 1999: *Phytophthora quercina* sp. nov., causing root rot of European oaks. Mycol. Res. **103**, 785-798.

Klotz, L.J.; De Wolfe, T.A.; Wong, P.P., 1958: Decay of fibrous roots of citrus. Phytopathology **48**, 616-622.

Kreutzer, K., 1991: Zusammenfassung der Ergebnisse aus der Höglwaldforschung 1984-1989/90. In: Ökosystemforschung Höglwald, Parey, Hamburg, S. 252-261.

Luisi, N.; Lerario, P. and Vannini, A., 1993: Recent advances in studies on oak decline. Proc. Int. Cong., Selva di Fassano (Brindisi), Italy - Sep. 13-18, pp. 541.

Malaisse, F.; Burgeon, D.; Degreef, J.; Deom, B. and Van Doren, B., 1993: Le dépérissement des chênes indigènes en europe occidentale. Belg. Journ. Bot. **126**, 191-205.

Manion, P.D.; Lachance, D., (Eds.), 1992: Forest decline concepts. The American Phytopathological Society, St. Paul, pp. 249.

Matheron, M.E.; Mircetich, S.M., 1985: Pathogenicity and relative virulence of *Phytophthora* spp. from walnut and other plants to rootstocks of english walnut trees. Phytopathology **75**, 977-981.

Meyer, F.H., 1987: Der Verzweigungsindex, ein Indikator für Schäden am Feinwurzelsystem. Forstw. Cbl. **106**, 84-92.

Mohr, H., 1994: Stickstoffeintrag als Ursache neuartiger Waldschäden. Spektrum der Wissenschaft, 48-53.

Näveke, P.; Meyer, H., 1990: Feinwurzelsysteme unterschiedlich geschädigter Eichen im Lappwald. AFZ **33**, 382-384.

Newhook, F.J.; Podger, F.D., 1972: The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. Annu. Rev. Phytopathol. **10**, 299-326.
Nienhaus, F., 1987: Viren und primitive Prokaryonten in Eichen. Österr. Forstz. **98**, 64-65.
Nihlgard, B., 1985: The ammonium hypothesis - an additional explanation to the forest dieback in Europe. Ambio **14**, 2-8.
Oleksyn, J.; Przybyl, K., 1987: Oak decline in the Soviet Union: Scale and hypotheses. Eur. J. For. Path. **17**, 321-336.
Oosterbaan, A.; Nabuurs, G.J., 1991: Relationships between oak decline and groundwater class in The Netherlands. Plant and soil **136**, 87-93.
Ragazzi, A.; Fedi, I.; Mesturino, L., 1989: The oak decline: a new problem in Italy. Eur. J. For. Path. **19**, 105-110.
Ragazzi, A.; Mugnai, L.; Moricca, S.; Vagniluca, S. and Dellavalle, I., 1993: Requirements and biological aspects of *Fuarium eumartii* and ist possible role in oak decline in North-eastern Italian oak forests. Eur. J. For. Path. **23**, 171-177.
Rapp, J.; Schönwiese, C.D., 1995: Trendanalyse der räumlich-jahreszeitlichen Niederschlags- und Temperaturstruktur in Deutschland 1891-1990 und 1961-1990. Annalen der Meteorologie **31**, 33-34.
Ribeiro, O.K., 1983: Physiology of asexual sporulation and spore germination in *Phytophthora*. In: Erwin, D.C.; Bartnicki-Garcia, S.; Tsao, P.H. (Hrsg.): *Phytophthora*. Its biology, taxonomy, ecology, and pathology. The American Phytopathological Society, St. Paul, Minnesota, S. 55-70.
Robin, C.; Desprez-Loustau, M.L.; Capron, G.; Delatour, C., 1998: First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. Ann. Sci. For. **55**, 869-883.
Schlag, M.G., 1994: Das europäische 'Eichensterben' und seine Ursachen - von einem phytopathologischen Standpunkt aus gesehen. Centralbl. f. d. ges. Forstw. **111**, 243-266.
Schlag, M., 1995: The condition of the phloem in declining oaks. Eur. J. For. Path. **25**, 83-94.
Schmitthenner, A.F.; Canaday, C.H., 1983: Role of chemical factors in development of *Phytophthora* diseases. In: *Phytophthora*. Its biology, taxonomy, ecology, and pathology. Ed. by Erwin, D.C.; Bartnicki-Garcia, S.; Tsao, P.H., The American Phytopathological Society, St. Paul, Minnesota, pp. 189-196.
Scortichini, M.; Stead, D.E.; Rossi, M.P., 1993: Oak decline: Aerobic bacteria associated with declining *Quercus cerris* in Central Italy. Eur. J. For. Path. **23**, 120-127.
Siwecki, R.; Liese, W., 1991; Oak decline in Europe. Proc.Int. Symp., Kornik, Poland, May 15-18, 1990, pp. 360.
Skoric, V., 1929: Das massenhafte Eingehen der slavonischen Eiche. In: Petrini, S. (Hrsg.): Proceedings of the International Congress of forestry experimental stations. Stockholm, Centraltrychriet., S. 357-362.
Thomas, F.M.; Kiehne, U., 1995: The nitrogen status of oak stands in Northern Germany and its role in oak decline. In: Nilsson, L.O.; Hüttl, R.F.; Johansson, U.T. (Eds.): Nutrient uptake and cycling in forest ecosystems, U.T. Kluwer Academic Press, Netherlands, pp. 671-676.
Thomas, F.M.; Hartmann, G., 1998: Tree rooting patterns and soil water relations of healthy and damaged stands of mature oak (*Quercus robur* L. and *Quercus petraea* [Matt.] Liebl.). Plant and Soil **203**, 145-158.
Utkhede, R.S.; Smith, E.M., 1995: Effect of nitrogen form and application method on incidence and severity of *Phytophthora* crown and root rot of apple trees. Eur. Jour. Plant Path. **101** (3), 283-289.
Vincent, J.M., 1991: Oak decline: Alteration of the fine root biomass with the progress of the disease. In: Oak decline in Europe. Proc.Int. Symp., Kornik, Poland, May 15-18, 1990 Ed. by Siwecki, R.; Liese, W. pp. 173-175.
Zak, B., 1961: Littleleaf disease of shortleaf pine (Pinus echinata Mill.). In BAILEY, D.L. (ed.): Recent Advances in Botany (IX. Intern. Bot. Congress, Montréal, Canada, 1959), University of Toronto Press, Canada, 1525-1528.
Zak, B., 1964: The role of mycorrhizae in root disease. Annu. Rev. Phytopathol. **2**, 377ff.
Zare-Maivan, H., 1983: Root and mycorrhizal distribution of healthy and declining English oaks (*Quercus robur* L.). M.Thesis. Western Illinois University, Illinois, USA.

RECENT ADVANCES IN STUDIES ON *PHYTOPHTHORA* SPECIES ASSOCIATED WITH *CASTANEA SATIVA* AND *QUERCUS CERRIS* IN ITALY

A.M. Vettraino, G. Natili, N. Anselmi and A. Vannini*

Department of Plant Protection, University of Tuscia, Viterbo Italy
*Corresponding author: vannini@unitus.it

SUMMARY

Presence of *Phytophthora* spp. in the rhizosphere of healthy looking and declining *Castanea sativa* and *Quercus cerris* trees was investigated in different forest sites in Central Italy.

Five different species of *Phytophthora* have been isolated from soil in chestnut stands affected by "ink disease". Among these, only *P. cambivora* was constantly associated with symptomatic trees and isolated from infected tissues.

Surveys carried out in oak stands in Central Italy revealed the presence of a number of *Phytophthora* species present in the rhizosphere of both healthy looking and declining trees. Among the species isolated *P. citricola, P. gonapodyides, P. cactorum, P. cinnamomi* and *P. cambivora* were identified.

INTRODUCTION

The role of *Phytophthora* species in decline and death of broad-leaved trees has been widely investigated in different areas of the world. *P. cinnamomi* Rands is cause of the decline of Jarrah (*Eucalyptus marginata*) in Australia (Old, 1979), "ink disease" of red oak (*Quercus rubra*) in Europe (Moreau and Moreau, 1952), decline of *Q. ilex* and *Q. suber* in Spain and Portugal (Brasier and Ferraz, 1993). Lately a new species of *Phytophthora* has been found to be responsible of alder decline in Europe (Brasier *et al*, 1995).

In Italy high mortality of sweet chestnut (*Castanea sativa* Mill.) by "ink disease" has been recently reported from different areas, suggesting a new epidemic spread of the disease (Anselmi *et al.*, 1996). The disease, probably carried by humans and animals, commonly starts from trees along roads and trails. Due to the high economic and environmental value of sweet chestnut in Italy, regional and local authorities decided to dedicate research funding to study the etiology and epidemiology of the disease and to define efficient prevention and control methods. Beside this alarming epidemic on chestnut, particular attention has been put in the recent years on the role of *Phytophthora* spp. in the decline of *Quercus* spp. in Europe (Jung *et al*., 1996). Since Italy is one of the countries where oak decline is present with the highest incidence, particularly on *Q. cerris* L., it has been decided to investigate the association of *Phytophthora* spp. with the syndrome of decline in different oak stands.

With the present work we report the recent advances in studies on the presence of *Phytophthora* spp. in the rhizosphere of healthy looking and declining *C. sativa* and *Q. cerris* trees from different forest sites in Central Italy

MATERIALS AND METHODS

Areas investigated

Chestnut

The chestnut areas investigated are localized in Central Italy, the first in the Northern Latium on the Monti Cimini, one of the largest chestnut areas in Italy; the second in the Eastern Latium on the Monti del Cicolano. Both the areas are characterized by extensive coppice stands and orchards devoted to fruit production that extend at an altitude ranging from 400 to 1000 msl. Surveys have been carried out in the period March – July 1998 and 1999 in the Monti Cimini and Monti del Cicolano chestnut areas by collecting soil samples from different forest sites where the disease was previously recorded (Anselmi *et al.*, 1996). A total of 99 soil samples were collected under symptomatic and healthy looking trees, from areas devoted as deposit for chestnut logs and from seasonal streambeds crossing chestnut forest.

Oak

Sampling of soil in oak forests was performed with a different design. A 600 square meter plot, representative of the average vegetative condition of the trees in the stand, was delimited in each of 6 oak forest sites in Central Italy. Six more plots were delimited, 2 for each of 3 sites, representing one of the most declining and one of the healthiest situations of the stand. Totally 12 plots distributed in 9 sites were considered. From each plot soil under 4 *Q. cerris* trees was sampled. Seven plots over 9 sites have been surveyed twice for a total of 84 soil samples.

Phytophthora isolation

Soil samples were moistened and incubated at 20°C for 3 days. About 200 ml of soil was then flooded with 500 ml of distilled water in plastic containers. Five fresh picked leaves of *Rhododendron* spp. or 2-4 days old leaves of *Q. robur* were placed directly on the water surface and incubated at 20°C up to the development of spots or discoloration on the leaves but not longer than one week. The leaves were then blotted on filter paper, cut in small pieces and placed on PARBhy (Robin, 1991). *Phytophthora* isolates were maintained on carrot agar (CA) (Brasier, 1969) at 20° in darkness and sub-cultured at 4 wk intervals.

Isolate identification

Colony morphology assessment was carried out on 10 d cultures grown in 9 mm Petri dishes at 20°C in darkness. Sporangia were produced in soil extract according to Chee and Newhook (1965).

Tests for sexual compatibility type were carried out directly on microscope slides by placing a plug of the "unknown" isolate in contact with a plug of the A1 or A2 tester isolates. The slides were incubated in the dark at 20°C and RH near saturation and scored by optical microscope for oogonial formation after 10-15 d. Oogonia and antheridia of homothallic species were produced directly on CA.

RFLP patterns of ITS1- 5,8S - ITS2 region of rDNA were generated according to the methodology reported by Cooke and Duncan (1997) with some modifications, among which the use of *Rsa* I instead of *Taq* I restriction enzyme.

RESULTS

Chestnut

Phytophthora spp. was recovered in the chestnut forests investigated from 26 soil samples over 99 attempted (25.7%). In particular, isolation of *Phytophthora* spp. was obtained from 21 soil samples collected under symptomatic trees, over 68 attempted (30,3%); from 2 soil samples collected under healthy looking trees, over 23 attempted (8,7%). Moreover positive isolation was obtained from 1 (25%) and 2 (50%) soil samples collected respectively under chestnut log material and in streambeds crossing chestnut forests.

Five species of *Phytophthora* were recognized. According to colony, sporangia, oogonia and antheridia morphology and presence or absence of hyphal swelling and chlamidospores, they were preliminarily identified as *P. cambivora*, *P. citricola*, *P. cactorum*, *P. gonapodyides* and *P cryptogea*. Comparison of RFLP patterns of isolates with standard strains confirmed the morphological classification with the exception of the isolates referred as *P. cryptogea* whose classification remains to be confirmed.

All the *P. cambivora* isolates belonged to the A2 sexual compatibility type; the 3 isolates referred as *P. cryptogea* belonged to the A1 sexual compatibility type. No oogonia were obtained from the isolate classified as *P gonapodyides.*

As showed in Table I, isolates of *P. cactorum* were obtained from symptomatic (33) and healthy looking (2) trees, and from streambeds (12); isolates of *P. citricola* were obtained from symptomatic (60) and healthy looking (17) trees, streambeds (17), and areas devoted as deposit of log material (6). *P. cambivora* was isolated only from symptomatic trees (93 isolates totally). Finally the three isolates related to *P. cryptogea* and the single isolate of *P. gonapodyides* were obtained from soils in an area devoted to deposit of log material.

TABLE I. Number of isolates of the 5 species of *Phytophthora* obtained from different sources in chestnut stands.

		N° of isolates				
Phytophthora Species	Period of sampling	Sympto-matic trees	Healthy looking trees	Logging areas	Stream bed	Total
cactorum	March-July 1999	33	2	0	12	47
citricola	March-July 1999	60	17	6	17	100
cambivora	May 1999	93	0	0	0	93
cryptogea ?	June 1998	0	0	3	0	3
gonapodyides	June 1998	0	0	1	0	1

Oak

Phytophthora spp. were recovered in 4 oak sites of 9 investigated in Central Italy. Furthermore 11 soil samples of 84 attempted (13,1 %) gave positive isolation. In particular isolation of *Phytophthora* spp. was obtained from 5 soil samples collected under symptomatic trees, of 28 attempted (17,8%); from 6 soil samples collected under healthy looking trees, of 56 attempted (10,7%).

A total of 22 isolates of *Phytophthora* spp. were recovered from the baiting. According to the methodology reported above for chestnut, 1 was classified as *P. cactorum*; 13 *as P. citricola*; 2 as *P. gonapodyides*; 1 as *P. cambivora*, mating type A2; 1 as *P. cryptogea*, mating type A2; 2 isolates from declining trees in Ladispoli were classified as *P. cinnamomi* Rands. both mating type A2; finally 2 isolates belong to Group VI according to Waterhouse, but still have not been assigned to any species.

As shown in Table II, the single isolate of *P. cactorum* was obtained from an healthy looking tree; the isolates of *P. citricola* were obtained from symptomatic (11) and healthy looking (2) trees; the isolate of *P. cambivora* was recovered from an healthy looking tree as well as the isolate of *P. cryptogea*, the 2 isolates of *P. gonapodyides* and the 2 *Phytophthora* spp. isolates. Finally the two isolates assigned to *P. cinnamomi* were recovered from 2 distinct declining trees in the same area in Ladispoli.

TABLE II. Number of isolates of the 6 species of *Phytophthora* from declining and healthy looking *Q. cerris* trees

		N° of isolates		
Species	Period of sampling	Declining trees	Healthy looking trees	Total
P. cactorum	June 1998	0	1	1
P. citricola	March & June 1998	11	2	13
P. cambivora	May 1999	0	1	1
P. cryptogea ?	December 1998	0	1	1
P gonapodyides	March 1998	0	2	2
P. cinnamomi	December 1998	2	0	2
Phytophthora spp.	March 1998	0	2	2

DISCUSSION

Phytophthora spp. frequently occur in the rhizosphere of both healthy looking and declining chestnut and oak trees in Italy. The same species were recovered under chestnut and oak trees but at different frequencies. One exception is represented by *P. cinnamomi* that surprisingly was not isolated from chestnut stands where it is constantly associated with ink disease in Europe.

The frequency of isolation of *Phytophthora* spp. from chestnut soils was much higher under symptomatic trees then under healthy looking trees. *P. cambivora* was constantly associated with diseased trees and it was the only species isolated from symptomatic tissues also (Vannini, unpublished). However *P. citricola* and *P. cactorum* also were mainly recovered from soil samples under symptomatic trees. *P. citricola* together with *P. cactorum*, do not seem to be able to cause serious damage when present alone. However both the species were often present in soil samples and associated with *P. cambivora* in May when the latter species could be isolated. The possibility of a synergism among *Phytophthora* species in inducing “ink disease” on chestnut should be further investigated.

The difficulty to isolate *P. cambivora* from soil and streambeds during most of the year could be associated with the lack of resting structures. In fact the presence in the population of a single mating type (A2) suggests a prevalently clonal population and the absence of oospore formation. Furthermore from the 93 isolates collected, no chlamydospores have been ever detected. The above considerations would suggest that *P. cambivora* has the opportunity to spread from infected trees only for a limited period of the year when the climatic conditions are favorable to the zoospore surviving in the environment.

Particularly alarming is the presence of *Phytophthora* spp. in areas devoted to deposit of log material that could act as source of inoculum.

Concerning oak, it should be highlighted that *Phytophthora* spp. were present but not abundant in soils from oak stands. At the moment it is difficult to find any association between isolation of *Phytophthora* spp. from soil and health status of the trees in Central Italy. As reported before, 5 of the classified species were also found in chestnut soils. However *P. cambivora*, considered the most aggressive to chestnut, was isolated under a healthy vigorous *Q. cerris* tree suggesting a different behavior of this species on oak.

P. cinnamomi, generally reported as the main species causing *Q. suber* and *Q. ilex* decline in Spain and Portugal (Brasier and Ferraz, 1993) and *Q. rubra* "ink disease" in France (Moreau and Moreau, 1952), was the only species isolated under clearly declining trees in the Ladispoli site. However this site is seasonally subjected to natural flooding since the water table level increases during spring and fall forming natural pods called "piscine". Tests on pathogenicity of these *Phytophthora* species on *Quercus* spp. are actually ongoing.

In conclusion, the high mortality of sweet chestnut in Central Italy by "ink disease" is particularly alarming. Localization of infected trees mainly in proximity of roads suggests a possible start of a new epidemic. Different species of *Phytophthora* other then *P. cambivora* should be carefully studied to understand their involvement in the development of the disease. We trust in the possibility to control this disease taking in account the behavior of the most pathogenic species

present, *P. cambivora*, whose spreading in the environment seems to be restricted to a short period of the year.

No conclusions can be drawn at the moment on involvement of *Phytophthora* spp. in oak decline in Central Italy, except that a number of potentially dangerous species are naturally present in oak soils although at low rate of inoculum.

ACKNOWLEDGEMENTS

This study is being carried out with the financial support from the project EC - Latium Region DOCUP Objective 5b 1994-1999 I.1.3.07.002 "Development of control strategies against “ink disease” caused by *Phytophthora* spp. in Italy", and from the Commission of the European Community, Agriculture and Fisheries (FAIR) specific RTD program, CT 97-3926, "Long term dynamics of oak ecosystems: assessment of the role of root pathogens and environmental constraints as interacting decline inducing factors" (PATHOAK). It is the sole responsibility of its publishers and in no way represents the views of the Commission or its services or their future policy in this area.

Special thanks to C. Brasier, C. Delatour, G. Magnano di San Lio, C. Refforld, J. Rose, T. Jung and S. Werres for providing the strains of *Phytophthora* spp. used as standards in the present study.

REFERENCES

Anselmi, N., Giordano, E., Vannini, A., Troiani, L., Napoli, G., and Crivelli, L., 1996. Il mal dell’inchiostro del castagno in Italia : una vecchia malattia ritorna attuale. Linea Ecologica XXVIII, 39-44.

Brasier, C.M., 1969. The effect of light and temperature on reproduction *in vitro* in two tropical species of *Phytophthora*. Transactions of the British Mycological Society 52, 105-113.

Brasier, C.M. and Ferraz J.F.P., 1993. Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. Plant Pathology, 42: 140-145.

Brasier, C.M., Rose, J., Gibbs, J., 1995. An unusual *Phytophthora* associated with widespread alder mortality in Britain. Plant Pathol. 44: 999-1007

Browne, G.T. and Mircetich, S.M., 1993. Relative resistance of thirteen apple rootstocks to three species of *Phytophthora*. Phytopathology, 83:744-749

Chee, K.H. and Newhook. F.J., 1965. Improved methods for use in studies on *Phytophthora cinnamomi* Rands and other *Phytophthora* species. N.Z.J. Agric. Res. : 88-95.

Cooke, D.E.L. and Duncan J.M., 1997. Phylogenetic analysis of Phytophthora species based on ITS1 and ITS2 sequences of the ribosomal RNA gene repeat. Mycol. Res. 101: 667-677

Jung, T., Blaschke, H. & Neumann, P., 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. European Journal of Forest Pathology 26, 253-272.

Moreau, C. and Moreau, M. 1952. Etude mycologique de la maladie de l'encre du chêne. Rev. Pathol. Veg. 31: 201-231.

Old, K.M. ed., 1979. *Phytophthora* and Forest Management in Australia. C.S.I.R.O. Melbourne, Australia. 114 pp.

Waterhouse, G.M., 1970. The genus *Phytophthora* de Bary. Mycological Papers 122, Commowealth Mycological Institute, Kew, Surrey , England.

White, T.J., Bruns, T., Lee, S.B. and Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomial RNA genes for phylogenetics. In: PCR protocols, a guide to methods and applications, Innis. M.A.; Gelfand, D.H.; Sni nsky, J.J. and Whit, T.J., eds.. Academic Press: San Diego, USA. pp.315-322.

ALDER *PHYTOPHTHORA* IN FRANCE AND THE UNITED KINGDOM : SYMPTOMS, ISOLATION METHODS, DISTRIBUTION, AND DAMAGE

J.-C. Streito* and J. N. Gibbs**

* : Laboratoire National de la Protection des Végétaux , 38 rue Sainte Catherine 54043 Nancy cedex, France
** : Forestry Commission Research Agency, Alice Holt Lodge, Farnham, Surrey GU10 4LH, United Kingdom.

A previously unknown disease of common alder *Alnus glutinosa* (L.) Gaertn. was discovered in 1993 in southern United Kingdom (Gibbs, 1995). As described by Brasier *et al*. (1995), it is caused by a fungus resembling *Phytophthora cambivora* but differing from it in some important particulars. This pathogen is now known to comprise a range of interspecific hybrids involving *P. cambivora* and an unknown taxon similar to *P. fragariae* (Brasier *et al*., 1999). At the present time this *Phytophthora* is known in United Kingdom, France, Germany, Netherland, Sweden and Austria. Damage is significant in United Kingdom and in France, but less so in other countries. This paper presents French and English studies concerning detection, distribution, damage and biology of the alder *Phytophthora*.

1. Experience of the Phytophthora disease in France : symptoms, isolation method and distribution.

Before 1993 no virulent disease had been observed on common alder in France, although cases of decline, principally caused by environmental factors, had been reported from time to time.

Studies on the 'alder *Phytophthora*' began in 1996 with confirmation that the disease occurred in the country. The main problem that was encountered was the frequent failure of isolation attempts. In 1997, 151 samples from necrotic bark lesions were processed but only 15 isolates of the alder *Phytophthora* were obtained. In 1998 several aspects of the isolation procedure were studied in order to improve its success. As shown below the choice of sample material and the activity of the fungus within the sample appeared to have the greatest influence. Success rates improved markedly and from 173 bark samples, 108 alder *Phytophthora* isolates were obtained. In addition to sampling from diseased alders, an attempt was made to bait for the fungus in rivers bordered by diseased trees. A more complete account of some aspects of the work described here has already been published. (Streito *et al*., 1999)

1.1. Tree symptoms.

From a distance diseased alders attract attention because the leaves are abnormally small, yellow and sparse. The stem base is often marked with tarry or rusty spots sometimes occuring up to 2 meters above ground level; these being formed through the gradual drying of drops of black fluid that have exuded from the bark. Under the tarry spots, necrotic lesions can be found in the inner bark. Usually these lesions are connected with necrotic roots but on one occasion the alder *Phytophthora* was obtained from an isolated bark lesion on a lower branch. When found in combination, tarry spots associated with a continuous basal lesion and the presence of the crown symptoms described above are highly characteristic of the disease.

The condition of the necrotic lesions is very important for isolation success. Tarry spots can remain for a long time on the trunk and often overlie lesions which are old and dry: isolation from such tissues is very difficult. By contrast, isolates of *Phytophthora* are obtained easily from fresh active lesions. In these the necrotic tissues are moist and are continuous with healthy tissues. Commonly, old necrosis and fresh necrosis occur together on the same lesion.

In 1997 active lesions were not observed in the North-East of France before July and the best period for sampling was in September and October. In 1998 fresh bark killing was observed as early as May and successful isolations were made at this time. The success rate continued high until the end of October. This suggests that the activity of the pathogen can vary from year to year. In 1999 it was isolated from Charentes (West of France) in January.

1.2. Method of isolation from bark lesions.

If the necrosis is fresh and the pathogen active, isolation is easy and several techniques (apple baiting, direct isolation on agar etc.) can be used. The method used by the LNPV (Laboratoire National de la Protection des Végétaux), and described below, is simple and efficient :

Sampling.
As indicated above there is no particular time of year for taking samples, as the activity of the fungus varies from year to year. However, active lesions are usually present in the autumn. The location of fresh necrotic lesions is determined by cutting into the inner bark with a knife. A piece of bark and attached wood of 10 cm x 10 cm x 3 cm is taken from the outer edge of the necrotic lesion, placed in a plastic bag and brought to the laboratory at ambient temperature.

Storage.
Samples are usually wrapped in damp paper, left at room temperature (25±5°C) overnight and analysed the next day. However, it is possible to keep samples in the fridge at 7±4°C for up to a month.

Washing and disinfection.
The outer bark is removed with a knife, and the sample is then washed for a few minutes under tap water and dried with filter paper. The whole surface is disinfected quickly with 70 % ethanol and dried again under sterile conditions.

Isolation.
Very small pieces of inner bark tissue from the outer edge of fresh necrotic lesions are plated directly onto a selective medium (composition in Table 1) or corn meal agar (15g/liter).

TABLE 1. Composition of culture media (for one liter) :

Corn Meal Agar	Selective media
17 g Corn Meal Agar per liter	17 g Corn Meal Agar per liter 0.1 mg Pimaricin 10 mg Rifampicin 250 mg Ampicilin 15 mg Benomyl (Benlate 50 %) 50 mg Hymexazole (Tachigaren 75 %)

Incubation.
Plates are incubated under the following conditions : 12 hours day light 20±3°C, 12 hours dark 18°C±3°C. Colonies are observed after 3 days, by direct observation through the bottom of the plate (magnified X 100), and then once a week for three weeks. The alder *Phytophthora* is easily recognised by its gametangial morphology (Brasier *et al*., 1995).

1.3. Baiting method to detect the alder *Phytophthora* in river water.

Two kinds of baits were tested: alder leaves and alder twigs. Successful isolations were made using twigs but not leaves. The technique is as follows :

Twigs, about 1 cm in diameter and about 10 cm long, are joined together to form a raft, moored to the bank and left to float on the surface of the river. After about one week (in summer) and about four weeks (in winter),necrosis appears in the bark at the ends of the twigs and around injuries. Isolation for *Phytophthora* species is then conducted using the method described above.

In 1998 the alder *Phytophthora* was isolated once from the river Sarre and several times from the Moselle (between May and September). However, the number of positive isolations was very low: in total less than 1% of fragments of necrotic bark yielded the alder *Phytophthora*. Other fragments yielded a variety of *Phytophthora* species : principally a species of the 5th or 6th group (Waterhouse,1963) with non-papillate sporangia, internal and external proliferations, and no oogonia in single culture. Athough the successful isolation of the pathogen from river water is of interest, it seems that the alder twig tissues may not be selective enough for much use to be made of the 'raft technique' in ecological and pathological studies of the disease.

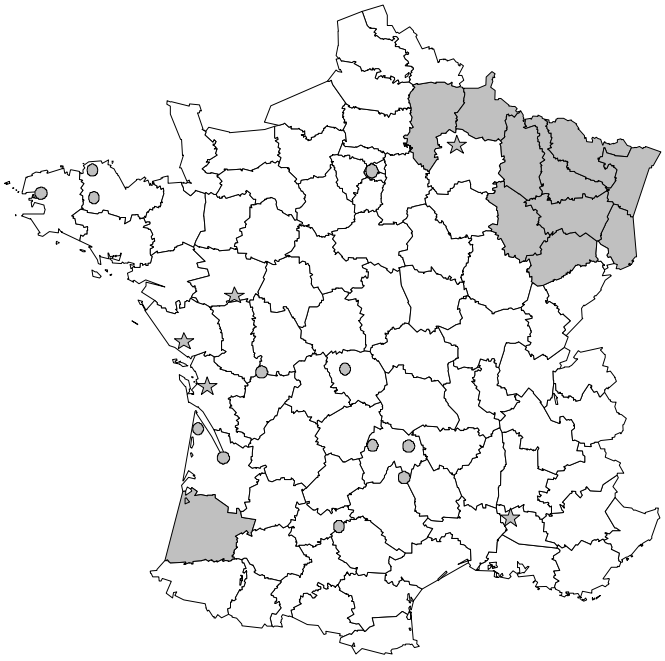


FIGURE 1. Distribution of alder disease in France :
several sites with alder *Phytophthora* in these departments;
one site with alder *Phytophthora*;
alder decline without alder *Phytophthora* detected

1.4. Distribution of the disease in France

The known distribution of the disease across France is shown in Figure 1. The alder *Phytophthora* is evidently very widely distributed in the north-east of France. It has been isolated from more than 110 different sites, and all the main rivers and most of their tributaries are affected. On the Sarre system, the decayed condition of the dead trees indicates that the disease may have been present for a long time and damage levels are very high. In some places more than 50 % of trees are diseased or dead. On the Moselle, Meurthe and Meuse the damage is less severe but can be locally important. In Alsace, the alder *Phytophthora* has been found on some of the Rhine tributaries, but there has been no 'scouting' for the disease along the Rhine itself. The alder *Phytophthora* is also present in the Oise and Marne systems. In north-east France, only upper regions of the Vosges mountains seem to be free from disease.

Although the majority of records have concerned riparian alders, some cases of disease on lake banks and in seasonally-flooded forest stands have also been detected. All examples have involved *A. glutinosa*; other alder species not being common in this region. Trees of all ages can be affected - from three to more than 60. On the same river-bank dead, diseased and healthy trees are often found side by side.

Elsewhere in France the alder *Phytophthora* has been isolated in the Landes, near Bordeaux (C. Robin, personal communication). Several cases of damage associated with the alder *Phytophthora* have been observed in the west of the country, along the Charente river and in Deux-Sèvres. In addition the fungus has been recently isolated on the Rhône river near Avignon. Several cases of decline in Brittany, and in the west and centre of France were observed by staff of the " Département de la santé des Forêts " but the *Phytophthora* has not been isolated yet. No data are available for the rest of the country.

2. The alder *Phytophthora* in the United Kingdom : distribution, impact on the riparian population of alders.

2.1. Distribution of the disease in the United Kingdom.

As has been made clear in the introduction, it was in the United Kingdom that the disease was first recognised and the alder *Phytophthora* isolated. It was soon established that it was widespread in England and Wales (Gibbs, 1995) and it has since been found in Scotland, at two sites from the River Spey.

The vast majority of diseased trees are to be found on the banks of streams and rivers or on sites subject to flooding from adjacent watercourses. However it has also been found in orchard shelterbelts and occasionally in new woodland plantings. In these site-types there is no possibility of flooding. Most of the cases have involved the native *A. glutinosa* but the disease has also recorded on the grey alder *A. incana* (L.) Moench and Italian alder *A. cordata* Desf. (Gibbs,1995).

2.2. Impact of the *Phytophthora* disease in riparian population of common alder.

In 1994 a survey was established to obtain quantitative data on the impact of the disease on riparian alder and on its subsequent development. A full description of the work has recently been published (Gibbs et al.,1999) and only a brief account will be given here.

Within an area of 70 000 km², 63 observation plots were set up on stretches of river over 8 m wide. Average alder density varied widely in different parts of the survey area, from 0.7 to 22.2 trees per 100 m of river. From the density figures and from data on the total length of rivers over 8 m wide within the survey area, it was estimated that there were approximately 585 000 alder trees growing on the banks of such rivers.

At the first survey, 1.3% of trees were classified as dead i .e. they possessed no living stems over 7 cm diameter. Not all of these had been killed by *Phytophthora* although close association with trees showing crown symptoms suggested that most of them had been. 3.6% of the trees had at least one stem with *Phytophthora* crown symptoms and approximately half of these showed clear evidence of tarry spots or bark death at the stem base. The largest number of trees with *Phytophthora* crown symptoms was found in the south-east of England. If these measures of disease are related to the total estimated population of 585 000 an estimate of 7000 dead trees and 22800 trees with *Phytophthora* crown symptoms is produced. A limited study suggested that disease incidence on smaller water courses was only between 10% and 20% of that found on larger rivers.

Disease development from 1994 to 1998.

All the plots containing alder at the first survey in 1994 were resurveyed in subsequent years. However in 1998 a change was made to make better use of staff resources and only the 31 plots containing at least 15 alders were surveyed. Table 2 contains the data from those plots for each of the years 1994 to 1998. It can be seen that the number of trees with *Phytophthora* crown symptoms increased from

51 to 112 over the five years and that the number of dead trees increased from 22 to 54. Examination of the data showed that the increase was entirely due to the death of trees that had shown crown symptoms of the disease in previous years. If the data are expressed in relation to the alder population at the time of assessment, it is found that the percentage of the alder population affected by the disease increased from 4.3% in 1994 to 9.7% in 1998. If one takes account of the missing diseased trees, between 1.09 % and 2.51% of previously asymptomatic trees became diseased each year between 1994 and 1998.

Disease incidence in relation to various host attributes and site factors.

There was no difference between single stem trees and multiple-stemmed trees in disease incidence. However, when the incidence of symptoms was examined in relation to the distance of the trees from the water edge, a strong negative association was found. Among trees within 1 m of the the river bank 5.4 % showed disease while among those further away (1-10 m) only 0.7 % were affected.

The relationship between disease incidence and water quality was examined because of the possibility that the prominence of the disease in some areas might be linked in some way to pollution. A significant positive association was found with total oxidized nitrogen but not with other indices of water quality. This association is intriguing because of evidence that nitrogen fertilization can enhance some well-known *Phytophthora* diseases such as crown rot of apples caused by *P. cactorum*. However, the severity of other *Phytophthora* diseases is reduced by increased nitrogen and much more research is required before a causal relationship can be inferred.

TABLE 2. Summary of data on *Phytophthora* disease from riparian plots in southern England and east Wales.

	Data from 31 plots (Plots with a minimum of 15 trees)				
	1994	1995	1996	1997	1998
Category of alder					
Number of trees assessed (n)	1679	1716	1717	1720	1712
Number missing since the last survey	-	5	46	53	37
Number with *Phytophthora* crown symptoms	51	62	86	101	112
Number dead (of which no. Long dead)	22	28	40	44	54
Number with *Phytophthora* crown symptoms or dead (d)	73	90	126	145	166
Number missing since last survey which had *Phytophthora* crown symptoms and were dead	-	1	4	7	5
Percentage of symptomatic and dead trees	4.3	5.2	7.3	8.4	9.7
Annual incidence of disease derived from : $\frac{d_i + m_i - d_{i-1}}{n_i + m_i - d_{i-1}} \times 100$	-	1.09	2.51	1.62	1.65

PROGNOSIS

In Europe, common alder (*A. glutinosa*) is an important component of the riparian ecosystem, and has great conservation value. It provides food and protection for many organisms, from birds and fish to insects and fungi. In association with actinomycetes of the genus *Frankia*, alder is strongly nitrogen fixing and its root system helps to stabilize the river banks. The loss of alder would affect the appearance of the landscape and there would be a wide range of biological repercussions. Even if, in the long term, other tree species took over the places previously occupied by alders, the nature of the ecosystem would be different.

Given the nature of the host and of the pathogen, a sanitation approach to control is not considered feasible. Experiments have been established in the United Kingdom and in France to determine if there are any benefits to be gained from the early coppicing of diseased trees. Also in the United Kingdom, a range of European alder provenances is being evaluated to determine if differences in disease resistance are present. However, at present no technique can be proposed for disease management and control.

ACKNOWLEDGEMENTS

We would like to thank The European Community for financing the Concerted Action " *Phytophthora* Disease Of Alder ": this has provided valuable opportunities for the exchange of information. In addition, we would thank the Agence de l'eau Rhin-Meuse for financing part of our studies in France and the Forestry Commission and the Environment Agency for financing the research in the UK. We are grateful to Anne Giraud and Steven Hendry for critically reading the manuscript.

REFERENCES

Brasier C.M., Rose J., Gibbs J.N., 1995. An unusual *Phytophthora* associated with widespread alder mortality in Britain. Plant Pathol. 44, 999-7007.

Brasier C.M ., Cooke D.E.L., Duncan J.M., 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridization. Proc. Natl. Acad. Sci. USA Vol. 96, 5878-5883.

Gibbs J.N., 1995. *Phytophthora* root disease of alder in Britain. EPPO Bull. 25, 661-664.

Gibbs J.N., Lipscombe M.A., Peace A.J., 1999. The impact of *Phytophthora* disease on riparian populations of common alder (*Alnus glutinosa*) in southern Britain. Eur. J. For. Path. 29 39-50.

Streito J-C., de Villartay G. and Tabary F.,1999. Une nouvelle espèce ; ce de *Phytophthora* s'attaque à l'aulne. *Phytoma* **519**, 38-41

Waterhouse G.M., 1963. Key to the species of Phytophthora de bary. Mycological Paper 92, Commonwealth Mycological institute, Kew.

REGENERATION AFTER DIEBACK DUE TO *PHYTOPHTHORA CINNAMOMI* – ARE SUPPRESSIVE SOILS INVOLVED?

Tonia Walchhuetter, Gretna Weste, David Guest.

School of Botany, University of Melbourne, Parkville, Victoria, Australia 3052

ABSTRACT

Regeneration of highly susceptible plant species was observed 20-30 years after infection of native forest that resulted in epidemic dieback disease due to *Phytophthora cinnamomi* lasting approximately twenty-thirty years. We hypothesise that an increase in antagonistic soil microbe species was involved in this regeneration. This was tested with bioassays of suppressive and conducive soils using *Lupinus albus*, and inhibition tests involving antagonists isolated from the soils over a two year period. Soils were found to be suppressive to disease due to *P. cinnamomi* in lupins, as indicated by significantly higher seedling emergence rates, lower mortality, and a higher frequency of healthy plants in non-sterilised inoculated soils compared with sterile inoculated soils. Many antagonistic microbes were present in the soil, with fungi the most antagonistic group. A model of microbial succession is proposed for the involvement of microbes in the regeneration of highly susceptible species in this area.

INTRODUCTION

Dieback due to *Phytophthora cinnamomi* Rands is a serious problem in both native vegetation and crops worldwide. It is the pathogen responsible for jarrah dieback in Western Australia and is estimated to cause losses of up to $200 million annually in Australia (Irwin et al. 1995). A significant proportion of Australian species is susceptible to the pathogen, making it a serious threat to timber supplies and native flora, particularly endangered susceptible species.

Control of the disease is difficult as it has a wide host range and is able to survive in non-hosts for long periods of time (Zentmyer 1980; Weste 1994). Disease management aims to prevent spread of the pathogen from infested areas into uninfested areas. However, biological control and phosphonate are emerging as promising methods of disease management in areas where the pathogen has been introduced.

The Pathogen

P. cinnamomi has a disease cycle that makes it a particularly serious threat to vegetation. The disease is often introduced into areas in the form of thick walled chlamydospores; these can be introduced in infested soils, gravels, or plant material. Chlamydospores are formed in soil or plant tissue during dry conditions, and are capable of surviving in gravel for up to five years (Weste and Vithanage 1979a). Chlamydospores are vulnerable to soil microbes; high soil microbe populations may result in suppression of chlamydospore germination or their eradication.

Chlamydospores germinate in moist conditions to form mycelia, sporangia and zoospores, which allow *P. cinnamomi* to move within a host and between hosts through root-root contact. Mycelia are capable of surviving in moist soils for up to six years (Zentmyer and Mircetich 1966). The persistence of mycelia in soil is reliant on soil microbial populations; if large numbers of microbes are present, rapid lysis of mycelium occurs (Kuhlman 1964; Mircetich and Zentmyer 1967). Lysis of mycelium is frequently associated with formation of chlamydospores and sporangia (Tsao 1969).

Sporangia production and subsequent zoospore release are affected by temperature changes, moisture levels, chemical factors, and soil microorganisms. In forest soils, *P. cinnamomi* is dependent on the presence of microbes to stimulate production of sporangia (Zentmyer 1965); all forest soils tested stimulate sporangial production (Weste and Vithanage 1979). High microbial populations may result in antagonism of sporangia, resulting in suppression of sporangial production, abortive sporangia, or suppression of zoospore release (Tsao 1969). Under favourable conditions, sporangia release biflagellate zoospores.

Zoospores are capable of being moved great distances by the movement of water through soil. In addition, zoospores are chemotactically attracted to root exudates (Hinch and Weste 1979). When a zoospore reaches a root surface, it attaches itself and encysts. The cyst is resistant to microbes but germinates on any root to form a germ tube, or under unfavourable conditions, a sporangium and secondary zoospore. In close proximity to a root, a germ tube will arise from the cyst and penetrate the root, initiating infection. By nature of their motility and their ability to be transported in water moving through soil, zoospores are the main infective propagule of *P. cinnamomi*. However, as zoospores have only a cell membrane and are short-lived, they are more vulnerable than chlamydospores to microbial antagonism and predation.

Host plants

Many Australian plants are susceptible to the pathogen. In one study, all hosts and non-hosts investigated could be infected by *P. cinnamomi* zoospores, with resistant plants able to contain infection or replace infected roots (Hinch and Weste 1979). Susceptible plants show characteristic secondary symptoms of chlorosis, wilting, dieback, and death. Infected plants may collapse rapidly, but depending on climate, may persist for several years.

There is a range of responses to this pathogen, ranging from fully resistant species that do not show symptoms of infection under any environmental conditions to highly susceptible species in which death occurs rapidly after infection.

Suppressive soils

The term "suppressive soils" refers to soils where disease is limited or absent despite the presence of the pathogen and of conditions favourable to disease. Soils suppressive to various diseases have been found in many forest ecosystems (Broadbent and Baker 1974) as well as in many monoculture crops, e.g. take-all disease of wheat (Cook and Baker 1983). In many cases, the suppressive nature of soil is believed to be of microbial origin, as sterilisation of suppressive soil renders the soil conducive to disease (Broadbent and Baker 1974). Higher populations of antagonistic microbes are present in suppressive than in conducive soils (Malajczuk 1983).

The Brisbane Ranges National Park (BRNP)

The BRNP is located 80km southwest of Melbourne CBD, Victoria, Australia, and was first infected with *P. cinnamomi* in the 1960s following the construction of a road through the park. The gravel used for the road was evidently infested with *P. cinnamomi*, which during subsequent rains, was washed into surrounding vegetation. Approximately 75% of the vegetation in this forest is susceptible to *P. cinnamomi*; as a result, a disease epidemic occurred during which

large areas of the understorey and some species of the overstorey were destroyed. Highly susceptible species were killed and replaced with resistant colonisers such as grasses and sedges. In particular, the dominant understorey species *Xanthorrhoea australis* (Austral Grass Tree) was nearly eradicated from areas infested with the pathogen. This species is highly susceptible to the pathogen, and no record of resistance has been found in the species to date.

In the mid-1980s, regeneration of this species was recorded in an area previously devastated by the disease. Between 1997 – 1998, increased and highly significant regeneration of this species was recorded in some areas despite the continued presence of *P. cinnamomi* in the soil (Weste et al. 1999). Scattered isolations of *P. cinnamomi* were made from soil and roots surrounding the regenerating plants. The majority of plants were not diseased, despite environmental conditions being favourable to disease.

One hypothesis for this regeneration was that over time the microbial population of soils in the area had altered such that disease was suppressed. The aim of the work presented here was to investigate whether suppressive soils were implicated in the regeneration of highly susceptible species after dieback, and to propose a model of microbial succession to account for development of suppressive soils.

METHODS

Soil suppression bioassay (1)

A total of forty 2 x 1 metre quadrats were established in two adjacent areas of the BRNP. The first area was an uninfested area from which *P. cinnamomi* had not been isolated. The second area was an infested area in which regeneration was occurring. Nine soil samples were collected from beneath regenerating *X. australis* plants in the regenerating area. For comparison, soil was also collected from the uninfested area, which had never been exposed to the disease. Half of each soil sample was autoclaved twice (at 121ºC for 20 minutes) with a week in between each sterilisation.

The inoculum for the bioassay was an isolate of *P. cinnamomi* (18d) that had proven in pathogenicity bioassays to be the most virulent isolate on lupins compared with other isolates obtained from the Brisbane Ranges. Inoculum was prepared by placing lupin seeds (*Lupinus albus*) into 9cm glass petri dishes. Distilled water was added and the lupins autoclaved twice with a day in between each sterilisation. Seeds were inoculated with seven 0.5cm squares of carrot agar on which *P. cinnamomi* had been grown for 5 days. Controls consisted of lupin seeds inoculated with uncolonised carrot agar. Inoculated seeds were incubated for 2 weeks at 20ºC before use.

Seedling trays (15 x 10 x 5cm) were lined with a layer of soil. Ten *P. cinnamomi*-inoculated seeds were sown into each pot and covered with a layer of soil. Twenty 2-day old lupins with radicles of 5mm or less were sown into holes prepared using a sterile test tube. A final layer of soil was added and the trays watered. Trays were kept separate from each other. Soil samples were assigned random letters from A – J to remove bias.

Four trays were set up for each soil sample, with the following treatments:

Soil treatment	Inoculated with *P. cinnamomi*?
Sterilised	Yes
Sterilised	No
Non-sterile	Yes
Non-sterile	No

Trays were kept in a greenhouse at 20ºC and watered daily for 20 days, after which emergence of seedlings and health of seedlings were recorded.

Soil suppression bioassay (2)

A second bioassay was used to investigate the effect of increasing amounts of inoculum on soil suppressiveness. The method used was as described above, with one modification. Five trays were used for each soil sample. These five trays contained different levels of inoculum: 1, 2, 4, 8, or 16 *P. cinnamomi* -inoculated seeds were used respectively.

Isolation of antagonists

Soil samples from the regeneration area were collected seasonally for two years. Each soil sample was serially diluted and plated onto agar. Four selective agars were used:

AGAR	Organism isolated
PDA	Fungi
King's B	Fluorescent pseudomonads
Water agar	Actinomycetes
Nutrient agar	Endospore-forming bacteria

Dilutions used for endospore-forming bacteria were heated to 80ºC for 10 minutes before plating. Agar plates were incubated until colonies formed. Colonies were isolated into pure culture and tested against *P. cinnamomi* in agar plate inhibition tests. This involved placing four 0.5cm plugs of agar on which the antagonist had been grown for four days onto a PDA plate centrally inoculated with a 0.5cm plug of *P. cinnamomi*. A control was created by sham-inoculating a plate with plain agar plugs and a central 0.5cm plug of *P. cinnamomi*. After four days, the diameter of the *P. cinnamomi* colony was determined and inhibition of growth rate determined as percentage of control diameter. Symptoms of antagonism were observed microscopically; these included increased hyphal swellings, abnormal growth patterns, formation of chlamydospores, and lysis of hyphae.

Fungal antagonists were identified with mycological keys and the assistance of Dr. Alan Woodgyer (Microbiological Diagnostic Unit, Parkville). Identification of other antagonist groups was not attempted.

Statistical analysis

All statistical analysis was performed on Minitab ™ using ANOVA and Fisher's or Tukey's multiple comparison method. Frequency data was subjected to Chi-square analysis based on contingency tables. All data was analysed at the 0.05 probability level unless otherwise stated.

RESULTS

Soil suppressiveness

There were significant differences in seedling emergence, health, and mortality of lupins exposed to sterile or non-sterile inoculated soils. Emergence was significantly higher in non-sterile inoculated soils compared with sterile inoculated soils (Figure 1).

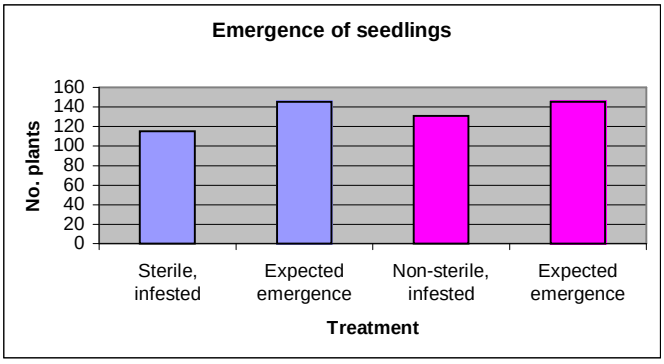


FIGURE 1: Average emergence of lupin seedlings in infested treatments. Shaded bars represent the expected emergence value for the preceding bar, based on Chi-square analysis.

There were significant differences ($p = 0.01$) in the frequencies of healthy, dying, and dead plants in sterile and non-sterile inoculated

soils, indicating disease suppression (Figure 2). Sterile inoculated soils contained fewer healthy plants and more dying and dead plants than expected, whereas the non-sterile inoculated soils contained more healthy plants and fewer dead/dying plants than expected. Mortalities were significantly higher in sterile inoculated soils than non-sterile inoculated soils (Fig. 2).

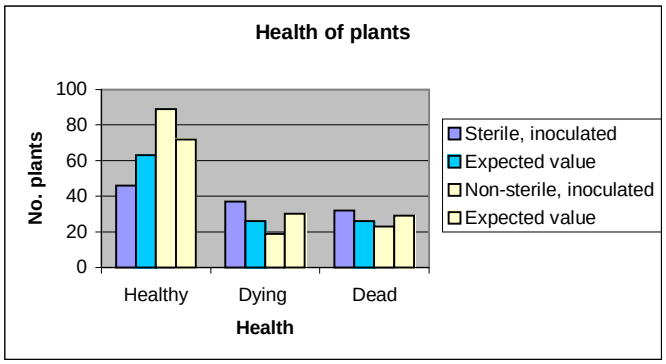


FIGURE 2. Average numbers of healthly, dying and dead lupin plants in each treatment. Shaded bars represent the expected seeding emergence value for the preceding bar, based on Chi-squared analysis.

When increasing inoculum was added to soil, the suppressive nature of the soil was overcome at the 8-seed inoculum level (Figure 3). This is based on a comparison of the observed frequency of healthy or dying/dead plants and the expected value predicted by Chi-square analysis.

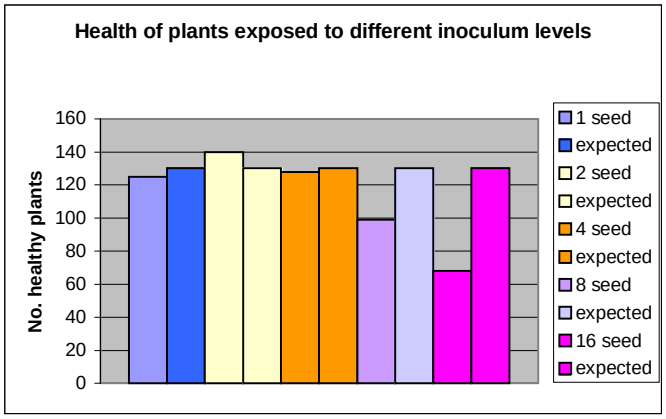


FIGURE 3**:** Number of. healthy plants in treatments exposed to different levels of inoculum.

Isolation and effect of antagonists

In agar plate inhibition tests, fungi were the most antagonistic microbe group of all isolated. Generally, fungal antagonists caused extreme inhibition of *P. cinnamomi* growth as well as causing severe symptoms such as lysis of hyphae. Other groups of microbes (bacteria, actinomycetes) were not as effective at inhibiting *P. cinnamomi* growth or causing symptoms of antagonism *in vitro*.

Penicillium and *Aspergillus* were the most commonly isolated fungal genera (Table 1). These genera usually caused complete lysis of *P. cinnamomi* hyphae in agar plate inhibition trials. Other common genera isolated included *Absidia*, *Trichoderma* and *Paeciliomyces* (Table 1).

DISCUSSION

Soils in the regeneration area are suppressive to disease caused by *P. cinnamomi* in lupin seedlings. Autoclaving abolished the suppressiveness. Mortality was significantly lower in non-sterile inoculated soils than in sterile inoculated soils. Disease suppression appears to occur at all stages of lupin development, ie at juvenile stages (emergence data) and post-juvenile stages (mortality/health data).

When antagonists were isolated from the soil and tested against *P. cinnamomi* in *in vitro* tests, fungi were found to have the greatest effect on *P. cinnamomi* growth and behaviour. Many fungi caused complete lysis of the *P. cinnamomi* colony, while others caused extensive chlamydospore formation. The most common fungi isolated were species of *Penicillium* and *Aspergillus*, which caused complete lysis of *P. cinnamomi* hyphae. In relating the result of these *in vitro* tests to *in vivo* conditions, it is necessary to consider the microhabitat occupied by the pathogen and the antagonist. Antagonists that exist in microhabitats disjunct from the pathogen are less likely to affect the growth and behaviour of the pathogen. Fungi may inhabit similar microhabitats to those occupied by *P. cinnamomi* and are therefore more likely than bacteria to affect the pathogen. As a result, it is likely that fungi are the most antagonist group of microbes *in vivo* as well as *in vitro*.

TABLE 1: Summary of fungal genera identified. Number of different species of each genera is shown.

GENERA	No. isolates obtained
Pestalotia	1
Rhizoctonia	1
Coniothyrium	2
Botrytis	2
Mucor/Aphophyces	1
Cunninghamella	1
Torulomyces	1
Oedocephalum	1
Monilia	1
Chaetophoma	1
Aureobasidium	1
Mycelia sterilia	1
Candida	2
Absidia	3
Phymatotrichum	1
Eupenicillium	1
Mortierella	1
Acremonium	1
Penicillium	26
Aspergillus	7
Paeciliomyces	5
Trichoderma	4
Unidentifiable	12

The suppressive soils in the regeneration area may have developed over time as a result of both direct and indirect changes caused by *P. cinnamomi*. This may have occurred in three stages:

In the first stage, *P. cinnamomi* invaded the area, changing the vegetation structure by reducing species diversity and % cover of susceptible species, resulting in increased soil temperatures and exposure to the elements. As susceptible hosts were eliminated, *P. cinnamomi* was less frequently isolated but dormant and fungistatic propagules of *P. cinnamomi* increased. Decomposition of plants killed by *P. cinnamomi* is associated with an increase in soil organic matter, microbial populations and biological activity.

In the second stage, a new vegetation structure emerged, as resistant plants colonised bare ground; there was an increase in quantity and quality of root exudates. Plant root exudates are known to have a selective effect on microbial populations (Yip and Weste 1985); it is likely that the new vegetation structure caused both qualitative and quantitative changes in soil microbial populations. The reduction in root diversity during the epidemic as species diversity decreased may have resulted in changes in microbial populations similar to those seen in intense monoculture over time. It is possible that the combination of reduced root diversity and the alteration in root exudates changed microbial populations, possibly leading to an increase in antagonistic soil microbes and the formation of suppressive soils.

In the third stage, highly susceptible plant species regenerated in the presence of an altered, more antagonistic microbe population. The root exudates of susceptible plants are known to stimulate *P. cinnamomi*; at this stage, dormant propagules of *P. cinnamomi* may have begun to germinate. However, the altered microbial environment may have resulted in antagonism of the germinating *P. cinnamomi* propagules, resulting in small scattered populations of *P. cinnamomi* and reduced plant infection. Regenerating plants may also vary in susceptibility to the pathogen and be able to tolerate low population levels.

It would appear that a dynamic equilibrium exists in the regenerating area of this forest such that soil microbes suppress populations of *P. cinnamomi* to a level low enough to be tolerated by potentially less susceptible regenerating species. This equilibrium is not uniform; disease still occurs in scattered plants, which may reflect the heterogeneity of soil microbe populations or a shift in the environmental conditions that favours the pathogen and disfavours the plant.

It is possible that the amplitude of the disease cycle is reduced as a result of the varying microbial numbers outlined above. Over time, it may be that *P. cinnamomi* becomes integrated into the soil microbiota, and that disease only occurs within a narrow range of disease-conducive conditions.

The results of this study support the idea that in investigating plant pathogens, it is essential to consider all sides of the "disease triangle". Disease epidemics are a product of the pathogen, environment, and host, and interactions between these factors. It is necessary when investigating disease epidemics to consider all interactions between the factors, as well as considering each factor individually.

It is not possible to categorically state that the recovery seen in this forest will occur in other ecosystems affected by the pathogen, due to the differences between forests in both biotic and abiotic factors. However, this is the first record of sustained regeneration after epidemic; investigating the mechanisms involved may lead to a better understanding of the disease and ultimately to rehabilitation of affected areas.

REFERENCES

Broadbent, P. and Baker, K.F. (1974). Behaviour of *P. cinnamomi* in soils suppressive and conducive to root rot. Aust. J. Agric. Res. 25: 121-37.

Cook, R.J. and Baker, R. (1983). *In* The nature and practice of biological control of plant pathogens.

Hinch, J. and Weste, G. (1979). Behaviour of *P. cinnamomi* zoospores on roots of Australian forest species. Aust. J. Bot. 27: 679-91.

Irwin, J.A.G., Cahill, D.M. and Drenth, A. (1995). Phytophthora in Australia. Aust. J. Agric. Res.: 1311-37.

Kuhlman, E.G. (1964). Survival and pathogenicity of *P. cinnamomi* in several western Oregon soils. For. Sci. 10: 151-58.

Malajczuk, N. (1983). Microbial antagonism to Phytophthora. In Phytophthora, its Biology, Taxonomy, Ecology and Pathology. Eds D.C. Erwin, S. Bartinicki-Garcia and P.H. Tsao. pp197-218. American Phytopathological Society, Minnesota, USA.

Mircetich, S.M. and Zentmyer, G.A. (1967). Existence of *P. cinnamomi* as chlamydospores and oospores in roots and soil. Yearbook Calif. Avocado Soc. 51: 117-24.

Tsao, P.H. (1969). Studies on the saprophytic behaviour of *Phytophthora parasitica* in soil. Proc. 1st Int. Citrus Symposium. 3: 1221-30.

Weste, G. (1994). Impact of Phytophthora species on native vegetation of Australia and Papua New Guinea. Australasian Plant Pathol. 23: 190-209.

Weste, G. and Vithanage, K. (1979). Production of sporangia by *P. cinnamomi* in forest soils. Aust. J. Bot. 27: 693-791.

Weste, G., Walchhuetter, T. and Walshe, T. (1998). Regeneration of *Xanthorrhoea australis* following epidemic disease due to *Phytophthora cinnamomi* in the Brisbane Ranges, Victoria. Australas. Plant Pathol. 28: 162-169.

Yip, H.Y. and Weste, G. (1985). Rhizoplane mycoflora of *Gahnia radula* and *Isopogon ceratophyllus* in sols infested and free from *Phytophthora cinnamomi*. *In* Ecology and management of soilborne plant pathogens. Ed. Parker, C.A, Rovira, A.D, Moore, K.J. and Wong, P.T.W. American Phytopathological Society. Pp 92-98.

Zentmyer, G.A. (1965). Bacterial stimulation of sporangium production in *P. cinnamomi*. Science 150: 1178-79.

Zentmyer, G.A. (1980). *Phytophthora cinnamomi* and the diseases it causes. Monogr. 10. Am. Phytopathol. Soc., St. Paul, MN. 96pp.

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

IDENTIFICATION OF *PHYTOPHTHORA CINNAMOMI* BASED ON CINNAMOMIN GENE TARGETTING

Coelho, A.C.[1]; Sancho, R.[2]; Graça, J.[2]; Neves, D.[2]; Duclos, J.[4]; Fauconnier, A.[3]; Godfroid, E.[3] & Cravador, A.[2*]

[1]Escola Superior de Educação (ESE), Universidade do Algarve, Campus da Penha, 8000 Faro.
[2]Unidade de Ciências e Tecnologias Agrárias (UCTA), Universidade do Algarve, Campus de Gambelas, 8000 Faro.
[3]Service de Génétique Appliquée, Université Libre de Bruxelles, IBMM, ULB, Rue des Professeurs Jeener et Brachet,12 B-6041 Gosselies, Belgium.
[4]Instituto de Tecnologia Química e Biológica, Quinta do Marquês, 2780 Oeiras.
[*]Corresponding author. Electronic mail address: acravad@ualg.pt

INTRODUCTION

Quercus suber ecosystems in Portugal present symptoms of decline resulting each year on the death of thousands of trees. The causes have been ascribed to several factors, like drought, damage of the roots by the ploughing techniques, insect attack or diseases caused by fungus and also to the poverty of the soil in organic matter. Although some of these factors seem to contribute to the decline, the hypothesis of their involvement is based on mere assumptions that are not supported by any serious scientific study.

Since 1993, several researchers made the hypothesis that the aggressive filamentous soilborne fungus *Phytophthora cinnamomi* was probably the major contributory factor for cork oak decline in Portugal (Brasier *et al.*, 1993). This hypothesis was supported by the isolation of this fungus from roots of diseased trees and associated soil, and also by the characterization of disease symptoms exhibited by the trees (Ferraz & Moreira, 1996).

Traditional methods of isolation and detection of *Phytophthora* in oak roots, based mainly on baiting methods are of relatively low effectiveness and reliability. They also require knowledge of fungal taxonomy.

Other methods for identification of *P. cinnamomi* on roots of eucalyptus and avocado using hybridisation techniques with DNA probes and/or PCR have been developed by Dobrowolski & O'Brien (1993) and Judelson & Messenger-Routh (1996). The targets for DNA amplification were the regions ITS I and ITS II of ribosomal DNA. All these systems turn to the labelling of DNA probes with radioisotopes.

Commercial diagnostic kits based on immunological techniques have been offered in recent years for the rapid diagnosis of several fungi in plant tissue, soil and water. Targets for kits include *Phytophthora spp.*, *Pythium spp* and others. The sensitivity of these kits for analysing infected plant samples depends on the host/pathogen-system. Ali-Shtayeh *et al.* (1991) applied these commercial kits to the detection of *Phytophthora spp.* in soil and water samples and observed that some kits could not distinguish among individual fungal species. They show some cross reactivity. This author reports also that the sensitivity of the detection can change with the fungal structure and that different fungal structures can have different quantities of detectable antigens. The intensity of the reaction can be different between species and also between isolates of the same species.

Highly specific and sensitive non-radioactive molecular method to identify *P. cinnamomi*

In response to the need for a faster and more reliable method for identifying *Phytophthora cinnamomi* we have developed a colorimetric detection system, which targets a 349 bp amplified DNA product, partially covering the 3´-translated and 3´-untranslated regions of the cinnamomin gene (Coelho *et al*., 1997). Our approach is based on an assay that has been developed for the identification of infectious agents in clinical specimens (Mansy *et al.,* 1996 and Fauville-Dufaux *et al.,* 1995).

This colorimetric detection system combines an amplification assay with a sandwich hybridisation reaction where the target DNA is captured by a specific oligonucleotide probe, covalently linked onto the surface of an activated microplate. The hybridised DNA is then recognised by a multi fork-like oligonucleotide probe carrying eight biotin groups. This structure composed of three single-stranded hybridising DNA molecules (capture probe, target DNA, multi-biotinylated detection probe) is complexed by the streptavidin-horseradish peroxidase conjugate that reacts with a chromophore. The optical density can then be measured in a microplate reader. The target DNA is a 349 bp DNA fragment partially covering the 3'-translated and 3'-untranslated regions of the cinnamomin gene. This target was defined by sequencing the cinnamomin gene and its 3' downstream region. Our detection system has the advantage of providing an additional level of specificity since it uses capture and detection probes internal to the amplified PCR product, delimited by the position of the specific primers.

The specificity of the detection system was evaluated on DNA isolated from four *P. cinnamomi* isolates and eight other reference species. The results were considered positive when the OD was greater than the cut off value, defined as 3 standard deviations above the mean of the blank samples, consisting of PCR mixtures without target DNA. Only *P. cinnamomi* isolates gave a positive signal, whereas all the isolates from other species of *Phytophthora* gave a negative signal. The assay has the sensitivity of about 2-5 genome equivalents of *P. cinnamomi* and is about hundred times more sensitive than the widely used agarose gel electrophoresis method (Coelho *et al.*, 1997).

The evaluation of the performance of the detection system was first carried out on thirty isolates previously identified as *P. cinnamomi* isolated from roots of *Q. suber* and associated soils of affected areas from the Algarve region (Portugal). Twenty-six samples out of thirty gave a clear positive response. The *P. cinnamomi* isolates named VI-3RfM, V-4SM, IV-3SM and II-1RMS gave a clear negative response corresponding to that of the cut-off. The four negative isolates were rechecked for affinity to *P. cinnamomi* using a range of routine diagnostic tests. They showed no evident differences from the other twenty-six *P. cinnamomi* isolates and were again considered to fall within the normal range of variation for the traditional *P. cinnamomi* morphospecies (Coelho *et al.*, 1997).

The specificity of the identification method was further evaluated with twenty-eight isolates from other regions of the world. The analysis was performed on dehydrated mycelium from isolates supplied dead and without identification of the species (Table 1). All the isolates from species other than *P. cinnamomi*, including a new recently isolated species (Brasier *et al*., 1999), gave negative responses. Eighteen out of twenty *P. cinnamomi* isolates gave a positive response (fig.1). These results show that our method is specific for the identification of *P. cinnamomi*.

Table 1. Isolates of *Phytophthora* from other part of the world tested by the method.

Isolate number	Code number	Species	Mating Types	Result	Host	Country
P 118	1	*P. cinnamomi*	A2	+	*Taxus*, roots/soil	U.K.
P 216	2	*P. cinnamomi*	A2	+	*Pinus*, roots	U.K.
P 402	3	*P. cinnamomi*	A2	+	Avocado	Canary Islands
P 404	4	*P. cinnamomi*	A2	+	Clove	Malaysia
P 416	5	*P. cinnamomi*	A1	Neg	*Robinia pseudoacacia*	China
P 596	6	*P. cinnamomi*	A2	+	*Quercus ilex*, roots/soil	Spain
P 612	7	*P. cinnamomi*	A1	+	*L. comosum*	South Africa
P 613	8	*P. cinnamomi*	A2	+	Avocado	South Africa
P 709	9	*P. cinnamomi*	A2	+	*Quercus suber*, soil	Portugal
P 799	10	*P. cinnamomi*	A2	+	*Quercus* sp., soil	France
P 825	11	*P. cinnamomi*	A2	+	*Arbutos unedo*	Portugal
P 900	12	*P. cinnamomi*	?	+	*Rhododendron*, soil	Japan
P 901	13	*P. cinnamomi*	A1	Neg	*Nothofagus* sp., soil	Papua New Guinea
P 902	14	*P. cinnamomi*	A1	+	Unknown, soil	Papua New Guinea
P 903	15	*P. cinnamomi*	A2	+	*Araucaria cunninghamii, soil*	Papua New Guinea
P 904	16	*P. cinnamomi*	A1	+	*Eucalyptus marginata*	Westen Australia
P 905	17	*P. cinnamomi*	A2	+	*E. pilularis*, soil	New South Wates, Australia
P 907	19	*P. cinnamomi*	?	+	Jarrah	Australia
P 909	20	*P. cinnamomi*	?	+	*Banksia attenuata*, soil	South Australia
P 966	21	*P. cinnamomi*	?	+	*Persea* sp.	California, USA
P 820	22	*P. camvivora*	-	Neg	Raspberry	U.K.
P 821	23	*P. cambivora*	-	Neg	*Castanea sativa*	Italy
P 822	25	*P. fragariae*	-	Neg	Raspberry	France
P 772	26	*P.* sp. *alder*	-	Neg	Alder bam	U.K.
P 770	27	*P.* sp. *alder*	-	Neg	*Alnus glutunosa*	Netherlands
P 535	28	*P. drechsleri*	-	Neg	Cherry	California, USA
P 426	29	*P. megasperma*	-	Neg	Poplar	U.K.
P 714	30	*P. gonapodyides*	-	Neg	*Quercus suber*, soil	Portugal

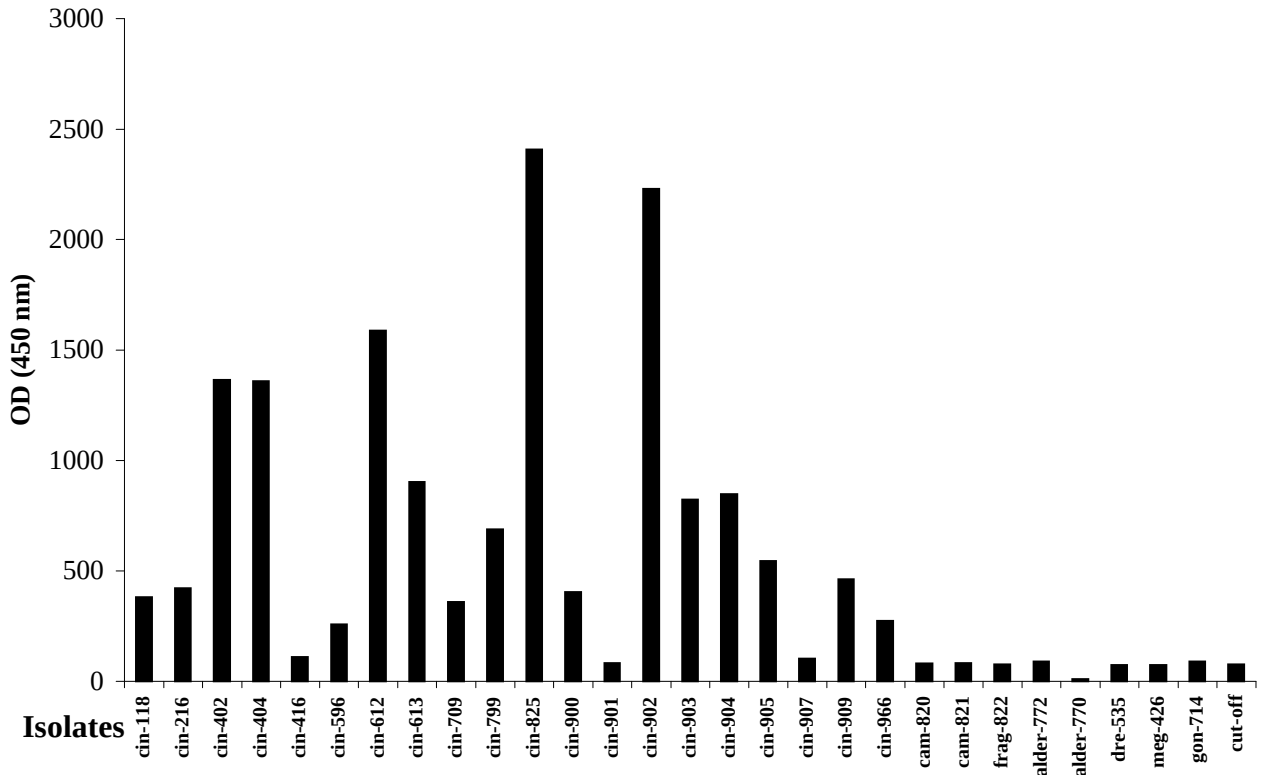


FIGURE 1. Specificity analysis of P. cinnamomi isolates from various regions of the world by the colorimetric hybridisation assay. The origin of the isolates is shown on Table 1. The cut-off value was defined as 3 standard deviations above the mean for the blank PCR mixtures.

Analysis of *P. cinnamomi* isolate II-1RMS

In order to elucidate whether there is production of cinnamomin by the *P. cinnamomi* isolate II-1RMS that is negative in our detection assay, the culture growth medium of this isolate was submitted to a western immunoblotting analysis. Polyclonal antibodies raised against cryptogein (anti-β cryptogein, Tercé-Laforgue *et al.*, 1992) and polyclonal antisera against β-cinnamomin and against recombinant β-cinnamomin were used. A positive reaction was detected in all cases showing the presence of cinnamomin or of a cinnamomin-like polypeptide in the growth extract (fig. 2).

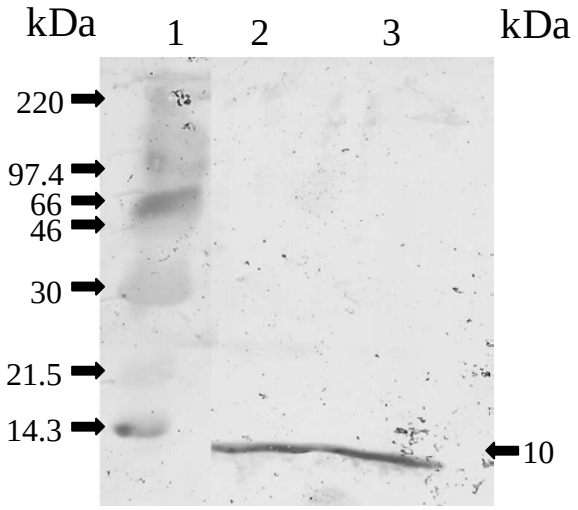


FIGURE 2. Western blot performed with polyclonal rabbit antibodies anti-beta-cryptogein. Lane 1, protein molecular weight marker; lane 2, crude extract of the total secreted proteins from *P. cinnamomi* isolate P910555; lane 3, crude extract of the total secreted proteins from *P. cinnamomi* isolate II-1RMS.

However, genomic DNA analysis by AFLP (Amplified Fragment Length Polymorphism's) showed that this isolate has a distinct pattern from that of three other isolates positive to the detection assay (P910555, XIV-4R and XI-4S). AFLP is a recently described technology for DNA fingerprinting that is based on selective amplification of a restricted number of genomic fragments (Vos *et al.* 1995).

Fingerprint analysis was performed with two *Eco*RI/*Mse*I primer combinations (*Eco*RI-AAG, *Mse*I-CAG; *Eco*RI-AAG, *Mse*I-CAC). The AFLP patterns of the isolates P910555, XIV-4R and XI-4S showed to be very similar between each other presenting a low level of restriction polymorphism while twenty one polymorphic fragments were identified between the isolate II-1RMS and the other three positive to the detection assay (fig. 3). These results suggest that the isolate II-1RSM may have a polymorphism associated to the nucleotide sequence of the cinnamomin gene that inhibits recognition by the primers of the PCR reaction, giving a negative response to the detection assay. The high number of polymorphic fragments shown in the AFLP pattern of the isolate II-1RMS, compared to the other three *P. cinnamomi* isolates suggests that II-1RMS could be a hybrid derived from an interspecific cross.

Does *P. cambivora* produce an elicitin cambivorin?

The nucleotide sequence deduced from the published amino acid sequence of cinnamomin (Huet & Pernollet, 1989) is not a specific target for the identification of *P. cinnamomi.* We showed that it is also a target for *P. cambivora* and that the 257 bp regions amplified in each of those species with the same pair of degenerated primers are 96 % homologous (Coelho *et al*, 1997). This observation suggests that *P. cambivora* contains nucleotide sequences coding for a hypothetical "cambivorin".

In order to investigate whether *P. cambivora* produces "cambivorin" or not the extract from the growth culture medium was analysed by western immunoblotting. Antiserum raised against recombinant β-cinnamomim and against β-cryptogein did not recognise proteins with the expected molecular weight of 10 kDa. The reaction with these antisera revealed the presence of two proteins with molecular weights of 25 and 35 kDa (fig.4). Whether these proteins correspond to other elicitors sharing amino acid sequences with cinnamomin remains to be shown.

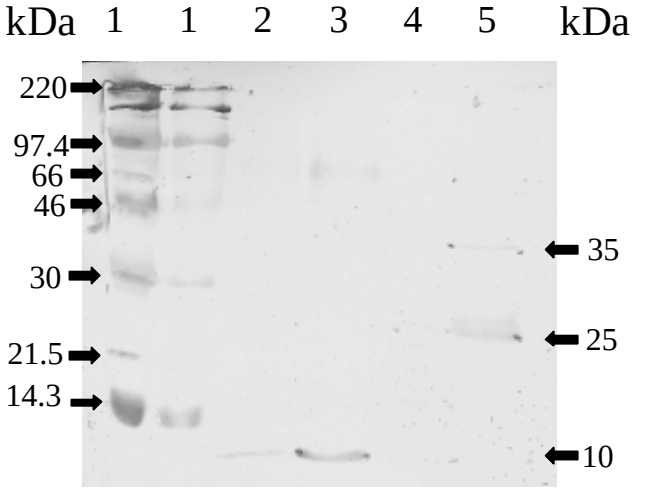


FIGURE 4. Western blot performed with polyclonal rabbit antibodies anti-recombinant-beta-cinnamomin. Lnae 1; protein molecular weight marker; lanes 2 and 3, total secreted proteins from P. cinnamomi isolate P910555; lanes 4 and 5, total secreted proteins from P. cambivora isolate IMI-340633. Lanes 3 and 5 refer to a 50 fold concentration from the original culture medium.

Elicitin gene cluster in *P. cinnamomi*

The presence of other elicitin genes in *P. cinnamomi* genome was further studied using the "vectorette" PCR method (Arnold & Hodgson, 1991). The application of this method using initiating primers directed to the internal region of the cinnamomin gene led to the production and isolation of four DNA fragments Cl 6, Cl 13, Cl 16 and Cl 17. Cl 6 is a 646 bp fragment composed of a part of the coding (256 bp) and the 3' non coding region (390 bp) of the cinnamomin gene. It has 99.6% homology with the fragment obtained by PCR amplification with the degenerated primers. The deduced amino acid sequence of the coding region perfectly matches with residues from 15 to 98 of the published amino acid sequence of cinnamomin.

The Cl 6 fragment was used as a probe to screen a genomic library of *P. cinnamomi* constructed in l-FIX II (Duclos *et al.*, 1998). One positive phage was isolated and extensively studied by restriction enzyme and southern hybridisation analyses. Several restriction DNA fragments of the positive phage insert hybridised to the Cl 6 probe, suggesting that several copies of the gene encoding cinnamomin or related elicitin genes are present in the genome of *P. cinnamomi*.

The sequence analyses of the insert DNA showed that it exhibits four open reading frames that are identical to the sequences that we had previously isolated, namely Cl 6, Cl 13, Cl 16 and Cl 17.

These four genes are clustered in tandem pairs. Each pair contains an elicitin isoform gene followed by a highly acidic elicitin gene (fig. 5). This positioning is similar to that observed in *P. cryptogea*, suggesting a conservation of the elicitin cluster structure through the *Phytophthora* species (Panabières *et al.*, 1995).

The Cl 6 and Cl 16 genes may encode proteins of 118 aa, including the signal peptide. The protein encoded by the Cl 6 gene is identical to cinnamomin. The deduced amino acid sequence of Cl 16 ORF is more closely related to the acidic elicitins. The Cl 13 and Cl 17 genes would encode distinct proteins of 124 and 123 amino acids,

respectively. Moreover, the deduced amino acid sequences of these two genes show higher identities to the HAE proteins of *P. cryptogea* (Duclos *et al.*, 1998).

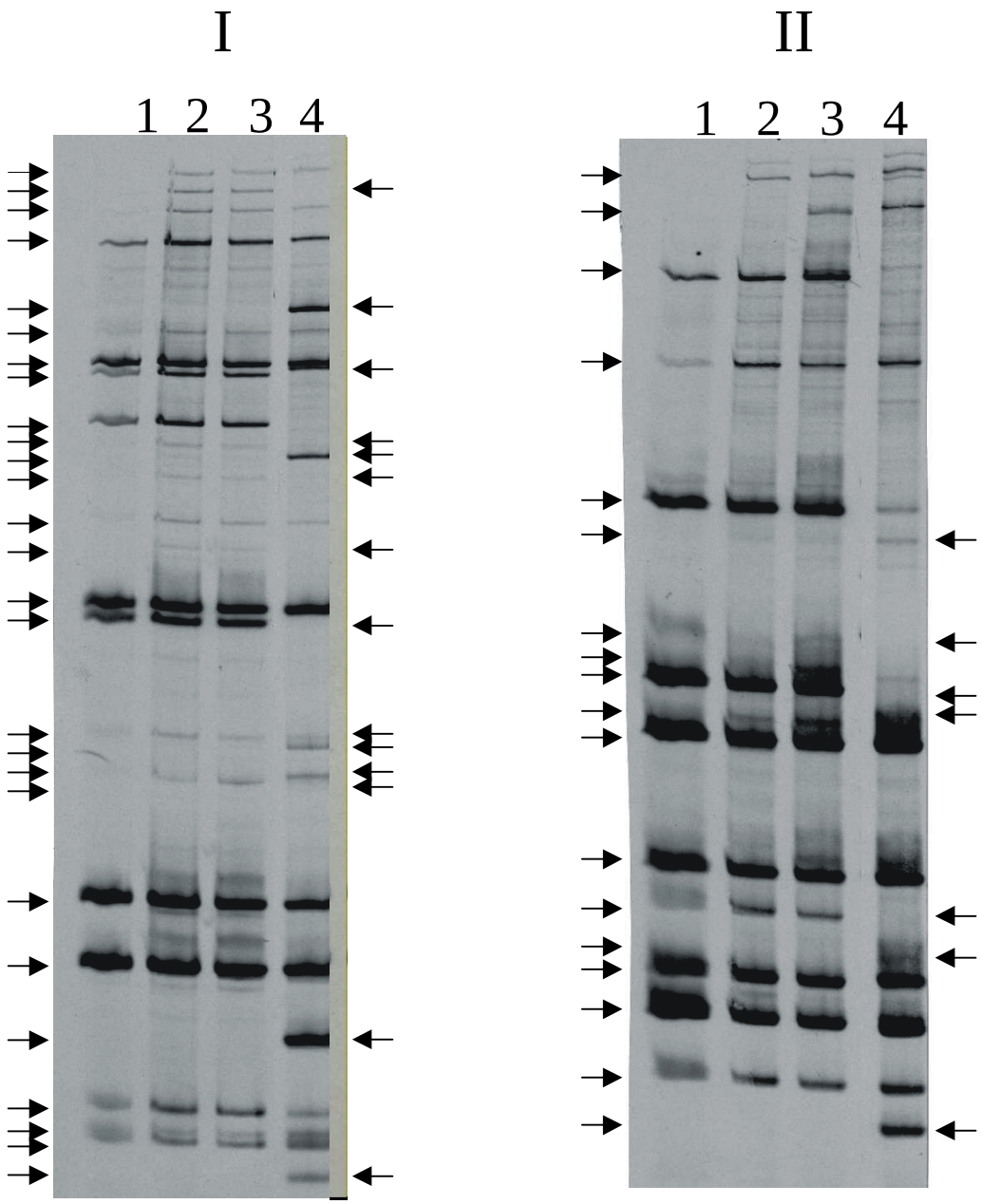


Fig. 3. Analysis of *P. cinnamomi* isolates by AFLP. Lanes 1, 2 and 3 correspond to templates of the isolates P910555, XIV-4R and XI-4S respectively, positive to the identification system; lane 4 corresponds to the isolate II-1RMS that gave a negative response. I and II are primer combinations *Eco*RI-AAG, *Mse*I-CAG and *Eco*Ri-AAG; *Mse*I-CAC, respectively.

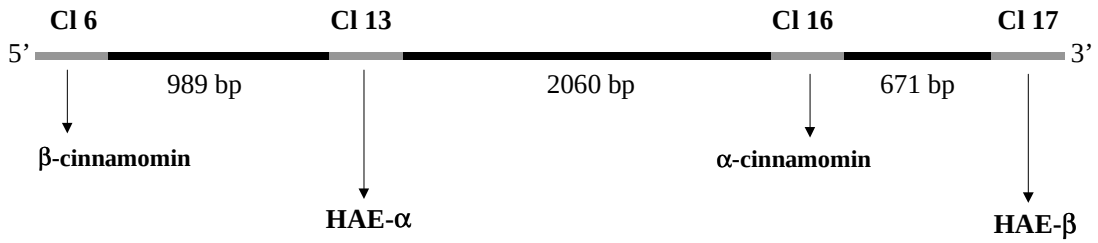


Fig. 5. Cluster organisation of the elicitin genes from *P. cinnamomi*. The four sequences are clustered in tandem pairs and each pair contains an elicitin isoform gene (β-cinnamomin/∇-cinnamomin) followed by highly acidic elicitin gene (HAE-∇/HAE-β). Numbers refer to regions between the coding regions.

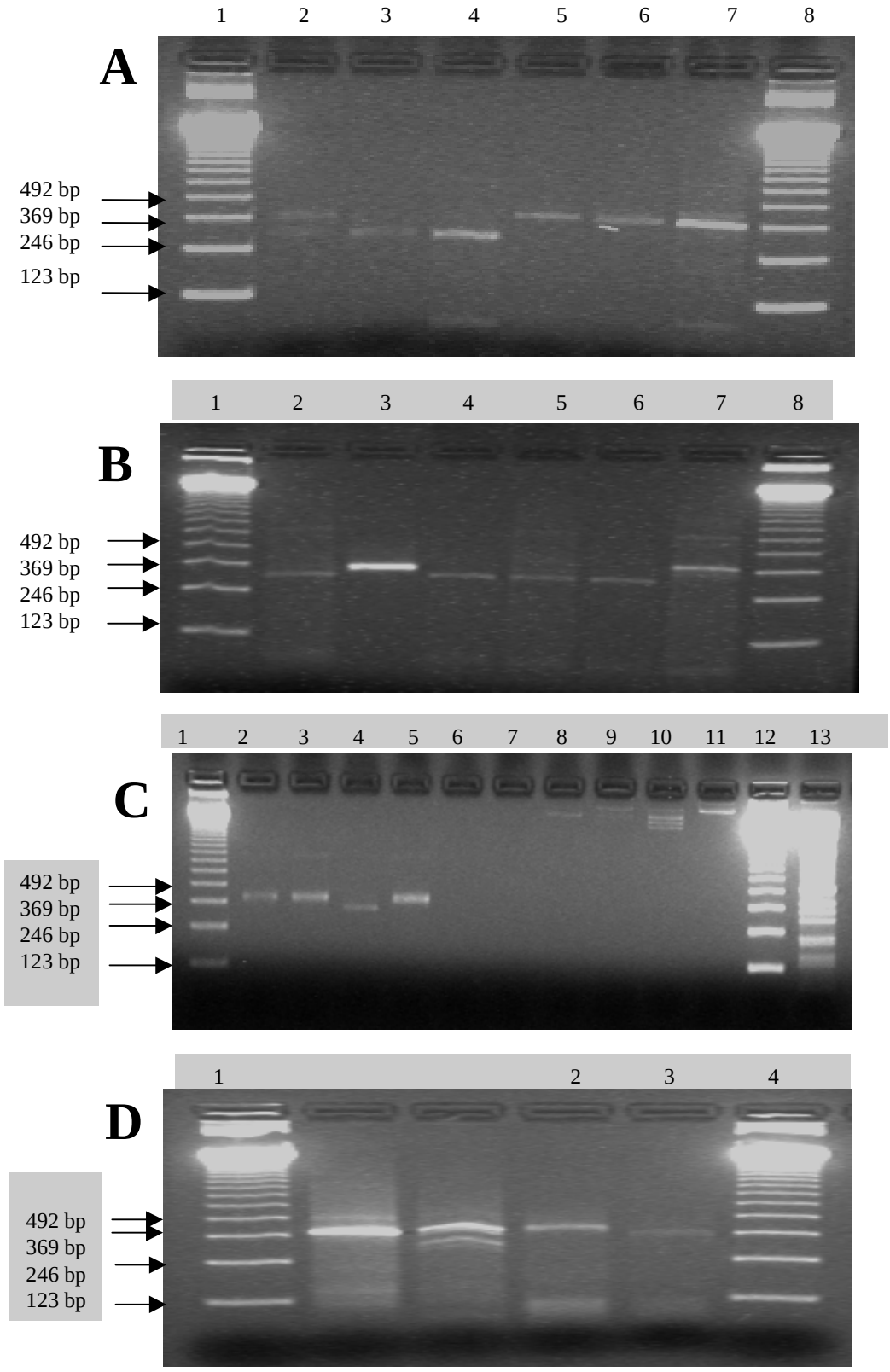


FIGURE 6.Restriction analysis of cDNAs of the four elicitins isoforms genes from *P. cinnamomi.* Separation on 2% agarose gel electrophoresis stained with ethidium bromide.

A. α-cin: lanes 1,8 – molecular weight marker (123 DNA ladder); lane 2 – product of amplification digested with *Pml*I, incomplete digestion (1U); lanes 3,4 - product of amplification digested with *Pml*I (20U); lanes 5,6,7– cDNA not digested (negative control).

B β-cin: lanes 1,8 – molecular weight marker (123 DNA ladder); lanes 2,4,5,6 – product of amplification digested with *Sfi*I (2 U); lanes 3, 7 – cDNA not digested (negative control).

C hae-α: lanes 1,12 – molecular weight marker (123 DNA ladder); lane 13 - molecular weight marker (1kb DNA ladder); lane 4 – product of amplification digested with *Sca* I (3U); lane2 3,5 - cDNA not digested (negative control); lanes 6,8,10 – plasmidic clones containing cDNA digested with *Sca*I (1U); laness 7,9,11 - plasmidic clones not digested (negative control).

D hae-β: lanes 1,4 - molecular weight marker(123 DNA ladder); lane 2 – cDNA not digested (negative control); lane 3 – product of amplification digested with *Sac*II (1 U).

The four elicitin isoforms of *P. cinnamomi* are expressed

The expression of the four elicitin isoforms was evaluated by RT-PCR using primers specific for each isoform gene and restriction analysis of the four products. Briefly, total RNA was isolated from *P. cinnamomi* grown in *in vitro* culture medium. cDNA was synthesised by RT-PCR using primers specific for each isoform gene. The cDNA corresponding to each of the mRNA was cloned into a plasmidic vector. To confirm their identity, we raised the restriction site map of the four elicitin genes and carried out a restriction analysis with enzymes that recognise sites specific for each of the four genes. Enzymes *Pml*I, *Sfi*I, *Sca*I, and *Sac*II specifically digest α-cin, β-cin, hae-α and hae-β, yielding fragments of 62 bp, 276 bp and 16 bp (α-cin), 70 bp and 287 bp (β-cin), 319 bp and 56 bp (hae-α) and 32 bp and 337 bp (hae-β) respectively. (fig. 6).

Our results indicate that all of these elicitins isoforms are expressed in *in vitro* culture of *P. cinnamomi*.

Work is underway that will establish the expression level of these genes.

ACKNOWLEGEMENTS

We are grateful to Professor C. M. Brasier for kindly providing *Phytophtora* isolates and for information about their identification.

We acknowledge Fundação para a Ciência e Tecnologia (FCT) (PRAXISXXI 3/3.2/FLOR/2112/95, INTERREG 14/REG II/6/96) and NATO (SfS PO-CORKOAKS II) for financial support. ACC thanks Fundação Luso Americana para o Desenvolvimento and Fundação Calouste Gulbenkian for her travelling grant and MGH thanks FCT for their PRAXISXXI research grant (BIC J/17089/98).

REFERENCES

Ali-Shtayeh, S.M. (1991). A method for using commercial ELISA tests to detect zoospores of *Phytophthora* and *Pythium* species in irrigation water. *Plant Disease*, **75** (3), 305-311.

Arnold, C. & Hodgson, I.J. (1991). Vectorette PCR: a novel approach to genomic walking. *PCR Methods and Applications* **1**, 39-42.

Brasier, C.M., Robredo, F. & Ferraz, J.F.P. (1993). Evidence for *P. cinnamomi* involvement in Iberian oak decline. *Plant Pathology*, **42**, 140-145.

Brasier, C.M., Cooke, D.E.L. & Duncan, J.M. (1999). Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proc. Natl. Acad. Sci. USA*, **96**, 5878-5883.

Coelho, A.C., Cravador, A., Bollen, A., Ferraz, J.F.P., Moreira, A.C., Fauconnier, A. & Godfroid, E. (1997). Highly specific and sensitive non-radioactive molecular identification of *Phytophthora cinnamomi*. *Mycological Research*, **101**, (12), 1499-1507.

Dobrowolski, M. P. & O'Brien, P. (1993). Use of RAPD-PCR to isolate a species specific DNA probe for *Phytophthora cinnamomi*. *FEMS Microbiology Letters*, 113, 43-48.

Duclos, J., Fauconnier, A., Coelho, A.C., Bollen, A., Cravador, A. & Godfroid, E. (1998). Identification of na Elicitin Gene Cluster in *Phytophthora Cinnamomi*. *DNA Sequence*, **9**, (4), 231-237.

Ferraz, J.F.P. & Moreira, A.C. (1996). Le dépérissement des forêts de *Quercus suber* au Portugal associé au champignon *Phytophthora cinnamomi*. Oral communication, *Colloque Nacional sur le Dépérissement des Forêts au Maroc*, 28 and 29 February, Rabat-Maroc.

Fauville-Dufaux, M., Maes, N., Severin, E., Farin, C., Serruys, E., Struelens, M., Younes, N., Vincke, J.-P., De Vos, M.-J, Bollen, A. & Godfroid, E. (1995). Rapid identification of *Mycobacterium xenopi* from bacterial colonies or "Batec" culture by the polymerase chain reaction and a luminescent sandwich hybridization assay. *Research in Microbiology*, **146**, 349-356.

Huet, J.-C. & Pernollet, J.-C. (1989). Amino acid sequence of cinnamomin, a new member of the elicitin family, and its comparison to cryptogein and capsicein. *FEBS Letters*, **257**, 302-306.

Judelson, H.S. & Messenger-Routh, B. (1996). Quantification of *Phytophthora cinnamomi* in avocado roots using a species-specific DNA probe. *Phytopathology*, 86, 763-768.

Mansy, F., Hoyois, B., De Vos, M.-J., Van Elsen, A., Bollen, A. & Godfroid, E. (1996). A colorimetric solid phase capture hybridization assay for detection of amplified *Borrelia burgdoferi* DNA. *BioTechniques*, **21**, 122-125.

Panabières, F., Marais, A., Le Berre, J.-Y., Penot, I., Fournier, D. & Ricci, P. (1995). Characterisation of a Gene Cluster of *Phytophthora cryptogea* which codes for elicitins, proteins inducing a hypersensitive-like response in Tobacco. *Molecular Plant-Microbe Interactions*, **8**, (6), 996-1003.

Tercé-Laforgue, T., Huet, J.-C. & Pernollet, J.-C. (1992). Biosynthesis and secretion of Cryptogein, a protein elicitor secreted by *Phytophthora cryptogea*. *Plant Physiology*, **98**, 936-941.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, (21), 4407-4414.

VARIATION IN *PHYTOPHTHORA LATERALIS*

Michael G. McWilliams

Forest Health Management, Insects/Diseases, Oregon Department of Forestry, Salem, Oregon 97310 USA

INTRODUCTION

Phytophthora lateralis (Tucker and Milbrath) is a soilborne fungal pathogen that causes a serious root disease and mortality of Port-Orford-cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl) in the Pacific Northwest. The disease was first reported in a letter from a commercial ornamentals nursery in 1923. In the original report it was noted that "…The disease prevails throughout the whole Northwest, killing cypress in the private gardens as well as in all nurseries, causing vast loss, as this Lawson cypress is used very much here…" (Hunt, 1957). Although perhaps overstated in this communication, it is clear that the fungus was present in the Pacific Northwest for some time before 1923.

The fungus has been reported twice outside North America. *P. lateralis* and three other *Phytophthora* species were reported associated with root, crown, and collar rots on Kiwifruit (*Actinidia chinensis*) in New Zealand (Robertson 1982). Inoculations using macerated mycelium mixed with soil were attempted, but none of the *Phytophthora* species produced "significant levels of infection" although some vines showed root rot symptoms. Zoospores were difficult to produce in the isolate of *P. lateralis*. More recently, *P. lateralis* was reported from France (Hansen 1999), where it had been isolated from symptomatic *C. lawsoniana* growing in nurseries. The two French isolates and North American isolates were reported to have identical ITS-1 sequences and colony morphology in culture.

Within North America *P. lateralis* has been reported twice outside the Pacific Northwest. In North Carolina *P. lateralis* was isolated from diseased creeping juniper (*Juniperus horizontalis*), azalea (*Rhododendron* spp.), Frasier photinia (*Photinia* X *fraseri*), mountain laurel (*Kalmia latafolia*), and Madagascar periwinkle (Abad et al. 1994). *P. lateralis* was isolated from *Rhododendron* crowns in Ohio and Pennsylvania (Hoitink and Schmitthenner 1974) and identified on the basis of laterally attached, thin walled chlamydospores. It was noted that these isolates differed from the original description in that they grew well at high temperatures, and did not form sporangia when flooded with nonsterile soil extract water. Isolates from the Pacific Northwest generally form sporangia readily and abundantly. Inoculations on Rhododendron caused "only slight root damage". Only the report from France has been confirmed. All other reports from beyond western North America are apparently based on misidentifications.

Limited variation has been found in previous studies of *P. lateralis*. In a comparison of sporangia production in ten isolates from Oregon, nine isolates were similar and one isolate produced fewer sporangia when grown on pea broth and then rinsed and flooded with distilled water (Trione 1957). Chlamydospore production varied greatly among isolates, and all isolates produced oospores equally well. Isozyme banding patterns of 11 isolates from Oregon and California were identical (Mills et al. 1991). Total protein banding patterns of several isolates collected in Oregon and California were identical (Hamm and Hansen, unpublished).

The present study focused on variation in growth rate, virulence, and DNA fingerprint among isolates collected from Oregon, Washington, California, and British Columbia (Table 1). Isolates were from 3 hosts: *Chamaecyparis lawsoniana* (Port-Orford-cedar), *C. nootkatensis* (yellow cedar), and *Taxus breviforlia* (Pacific yew).

TABLE 1. Host, origin, and date of isolation of isolates used in this study. (All isolates are *Phytophthora lateralis* unless otherwise stated.)

Isolate	Host	Origin	Date Isolated
366	*Chamaecyparis lawsoniana*	Gasquet R.D., Shelly Crk., CA	?
POC-2	*Chamaecyparis lawsoniana*	Kalmiopsis Wilderness, OR	Aug-95
YEW-2	*Taxus brevifolia*	Kalmiopsis Wilderness, OR	Aug-95
1-1	*Chamaecyparis lawsoniana*	Vancouver, B.C.	Oct-95
1-10	*Chamaecyparis lawsoniana*	Vancouver, B.C.	Oct-95
20-1	*Chamaecyparis lawsoniana*	Bellingham, WA	Oct-95
21-2	*Chamaecyparis lawsoniana*	Bellingham, WA	Oct-95
6187	*Chamaecyparis lawsoniana*	Johnson Mt., OR	Jun-94
6248	*Chamaecyparis nootkatensis*	Quosatana Crk., OR	Jun-94
6900	*Chamaecyparis nootkatensis*	Quosatana Crk., OR	Jun-94
7144	*Chamaecyparis lawsoniana*	Johnson Mt., OR	Jun-94
7354	*Chamaecyparis nootkatensis*	Quosatana Crk., OR	Jun-94
8041	*Chamaecyparis nootkatensis*	Quosatana Crk., OR	Jun-94
Plat1	*Chamaecyparis lawsoniana*	France	?
980093.1	*Chamaecyparis lawsoniana*	France	?
Phytophthora cryptogea	*Chamaecyparis lawsoniana*	Oregon	?
Phytophthora cinnamomi	*Chamaecyparis lawsoniana*	Cottage Grove, OR	Jun-94

MATERIALS AND METHODS

Growth Rate

Fifteen isolates of *P. lateralis* , including two from France, were transferred from storage tubes to 100mm petri plates containing corn meal agar (Difco) amended with 20 ppm *B*-sitosterol. After 9 days of growth at 18C, plugs were cut with a #2 cork borer and placed on 100mm petri plates containing 20 ml of corn meal agar amended with 20ppm *B*-sitosterol. After 3 days of growth at 20C, the edge of the advancing colony margin was marked on 4 radii per plate, and 2 plates of each isolate were placed in each of 3 covered plastic boxes. Boxes were placed in Sorvall constant temperature incubators held at average temperatures of 12C, 18C, and 24C. The edge of the advancing colony was marked at 4, 7 and 11 days, and a growth rate per day was calculated from these measurements. The experiment was repeated once, and small electronic temperature loggers were included in each box. The measured average temperature in each incubator was 12.2C, 18.8C, and 24.8C in the second experiment.

Growth rates were analysed using ANOVA (SAS Institute Inc., 1989), and differences among isolates were determined using Duncan's multiple range test.

Virulence Comparisons Using Stem Inoculation with Mycelium

Virulence of isolates of *P. lateralis* was compared in 3 tests. In the first test cuttings of individual Port-Orford-cedar trees were made and

rooted under mist. After root formation, cuttings were potted in 3 gallon round plastic pots in a standard soilless mix containing approximately 2 parts Douglas-fir bark, 1 part peat, and 1 part pumice and grown for an additional year with periodic fertilization with an encapsulated time release fertilizer. All cuttings were produced and grown by the USFS Dorena Tree Improvement Center near Cottage Grove, Oregon. Trees were moved to Corvallis, Oregon and inoculated on January 29 and 30, 1997. All cuttings from a single tree were analyzed as a family.

Ten isolates of *P. lateralis* and one isolate *P. cinnamomi* were grown for one week on cornmeal agar amended with 20ppm *B*-sitosterol. Plugs cut from the advancing edge of colonies were then transferred to pea broth (150 g dry split peas per liter, autoclaved for 3 min and strained through a double layer of cheesecloth, then autoclaved for 20 min at 121C). Colonies were grown for an addtional 7 days (5 days for *P. cinnamomi*), rinsed in deionized water, and used for inoculation.

Trees were inoculated by cutting a longitudinal 1.5 cm slit in the bark, and placing a small weft of mycelium (approx $1mm^3$) under the bark. Slits were then sealed with sterile petroleum jelly. Tree number, family, isolate, height of inoculation, caliper of stem at inoculation site, and total tree height were recorded for each inoculation. Mean height of inoculation was 32 cm (range 13-57), and mean caliper at inoculation site was 7 mm (range 2.5-12) Each tree was inoculated with one isolate, and there were three replications per isolate per family. Eleven tree families were used for a total of 341inoculations. Total extent of necrosis was measured after 7 weeks. Lesion length was analyzed using the General Linear Model procedure (SAS Institute Inc., 1989) rather than ANOVA because of unequal cell sizes.

Virulence Comparison Using Zoospore Inoculum

Two additional tests were carried out to compare virulence among isolates using zoospore inoculation of stems and roots. Five isolates of *P. lateralis* were grown for one week on cornmeal agar amended with 20ppm *B*-sitosterol. 3X3mm plugs cut from the advancing edge of colonies were then transferred to pea broth and grown for 7 days. Medium was then removed, plates were rinsed with deionized H2O, and sporangium formation and zoospore release was induced by filling plates with filtered water from a local creek. One day later the water containing the colonies and zoospores was poured from plates and placed in 1 quart glass jars. Water from one plate of each isolate along with an additional 40ml deionized water was combined in each jar to an approximate depth of 1 cm.

Stem Inoculations with Zoospores

In the second test, Port-Orford-cedar seedlings were grown for one year by the U.S. Forest Service Dorena Tree Improvement center from open-pollinated seed collected from trees with potential resistance to *P. lateralis*. These seedlings were excised at the ground line, wrapped in plastic bags, and stored in a styrofoam box for transportation. On the day following excision, 10 excised seedlings were placed in each inoculation jar containing freshly prepared zoospore suspension for 24 hours, and then were placed in 4X20 cm plastic tree growing tubes filled with perlite. The excised seedlings were watered every other day for 20 or 21 days. Length of necrosis was recorded by measuring the distance between the excised end and upper limit of discoloration inside bark, and mm per day lesion extension was calculated for the analysis. Twenty seven tree families were used for a total of 386 inoculations.

Root Inoculation with Zoospores

In the third test, Port-Orford-cedar seedlings were grown and inoculum was prepared as stated above. Intact seedlings in 4X20 cm plastic tree growing tubes had projecting roots trimmed at the bottom of the container, and the growth medium was fully hydrated at the time of inoculation. The distal1 cm of the seedling container was immersed in zoospore suspension in plastic cups for 24 hours. Seven seedlings were inoculated in each cup. Tree containers were replaced in plastic racks and watered every other day until measurements were made. The time until measurement 65 or 66 days. Lesion length was recorded by measuring the distance between the bottom of the root mass and the upper extent of discolored roots, and mm per day lesion extension was calculated for this analysis. Twenty six tree families were used for a total of 339 inoculations.

Lesion length was analyzed using the General Linear Models procedure (SAS Institute Inc., 1989).

DNA Fingerprint Analysis

DNA fingerprints were obtained from 13 isolates of *P. lateralis*, and one isolate each of *P. cinnamomi* and *P. cryptogea*. DNA extraction was done twice using different methods from isolates grown in different media.

For the first extraction, isolates were grown for 10 days on cornmeal agar amended with 20ppm *B*-sitosterol. 3X3mm plugs were cut from the advancing edge of colonies and placed in 125ml Erlenmeyer flasks containing approximately 50ml of clarified V-8 juice broth (125ml V-8 juice, mixed with 1.8g $CaCO_3$, centrifuged at 12000 rpm, supernatant removed and brought to 1 L with deionized water and autoclaved for 20 minutes at 121C). Cultures were grown at room temperature for 15 days. The contents of each flask was then poured in a Buchner funnel with a miracloth filter, vacuum was applied and colonies were rinsed with sterile deionized water. The miracloth filter was removed, dried on paper towels, and the mycelium scraped off and placed in an eppendorf tube. Each tube contained 0.1g to 0.17g fresh weight of mycelium. Tubes were then lyophyllized.

DNA was extracted by placing lyophillized mycelium in a mortar with about 5ml of liquid N_2, grinding, then adding a small amount of autoclaved sand and grinding in 7 ml of an extraction buffer containing 3% CTAB, 2% NaCl, and 1% PVP, 100mM Tris-HCl, 20mM EDTA pH 8, 1% 2-mercaptoethanol. Ground mycelium and buffer was placed in a 15ml plastic conical bottom tube to a final volume of 7ml and incubated at 37C for 3.5 hours. An equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed, then centrifuged at 7000 rpm for 5 min. The aqueous layer was removed, an equal volume of chloroform:isoamy alcohol was added, mixed, centrifuged, and the aqueous layer removed. This was repeated one additional time. After the removal of the aqueous layer, 2/3 volume of isopropanol was added, and tubes were stored in a refrigerator for 9.5 hours. Tubes were then centrifuged at 7000 rpm for 10 min, the supernatant was removed, and 8ml 80% ethanol added. After 5 min. tubes were centrifuged at 7000 rpm for 5 min, liquid was removed, and the resulting pellet was dried in a laminar flow hood. After all ethanol had evaporated, the pellet was redissolved in 100*u*l of TE buffer , placed in an eppendorf tube, and stored until used for reactions in a -20C freezer.

For the second extraction, isolates were grown for 10 days on cornmeal agar amended with 20ppm *B*-sitosterol. 3X3mm plugs were cut from the advancing edge of colonies and placed in 125ml Erlenmeyer flasks containing approximately 50ml of glucose-yeast-peptone-broth. Cultures were grown at room temperature for 21 days. Mycelium was harvested as stated above.

Mycelium was placed in a 15ml conical bottom plastic tube with 3 6mm glass beads and about 5ml liquid N_2, and vortexing about 90 sec to grind mycelium. 7 ml of JEB buffer (100mM Tris-Hcl pH 7.5, 12.5 mM xanthogenate, 10 mM EDTA, 700 mM NaCl) was added and tubes were incubated at 65C for 40 min. Then 2/3 volume choroform: isoamyl alcohol (24:1) was added, tubes were inverted to mix, and then centrifuged at 7000 rpm for 15 min. Supernatant was removed, and 70*u*l RNAse (10mg/ml) was added and incubated at 35C for 15 min. An equal volume of chorform:isoamyl alcohol was added, and tubes were centrifuged at 7000 rpm for 10 min. Supernatant was removed and 3.25 ml isopropanol was added, and tubes were incubated at room temperature for 19.5 hours. Tubes were centrifuged at 5000 rpm for 5 min, then 7000 rpm for an additional 5 min. Supernatant was removed, and 6 ml ice cold 70% ethanol was added, incubated for 5 min at room temperature, centrifuged for 5 min at 7000 rpm, supernatant was removed and

pellet was dried for 12 hours. Pellet was then resuspended in 60*u*l TE buffer.

PCR primers for DNA fingerprints were based upon inter-simple-sequence-repeats (ISSR) and obtained from the University of British Columbia (UBC Primer Set #9) One hundred primers were screened for ability to amplify sequences and produce readable bands with *P. lateralis* DNA. Forty five primers were chosen and used to amplify DNA from 13 isolates of *P. lateralis* and one isolate each of *P. cinnamomi* and *P. cryptogea*. Amplifications were performed in 28*u*l reaction volumes containing 1.4mM MgCl, 0.7X amplification buffer, 112 *u*M dNTPs, 0.04% DMSO, 18*u*g BSA, 0.38 unit Tfl DNA polymerase (Epicentre Technologies), 0.6*u*M primer, and approximately 80ng DNA in 2 *u*l TE buffer. Amplifications were run on an MJ Research PTC-100 thermocycler with a 1 minute initial denaturation at 94C, 40 cycles of 45 second denaturation at 94C, 30 sec annealing at 52C, and 1 min extension at 72C, followed by a final extension step of 7 minutes at 72C. Products were run on 20X20cm 2% agarose gels containing 0.57*u*g ethidium bromide for approximately 1 hour at 148 volts. Gels were then visualized on an ultraviolet transilluminator, and bands were read from photographs.

Molecular weight of bands was estimated by comparison to a 100 kilobase DNA ladder, and scored for presence or absence of each band. A total of 189 bands were scored. A distance matrix was constructed using Jaccard's similarity coefficient which only takes positive matches into account. From the distance matrix, a dendrogram was constructed using the neighbor-joining algorhythm in PAUP (Swofford).

RESULTS

There were significant differences in growth rates among isolates grown on cornmeal agar (Figure 1). For any specific temperature or time period there were at least two and as many as four groups of isolates that differed significantly from other groups. Although relative growth rates of most isolates differed among temperatures and growth period, two isolates (980093.1 and 20-1) were always among the slowest growing, and Yew2 was usually among the fastest. Some isolates seemed able to tolerate 24C, while other isolates had reduced growth rates at this high temperature. Most isolates grew faster at 24C than 18C during the 0 to 4 day time period, and slower at 24C during the 4 to 7 day time period. This was likely due to drying of the medium or nutrient depletion as the test progressed.

Comparisons of virulence among isolates

Lesion length differed significantly among isolates in both inoculation tests (Figure 2 and 3). In the stem mycelial inoculations one isolate, Yew2, caused significantly shorter lesions than other isolates. All *P. lateralis* isolates caused significantly longer lesions than the one isolate of *P. cinnamomi* tested. In the tests using zoospore inoculum on roots, Yew2 caused significantly shorter lesions than other isolates tested. On excised stems Yew2 caused the shortest lesions, although these were not significantly different from 2 other isolates. The differences among other isolates, although statistically significant, were small. There was no evidence of isolate X family interactions. There were more marked differences among isolates in the root inoculation than in either of the stem inoculation tests.

DNA Fingerprints

All *P. lateralis* isolates had nearly identical banding patterns, with only two bands polymorphic. All *P. lateralis* isolates that had an additional band with primer 808 also had an additional band with primer 835. The *P. lateralis* isolates had no bands in common with the *P. cinnamomi* or *P. cryptogea* isolates. This causes all *P. lateralis* isolates to group together very closely when analyzed by the neighbor joining algorhythm of PAUP (Figure 4). DNA from the two French isolates were amplified with 8 primers and had fingerprints identical to the North American isolates (data not shown).

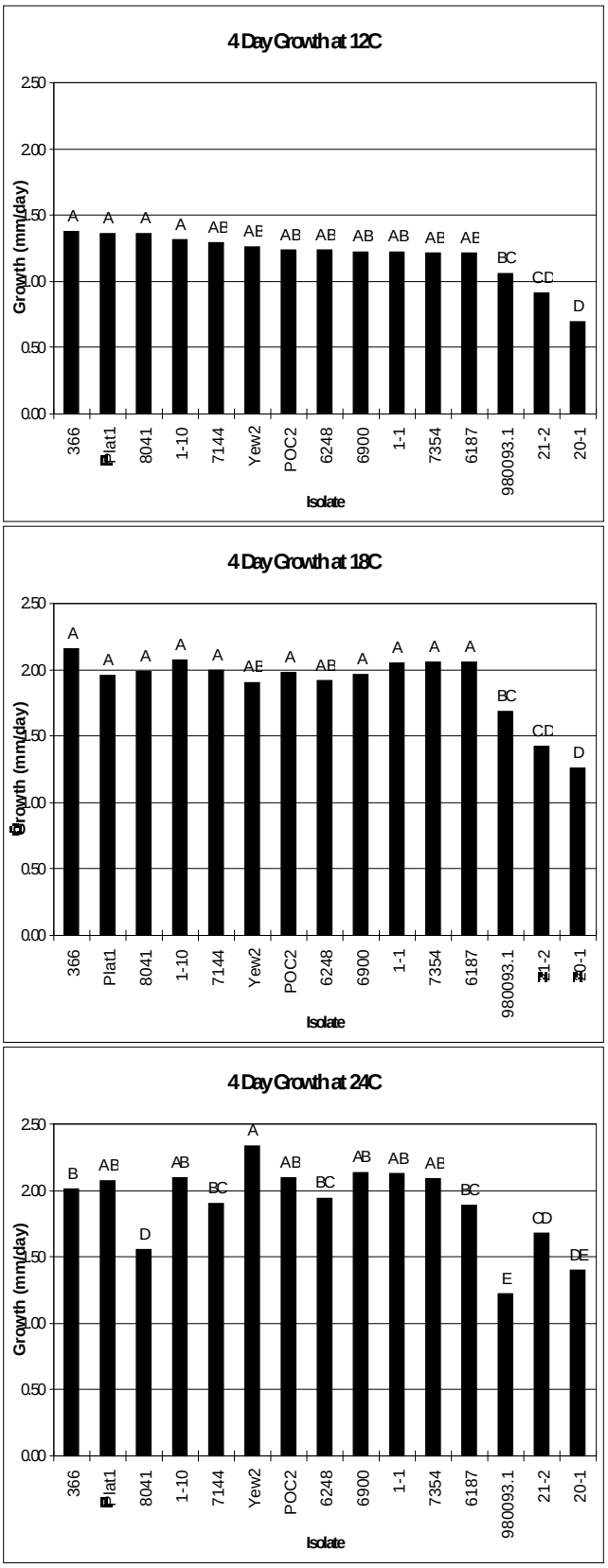


FIGURE 1. Comparison of growth rates of 15 isolates at 3 temperatures on Cornmeal Agar. Bars with the same letter are not significantly (p=.05) different.

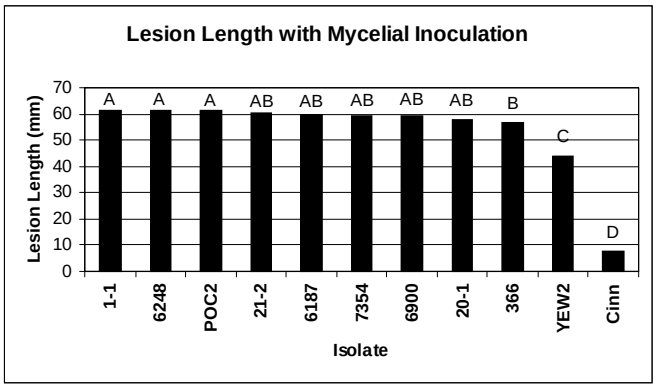


Figure 2. Comparison of isolates when stems were inoculated with mycelium. Bars with the same letter are not significantly (p=.05) different

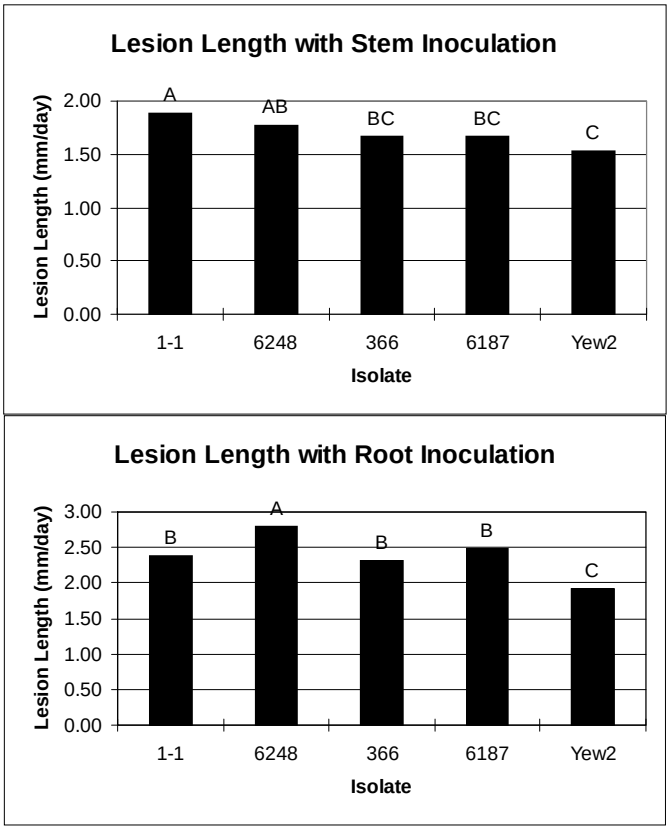


Figure 3. Comparison of stem and root inoculation with zoospore suspernsion. Bars with the same letter are not significantly (p=.05) different.

DISCUSSION

The heterogeneity among isolates of *P. lateralis* is striking. Although some differences in growth rate on a weak artificial medium were detected, the importance of growth rate on artificial media is questionable, and may be due to such factors as age of culture, storage conditions, and stochastic factors as much as genetic factors affecting potential growth rate. There was much more variation in growth rate on cornmeal agar than on V-8 juice agar (data not shown). In *P. cinnamomi* growth rate on PDA was positively correlated with virulence in field inoculation tests (Linde et al. 1999), and isolates that had been in culture for longer periods had slower growth rates than isolates that had been more recently isolated. This does not appear to be the case with *P. lateralis*. The isolate that caused the shortest lesions (Yew2) in three inoculation tests was among the fastest growing isolates at all temperatures.

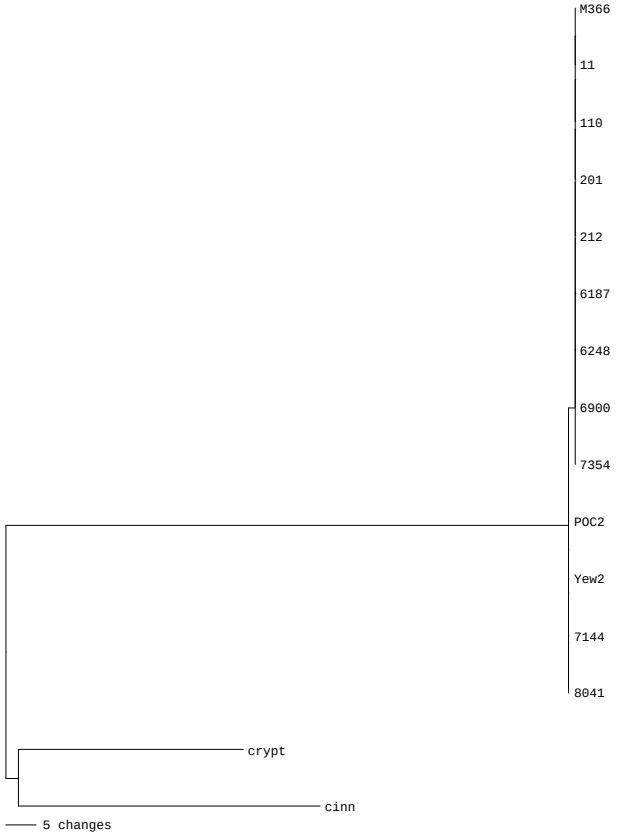


Figure 4. PAUP Neighbor Joining dendrogram showing relationships among isolates of *P. lateralisI.*

The near uniformity of ISSR profiles among all isolates indicates an extremely low level of genetic variation. These data, combined with the previously published uniformity of isozyme profiles (Mills et al. 1991), and the complete homology of ITS1 sequences among four isolates (Winton, this volume), suggests very limited genetic variability in the species (if this is a native fungus), or a single or limited number of introductions (if this fungus was, in fact, introduced to North America). Other studies using ISSR amplification (sometimes referred to as RAMS for Randomly Amplified Microsatellites) have shown considerable variation among isolates of *P. cactorum* in Europe (Hantula et al. 1997) as well as among isolates of other fungi (Hantula and Muller 1997, Vainio et al. 1998). When lack of variation was found among isolates of *P. cactorum* from strawberry it suggested existence of a single clone on that host (Hantula et al. 1997).

Given the extreme genetic uniformity of this fungus, it is interesting that there are significant differences in virulence among isolates on the primary host. In well controlled experiments only one isolate shows decreased virulence, even though this isolate was among the isolates with the fastest growth rates at all temperatures tested. This difference may be due to attenuation because of storage conditions or other factors, or some change in virulence caused by growing in Pacific yew. More isolates from yew should be tested for pathogenic ability to determine whether this phenomenon is common.

The existence of variation in virulence suggests that isolates of known pathogenic ability should be chosen when performing tests to detect resistance in Port-Orford-cedar. The differences among isolates when roots and shoots are exposed to zoospore inoculum may be due to differences in the susceptibility of roots and stems, differences in host mechanisms to limit growth in the different plant

tissues, or because of variations in the inoculation technique or number of zoospores in the inoculum. In testing *Eucalyptus* for resistance to *P. cinnamomi* family ranking based on survival in field plantings and lesion length with stem inoculation tests were equal, and stem tests were recommended for screening for resistance (Stukely and Crane, 1994). Given the greater variation among isolates using a root inoculation, and the difficulty of measuring lesions on root systems, stem inoculation using isolates of known pathogenic ability is most likely to yield reproducible and reliable results. However, the correlation between stem lesion length and survival in infested sites must be determined.

LITERATURE CITED

Abad, Z.G., T. Cresswell, R.K. Jones, and H.D. Shew. 1994. Occurrence of *Phytophthora* species on various hosts in North Carolina Plant Disease 78:830.

Hansen, E.M., J.-C. Streito, and C. Delatour. 1999. First confirmation of *Phytophthora lateralis* in Europe. Plant Disease 83:587.

Hantula, J, and M.M. Muller. 1997. Variation within *Gremmeniella abietina* in Finland and other countries as determined by Random Amplified Microsattelites (RAMS). Mycol. Res. 101:169-175.

Hantula, J., A. Lilja, and P. Parikka. 1997. Genetic variation and host specificity of *Phytophthora cactorum* isolated in Europe. Mycol Res. 101:565-572.

Hoitink, H.A.J. and A.F Schmitthenner. 1974. Relative prevalence and virulence of *Phytophthora* species involved in Rhododendron root rot. Phytopath. 64:1371-1374.

Hunt, J. 1957. Early history of Port-Orford-cedar root rot. Office Report, U.S. Forest Service, Division of Forest Disease Research, Portland, Oregon.

Linde, C, G.H.J. Kemp, and M.J. Wingfield. 1999. Variation in pathogenicity among South African isolaes of *Phytophthora cinnamomi*. European J. Plant Pathology 105:231-239.

Mills, S.D., H. Forster, and M.D. Coffey. 1991. Taxonomic stucture of *Phytophthora cryptogea* and *P. drechsleri* based on isozyme and mitochondrial DNA analyses. Mycol. Res. 95:31-48.

Robertson, G.I. 1982. Kiwi fruit can tolerate *Phytophthora*, but not 'wet feet'. Orchardist of N.Z. 55:148-152.

Stukely M.J.C. and C.E. Crane. 1994. Genetically based resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*. Phytopathology 84:650-656.

Trione, E.J. 1957. The physiology and pathology of *Phytophthora lateralis* on native *Chamaecyparis Lawsoniana*. Ph. D. Thesis, Oregon State University, Corvallis, OR.

Vainio, E.J., K. Korhonen, and J. Hantula. 1998. Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite (RAMS) markers. Mycol Res. 102:187-192.

RISKS AND THREATS DUE TO GENETIC VARIATION IN *PHYTOPHTHORA CINNAMOMI* FOR DISEASE MANAGEMENT IN NATURAL VEGETATION ECOSYSTEMS

I. C. Tommerup[1], M.P. Dobrowolski[2], D. Hüberli[2], P.A. O'Brien[2], G E. St. J. Hardy[2] and B.L. Shearer[3]

[1]Forestry and Forest Products, CSIRO Perth, PO Box 5 Wembley 6913, Western Australia
[2]Biology and Biotechnology, Murdoch University, Murdoch 6150, Western Australia
[3]CALM Science, 50 Hayman Rd., Como 6152 Western Australia.

SUMMARY

Phytophthora cinnamomi is often genetically diverse in disease fronts and diseased areas. *P. cinnamomi* has considerable ability to produce a wide range of pathogenic phenotypes measured from 'plant clone by isolate by environmental factor interactions' in each of the three pathogen clonal lineages of *P. cinnamomi* isolated in Australia. *P. cinnamomi* lineages are defined by microsatellite types. In a few disease areas, despite both mating types occurring in the same square metre of soil, or 50 g soil sample or bait plant, no evidence of sexual reproduction (genomic recombination) has been found in the field. Isolates are sexually competent in the laboratory and all lineage combinations are strongly outcrossing. Hence the substantial variation in lineages in a range of traits associated with disease development must be arising asexually. Preventing movement of any *P. cinnamomi* contaminated material is critical to maximising disease control and minimising risks of introducing new strains which may threaten plant communities, their diversity and the integrity of these ecosystems. Phosphite or other intended phytophthoracide usage, which does not eliminate *P. cinnamomi* in plant nurseries can, by camouflaging disease, increase potential risks and threats by spreading different strains in diseased materials.

INTRODUCTION

Phytophthora diseases are major problems in wildlands where the pathogen is introduced, or as in the case of the alder *Phytophthora*, it is a hybrid between introduced species (Brasier *et al.* 1999; Hansen 2000). Some other major forest diseases, such as white pine blister rust and *Dothiostroma* disease of *Pinus radiata*, are also the result of recent host jumps. None of these devastating diseases have simple control measures.

Disease caused by *P. cinnamomi* has often been called 'dieback', but it is more aptly termed '*Phytophthora* root and collar rot' (Colquhoun and Hardy 2000). *P. cinnamomi* is a pan global pathogen which has most probably been dispersed by trade and with human migration. Most of its host plant species are non-coevolved associations and the number and diversity of hosts are very large (see e.g. Gerrettson-Cornell 1973; Brown 1976; Zentmeyer 1980; Broembsen 1984; Broembsen and Kruger 1985; Weste and Marks 1987; Podger and Brown 1989; Wills and Keighery 1994). The host range of *P. cinnamomi* for Australian plant species is especially large including many woody perennial species, and some rare and endangered species for which *P. cinnamomi* is a major threat to their continuing existence in their 'native vegetation' (Peters and Weste 1997; Weste 1994; Shearer and Tippett 1989; Wills 1993; Shearer and Dillon 1995). The term 'wildlands' equates to 'natural vegetation' in European usage or 'native vegetation' in Australian usage and as this paper is substantially about impacts of *P. cinnamomi* on Australian vegetation, 'native vegetation' will be used frequently. In south western Australia, it is estimated that approximately 2000 of the 9000 plant species are susceptible (Wills 1993). In plant communities in this region, disease is a major disaster because there are up to 40 susceptible species per ha (Shearer 1990). Rare and endangered animal species are also put at increased risk by *P. cinnamomi* when their habitats have a predominance of susceptible host plants.

Disease impacts are large on susceptible plant communities and some landscapes due to losses of genetic resources, plant community structure and key species which are animal food (Figs 1, 2). As well as high species diversity, genetic diversity within species is high and exists as a mosaic within landscapes of south western Australian *P. cinnamomi* susceptible flora (Hopper 1992; Keighery *et al.* 1994). This may also apply to communities in Tasmania. The consequences of *P. cinnamoni* disease in native ecosystems for biodiversity are far greater than just the losses of plants and larger animals (Wilson *et al.* 1994). For the myriads of small organisms, (fungi, bacteria or insects) associated with shoots, roots and litter, which are the major components of biodiversity, the repercussions are largely unknown. Indications of the possible extent of decline in small organism biodiversity can be gauged by analogy. These include insects associated with individual *Eucalyptus* tree canopies (Majer *et al.* 1999) and the diversity of ectomycorrhizal associates in the vegetation of 3 ha of *E. marginata* forest, *Eucalyptus* woodlands or heathlands (Bougher and Tommerup 1996; Tommerup and Bougher 1999; Glen *et al.* 2000a, b). *P. cinnamomi* disease centers may have low impact on ground dwelling insects in south eastern Australia, however effects are largely unknown in south western Australia where the disease impacts are often greater than in the south east (Wills 1993; Shearer 1994; Shearer and Dillon 1995, 1996; Newell 1997).

Figure 1. Death of *Banksia* and many understorey shrubs due to a *Phytophthora cinnamomi* diseasefront on either side of a track in a Bassendean sand community

In wildlands, considerable management challenges are raised when *P. cinnamomi* has large impacts on highly diverse communities and threatens ecosystem integrity. It is a soil borne disease and the early development stages in roots and butts are often camouflaged. This increases the challenges for broadscale management over large tracts of land. Effective means of reducing the spread of the pathogen are quarantine, restricting access, including for logging operations, to only dry seasons, and adherence to hygiene with a high level of public cooperation (Brandis and Batini 1985; Shearer and Tippett 1989; Colquhoun, 2000). More recently the strategic application of phosphite has been used to control *P. cinnamomi* disease in some rare and endangered plant communities (Hardy *et al*. 2000). The possibility of introducing naturally resistant lines of some susceptible

plant species is being investigated (McComb *et al.* 1994; Colquhoun, 2000). Plant breeding programs selecting for resistance to *P. cinnamomi* should take account of the considerable genetic and pathogenic phenotypes in the pathogen (Tommerup 1995; Tommerup *et al*. 1997; Dobrowolski *et al.* 1998c; Hüberli *et al.* 2000b). Fundamental factors to disease management and control are (i) how much genetic variability is in a pathogen population, (ii) how and when that variability flows through the population and (iii) how it affects the pathogen's capacity to cause disease, survive and reproduce.

Figure 2. Spasmodic seedling regeneration of *Eucalyptus marginata* (jarrah), *Banksia* species. and other susceptible understorey species sometimes occurs after *Phytophthora cinnamomi* disease.

We hypothesise that the basis of the adaptive variability of *P. cinnamomi* is that it has a large amount of genetic variation in pathogenesis characters relative to other characters (Tommerup 1995; Tommerup *et al*. 1997). In order to identify genetic diversity and relationships the development of markers is a prerequisite to discriminate amongst strains and make comparisons among local, regional and national isolates of *P. cinnamomi*. Unequivocal markers are also a prerequisite for examining mechanisms of genetic exchange, determining the extent of genetic variation due to asexual and sexual reproduction and providing distinct characters linked to quantitative traits.

GENETIC MARKERS IN *P. CINNAMOMI*

Old *et al.* (1984, 1988) showed that the A1 mating type of *P. cinnamomi* had nine different isozyme types and the A2 had two. Oudemans and Coffey (1991) examined more world-wide isolates and probably found additional variation in the A1 isozyme type and still only two isozyme types for A2. Whether there is additional variation in the South African *P. cinnamomi* isolates can not be discerned as reference isolates were not used (Linde *et al.* 1997).

As an initial class of markers for analyzing variation in populations of *P. cinnamomi* in native vegetation we used RAPD–PCR (random amplified polymorphic DNA-polymerase chain reaction). We used 700 arbitrary primers including primers with and without microsatellite sequence anchoring at the 3' or 5' end to analyse variation in 100 isolates from all known Australian and Papua New Guinea and a few Asian and USA isozyme types (Fig. 3; Tommerup 1995). The Papua New Guinea A1 isolates, which are distinctly isozymically different to the Australian A1, (Old *et al.* 1988) had distinctly different RAPD-PCR patterns and they also differed from isolates from other regions. The RAPD–PCR investigations have (i) revealed considerable homology amongst all isozyme types; (ii) shown unique bands consistently associated with each isozyme type and other unique bands associated with mating type; (iii) raised the possibility that some genome regions of A1 isozyme type 1 and A2 isozyme type 2 may be more closely related to each other than they are to A2 isozyme type 1; (iv) revealed bands associated with some pathogenesis factors and supported the hypothesis that pathogenicity factors and isozyme types are not closely linked; (v) demonstrated a substantial genetic variability within each mating and isozyme type in some regions of the genome and indicated that variation due to asexual and/or possibly infrequent sexual mechanisms have occurred; and (vii) provided evidence from 2000 markers suggesting clonal relationships among some isolates within isozyme types in Australia (Tommerup 1995; Fig. 3). However, this study showed RAPD-PCR would most probably not provide sufficient genetic information for fine scale population genetic analysis.

Taiwanese isolates of *P. cinnamomi* examined for genetic diversity with RAPD–PCR DNA appear to have similar patterns of variation and to be genetically limited, although distinction at the isozyme type level was not analyzed. Taiwanese isolates did not cluster into groups corresponding to their mating types, however, bands associated with A1 and A2 mating types were found (Chang *et al.* 1996). Genetic differentiation among isolates from the same location was significantly high between the mating types, indicating that no hybridization occurred between A1 and A2 mating types in that situation. Although genetic differentiation between isolates from avocado and those from other sources was significantly strong indicating that host specified races might occur in *P. cinnamomi* associated with some crop species Chang *et al.* (1996). An unexplored possibility is that particular isozyme types have developed in avocado plantations, probably due to anthropocentric induced founder events, as in Papua New Guinea (Arentz and Simpson 1986; Old *et al.* 1988).

Due to the possibility that isozyme types of *P. cinnamomi* in native vegetation represented clonal lineages (Tommerup 1995), we chose to develop microsatellite markers. Unlike RAPD–PCR markers, they are codominant (i.e. can distinguish heterozygotes from homozygotes), which is important for a diploid organism. Once developed, they are easily used and require minimal material unlike RFLP markers. They are more polymorphic than isozymes and RFLPs and being PCR markers they are efficient to use in large populations (Dobrowolski *et al*. 1997, 1998a).

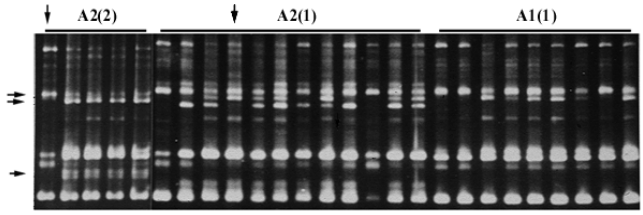


Figure 3. RAPD–PCR variability within isozyme types of Australian isolates of *P. cinnamomi*. Arrows indicate variable bands (→) within isozyme types and isolates representing distinct patterns (↓) (after Tommerup 1995).

SEXUAL RECOMBINATION IN OOSPORES.

Old *et al.* (1984, 1988) found both mating types of *P. cinnamomi* in southern and eastern Australia and they co-occurred in the same patches of native vegetation. Indeed they were found in the same 50 g soil sample. Their isozyme analysis showed no evidence of sexual recombination in isolates from agricultural or native ecosystems. However, isolates of these isozyme types are sexually competent (Dobrowolski *et al.* 1997, 1998b). Dobrowolski *et al.* (1997, 1998b) studied the inheritance of microsatellite markers and demonstrated unequivocally for the first time that genetic exchange and recombination occurred in single oospore progeny. They used progeny (Fig. 4) from four controlled crosses of the A1 isozyme type 1 with two isolates of the A2 type 1 and two of A2 type 2 produced and germinated axenically (Tommerup and Catchpole 1997).

Analysis of sexual progeny also allowed them to test if the markers were inherited in a Mendelian fashion, prior to analysing field populations. The majority of progeny inherited alleles at the microsatellite loci in a non-Mendelian manner, that is, showed aberrant sexual recombination sometimes at all loci (Fig. 5). In

Phytophthora infestans genetic markers linked to mating type were prone to duplication, transposition, deletion and other aberrant sexual recombination (Judelson 1996). In their study, mating type was not linked to any microsatellite locus so it is unlikely to explain the aberrant sexual recombination (Dobrowolski *et al.* 1998b).

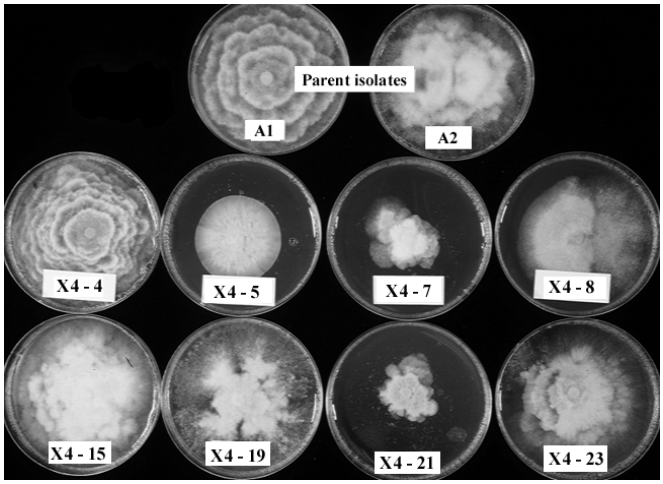


Figure 4. Phenotype variation in growth of eight single oospore progeny of a pair of A1 and A2 parent isolates of *Phytophthora cinnamomi* (after Tommerup and Catchpole 1997).

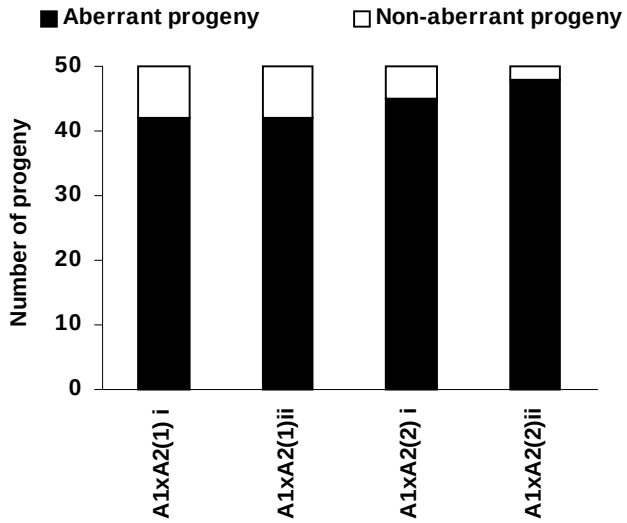


Figure 5. Histogram of numbers of progeny of *Phytophthora cinnamomi* from each cross having aberrant and non-aberrant inheritance of alleles at the microsatellite loci tested (after Dobrowolski *et al*. 1998b).

All 200 progeny analyzed in detail were outcrossed as determined by the inheritance of alleles at the microsatellite loci tested (Dobrowolski *et al.* 1998b). High levels of heterozygosity at these loci in the parents and progeny show that *P. cinnamomi* is basically diploid and this supports the cytological evidence of Brasier and Sansome (1975) and Sansome (1980). Dobrowolski *et al.* (1998b) concluded that the high level of aberrant sexual recombination in this diploid pathogen could be explained by a high frequency of imperfect meiosis (e.g. nondisjunction, unequal crossing over) leading to additions and deletions in the chromosome complement of the sexually derived progeny.

Dobrowolski *et al.* (1998a) also developed microsatellite mitochondrial markers for *P. cinnamomi* to determine if and how they vary in Australian and non-Australian isolates. For the loci examined, the Australian isolates were uniform for all mating and isozyme types. Interestingly, all the A2 isozyme type 1 and type 2 isolates from throughout the world had no variation in the microsatellite loci. The only variation found was in A1 isolates from Japan and Papua New Guinea. Because the loci are uniform in the Australian populations they are not valuable for analysis of mitochondrial inheritance in sexual progeny of the three isozyme types. They obviously may be valuable for analyzing recombination amongst pairs of isozyme types differing at these loci.

The potential exists for oospore production in the wild. This could be due to interaction between A1 and an A2 mating types, or selfing including for the *Trichoderma* effect, or by intraspecific interactions (Brasier 1971; Shepherd 1978; Zentmeyer 1980; Old *et al.* 1984; Chambers *et al.* 1995). Lack of evidence for sexual reproduction in the field (Old *et al.*1984, 1988; Dobrowolski *et al.* 1998c) may not necessarily indicate its failure to ever occur. The progeny may not persist because they are may be less ecologically fit than their parents or unable to compete with the vast numbers of propagules of the parent types and not persist (Dobrowolski *et al.* 1998c, 2000).

ASEXUAL VARIATION: A PREDOMINANT MECHANISM OF CHANGE IN *P. CINNAMOMI* IN WILD POPULATIONS?

Two factors contributed to our hypothesis that considerable variation in Australian populations of *P. cinnamomi* may be asexually derived: (i) the pattern of variation in RAPD–PCR seen amongst isozyme types; and (ii) the lack of sexual reproduction in wild populations (Dudzinski *et al.* 1993; Old *et al.* 1984, 1988; Tommerup 1995; Dobrowolski *et al.* 1999). Morphological variation in isozyme lineages is continuous e.g. colony morphology under defined conditions, asexual and sexual reproductive structures (Hüberli *et al.* 1997a, b, 2000b). We have not found any of these phenotypic characters to be sufficiently distinct for genetic analysis based on studies of either field populations in Australia or isolates from most of the isozyme types identified world-wide, or of 200 single spore progeny of crosses from all Australian isozyme types. Clearly genetic analysis of field populations was needed to test our hypothesis that variation is derived asexually. The microsatellite markers developed on sexual progeny could be used to distinguish clonal lineages (Dobrowolski *et al.* 1999, 2000)

Genetic diversity of *P. cinnamomi* in disease fronts of native vegetation

To survey the genetic structure of *P. cinnamomi* in disease fronts of south western Australia and assess the potential for sexual reproduction Dobrowolski *et al.* (1998c, 1999) hierarchically sampled three disease fronts. Tissue (bark or lesion in wood) and adjacent soil samples were taken from diseased plants; up to 100 per disease front. *P. cinnamomi* was isolated by direct plating of tissue on selective agar and baiting of flooded soil samples with *Eucalyptus sieberi* cotyledons. Mating type was determined by pairing with known A1 and A2 tester isolates. DNA extractions of 640 isolates were analyzed with four microsatellite loci.

The isolates grouped into three clonal types as indicated by their multilocus microsatellite genotypes, two of A2 and one of A1 mating type. One clonal type was common to all three disease fronts, and the other two were found only at site three. Because no recombinants were found, Dobrowolski *et al.* (1998c) concluded that any variation was asexual in origin, despite recovering an A1 isolate from tissue and an A2 isolate from the adjacent soil sample on one occasion, and A1 and A2 isolates were obtained from soil samples within 1m proximity. A size change in one allele (but no recombination) distinguished the site one isolates from site two and some site three isolates. With few exceptions, our hierarchical sampling showed that multiple isolates from a single tissue or soil sample were identical. No association was evident between host species and *P. cinnamomi* clonal type at any disease front.

Dobrowolski *et al.* (1998c) concluded that founder effects gave rise to the genetic diversity of *P. cinnamomi* in the three disease fronts. Sites one and two were the result of single introductions of *P. cinnamomi* which are clonaly related but asexually divergent. The disease front at site three was caused by the introduction of three

clonal types. Although the potential for sexual reproduction exists in disease fronts, we found no evidence for it. However, we have found genetic variation within clonal types (Dobrowolski *et al.* 2000). The significance of this to management in wildlands is that every new introduction of the pathogen has the potential to be genetically different and with new capacities to cause disease.

PATHOGENESIS: A QUANTATIVE TRAIT

Dudzinski *et al.* (1993) unequivocally showed pathogenesis varied among isolates and that the range in phenotypic diversity in pathogenesis was unrelated to mating or to both A2 isozyme types and an A1 isozyme type. They found a greater variation among the A2 isozyme types than the A1 type. This confirmed earlier research indicating variation in pathogenesis among isolates (Zentmeyer 1980; Shearer *et al.* 1988) and it has been reconfirmed in Australia, France and South Africa (Hüberli *et al.* 1998, 2000b; Robin and Desprez-Loustau 1998; Linde *et al.* 1999). Variation in pathogenesis is broad and continuous within mating types and within isozyme types indicating that the trait is quantitative (Fig. 6) (Dudzinski *et al.* 1993; Tommerup *et al.* 1997; Tommerup 1998; Hüberli *et al.* 2000a,b).

A series of disease associated phenotypes have been described involving variation in isolate pathogenesis, physiology and inoculum production. Presently, no trait has been shown to be unequivocally a genetic one and no genetic marker has been unequivocally associated with any phenotype variation. *P. cinnamomi* isolate phenotypes vary from a biotroph to an aggressive necrotroph (Hüberli *et al.* 2000a, b). This has now been shown for 120 isolates in 1-5 different clones of *E. marginata* in field experiments with 5-7 year old trees, controlled environment and glasshouse experiments (Dudzinski *et al.* 1993; Hüberli *et al.* 2000a,b). Whether the variation is arising by episodic or progressive evolution is as yet unknown. Host variation impacts on disease expression so that some isolates in host clonal lines or seedlings produce a 'biotrophic' response with large areas of colonized tissue with no macroscopically visible lesions (O'Gara *et al.* 1997; Hüberli *et al.* 2000a). This behavior, if it occurrs in a wide range of species, is significant to recognising disease in wildlands and to quarantine procedures where accurate disease assessment and disease free certification are critically important.

Environment-by-pathogen isolate combinations produce variable interactions which are part of the total complexity. Large shifts in the behavior of isolates due to interactions changing with environmental factors can pose difficulties for diagnosing disease levels because the host response may vary from nil to a large disease expression. That individual isolates from a population have relatively large variation in disease expression in *E. marginata* clones under standardized conditions means that there are likely to be concomitantly large interactions between environment and disease expression in the forests. Which environmental factors are most influential is more difficult to define in native ecosystems than in controlled environment interactions.

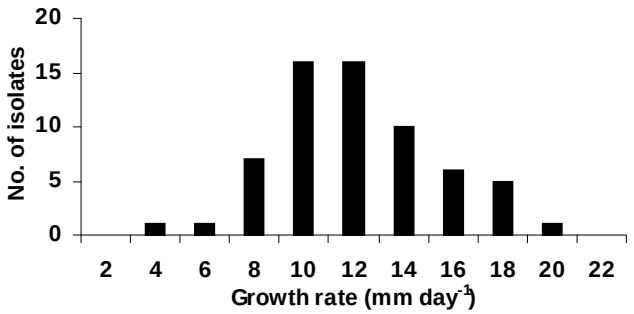


Figure 6. Pathogenesis phenotypes of *Phytophthora cinnamomi* A2 mating type isolates from the northern *Eucalyptus marginata* forest grow at up to five-fold different rates in *E. marginata* (after Hüberli *et al.* 2000b).

Inheritance of resistance to *P. cinnamomi* is also probably quantitative (Tommerup *et al.* 1997). It has long been recognized that species of *Eucalyptus* vary in their resistance to *P. cinnamomi* (Marks *et al.* 1972). Variation in resistance of half sib families of *Eucalyptus regnans* and *E. marginata* is continuous (Harris *et al.* 1983; Harris *et al.* 1985; McComb *et al.*1990; Stukely and Crane 1994). Major differences among species in susceptibility to *P. cinnamomi* occur in many genera including *Eucalyptus*, *Banksia* and *Acaia* and between codominant woody species in plant communities (Marks *et al.* 1981; McCredie *et al.* 1985; Tippett *et al.* 1985; Wardlaw and Palzer 1988; Noble 1989).

Defining resistance-susceptibility responses in clonal *E. marginata* lines is strongly dependent on environmental factors and pathogenesis-by-environment interactions. Host water availability and temperature are two factors for which interaction effects have been defined to some extent. Temperature changed resistance of clonal *E. marginata*, seedling *E. marginata* and seedling *E. calophylla*, and the pathogenesis phenotypes of several *P. cinnamomi* isolates (Grant and Byrt 1984; Hüberli *et al.* 1998). One-year old *E. marginata* clones selected as resistant or susceptible at 20º C using one isolate were (i) equally susceptible at 25-30º and at 15º C and, (ii) for clone 2 susceptible not resistant at 20º C when tested against another isolate (Fig. 7) (Hüberli *et al.* 1998). Lesion size in roots of *E. marginata* in the forest is associated with temperature (Shearer *et al.* 1987a,b; Tippett *et al.* 1983). Cold soils were unfavorable to inoculum build up and to disease development by *P. cinnamomi* in susceptible tree species, and disease was markedly reduced below 15º C (Fagg *et al.* 1973).

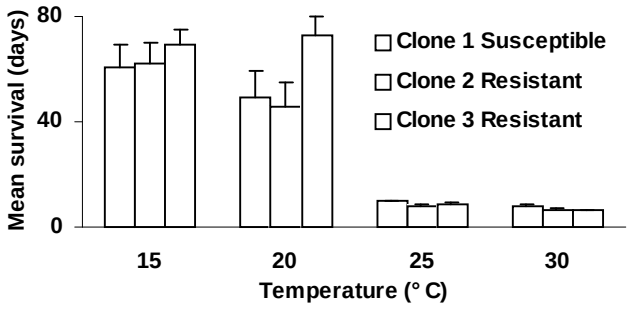


Figure 7. Temperature changes resistance of susceptible and resistant *E. marginata* clones to *P. cinnmamomi* (after Hüberli *et al.* 1998).

Many different forest and laboratory studies involving inoculation of moderately resistant *Eucalyptus* species stems and roots have shown that high bark and phloem moisture favors lesion development by *P. cinnamomi* (Tippett and Hill 1983; Smith and Marks 1985, 1986; Tippett *et al.* 1987; Bunny *et al.* 1995) Also viability of *P. cinnamomi* in 10-week-old and 1-year-old lesions was decreased as tree water deficits increased (Bunny *et al.* 1995). Impeded drainage and any form of water-logging can exacerbate predisposition of susceptible tree and shrub species to *P. cinnamomi* and increase *Phytophthora* root and collar rot disease hazard rating for sites (Christensen 1975; Marks *et al.* 1975; Shea *et al.* 1983; Colquhoun 2000; Old and Dudzinski 2000). Management of soil moisture and soil temperature have implications for land hygiene measures during logging and clearing operations (Kassaby *et al.* 1977). Under a closed canopy, low soil temperatures resulted in poor establishment of *P. cinnamomi* and low disease expression.

Increased disease in resistant hosts at higher temperatures and high phloem and bark moisture has large implications for disease control with global warming, especially if summer rainfall increases. Disease management strategies in wildlands may need to be revised based on long term predictions.

Undefined soil factors have an effect on disease development in native vegetation (Marks and Smith 1983). Susceptible host species in some soil types very rarely have disease, as in the Quindulup or

Spearwood dune systems of the Swan coastal plain (west coast of S. W. Western Australia) where no disease centres were found. *Banksia attenuata* was apparently disease free yet on the adjacent Bassendean dune system 60% of *B. attenuata* was devastated by disease (Hill *et al.* 1994; Shearer and Dillon 1996). The reasons for the low disease impact are currently unknown and they are clearly important to disease management.

CONTROL OF *P. CINNAMOMI* IN LONG LIVED WOODY PLANT SPECIES

Disease control and rehabilitation pose considerable challenges in wildlands where disease impact is greatest such as in Bassendean dune systems or Tasmanian or Victorian heath communities. Disease alters ecosystem functions and the pathogen persists for several years after plants die (Hill *et al*. 1994, 1995). *P. cinnamomi* will probably not be eliminated from diseased wildlands. Resistant hosts and strategic use of chemicals may reduce its impacts.

Breeding and selecting for resistance

Resistance selection has been successful in several species including *E. marginata*, *E. regnans*, *Castanea sativa* and *Pinus echinata* (Ruehle *et al.* 1984; Harris *et al.* 1985; Salesses *et al.* 1993; Colquhoun 2000). Breeding and selecting for resistance may be more durable if screening stages take account of *P. cinnamomi* pathogenesis variation (Tommerup *et al.* 1997; Tommerup 1998). The cryptic nature of lesion development by some isolates, even in pathogen clonal lineages, and the host-pathogen interaction variation induced by some environmental conditions are a particular challenge to experimental design (Dudzinski *et al.* 1993; Dobrowolski *et al.* 1998c, 1999; Hüberli *et al.* 2000a,b). If resistance is sufficient to enable reseeding and regeneration then not only will rehabilitation be improved but the possibility of natural selection for enhanced resistance by geneflow through the community will be enhanced. Geneflow in disjunct and widely separated populations of *Eucalyptus* species may have occurred for up to a maximum of 50 km, however, in forests it may occurr over greater distances (Sampson *et al*. 1995 Adams 1997). Future tree generations may evolve sufficient resistance to not only complete a life cycle but to perpetuate. Only then will it be known if resistance is durable. Development of mature plants of dominant species is important to ecosystem rehabilitation.

Natural regeneration of some susceptible plant species, following a major decline in the population due to *P. cinnamomi*, has occurred in open forest, woodland and heathland of the *E. marginata* forest in Western Australia and the Grampians, Victoria, Australia. Whether the host resistance in reseeding species has increased or whether it is disease escape is not known (Weste and Kennedy 1997; McDougall 1998; Weste *et al*.1999). As for outplanting of deliberately selected resistant individuals, the potential for naturally evolving resistance offers hope of increasing the diversity of indigenous species in disease sites. Rehabilitation of plant community structure has benefits which flow on to other biodiversity that depend on the plants for survival. Self reproducing and sustaining communities offer the greatest long-term protection of genetic diversity within species.

Chemical control

Phosphite (phosphonate) has been successfully used to control *P. cinnamomi* in *E. marginata* and *Banksia* species in forests and woodlands (Komorek *et al*. 1997; Shearer and Fairman 1997a,b). Recent research with other native vegetation species in different plant commuities has indicated two emerging complexities. Firstly the chemical may need to be applied every 1-3 years, as a spray treatment, to control the pathogen in some species on some sites rather than only every four or more years as found for injection of *E. marginata* and *Banksia* species (Komorek *et al*. 1997; Shearer and Fairman 1997a,b;Wilkinson *et al*. 1999a, 2000; Hardy 2000). Secondly, evidence of *P. cinnamomi* resistance to phosphite treated plants is increasing among isolates from native vegetation which has not been exposed previously to phosphite (Wilkinson *et al*. 1999b; Hüberli *et al.* 2000b) and among isolates from phosphite treated orchards (Duvenhage 1994). Phosphite does not kill *P. cinnamomi* in soil and does not always kill the pathogen in plants (Ali and Guest 1998). Phosphite or intended phytophthoracide usage which does not eliminate *P. cinnamomi* in plant nurseries can, by camouflaging disease, increase potential risks and threats to native plant communities. These increased risks and threats are caused by spreading *P. cinnamomi*, having different pathogenic phenotypes or strains with other ecological adaptations, in diseased materials. Should phosphite and any phytophthoricide which does not kill the *P. cinnamomi* be banned from use in nurseries unless there is adequate testing to demonstrate that the pathogen has been eliminated? Or should those chemicals be banned from plant nurseries if they allow *P. cinnamomi* to evolve strains which are more phosphite resistant?

Disease prevention and minimising its spread

Rapid dispersal of *P. cinnamomi* occurs when infested soil is carried from infested to uninfested sites. Hygiene management can reduce spread of *P. cinnamomi* (Batini and Cameron 1971; Kassaby *et al*. 1977; Colquhoun 2000). Any new introductions of the pathogen have the potential to introduce new genetic variation (Dobrowolski *et al*. 2000) including new pathogenicity types and increase disease impact on remaining or regenerating vegetation.

Quarantine

In wildlands at the local, regional, national and international scale quarantine deals with similar issues, namely minimizing risks of introducing new *P. cinnamomi* strains. That leads to reduced threats to plant communities, their diversity and the integrity of their ecosystems.

Lack of evident lesions due to biotrophy or phosphite treatment are a threat to quarantine and disease free certification. These threats could be minimised in the plant nursery trade but may be less tractable to management in wildlands (Huberli *et al.* 2000a). Visual inspection as the main criterium for assessment as 'disease-free' is inadequate for resistant and susceptible plant species. Testing for disease-free certification needs to be augmented by molecular probe methods with or without baiting and plating (Dobrowolski and O'Brien 1994; Brasier *et al.* 1999; Cooke *et al.*2000; Hüberli *et al.* 2000a). Diagnostics are virtually available for routine use to detect importation of these pathogens. Diagnostics to detect current pathogens and new pathogens introduced by global trade are available in research laboratories. They have enabled e.g. detection of hybridization between *Phytophthora* species and the resulting disease in trees which previously had no known *Phytophthora* disease (Brasier *et al.* 1999).

Scale of the management problem

The cost of disease caused by *P. cinnamomi* in native vegetation or wildlands is probably in the billions. It has colonized a vast range of woody plants; in a wide range of ecosystems world-wide (Zentmyer 1980; Hansen 2000; Old and Dudzinski 2000). It threatens the survival of biodiversity in large areas of land and its control is difficult. There are economic costs adding to others due to its impact on ecosystems such as degradation and erosion. Forest, nature conservation, tourism and wild honey harvesting are other examples of industries negatively impacted upon by the pathogen.

Opportunities for *P. cinnamomi* to vary in Australian native vegetation, and probably other wildlands, are abundant. The pathogen has a mosaic distribution in forests, woodlands and woody heaths, all of which are very genetically diverse. These host communities inhabit a range of soils and climates. Host resistance in some *Eucalyptus* species, interacts with climate, such as in drought induced resistance, and possibly soil nutrition and may lead to evolution in *P. cinnamomi*. We have shown the pathogen produces vast numbers of zoospores in disease fronts under conducive conditions e.g. at least one per 40-200 µm sand grain (Tommerup and

Dobrowolski pers. comm.). This creates a huge potential for fostering rare pathogen variation in host-environment circumstances new to the pathogen. Has *P. cinnamomi* evolved with new hosts, and in new environments? If it has what are the implications for disease management? We have developed a suite of microsatellite tools to test these hypotheses and are in the process of developing additional suites of them to begin mapping the *P. cinnamomi* genome and linking pathogenicity phenotypes and chemical resistance traits to markers.

ACKNOWLEDGEMENTS

Janine Catchpole for technical assistance; Land and Water Research and Development Corporation and Alcoa World Wide Alumina for funding part of this work; M. P. D. for a Murdoch Scholarship and D. H. for an APG award.

REFERENCES

Adam, P. J. (1997). 'Analysis of genetic variation within and between populations of *Eucalyptus marginata* (Donn ex Sm.) in the South-West of Western Australia' Honours thesis Murdoch University.

Ali, Z. and Guest, D. L. (1998). Potassium phosphonate controls root rot of *Xanthorrhoea australis* and *X. minor* caused by *Phytophthora cinnamomi*. *Australasian Plant Pathology* **27,** 40-44.

Arentz, F. and Simpson, J. A. (1986). Distribution of *Phytophthora cinnamomi* in Papua New Guinea and notes on its origin. *Transactions of the British Mycological Society* **87,** 289-295.

Batini, F. and Cameron, J. N. (1971). Some aspects of logging hygiene. *Research Paper, Forests Department of Western Australia* pp. 1-7.

Bougher, N. L. and Tommerup, I. C. (1996). Conservation significance of ectomycorrhizal fungi in Western Australia: their coevolution with endogenous vascular plants and mammals. *Gondwanan Heritage, Past, Present and Future of the Western Australian Biota*. eds S. Hopper, J. Chappill and A.S. George. Surrey Beatty & Sons, Chipping Norton. pp. 299-308.

Brandis, A. J. and Batini, F. (1985). Dieback on the south coast. *Landscope* **1 (2),** 6-11.

Brasier, C. M. (1971). Induction of sexual reproduction in single A2 isolates of *Phytophthora* species by *Trichoderma viride*. *Transactions of the Britisn Mycological Society* **58,** 237-251.

Brasier, C. M. and Sansome, E. R. (1975). Diploidy and gametangial meiosis in *Phytophthora cinnamomi*, *P. infestans* and *P. drechsleri*. *Transactions of the British Mycological Society* **65,** 49-65.

Brasier, C. M., Cooke D. E. L. and Duncan, J. M. (1999). Origin of a new Phytophthora pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences* **96,** 5878-5883.

Brown, B. N. (1976). *Phytophthora cinnamomi* associated with patch death in tropical rain forests in Queensland. *Australian Plant Pathology Society Newsletter* **5,** 1-4.

Broembsen, S. von. (1984). Occurrence of *Phytophthora cinnamomi* indigenous and exotic hosts in South Africa, with special reference to the South-Western Cape Province. *Phytophylactica* **16,** 221-225.

Broembsen, S. von. and Kruger, F. J. (1985). *Phytophthora cinnamomi* associated with mortality of native vegetation in South Africa. *Plant Disease* **69,** 715-717.

Bunny, F. J., Crombie, D. S. and Williams, M. R. (1995). Growth of lesions of *Phytophthora cinnamomi* in stems and roots of jarrah (*Eucalyptus marginata*) in relation to rainfall and stand density in mediterranean forest of Western Australia. *Canadian Journal of Forest Research* **25,** 961-969.

Chambers, S. M., Hardham, A. R. and Scott, E. S. (1995). *In planta* immuno labelling of three peripheral vesicles in cells of *Phytophthora cinnamomi* infecting chestnut roots. *Mycological Research* **99,** 1281-1288.

Chang, T. T., Yang, W. W. and Wang, W. Y. (1996). Use of random amplified polymorphic and markers for the detection of genetic variation in *Phytophthora cinnamomi* in Taiwan. *Botanical Bulletin of Academia Sinica* **37,** 165 - 171 .

Christensen, P. (1975). Jarrah dieback - soil temperature and moisture regimes of some southern forest types. *Bulletin Forests Department Perth Western Australia* **No. 88,** 20.

Cooke, D. E. L. (2000). A Comparison of AFLP diversity in *Phytophthora quercina* and *Phytophthora citricola* populations This publication

Colquhoun, I. J. and Hardy, G. E. St J. (2000). Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the *Eucalyptus marginata* (jarrah) forest of Western Australia
Plant Disease **84,** 116-127.

Colquhoun, I. J. (2000). Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the Jarrah forest of Western Australia. This publication

Dobrowolski, M. P. and O'Brien, P. A. (1993). Use of RAPD-PCR to isolate a species specific probe for *Phytophthora cinnamomi*. *FEMS Microbiology Letters* **113,** 43-48.

Dobrowolski, M. P., Tommerup, I. C., and O'Brien, P. A. (1997). Microsatellite markers for pathogen population analysis. *Eleventh Biennial Australasian Plant Pathology Society Conference:* Perth. p. 133.

Dobrowolski, M. P., Tommerup, I. C., and O'Brien, P. A. (1998a). Microsatellites in the mitochondrial genome of *Phytophthora cinnamomi* failed to provide highly polymorphic markers for population genetics. *FEMS Microbiology Letters* **163,** 243-248.

Dobrowolski, M. P., Tommerup, I. C., and O'Brien, P. A. (1998b). Oospore progeny of *Phytophthora cinnamomi* show aberrant sexual recombination. *Sixth International Mycological Congress*, Jerusalem, Israel. p. 35.

Dobrowolski, M. P., Tommerup, I. C., Shearer, B. L. and O'Brien, P. A. (1998c). Genetic diversity of *Phytophthora cinnamomi* in disease fronts of natural vegetation. *Seventh International Conference of Plant Pathology*, Edinburgh, Scotland. p. 2.2.13.

Dobrowolski, M. P., Tommerup, I. C., Shearer, B. L. and O'Brien, P. A. (1999). Genetic diversity of *Phytophthora cinnamomi* in disease fronts of natural vegetation. *Australasian Plant Pathology Society* 12th *Biennial Conference*: Canberra. p. 85.

Dobrowolski, M. P., Tommerup, I. C., Shearer, B. L. and O'Brien, P. A. (2000). Mitotic recombination in continent-wide clonal lineages of *Phytophthora cinnamomi*. (submitted to *Proceedings of the National Academy of Sciences*)

Dudzinski, M. J., Old, K. M. and Gibbs, R. J. (1993). Pathogenic variability in Australian isolates of *Phytophthora cinnamomi*. *Australian Journal of Botany* **41,** 721-732.

Duvenhage, J. A. (1994). Monitoring the resistance of *Phytophthora cinnamomi* to fosetyl-Al and H_3PO_3. *South African Growers Association Year Book* **17,** 35-37.

Fagg, F. Y. Kassaby, F. Y.and Marks, G. C. (1973). Pytopathology. *Victoria Forests Commission, Research Activity* **72,** 26-30.

Gerrettson-Cornell, L. (1973). The recovery of *Phytophthora cinnamomi* Rands from Ourimbah State Forest (Wyong, Newcastle district, New South Wales, Australia) and its pathogenicity to eucalypt seedlings under controlled conditions. *Phyton, Argentina* **31,** 111-116.

Glen, M., Tommerup, I. C., Bougher, N. L. and O'Brien, P. A. (2000a). Specificity, sensitivity and discrimination of primers for PCR-RFLP identification of higher basidiomycetes and their applicability to identification of ectomycorrhizal fungi in *Eucalyptus* forests and plantations. *Mycological Research* submitted.

Glen, M., Tommerup, I. C., Bougher, N. L. and O'Brien, P. A. (2000b). Intraspecific differences and interspecific similarities in PCR-RFLP of higher basidiomycetes and implications for population structure and dynamics, taxonomy and detection of ectomycorrhizal fungi in *Eucalyptus* ecosystems. *Mycological Research* submitted

Grant, B. R. and Byrt, P. N. (1984). Root temperature effects on the growth of *Phytophthora cinnamomi* in the roots of *Eucalyptus marginata* and *E. calophylla*. *Phytopathology* **74,** 179-184.

Hansen, E. (2000). *Phytophthora* in America. This publication

Hardy, G. E. St J. (2000). Phosphite and its potential to control *P. cinnamomi* in natural plant communities and adjacent rehabilitated minesites in Western Australia. This publication

Harris, J. A., Kassaby, F. Y. and Smith, I. W. (1985). Variations in mortality in families of *Eucalyptus regnans* caused by *Phytophthora cinnamomi*, up to 5 years after planting. *Australian Forest Research* **15,** 57-65.

Harris, J. A., Kassaby, F. Y., Smith, I. W. and Marks, G. C. (1983). Intra-specific variation in resistance to *Phytophthora cinnamomi* in *Eucalyptus regnans*. *Australasian Plant Pathology* **12,** 20-22.

Hill, T. C. J., Tippett, J. T. and Shearer, B. L. (1994). Invasion of Bassendean Dune Banksia woodland by *Phytophthora cinnamomi*. *Australian Journal of Botany* **42,** 725-738.

Hill, T. C. J., Tippett, J. T. and Shearer, B. L. (1995). Evaluation of three treatments for the eradication of *Phytophthora cinnamomi* from deep, leached sands in south western Australia. *Plant Disease* **79,** 122-127.

Hopper, S. D. (1992). Patterns of plant diversity at the population and species level in the south-west of Australian mediterranean ecosystems. *Annual Review of Ecology and Systematics* **10,** 399-342.

Hüberli, D., Hardy, G. E. St J. and Tommerup, I. C. (1997a). Morphological and physiological characteristics of two *Phytophthora cinnamomi* populations *Eleventh Biennial Australasian Plant Pathology Society Conference* Perth. p. 307.

Hüberli, D., Tommerup, I. C. and Hardy, G. E. St J. (1997b). The role of paragynous and amphigynous antheridia in sexual reproduction of *Phytophthora cinnamomi*. *Mycological Research* **101,** 1383-1388.

Hüberli, D., Tommerup, I. C., Hardy, G. E. St J. and Colquhoun, I. J. (1998). Temperature changes resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*. *Seventh International Conference of Plant Pathology*, Edinburgh, Scotland. p. 2.5.4.

Hüberli, D., Tommerup, I. C. and Hardy, G. E. St J. (2000a). False-negative isolations or absence of lesions may cause misdiagnosis of *Phytophthora cinnamomi* diseased plants *Australasian Plant Pathology* (in press)

Hüberli, D., Tommerup, I. C. and Hardy, G. E. St J. (2000b). How variable are morphological and pathological phenotypes among *Phytophthora cinnamomi* isolates from two localised populations in Western Australia (to be submitted to *Mycological Research*).

Judelson, H. S. (1996). Genetic and physical variability at the mating type locus of the oomycete *Phytophthora infestans*. *Genetics* **144,** 1005–1013.

Kassaby, F. Y. Fagg , F. Y. and Marks, G. C. (1977). Survival in, and colonization of mountain forest soil by *Phytophthora cinnamomi* Rands in eastern Victoria. *Australian Forest Research* **7,** 173-183.

Keighery, G. J., Coates, D. J. and Gibson, N. (1994). Future ecosystems- ecological balance (ecological impact of disease causing fungi in south-western Australia). *Journal of the Royal Society of Western Australia* **77,** 181-184.

Komorek, B. M., Shearer, B. L., Blumberg, M. V. and Fairman, R. G. (1997). Potassium phosphite-effective chemical tool in the protection of native flora threatened by *Phytophthora*. Eleventh *Biennial Australasian Plant Pathology Society* Conference Perth. p. 34.

Linde, C., Drenth, A., Kemp, G. H. J., Wingfield, M. J. and Broembsen, S. L. von. (1997). Population structure of *Phytophthora cinnamomi* in South Africa. *Phytopathology* **87,** 822-827.

Linde, C., Kemp, G. H. J. and Wingfield, M. J. (1999).Variation in pathogenicity among South African isolates of *Phytophthora cinnamomi*. *European Journal of Plant Pathology* **105,** 231-239.

Majer, J. D., Recher, H. and Keals, N. (1999). Canopy arthropod faunas in fragmented agricultural landscapes. *In* 'Temperate Woodlands in Australia'. *Biology, conservation, management and restoration* eds Hobbs R. J. and Yates C. Surrey Beatty & Sons, Chipping Norton. pp. 235-247.

Marks, G. C. and Smith, I. W. (1983). Development of *Phytophthora cinnamomi* infection in roots of eucalypt species growing in a soil that suppresses *Phytophthora* root disease. *Australian Journal of Botany* **31,** 239-245.

Marks, G. C., Kassaby, F. Y. and Fagg , F. Y. (1975).Variation in population levels of *Phytophthora cinnamomi* in *Eucalyptus* forest soils in eastern Victoria. *Australian Journal of Botany* **23,** 435-439.

Marks, G. C., Kassaby, F. Y. and Reynolds, S. T. (1972). Die-back in the mixed hardwood forests of eastern Victoria: a preliminary report. *Australian Journal of Botany* **20,** 141-154.

Marks, G. C., Smith, I. W. and Kassaby, F. Y. (1981). Trunk infection of *Eucalyptus* species by *Phytophthora cinnamomi*: A preliminary report. *Australian Forest Research* **11,** 257-267.

McDougall, **K. L.** (1996). Vegetation patterns in the northern jarrah forest of Western Australia in relation to dieback history and the current distribution of *Phytophthora cinnamomi*. Murdoch University, 1996

McComb, J. A, Bennett, I., Stukely, M and Crane, C. E. (1990). Selection and propagation of jarrah for dieback resistance. *Combined Proceedings of the International Plant Propagators' Society* **40,** 86-90.

McComb, J. A., Stukely, M. and Bennett, I. (1994). Future ecosystems -use of genetic resistance. *Journal of the Royal Society of Western Australia* **77,** 179-180.

McCredie, T. A., Dixon, K. W. Sivasithamparam, K. (1985). Variability in the resistance of Banksia L.f. species to *Phytophthora cinnamomi* Rands. *Australian Journal of Botany* **33,** 629-637.

Newell, G. R. (1997). The abundance of ground-dwelling invertebrates in a Victorian forest affected by 'dieback' (*Phytophthora cinnamomi*) disease. *Australian Journal of Ecology* **22,** 206-217.

Noble, I. R. (1989). Ecological traits of the Eucalyptus L'Herit. subgenera Monocalyptus and Symphyomyrtus. *Australian Journal of Botany* **37,** 207-224.

O'Gara, E., McComb, J. A., Colquhoun, I. J. and Hardy, G. E. St. J. (1997). The infection of non-wounde and wounded periderm tissue at the lower stem of *Eucalyptus marginata* by zoospores of *Phytophthora cinnamomi*, in a rehabilitated bauxite mine. *Australasian Plant Pathology* **26,** 135-141.

Old, K. M. and Dudzinski, M. J. (2000). Phytophthora in forests and native vegetation in Australia and Eastern Asia. This publication

Old, K. M., Dudzinski, M. J. and Bell, J. C. (1988). Isozyme variability in field populations of *Phytophthora cinnamomi* in Australia. *Australian Journal of Botany* **36,** 355-360.

Old, K. M., Moran, G. F. and Bell, J. C. (1984). Isozyme variability among isolates of *Phytophthora cinnamomi* from Australia and Papua New Guinea. *Canadian Journal of Botany* **62,** 2016-2022.

Oudemans, P. and Coffee, M. D. (1991). Isozyme comparasions within and among world-wide sources of three morphologically distinct species of *Phytophthora*. *Mycological Research* **95,** 119-30.

Peters, D. and Weste, G. (1997). The impact of *Phytophthora cinnamomi* on six rare native tree and shrub species in the Brisbane Ranges, Victoria. *Australian Journal of Botany* **45,** 975-995.

Podger, F. D. and Brown, M. J. (1989). Vegetation damage caused by *Phytophthora cinnamomi* on disturbed sites in temperate rainforest in western Tasmania. *Australian Journal of Botany* **37,** 443-480.

Robin, C. and Desprez-Loustau, M. L. (1998). Testing variability in pathogenicity of *Phytophthora cinnamomi*. *European Journal of Plant Pathology* **104,** 465-475.

Ruehle, J. L., Sluder, E. R. and Bryan, W. C. (1984). Performance of control pollinated progeny from selected shortleaf pine on a littleleaf site in south Carolina. *Research Note, South Eastern Forest Experiment Station, USDA Forest Service* **No. SE-325,** 1-7.

Salesses, G., Ronco, L., Chauvin, J. E. and Chapa, J. (1993). Amélioration génétique du châtignier. Mise au point de tests d'évaluation du comportment vis-a-vis de la maladie de l'encre. *Arboriculture Fruitière* **No. 458,** 23-31.

Sampson, J. F., Hopper, S. D. and James, S. H. (1995). The mating system and genetic diversity of the australian arid zone mallee, *Eucalyptus rameliana*. Australian Journal of Botany **43,** 461-474.

Sansome, E. R. (1980). Reciprocal translation heterozygosity in heterothallic species of *Phytophthora* and its significance. *Transactions of the British Mycological Society* **74,** 175-185.

Shea, S. R., Shearer, B. L., Tippett, J. T. and Deegan, P. M. (1983). Distribution, reproduction and movement of *Phytophthora cinnamomi* on sites highly conducive to jarrah dieback in south western Australia. *Plant Disease* **67,** 970-973

Shearer, B. L. (1990). Dieback of native plant communities caused by *Phytophthora* species - a major factor affecting land use in south-western Australia. *Land and Water Research News***,** 15-26.

Shearer, B. L. (1994). The major plant pathogens occurring in native ecosystems of south-western Australia. *Journal of the Royal Society of Western Australia* **77,** 113-112.

Shearer, B.L. and Dillon, M. (1995). Susceptibility of plant species in Eucalyptus marginata forest to infection by *Phytophthora cinnamomi*. *Australian Journal of Botany* **43,** 113-134.

Shearer, B.L. and Dillon, M. (1996). Impact and disease centre characteristics of *Phytophthora cinnamomi* infection of plant species in *Banksia* woodlands on the Swan Coastal Plain, Western Australia. *Australian Journal of Botany* **44,** 79-90.

Shearer, B. L and Fairman, R. G. (1997). Foliar application of phosphite-delays and reduces the rate of mortality of three *Banksia* species in communities infested with *Phytophthora cinnamomi*. Eleventh *Biennial Australasian Plant Pathology Society* Conference Perth. p. 180

Shearer, B. L. and Fairman, R. G. (1997). Phosphite-inhibits lesion development of *Phytophthora cinnamomi* for at least four years following trunk injection of *Banksia* species and *Eucalyptus marginata*. Eleventh *Biennial Australasian Plant Pathology Society* Conference Perth. p. 181

Shearer, B. L., Michaelsen, B. J. and Warren, H. J. (1987a). Comparative behaviour of *Phytophthora* species in the secondary phloem of stems and excised roots of *Banksia grandis* and *Eucalyptus marginata*. *Australian Journal of Botany* **35,** 103-110.

Shearer, B.L., Shea, S. R. and Deegan, P. M. (1987b). Temperature-growth relationships of *Phytophthora cinnamomi* in the secondary phloem of roots of *Banksia grandis* and *Eucalyptus marginata*. *Phytopathology* **77,** 661-665.

Shearer, B.L., Michaelsen, B.J. and Somerford, P. J. (1988). Effects of isolate and time of inoculation on invasion of secondary phloem of *Eucalyptus* spp. and *Banksia grandis* by *Phytophthora* spp. *Plant Disease* **72,** 121-126.

Shearer, B. L. and Tippett, J. T. (1989). Jarrah dieback: the dynamics and management of *Phytophthora cinnamomi* in the jarrah (*Eucalyptus marginata*) forest of south-western Australia. Research Bulletin **No. 3**., Department of Conservation and Land Management, Western Australia.

Shepherd, C. J. (1978). Mating behaviour of Australian isolates of *Phytophthora* species. *Australian Journal of Botany* **26,** 123-138.

Smith, I. W. and Marks, G. C. (1985). Inhibition of *Phytophthora cinnamomi* lesion development in *Eucalyptus sieberi* through moisture stress. *Australasian Plant Pathology* **14 ,** 55-56.

Smith, I. W. and Marks, G. C. (1986). Effect of moisture stress in Eucalyptus sieberi on growth of lesions caused by *Phytophthora cinnamomi*. *Australian Forest Research* **16,** 273-279.

Stukely, M. and Crane, C. E. (1994). Genetically based resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*. *Phytopathology* **84,** 650-656.

Tippett, J. T., Crombie, D. S. and Hill, T. C. (1987). Effect of phloem water relations on the growth of *Phytophthora cinnamomi* in *Eucalyptus marginata*. *Phytopathology* **77,** 246-250 .

Tippett, J. T. and Hill, T. C. (1983). The relationship between bark moisture and invasion of *Eucalyptus marginata* by *Phytophthora cinnamomi*. *Australasian Plant Pathology* **12,** 40-41.

Tippett, J. T., Hill, T. C. and Shearer, B. L. (1985). Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. *Australian Journal of Botany* **33 ,** 409-418.

Tippett, J. T., Shea, S. R., Hill, T. C. and Shearer, B. L. (1983). Development of lesions caused by *Phytophthora cinnamomi* in the secondary phloem of *Eucalyptus marginata*. *Australian Journal of Botany* **31,** 197-210.

Tommerup, I. C. (1995). Genetic variation in isozyme groups of *Phytophthora cinnamomi*. *Tenth Biennial Australasian Plant Pathology Society Conference*, Lincoln University, New Zealand 28-30 August.p.

Tommerup, I. C. (1998). Diseases which Disrupt Natural Ecosystems. *Seventh International Conference of Plant Pathology*, Edinburgh, Scotland p. 4.4.1S.

Tommerup, I. C. and Bougher, N. L. (1999) The role of ectomycorrhizal fungi in nutrient cycling in temperate Australian woodlands. *In* 'Temperate Woodlands in Australia'. *Biology, conservation, management and restoration* eds Hobbs R. J. and Yates C. Surrey Beatty & Sons, Chipping Norton. pp 190-224.

Tommerup, I. C. and Catchpole, J. (1997). Cryopreservation of recalcitrant isolates and single oospore lines of *Phytophthora cinnamomi*. *Eleventh Biennial Australasian Plant Pathology Society Conference*: Perth. p. 171.

Tommerup, I. C., Hardy, G. E. St. J., Hüberli, D. and Colquhoun, I. J. (1997). Selecting plants resistant to *Phytophthora cinnamomi*. *Eleventh Biennial Australasian Plant Pathology Society Conference*: Perth. p. 89.

Wardlaw, T. J. and Palzer, C. (1988). Regeneration of *Eucalyptus* species in an eastern Tasmanian coastal forest in the presence of *Phytophthora cinnamomi*. *Australian Journal of Botany* **36,** 205-215.

Weste, G. (1994). Impact of *Phytophthora* species on native vegetation of Australia and Papua New Guinea. *Australasian Plant Pathology* **23,** 190-209.

Weste, G. and Kennedy, J. (1997). Regeneration of susceptible native species following a decline of *Phytophthora cinnamomi* over a period of 20 years on defined plots in the Grampians, Western Victoria. *Australian Journal of Botany* **45,**: 167-190.

Weste, G. and Marks, G. C. (1987). The biology of *Phytophthora cinnamomi* in Australasian forests. *Annual Review of Phytopathology* **25,** 207-229.

Weste, G., Walchhuetter, T. and Walshe, T. (1999). Regeneration of *Xanthorrhoea australis* following epidemic disease due to *Phytophthora cinnamomi* in the Brisbane Ranges, Victoria. *Australasian Plant Pathology* **28,** 162-169.

Wills, R. T. (1993). The ecological impact of *Phytophthora cinnamomi* in the Stirling Range National Park, Western Australia. *Australian Journal of Ecology* **18**, 145-159.

Wills, R. T. and Keighery, G. J. (1994). Ecological impacts of plant disease on plant communities. *Journal of the Royal Society of Western Australia* **77**, 127-131.

Wilkinson, C., Holmes, J., Tynan, K., Dell, B. McComb, J. and Hardy, G. (1999a). Does phosphite prevent zoospore production from *Phytophthora cinnamomi*-colonised plants in the field. *Twelth Biennial Australasian Plant Pathology Society Conference*: Canberra. p. 230.

Wilkinson, C., Shearer, B., Holmes, J., Tynan, K., Dell, B. McComb, J. Maradous, J. and Hardy, G. (1999b). A comparison of *invitro* and *inplanta* responses of *Phytophthora cinnamomi* isolates to phosphite. *Twelth Biennial Australasian Plant Pathology Society Conference*: Canberra. p. 231.

Wilkinson, C. J., Holmes, J. M., Dell, B. Tynan, K. M., McComb, J. A., Shearer, B. L., Colquhoun, I. J. and Hardy, G. (2000). The effect of phosphite on *Phytophthora cinnamomi* zoospore production *in Planta*. *Plant Disease* (in review).

Shearer, I. J. Colquhoun and G. E. St J. Hardy

Wilson, B. A., Newell, G., Laidlaw, W. S. and Friend, G. (1994). Impact of plant diseases on faunal communities. *Journal of the Royal Society of Western Australia* **77**, 139-143.

Zentmyer, G. A. (1980). *Phytophthora cinnamomi* and the diseases it causes. *American Phytopathological Society Monograph* **No. 10**, St. Paul MN, 96 pp.

IMPACTS OF ROOT INFECTION CAUSED BY *PHYTOPHTHORA CINNAMOMI* ON GROWTH AND WATER RELATIONS OF YOUNG CHESTNUTS GROWN IN A SPLIT-ROOT SYSTEM

M. Maurel[1], C. Robin[1], X. Capdevielle[1], D. Loustau[2] and M.L. Desprez-Loustau[1]

[1] Unité de Recherches Santé Végétale. Laboratoire de Pathologie Forestière. INRA Bordeaux. 33883 Villenave d'Ornon. France
[2] Unité de Recherches Forestières. Laboratoire d'Ecophysiologie. INRA Bordeaux. Domaine de L'Hermitage, Pierroton. 33610 Cestas. France.

INTRODUCTION

Alteration of plant water relations during root infection caused by *Phytophthora cinnamomi* has been reported in several studies (Dawson and Weste 1982). A decrease in predawn leaf water potential and stomatal conductance was observed in field studies in mature *Eucalyptus marginata* (Crombie and Tippett 1990), in *Eucalyptus macrorrhyncha* (Dawson and Weste 1982) and in avocado trees (Sterne et al 1978) infected by *P. cinnamomi*. The same results were obtained in infected *Eucalyptus sieberi* seedlings (Dawson and Weste 1984) in controlled conditions. As there is no available data on water relations of chestnuts infected by *P. cinnamomi*, the aim of our study was to investigate the water relations of young chestnuts infected by *P. cinnamomi* and to relate them to root infection levels. Plants were submitted to two watering conditions : normal watering (field capacity) or restricted water supply. Because of the difficulty to control the level of root infection in potted plants, particularly due to the production of secondary inoculum under moist conditions, chestnuts were grown in a split-root system with four compartments which allowed to inoculate only some parts of the root system.

MATERIALS AND METHODS

In mid-February 1998, chestnut seeds (*Castanea sativa*) were placed on wet filter paper to germinate. When taproot was about 2 cm long, it was cut to allow the formation of new roots. After one month of growth on perlite, seedlings were gently taken out and for each of them, four roots of equivalent length and diameter were selected, the others being cut. Four pots (each with a capacity of 2.5 l) were clipped together to constitute one split-root pot with four watertight compartments. Each root of a seedling was put into one compartment. Seedlings were grown in a greenhouse and were transferred into a plastic tunnel in June 1998.

Seedlings were inoculated with an aggressive isolate of *P. cinnamomi* (isolate 9) in mid-July 1998 and in mid-May 1999 by putting 5 ml of infected millet seeds per compartment into the substrate (2 $ml.l^{-1}$). Five inoculation levels were obtained as follows. One, 2, 3 or 4 compartments per seedling were soil infested, none for controls. After inoculation, pots were saturated with water to enhance the production of secondary inoculum.

All year in 1998, all the plants were maintained at field capacity. Watering was made by drip irrigation (two capillary tubes of 1 l. h^{-1} per compartment).

In 1999, half of the plants in each inoculation treatment were submitted to water stress (0S, 1S, 2S, 3S, 4S), in two periods. On June 15, a moderate water stress was applied for 20 days by providing half of the water given to the well watered plants (0W, 1W, 2W, 3W, 4W). One capillary tube was removed in each compartment for stressed plants (0S, 1S, 2S, 3S, 4S). Stressed plants were rewatered at the end of the stress.

On July 13, a second water stress was applied on the same plants by witholding watering for one week. Plants were then rewatered at the end of the experiment.

In 1998, predawn leaf water potential was measured every other week with a pressure chamber and stomatal conductance was measured twice a week with a steady-state porometer (" LI-1600 ", Li-Cor Inc., Lincoln, NE, USA).

In 1999, predawn and midday leaf water potential and stomatal conductance were measured on the same days twice a week. Plant transpiration per leaf area unit was measured once a week in 1998 and 1999 by the loss of weight of pots over three days. Transpiration was calculated on a leaf area basis by estimating the total leaf area of each plant by a length-area relationship. In 1999, shoot growth and stem diameter growth at 10 cm height were measured. Final leaf area was estimated by a linear weight-surface relationship.

Soil moisture was measured twice a week in the four compartments of each pot with a ThetaProbe ML2 (Delta-T Devices Ltd, Cambridge, U.K.).

At the end of the experiment, in July 1999, all the plants were harvested. For each compartment, roots were carefully washed and healthy and necrotic roots were separated. The percentage of root infection per plant was assessed by the ratio : dry weight of infected roots / total root dry weight.

RESULTS

At the end of the experiment, a wide range of root infection was obtained and the percentage of root infection increased with the number of infested compartments (Figure 1). The same range of root infection was obtained in the plants with one or two infested compartments (1-21%). For three out of four plants with three infested compartments, the percentage of root infection was approximately 40%. The percentage of root infection reached varied from 50% to 100% in all the plants with four infested compartments.

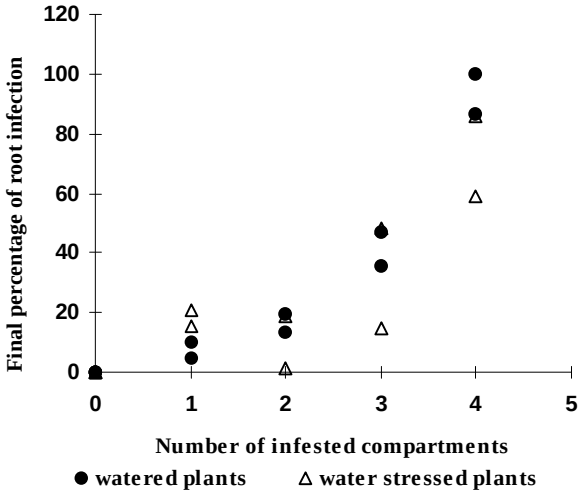


Figure 1. Final percentage of root infection (dry weight of infected roots/total root dry weight) of *C. sativa* plants grown in a split-root system and submitted to the following treatments : 0, 1, 2, 3 or 4 compartments infested with *P. cinnamomi* combined with two watering conditions : watered or stressed.

No aerial symptoms appeared except in the plants with four infested compartments that displayed a delay in bud break in 1999 and developed a low number of leaves with a reduced area. Only one plant with four infested compartments (4W treatment) died in 1999 on July 16. In this plant, root infection reached 100%. Yellowing of leaves and decline occurred just before dying.

TABLE 1. Soil moisture content of non infested compartments and compartments infested with *Phytophthora cinnamomi* of *Castanea sativa* plants grown in a split-root system and submitted to the following treatments : 0, 1, 2, 3 or 4 infested compartments combined with two watering conditions : watered (W) or stressed (S). Measurements were made on July 20 (1999). Values are means ± SD.

	0W	1W	2W	3W	4W	0S	1S	2S	3S	4S
Non infested compartments	26.4 ± 3.8 n = 16	24.1 ± 1.4 n = 6	22.1 ± 4.5 n = 4	20 ± 1.5 n = 2	_	8.9 ± 1.3 n = 16	7.8 ± 1.6 n = 6	5.4 ± 0.1 n = 4	5.3 ± 1.8 n = 2	_
Infested compartments	_	31.5 ± 1.8 n = 2	29.8 ± 1.7 n = 4	29.3 ± 2.3 n = 6	31.2 ± 4.9 n = 8	_	31.5 ± 0.9 n = 2	13.1 ± 12.1 n = 4	16.7 ± 3.8 n = 6	24 ± 1.1 n = 8

The soil moisture content of 0W plants and of non infested compartments of 1W, 2W and 3W plants was kept around 20% or higher (Table 1). 4W compartments (all infested) kept high values, around 30%. During the stress, the soil moisture content of 0S plants and of the non infested compartments of 1S, 2S and 3S plants decreased. The decrease was much lower in 4S plants which remained at approximately 25% soil moisture. In each treatment, soil moisture of infested compartments was always higher than soil moisture of non infested compartment. Soil moisture values were almost the same at all dates of measurements than values shown in Table 1.

The effects of root infection on growth were visible during the second year of the experiment. Root loss was compensated in plants with less than 20% final root infection as their final healthy root biomass was identical to that of non inoculated plants. Plants with more than 20% final root infection displayed a reduction in shoot and stem diameter growth. Above 50% final root infection, final leaf area was reduced. This reduction was a consequence of both the decrease in the number of leaves and the decrease in the mean leaf area.

In 1998, there was no effect of inoculation on predawn leaf water potential. In 1999, during all the measurement period, 0W, 1W, 2W and 3W plants kept their predawn leaf water potential above –0.2 MPa (Figure 2). The mean predawn leaf water potential of the 4W plants decreased sharply from June 29 to –2.5 MPa because of one individual which eventually died (July 16) with 100% root infection. The other 4W plant (with 86% final root infection) kept a high predawn leaf water potential until the end of the experiment. During water stress, mean predawn leaf water potential of all stressed treatments decreased. At the two peaks of stress (July 2 and 20), there was a significant effect of water stress and inoculation (with interaction) on predawn leaf water potential. Three groups could be differenciated : the watered treatments kept high predawn leaf water potential values whereas those of 0S, 1S and 2S decreased sharply. The decrease was less important for the 3S and 4S plants. After the second rewatering, all plants regained a good water status, except one 0S plant and the two 1S plants.

The same trends were observed for midday leaf water potential.

In 1998, a decrease in stomatal conductance in relation to the number of infested compartments was observed. Differences were significant between the non inoculated plants and plants with three or four infested compartments. Throughout June 1999, stomatal conductances remained at low and stable level (50-100 mmol. m^{-2}. s^{-1}) for all treatments except for the 4W plants which showed the lowest stomatal conductances during all the season (Figure 3). During the first half of July, the mean stomatal conductance of watered plants decreased in relation to the number of infested compartments. From July 13, the stomatal conductances of 3W and 4W plants were significantly different from those of 0W plants. On July 20, all the inoculation treatments (watered or stressed), except 1W plants, had stomatal conductances significantly lower than those of 0W plants. Stressed plants of all inoculation treatments kept low values of stomatal conductance (30-70 mmol. m^{-2}. s^{-1}), similar to those of 4W plants. Values were significantly different from those of 0W plants during the second stress period. There was a highly significant effect of treatment (inoc∗stress), of time and of the interaction between time and treatment on stomatal conductance.

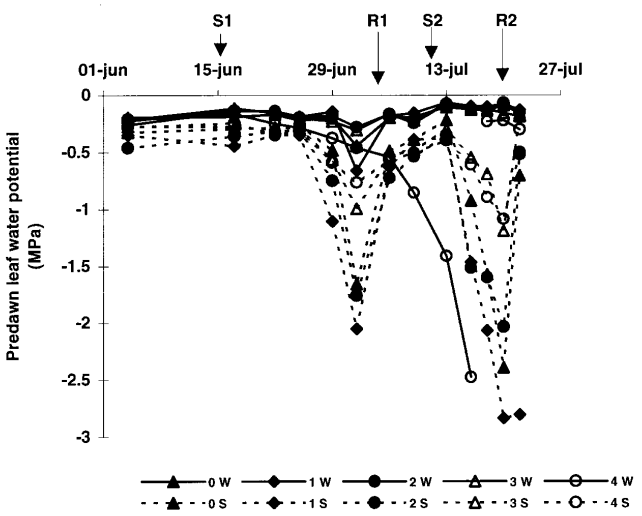


FIGURE 2. Time course of predawn leaf water potential of C. sativa plants grown in a split-root system and submitted to the following treatments : 0, 1, 2, 3 or 4 compartments infested with P. cinnamomi, combined with two watering conditions : watered (W) or stressed (S). S1 and S2 : dates of the beginning of the stress periods, R1 and R2 : dates of rewatering. n = 2 except for 0W and 0S n = 4. At the last measurement dates, n = 1 for 4W because one plant died (discontinuous curve).

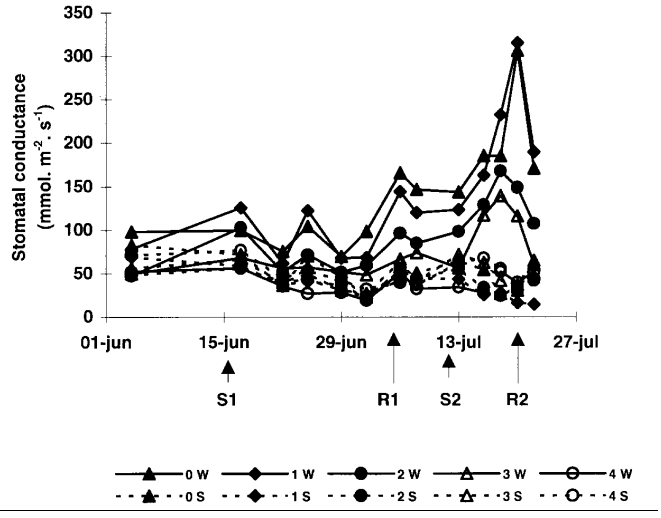


FIGURE 3. Time course of stomatal conductance of C. sativa plants grown in a split-root system and submitted to the following treatments : 0, 1, 2, 3 or 4 compartments infested with P. cinnamomi, combined with two watering conditions : watered (W) or stressed (S). S1 and S2 : dates of the beginning of water stress periods, R1 and R2 : dates of rewatering. n = 2 except for 0W and 0S n = 4. At the last measurement dates, n = 1 for 4W because one plant died (discontinous curve).

In 1998, a trend of decreasing transpiration per surface unit in relation to the number of infested compartments was observed although no significant difference between treatments could be demonstrated. During the second half of June 1999, transpiration of

all watered treatments remained stable at 0.2-0.37 mmol. m^{-2}. s^{-1} except for the 4W plants for which transpiration decreased on June 28 (Figure 4). A rising trend was observed during the first half of July for all watered plants except again 4W plants. Transpiration of inoculated plants tended to decrease with the number of infested compartments : the reduction of transpiration in comparison with the 0W plants was 58%, 53%, 65% and 80% in 1W, 2W, 3W and 4W plants, respectively, but differences were not significant. At the beginning of the stress, transpiration of all stress treatments decreased except in the 4S plants. These latter had the highest values at the first measurement. They kept high transpiration values during all the experiment. This could be explained by a border effect as they were exposed for longer to light and more to wind. Nevertheless, the transpiration course was quite similar to that of 4W plants. For the 3S plants, the reduction was about 55%. There was more than 80% reduction in transpiration at the last date for the 0S, 1S and 2S plants in comparison with the watered treatments. There was a significant effect of the interaction between time and stress on transpiration.

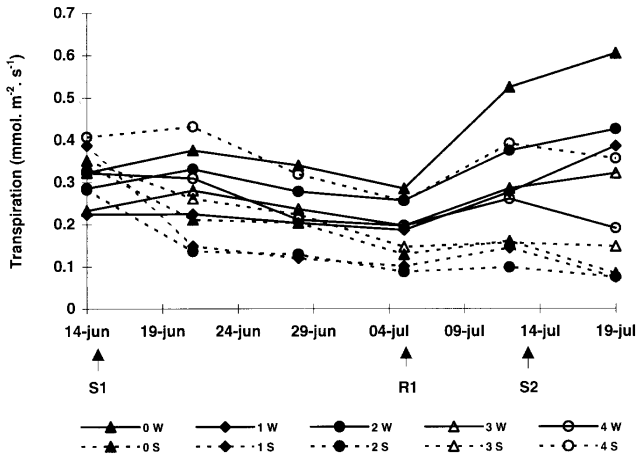


FIGURE 4. Time course of transpiration of C. sativa plants grown in a split-root system and submitted to the following treatments : 0, 1, 2, 3 or 4 compartments infested with P. cinnamomi, combined with 2 watering conditions : watered (W) or stressed (S). S1 and S2 : dates of the beginning of the stress periods, R1 and R2 : dates of rewatering. n = 2 except for 0W and 0S n = 4 and for 1W n = 1.

CONCLUSION

The split-root system allowed us to obtain a wide range of root infection (1-100%) and gave us a mean to relate, *a posteriori*, the effects observed on growth and water relations and the final percentage of root infection. Growth was reduced in plants with more than 20% final root infection.

In well watered plants, the first effect observed on water relations following root infection was a decrease in stomatal conductance. This decrease occurred in very early infection stages, as it was observed since the first year of the experiment. Moreover, this effect was observed even at very low final infection rates as it was observed in plants with one or two infested compartments. As expected, the decrease in stomatal conductance was related to a decrease in transpiration per surface area unit.

However, the stomatal closure was not associated with a decline in leaf water potential (predawn and midday). A decline in water status occurred only in the plant with 100% final root infection. The others kept a high predawn leaf water potential (above –0.2 MPa) until the end of the experiment, even in the plant with 86% final root infection.

When water supply was restricted, soil moisture content of the non inoculated plants and of the non infested compartments of plants with one, two or three infested compartments decreased. But soil moisture remained higher in most of the infested compartments. This is likely to be explained by the reduction (or the lack) in water absorption following root destruction by the pathogen. But some water absorption did occur in these compartments (at least in 2S, 3S and 4S plants) as shown by the reduction in soil moisture content (Table 1). The stomatal closure following root infection may also have contributed to keeping a better water status by the reduction of water loss. As a consequence, stress intensity was not the same for all inoculation treatments. Only non inoculated plants and plants with one or two infested compartments (1-21% final root infection) experienced severe water stress as shown by the sharp decrease in predawn and midday leaf water potential. No conclusion can be drawn on a possible lower resistance to water stress in inoculated plants because of the little number of plants. Moreover, it is likely that the 1S plants behaved in the same way as the 0S plants since they had the same healthy root biomass and no functional roots in infested compartments. Finally, no effect of inoculation on stomatal conductance and transpiration could be observed in stressed plants as effects induced by water stress were predominant.

The striking result of our study is the decrease in stomatal conductance from very low infection rates without a decrease in leaf water potential. Though the decrease in stomatal conductance had been previously observed in eucalyptus after *P. cinnamomi* infection (Dawson and Weste 1984, Crombie and Tippett 1990) and in avocado trees (Sterne and al. 1978), it was further accompanied by a decline in leaf water potential. As water status was not affected over a wide range of root infection, the processes inducing stomatal closure may not be the same as those involved in drought stress response. Furthermore, the reduction in stomatal conductance observed in plants with less than 20% root infection which had compensated for root biomass supports the hypothesis that stomatal closure may be triggered by root infection and not by root loss. The involvement of several mechanisms may be suggested : hormonal imbalance (Dawson and Weste 1982, Dawson and Weste 1984, Cahill and al. 1986), phytoalexins produced by the host in response to pathogen attacks (Willmer and Plumbe 1986).

REFERENCES

Cahill D. M., Weste G. M. and Grant B. R. 1986. Changes in cytokinins concentrations in xylem extrudates following infection of *Eucalyptus marginata* Donn ex. Smith with *Phytophthora cinnamomi* Rands. Plant Physiol., 81 : 1103-1109.

Crombie S. D. and Tippett J. T. 1990. A comparison of water relations, visual symptoms, and changes in stem girth for evaluating impact of *Phytophthora cinnamomi* dieback on *Eucalyptus marginata*. Can. J. For. Res., 20 : 233-240.

Dawson P. and Weste G. 1982. Changes in water relations associated with infection by
P. cinnamomi. Aust. J. Bot., 30 : 393-400.

Dawson P. and Weste G. 1984. Impact of root infection by *Phytophthora cinnamomi* on the water relations of two *Eucalyptus* species that differ in susceptibility. Phytopathology, 74 : 486-490.

Sterne R. E., Kaufmann M. R. and Zentmyer G. A. 1978. Effect of *Phytophthora* root rot on water relations of avocado : interpretation with a water transport model. Phytopathology 68 : 595-602.

Willmer C. M. and Plumbe A. M. 1986. Phytoalexins, water stress and stomata. In : Water, fungi and plants, Ayres P. G. and Boddy L. (Ed), British Mycological Society Symposium 11, pp. 207-222.

INVESTIGATIONS ON PHOTOSYNTHESIS OF OAK SEEDLINGS INFECTED WITH *PHYTOPHTHORA QUERCINA* AND CHARACTERIZATION OF THE *P. QUERCINA* TOXIN QUERCININ

W. Oßwald[1], M. Brummer[1], J. Fromm[4], A. Schlenzig[1], J. Koehl[2], T. Jung[1], I. Heiser[2] and R. Matyssek[3]

[1]Forest Botany, Section Phytopathology, Technical University of Munich, Am Hochanger 13, 85354 Freising, BRD
[2]Institute of Phytopathology, Technical University of Munich, 85350 Freising, Am Hochanger 2, BRD
[3]Forest Botany, Technical University of Munich, Am Hochanger 13, 85354 Freising, BRD
[4]Institute of Wood Research, Technical University of Munich, Winzererstr. 45, 80797 Munich, BRD

SUMMARY

Five week old oak seedlings were infected with *Phytophthora quercina* under controlled conditions. Gas exchange, root infection and root destruction were measured after each flooding event over a period of six months. Significantly reduced rates of CO_2 assimilation and transpiration as well as a decrease in stomatal conductance were detected after the second flooding. At that time, the root system was already heavily infected by *Phytophthora quercina* causing a severe loss of fine roots and root tips. During the last two months of the experiment the fungus was no longer detectable in fine roots. At that time strong regeneration of the root system started, and photosynthesis reached levels comparable with those of control plants.

We further showed that *P. quercina* released small proteins into the culture medium, which caused wilting and necrosis on tobacco leaves. Transmission electron microscopy studies clearly showed that membrane structures especially of chloroplasts were destroyed. The *Phytophthora quercina* proteins strongly crossreacted with an antibody raised against the *Phytophthora cryptogea* protein cryptogein. Therefore, the *P. quercina* peptides might belong to the family of Phytophthora leaf necrotic proteins called elicitins.

INTRODUCTION

Phytophthora spp. are among the most serious soilborne pathogens. In 1993 Brasier et al. reported the presence of *Phytophthora cinnamomi* Rands in a number of Iberian oak sites and suggested that this pathogen could be involved in decline of *Quercus suber* and *Quercus ilex*. Recently *P. cinnamomi* was also recorded on declining cork and holm oaks in France (Robin et al., 1998)

We showed that several Phytophthora root rot pathogens such as *Phytophthora quercina, Phytophthora citricola* and *Phytophthora cambivora* are the primary cause of oak decline in the field at certain stands (Jung et al. 1996; Jung et al., 1999 in preparation). These root rot pathogens restrict the uptake of water and nutrients finally causing wilting and pigment degradation of leaves in the crown of affected trees. Soil infestation tests proved that *P. quercina*, which was described by Jung et al. (1999) turned out to be the most aggressive pathogen towards oak (Jung and Oßwald, 1999 in preparation).

There are only some few reports in literature comparing alterations in root morphology caused by Phytophthora pathogens with biochemical and physiological changes in stems and leaves. Cahill et al. (1986) reported on changes of cytokinin concentrations in xylem exudates of Eucalyptus seedlings following infection with *Phytophthora cinnamomi*. They found a significant reduction of the zeatin-type and isopentyladenine-type cytokinin in the xylem exudate of susceptible *Eucalyptus marginata*, whereas no reduction was measured in the xylem exudate of the field resistant species *Eucalyptus calophylla*. The authors suggest that failure of cytokinin transport from the root system may be responsible for the failure in water transport and symptoms of *P. cinnamomi* infection observed in infected susceptible eucalyptus. Ploetz and Schaffer (1988) showed that root rot of avocado seedlings caused by the infection of *Phytophthora cinnamomi* resulted in a rapid reduction in net CO_2-assimilation, transpiration and stomatal conductance for CO_2 after flooding. Similar results were reported by Luque et al. (1999) infecting cork oak plants with *P. cinnamomi*.

A characteristic feature of *Phytophthora* species is the release of small proteins of 98 amino acids called elicitins into the culture medium (Yu 1995; Terce-Laforgue et al. 1992). Besides this several defense reactions were turned on in tobacco leaves treated with these peptides. Wendehenne et al. (1995) proved that specific, high-affinity binding sites for cryptogein, an elicitin of *P. cryptogea*, are localized within tobacco plasma membranes. The recognition of the elicitor by a plasma membrane receptor leads to a cascade of events known for other general and specific elicitors, including calcium influx, protein phosphorylation, activation of the NADPH oxidase and of mitogen-activated protein kinase homologues and finally gene activation (Lebrun-Garcia, 1999). Besides this, cryptogein was shown to induce leaf necrosis and a rapid break down of tobacco chloroplasts (Zanetti et al. 1992). We recently showed that *Phytophthora quercina* also released small peptides into the culture medium which strongly crossreacted with the antibody raised against cryptogein (Heiser et al., 1999). Therefore these peptides might belong to the family of Phytophthora leaf necrotic proteins called elicitins.

MATERIALS AND METHODS

1. The time shedule of the soil investation experiment.

The whole experiment was carried out as outlined in figure 1.

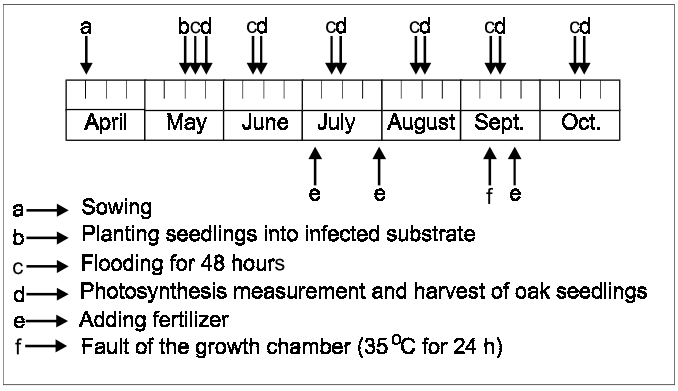


FIGURE 1: Soil infestation experiment of oak seedlings with *P. quercina*

The plants were grown seperately in root trainers filled with an autoclaved soil mixture consisting of peat, vermiculite and sand (1:1:1, v/v/v). The seedlings were infested with *P. quercina* inoculum during the flooding cycles as was described by Jung et al (1999). The plants were kept in a growth chamber at 18-22°C and 65% relative humidity. During the flooding procedure in September, a failure in phytotron control caused the air temperature to increase to 35 °C for 24 hours.

2. Assessment of leaf gas exchange

Within a period of two to eight days after each flooding event (cf. Fig. 1), measurements were conducted in the greenhouse with a CO_2/H_2O diffusion porometer (CQP 130, Walz, Germany), assessing the rates of net CO_2 uptake and transpiration as well as stomatal conductance by IR gas analysis (BINOS 100 analyzer, Leybold-Heraeus, Germany, operated in differential mode). During each measurement period, eight infected plants were compared with eight control plants, analyzing the gas exchange of two leaves per plant (each leaf being measured twice). Leaves were exposed during measurements to an artificial light source (Höhnle, Germany) which provided a stable photosynthetic photon flux density (PPFD) of about 780 µmol m^{-2} s^{-1} to ensure light saturation of photosynthesis. The relative air humidity in the greenhouse ranged between 33 and 65 %, while the air temperature was set to a constant level of 20 °C. Air from a neighboring greenhouse compartment was sucked into the porometer cuvette in order to provide a CO_2 level of about 380 µmol mol^{-1} during measurements. Readings were taken as soon as the gas analysis had stabilized for at least two minutes. Assessments were confined to the diurnal time interval of 8 a.m. to 2 p.m., and soil water supply was non-limiting during the measurements as an effect of the preceding flooding procedure. The rationale of the analysis was similar to that described in Maurer et al. (1997).

3. Quantification of the root system of oak seedlings

The total root system of each oak seedling was cut off and was carefully washed with tap water. Afterwards each root was scanned inside a plastic box which was filled up with water. The total root length of different root classes and the number of root tips was calculated with the Win Rhizo (3.10) software (Regent Instruments Inc., Quebec, Canada).

4. Enzyme immunoassay

The *Phytophthora quercina* infection of fine roots of oak seedlings was quantified using the Agri-Screen- Phytophthora detection Kit from Adgen (Agrifood diagnostic, Scotland). All fine roots (diameter 0-1.5 mm) of each seedling were cut off and carefully washed. Five milligram of the freeze dried powder were extracted with 1.0 ml of the extraction buffer for 20 min on ice. Afterwards the extract was centrifuged at 13.000 g for 5 min and 100 µl of a 1:100 dilution of each supernatant was used for the enzyme immunoassay carried out according to the instructions of the manufacturer.

5. Transmission electron microscopy

Approximately 1-2 mm^2 segments of leaf tissue were immersed in 6% glutaraldehyde in 50 mM cacodylate buffer pH 7.0, briefly evacuated and fixed for 90 min at 4 °C. Following fixation, the tissue was washed in four changes of cold buffer and postfixed in 2 % OsO_4 in 50 mM buffer overnight at 4 °C. For electron microscopy, the sections were dehydrated in a graded ethanol series and embedded in Spurr`s epoxy resin. Sections were cut with a diamond knife on a Reichert ultramicrotome and stained with 3 % uranyl acetate and lead citrate.

RESULTS

1. Net CO_2 uptake rates of control and *Phytophthora quercina* infected oak seedlings.

As shown in figure 2 the net CO_2 uptake rates of leaves of control plants increased from May to June and slowly decreased from August to October.

Leaves of *Phytophthora quercina* infected plants showed significantly reduced CO_2 uptake rates from May to September. The highest reduction in CO_2 uptake of about 30% was measured in infected plants in June, July and August, whereas differences between infected and control plants had diminished by October. In both treatments, the seasonal time course of photosynthesis was similar to that of the transpiration rate and stomatal conductance (data not shown).

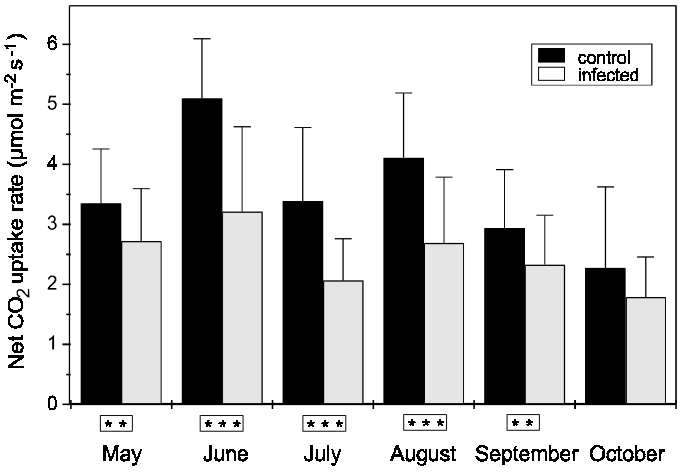


FIGURE 2: Net CO_2-uptake rate of control and *Phytophthora quercina* infected plants. Standard deviation was calculated by means of eight replications.

2. Root tip densities of control and *Phytophthora quercina* infected oak seedlings.

In parallel to photosynthesis measurements the root length and root tip densities of infected and control plants were measured (figure 3).

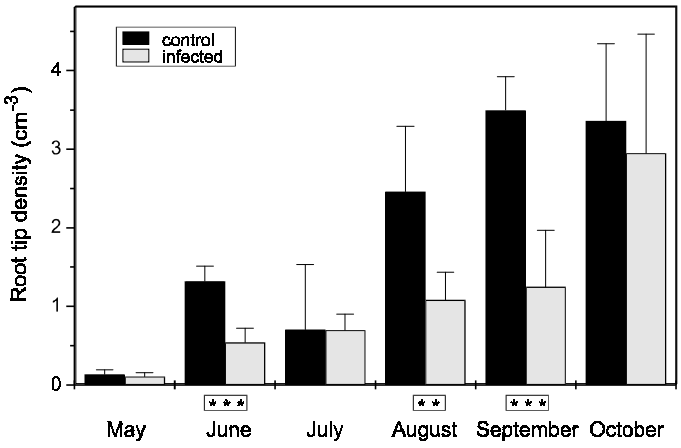


FIGURE 3: Root tip density of control and *Phytophthora quercina* infected plants. Standard deviation was calculated by means of eight replications.

Five days after the first flooding event in May no differences in the root systems of infected and control plants were observed. However, a strong reduction of the root tip density was measured for all infected plants compared to controls from June up to September. The *Phytophthora quercina* infection resulted in a fine root destruction between 50 to 60%. Surprisingly infected plants harvested in October after the last flooding cycle reached fine root densities comparable with those of control plants, indicating an apparently intense regeneration of root growth of infected plants in September and October. In parallel, the total root length density had recovered in infected and control plants (data not shown).

3. Quantification of *Phytophthora quercina* of infected oak roots over a time period of six months.

The quantification of *Phytophthora quercina* in oak roots was carried out with the Agri-Screen- Phytophthora detection Kit using ELISA-techniques (figure 4).

The highest infection level of oak roots was measured after the first, second and third flooding event in May, June and July. The infection level droped to almost zero in August. *Phytophthora quercina* was no longer detectable in roots harvested in September and October.

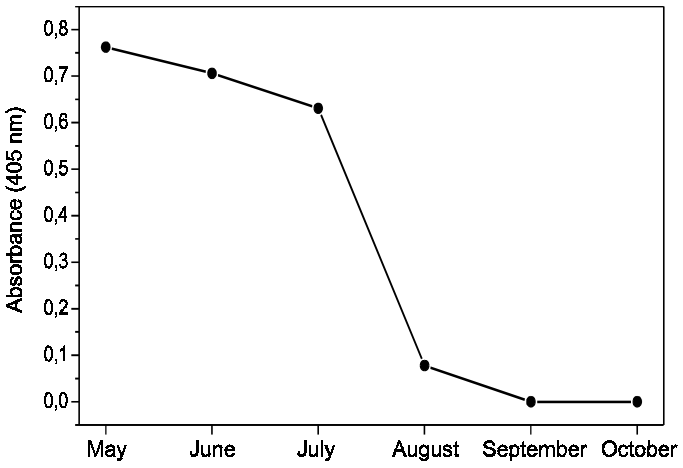


FIGURE 4: Infection status of roots of oak seedlings over a time period of six months. *Phytophthora quercina* infection of roots was quantified using a ELISA-Phytophthora kit. Eight plants were analysed at each time point.

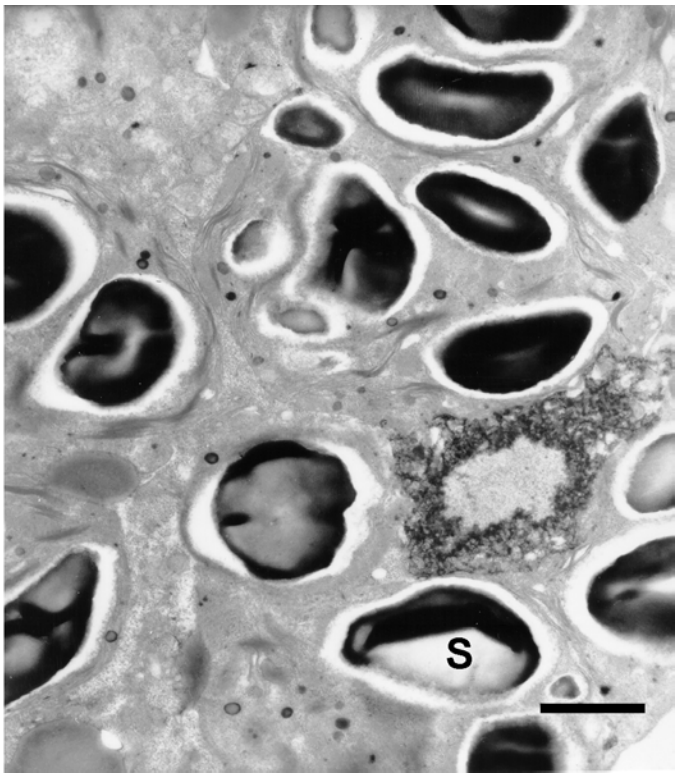


FIGURE 5: Transmission electron microscopy studies of tobacco leaves treated with *Phytophthora quercina* peptides called Quercinins. S, starch grain; Bar = 1 µm

4. Influence of *Phytophthora quercina* peptides on the ultrastructure of tobacco leaves.

As shown in figure 5 tobacco leaf cells treated with *Phytophthora quercina* proteins for one day in the light were severely destroyed. They lost their turgor and, especially, chloroplasts showed massive membrane degradation and less regularly stacked grana. There are almost no intact membrane systems in the cytoplasm. Only starch grains (S) and plastoglobuli still have some structure and seem therefore not very damaged.

5. Investigations on the nature of the *Phytophthora quercina* toxins.

Tobacco leaves, which were placed into the *P. quercina* toxin solution (0.5µg protein x mL^{-1}) showed severe wilting and necrosis after an incubation of three days in the light (Heiser et al. 1999). Only one major protein band with a molecular mass of 6.8 kDa was found for *P. quercina* when the concentrated M 1-medium was analysed on a 20% SDS gel. Cryptogein from *Phytophthora cryptogea* showed an almost identical molecular mass. The western blot experiments further demonstrated that the *P. quercina* peptides strongly crossreacted with the antibody raised against cryptogein. The peptides turned out to be very heat stable. Even boiling for 15 minutes did not destroy their activity. However, no wilting and necrosis was seen on tobacco leaves, when the protein solution was preincubated with pronase, indicating that the toxic effect is due to proteins released by *P. quercina* into the M1-medium. Isoelectric focusing experiments clearly showed that *P. quercina* produced three small peptides with isoelectric points of 3.6, 3.9 and 8.3 (Heiser et al., 1999).

DISCUSSION

In this paper we demonstrate that five week old oak seedlings, which were infested with *Phytophthora quercina*, showed a severe reduced root growth compared to control plants. This reduction was due to massive fine root loss caused by the pathogen. This severe root damage reflected in a significant reduction in photosynthesis, in transpiration and in stomatal conductance of infected plants. However, root damage was much more pronounced than reduction of photosynthesis. A root tip density reduced by 60 % related to a photosynthetic performance which had lost about one third of its capacity. Ploetz and Schaffer (1988) also found that net CO_2 assimilation declined rapidly after the onset of flooding of avocado plants that had been infected with *Phytophthora cinnamomi*. Transpiration and stomatal conductance had trends similar to those in CO_2 assimilation. The authors also showed that reduction in assimilation occured before the development of aboveground symptoms usually associated with advanced stages of root rot.

The ELISA- data clearly showed that *P. quercina* infection declined dramatically from July to August. During the next two months the fungus was no longer detecable in oak roots. The low infection rate in August might be due to the fact that all fine roots were already infected and rotten at that time. Therefore no more roots which could be infected were left. During the two day flooding cycle in September the temperature increased up to 35 ^{o}C for 24 hours in the growth chamber. Jung et al. (1999) recently showed that *Phytophthora quercina* was killed at these temperatures. This explains why there was no longer any fungus detectable in roots harvested end of September and October. The ceasing infection in September was the pre-requisite for the root systems to regenerate and to reach the same root tip densities and rates of photosynthesis as control plants. Recently Kennedy and Duncan (1995) also reported that growth of *P. citricola*, *P. idaei*, *P. syringae* and *P. cactorum* was dramatically inhibited at temperatures of 27^{o}C.

In our experiments we showed that photosynthesis of infected plants was already reduced five days after the first flooding event. However, there were no significant differences in root tip density and total root length detectable at that time, when infected and control plants were compared. Thus the question arises about the way root infection affects photosynthesis.

Studies of Dawson and Weste (1984) showed that susceptible eucalyptus seedlings grown under controlled conditions died when as little as 8 % of their root system was infected by *P. cinnamomi*, indicating that loss of root tissue alone is unlikely to be the cause of wilting or lethal effects on the host plant. This group also demonstrated a significant reduction in hydraulic conductivity of the root system of a susceptible species early after infection. This decline in root conductivity preceded reductions in leaf xylem water potential, leaf transpiration rate, and wilting in these plants. Additional histological examinations failed to show xylem blockage or extensive damage to the conducting system under these conditions. Cahill et al (1986) suggested that failure of cytokinin transport from the root system may be responsible for the symptoms observed in infected susceptible eucalypts. They proved that infection of the susceptible *Eucalyptus marginata* by *P. cinnamomi*, causing severe

root rot, resulted in a 26 % reduction of both cytokinin types compared to uninoculated controls.

We recently demonstrated that the root pathogen *Phytophthora quercina* released small proteins into the M1-medium which caused wilt symptoms and necrosis on tobacco leaves in the light (Heiser et al., 1999). Transmission electron microscopy studies clearly showed that plasma membranes, in particular chloroplast membranes, were degraded. Already 0.5 µg protein x mL-1 caused severe damage to tobacco leaves. Similar results were also reported by Ricci et al. (1989) for tobacco leaves treated with cryptogein and capsicein, proteins produced by *P. cryptogea* and *P. capsici*. The *P. quercina* proteins showed molecular masses comparable with that of cryptogein, the toxin produced by *Phytophthora cryptogea*. Furthermore the *P. quercina* peptides crossreacted with the antibody raised against cryptogein. Taken all together the *P. quercina* peptides will be grouped into the family of Phytophthora leaf necrotic proteins called elicitins. These proteins are also known to migrate from the stem into leaves where they can be dected by ELISA- techniques (Zanetti et al., 1992).

We suggest that these Phytophthora peptides, which we call Quercinins, may act in two ways: First they might interfere directly with fine root cells in the neighborhood of infection sites causing loss of turgor and decline in extension growth. Second they might also be transported to leaves where they may impair photosynthesis. Experiments with highly purified Quercinin are being currently conducted to elucidate the toxin effect on fine roots and on photosynthesis.

ACKNOWLEDGEMENT

The authors are greatful to the Deutsche Forschungsgemeinschaft, the Allianz Stiftung zum Schutz der Umwelt and to the Bavarian Ministry of Forestry for the financial support of this project.

LITERATURE CITED

Brasier C.M., Robredo F. and Ferraz J.F.P., 1993. Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. Plant Pathology 42: 140-145.

Cahill D. M., Weste G.M. and Grant B.R., 1986. Changes in cytokinin concentrations in xylem exudate following infection of *Eucalyptus marginata* Donn ex Sm with *Phytophthora cinnamomi* Rands. Plant Physiol. 81: 1103-1109.

Dawson P.D. and Weste G.M., 1984. Impact of root infection by *Phytophthora cinnamomi* on the water relations of two Eucalyptus species that differ in susceptibility. Phytopathology 74: 486-490

Heiser, I.; Fromm, J.; Giefing, M.; Koehl, J., Jung, T; Oßwald, W.; 1999: Investigations on the action of *Phytophthora quercina*, *P. citricola* and *P. gonapodyides* toxins on tobacco plants. Plant Physiol. Biochem. 37: 73-81

Jung T., Blaschke H. and Neumann P., 1996. Isolation, identification and pathogenicity of Phytophthora species from declining oak stands. Eur. J. For. Pathol., 26: 253-272.

Jung T., Cooke D., Blaschke H., Duncan J. and Oßwald W., 1999. *Phytophthora quercina* sp. nov, a new species causing root rot on European oak species. Mycol. Research 103: 785-798.

Kennedy D.M. and Duncan J.M., 1995. A papillate Phytophthora species with specificity to Rubus. Mycol. Research 99: 57-68

Lebrun-Garcia A., Bourque S., Binet M-N., Ouaked F., Wendehenne D., Chiltz A., Schäffner A. and Pugin A. (1999) Involvement of plasma membrane proteins in plant defense responses. Analysis of the cryptogein signal transduction in tobacco. Biochimie 81: 663-668

Luque J., Cohen M., Save R., Biel C. and Alvarez I.F. 1999. Effects of three fungal pathogens on water relations, chlorophyll fluorescence and growth of *Quercus suber* L. Ann. For. Sci. 56: 19-26

Maurer S., Matyssek R., Günthardt-Goerg M.S., Landolt W., EIinig W. 1997. Nutrition and the ozone sensitivity of birch (Betula pendula), I. Responses at the leaf level. Trees 12: 1-10

Ricci P., Bonnet P., Huet J.C., Sallatin M., Beauvais-Cante F., Bruneteau M., Billard V., Michel G. and Pernollet J.C. 1989. - Structure and activity of proteins from pathogenic fungi Phytophthora eliciting necrosis and acquired resistance in tobacco. *Eur. J. Biochem*. 183, 555-563,

Ploetz R.C. and Schaffer B., 1988., Effects of flooding and Phytophthora root rot on net gas exchange and growth of Avocado. Phytopathology 79: 204-208

Robin C., Desprez-Loustau M.L., Capron G. and Delatour C., 1998. First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. Ann. Sci. For. 55: 869-883

Terce-Laforgue T., Huet J.C. and Pernollet J.C. 1992. Biosynthesis and secretion of cryptogein, protein elicitor secreted by Phytophthora cryptogea. Plant Physiol. 98: 936-941.

Yu LM., 1995. -Elicitins from *Phytophthora* and basic resistance to tobacco. Proc. Natl. Acad. Sci. USA, 92: 4088-4094.

Zanetti A., Beauvais F., Huet C.J. and Pernollet J.C., 1992. Movement of elicitins, necrosis-inducing proteins secreted by *Phytophthora* sp. in tobacco. Planta, 187: 163-170.

HISTOLOGY OF THE ZONE LINE IN SECONDARY PHLOEM OF MEXICAN OAK TREES INFECTED WITH *PHYTOPHTHORA CINNAMOMI*

F.H.Tainter[1], L. Jolley[1], A. Hernandez[2], F. Orozco[3], E. P. Van Arsdel[4]

[1]Department of Forest Resources, Clemson University, Clemson, SC 29634-1003
[2]Secretaria de Medio Ambiente, Recursos Naturales y Pesca, Progreso No. 5, Col. De Carmen, Coyoacan, o.p. 04110, Mexico, D.F,
[3]Secretaria de Medio Ambiente, Recursos Naturales y Pesca, Victoria No. 360, Colima, Mexico
[4] P. O. Box 1870, Tijeras, NM 87059-1870

ABSTRACT

A zone line forms within secondary phloem of stems of *Quercus glaucoides*, *Q. peduncularis*, and *Q. salicifolia* colonized by the root destroying fungus, *Phytophthora cinnamomi*. The most evident feature of the zone line is a uniform yellow to orange discoloration of the contents of parenchyma cells within the oak tissues, shown by histochemical staining to be composed of polyphenolics. Fungal hyphae are abundant within these parenchyma cells. Parenchyma cells at the leading edge of fungal colonization contain numerous starch granules but the quantity is markedly less as the parenchyma cells become discolored and are colonized by fungal hyphae. This is followed by their disintegration. While the zone line may be a distinctive host reaction to fungal colonization, it appears to offer little resistance to invasion by *P. cinnamomi*, other than perhaps forcing the fungus to colonize host tissues in an advancing front with a uniform leading edge.

INTRODUCTION

Since it was identified and described as the causal agent of stripe canker of cinnamon (*Cinnamomum burmannii* Blume) in Burma in 1922 (Erwin and Ribeiro 1996), *Phytophthora cinnamomi* Rands has proven to be a serious root-destroying pathogen of over 1,000 plant species, including numerous woody plant hosts. It is now recognized as a destructive pathogen of oaks (*Quercus* spp.) wherever that genus grows naturally or has been introduced. *P. cinnamomi* causes a multiple basal stem canker, known also as ink disease, of northern red oak (*Quercus rubra* L.) in southwest France (Robin 1992b), and *Q. suber* L. and *Q. ilex* L. also in France (Robin et al. 1998), and is associated with decline of *Q. ilex* and *Q. suber* in southern Spain and Portugal (Brasier 1996, Tuset et al. 1996), *Q. suber* (Mircetich et al. 1977) growing in California, *Quercus* spp. in Italy, Morocco, and Tunisia (Brasier 1996), and a cause of decline and mortality of *Q. glaucoides* Mart. & Gal., *Q. peduncularis* Née, and *Q. salicifolia* Née in Mexico (Tainter et al. 1999).

The name ink disease was coined over 100 years ago by European researchers to describe a disease on chestnut trees (*Castanea sativa* Mill.), which was later diagnosed to be caused by *P. cinnamomi* (Crandall et al. 1945), because of an inky-blue exudate that stained the soil close to the root. Stem cankers on some woody hosts such as cinnamon, macadamia, plane tree (Zentmyer 1980), and oaks (Mircetich et al. 1977) often have black exudates. An additional characteristic of *P. cinnamomi* infections in oak and chestnut is a distinctive, dark zone line which delimits the canker lesion (Jordan and Tainter 1996). The gross morphology of basal stem canker lesions in oak has been described by Robin et al. (1992a) but little is known of the histological characteristics of these lesions in oaks. This research describes the histological changes in the secondary phloem of three Mexican oak species following colonization by *P. cinnamomi*.

MATERIALS AND METHODS

On February 2-3, 1999, eleven trees each of *Q. glaucoides*, *Q. peduncularis*, and *Q. salicifolia*, which had been inoculated 111 days previously with a locally procured isolate of *P. cinnamomi* Rands (Tainter et al. 1999), were observed for resulting canker lesion development. On three inoculated trees of each species, tissue samples approximately 1 cm square and 5 mm thick, which incorporated the zone line and adjacent tissues, were removed with a razor blade.

The samples were placed in small vials containing nonchilled 3.5% glutaraldehyde/cacodylate fixing buffer, labeled, and brought back to the laboratory at Clemson University. They were then dehydrated with a graded series of concentrations of aqueous ethanol from 25% to absolute (Gahan 1984). Tissues were embedded using a JB-4 Embedding Kit (Polyscience, Inc.) and sectioned using a JB-4 Microtome (Sorvall, Porter-Blum).

Sections were mounted in Permount either unstained or after staining with various histochemical stains to identity subcellular compounds, especially those associated with the darkly stained zone line. Specific targeted compounds for histochemical staining included 2% ferric chloride in 95 % ethanol for polyphenols, iodine (0.5%) in 5% aqueous potassium iodide for starch, 1% ferric chloride in 0.1 N HCL for tannins, and 1% aqueous toluidine blue for nucleic acids (Gahan 1984). Callose was stained with fast green (Pearce 1986), and fungal hyphae were stained with safranin and picro-anilin blue (Johansen 1940). Resulting sections were examined at 100X and 200X with a light microscope, characterized, and diagrams prepared which illustrated the major findings. Terminology used to describe the cell types within the zone line is that proposed by Trockenbrodt (1990).

RESULTS

Since the samples were collected during the dry season, no foliage was present and foliar symptoms could not be determined. Black exudations, and dried residues of these exudations, were present on the bark around many of the inoculated areas. Every inoculated tree produced a canker lesion, whereas none were produced by the inoculated controls. Except for the exudations, canker lesions were not visible until the outer bark was shaved off. Each oak species produced a canker lesion which was somewhat unique in visible appearance to that particular oak species.

Quercus glaucoides - This species, especially older trees, has a thick, fibrous bark. The canker lesion margin in secondary phloem, although visually detectable, was delimited by a zone line that was not readily visible, especially on larger trees (Figure 1A). The colonized portion of the canker lesion remained a light to moderate brown color, not attaining the dark brown, to reddish, to black discoloration typical of that occurring on the other two oak species. The canker lesion margin was detectable, however, for a few seconds after the outer bark was removed. Then, the healthy outer phloem tissues surrounding the canker lesion quickly oxidized to nearly the same brown discoloration as the colonized portion of the lesion.

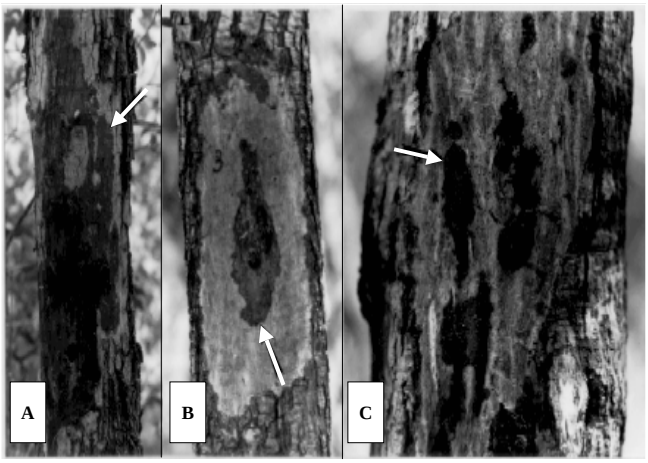


FIGURE 1 - Colonization of *Phytophthora cinnamomi* in Mexican oaks, showing resulting lesions in secondary phloem, on: A) *Quercus glaucoides*., B) *Q. peduncularis*, and C) *Q. salicifolia.* Arrows indicate zone line marking edge of lesion.

Microscopically, the secondary phloem, from the cambium outward to the rhytidome, was composed of parenchyma cells interspersed with visibly large clumps of sclerids and smaller clumps of fibers. In cross-sectional area approximately half of the cells were parenchyma cells, the remainder were equal amounts of sclerids and fibers and scattered sieve elements. Ray cells were generally obliterated or were sparse. There were occasional crystalliferous cells scattered throughout the secondary phloem.

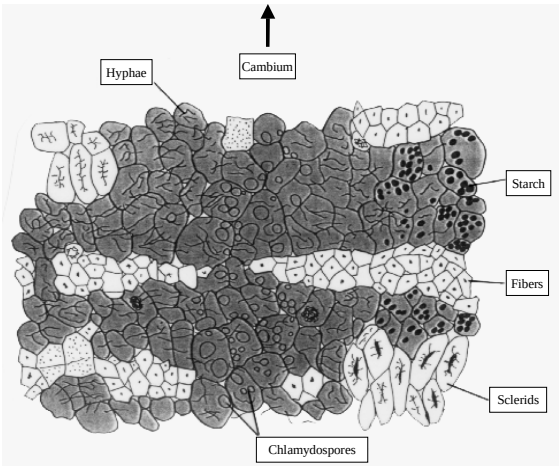


FIGURE 2 - Diagram of transverse section of portion of secondary phloem of *Quercus glaucoides*, showing deposition of starch, polyphenolics (shaded) in parenchyma cells, hyphae of *Phytophthora cinnamomi*, and chlamydospores, in relation to the pathway of the invading fungus which is moving from left to right

.

The zone line was approximately one mm wide and in its width contained only a few dozen affected cells. It was microscopically identifiable because the parenchyma cells were filled with a uniform yellow-to-orange-stained material, which seemed to be no less or more intense than a similar discolored zone in the other two oak species. The sclerid and fiber bundles and seive elements were not discolored. At the leading edge of fungal advancement, the parenchyma cells contained large numbers of starch granules (Figure 2). Behind this zone of advancement, starch markedly decreased and the incidence of hyphae increased. As the incidence of hyphae increased, large numbers of spherical swellings (chlamydospores) were present, often several per parenchyma cell. Immediately behind this zone the amount of fungal mycelium increased, there were few spherical swellings, and the cell walls of parenchyma cells appeared to become partially disintegrated.

The predominant host cells in the secondary phloem invaded by mycelium were the parenchyma cells and to only a limited extent ray cells, sieve elements, and companion cells. Cambial cells were also invaded. Mycelium was not observed in sclerids or fibers. Fungal colonization of the secondary phloem was effectively restricted from the bark by the first phellogen layer of the rhytidome but mycelium was able to cross the cambium and enter the xylem where it caused a compartmentalization type of reaction visible as a vertically extended bluish-black stain (Tainter et al. 1999) in the last-formed annual ring. The extent to which it invaded the xylem is not known as histological examination was not made of discolored xylem.

The histology of secondary phloem, and phloem lesions, in *Q. peduncularis* and *Q. salicifolia* was found to be very similar, hence the following description is for both with certain differences noted.

Quercus peduncularis - The outer bark was rough, moderately thick, but much thinner and not as fibrous as that of *Q. glaucoides*, but thicker than that of *Q. salicifolia*. After removal of the outer bark, the canker lesion appeared irregular in shape but always with a very dark zone line delimiting a moderately dark brown colonized area (Figure 1B) which contrasted with the lighter brown of the noncolonized secondary phloem. Noncolonized secondary phloem tissues did not oxidize upon exposure to the air as with *Q. glaucoides* and cankers remained visible during subsequent sampling. This species did not have a highly visible reddish colored rhytidome as did *Q. salicifolia*, otherwise the microscopic structure of the secondary phloem was very similar to that of *Q. salicifolia*. Fungal colonization within the zone line was also similar except that the parenchyma cells in the colonized portion of the zone line contained few to many spherical swellings.

Quercus salicifolia - The outer bark was smooth, tough, and fairly thin. After removal of the outer bark, the canker lesion was evident as a uniform, rich, dark chocolate-brown irregular area delimited by a distinct, nearly black zone line of uniform width (Figure 1C). The canker lesions were not very large in area on any given plane within the secondary phloem but appeared as separate islands of irregular shape. When succeeding layers of secondary phloem were shaved off, it became evident that the various islands were interconnected at different levels within the phloem. This characteristic was typical of the inoculated trees as well as naturally infected trees of this species which were observed during these sample collections.

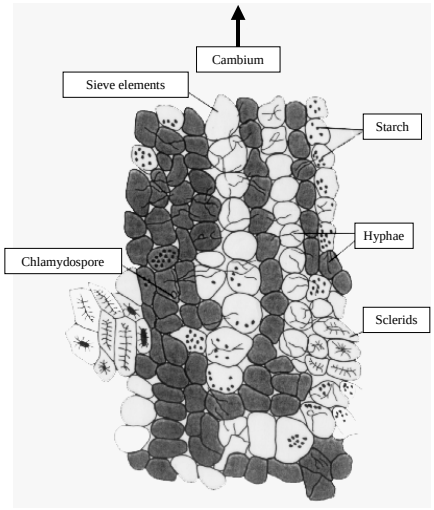


FIGURE 3 - Diagram of transverse section of portion of secondary phloem of *Quercus salicifolia*, showing deposition of starch, polyphenolics (shaded) in parenchyma cells, hyphae of *Phytophthora cinnamomi*, and chlamydospores, in relation to the pathway of the invading fungus which is moving from left to right.

The area examined microscopically included that portion of the secondary phloem from the cambium outward past the first phellogen

layer of the rhytidome. The dark zone line which delimited the area of fungal colonization indicated that portions of the canker lesion extended into areas of secondary phloem tissues which had remained extant between the newly formed arcs of rhytidome. In this species the rhytidome was a bright reddish color which contrasted with the tan color of the adjacent secondary phloem and was very visible.

In cross section the phloem tissues consisted mostly of parenchyma cells. In the nonfunctional secondary phloem these cells appeared to store large quantities of starch near to the zone line but then lost most of the starch as they were colonized by fungal hyphae. Microscopically the zone line discoloration was due to a uniform yellow-orange color deposited in a majority of these cells, also identified as polyphenolics. Also present in this region were clumps of sclerids interspersed amongst the parenchyma cells. Ray cells were present but usually colorless and often crushed by the maturation of adjacent cells. Sieve tubes, related companion cells, and fibers were relatively sparse.

The zone line was approximately one mm wide and was composed in width of only a dozen or so affected cells. The advancing mycelium was visible in the stained parenchyma cells and interspersed nonstained ray cells but did not appear to be abundant beyond that zone in the nonstained (healthy) parenchyma cells (Figure 3). There was a distinct diminution of starch granules in parenchyma cells as one followed fungal colonization across the zone line. Also, the incidence of starch granules was to some extent inversely related to the degree of discoloration in the parenchyma cells. As fungal mycelium passed through the zone of discoloration the yellowish contents of parenchyma cells became granular in appearance and the integrity of the tissues appeared to break down. Many more fungal hyphae were visible in this area. Mycelium did not appear to colonize the sclerid bundles at any time during this process and they continued to provide structural support for the affected tissues.

The yellow discoloration within parenchyma cells of all three oak species, and responsible for the darkly stained zone line, was identified as polyphenolics by the dark, blue-grey-green color imparted to their contents by the histochemical stain. The starch and nucleic acid stains were also useful as they enabled us to differentiate between host starch granules and spherical swellings produced by *P. cinnamomi*. The dark blue color produced by the starch stain in all three oak hosts indicated that these starches were long-chained. The safranin and picro-anilin stain for fungal hyphae enabled us to confirm the presence of fungal hyphae and also that the spherical swellings were fungal. The nucleic acid stain was also useful for differentiating fungal hyphae from host cytoplasm and in some instances between starch and spherical swellings. Callose was present in sieve elements within the zone line. Staining for gums and lignin did not produce any useful information.

DISCUSSION

There has been little published on histological aspects of phloem colonization by *P. cinnamomi* in oaks. The most complete description of stem lesion development in any oak species resulting from *P. cinnamomi* colonization are those of Robin et al. (1992b) but a brief unpublished histological description of stem lesions was limited (Robin 1992a). In *Q. rubra* stems inoculated with *P. cinnamomi*, necrotic lesions developed vertically in the secondary phloem and cambium, following phloem fibers and affecting all tissues up to the xylem, which was not colonized (Robin et al. 1992b). Xylem callus subsequently formed on the lateral margins of these necrotic lesions, thus limiting them, and allowed the affected tree to survive infection. The presence of tangential rings of sclerid fibers and sclerification of bark tissues were thought to be limiting to fungal colonization and it was the presence of these tissues that caused the pattern of fungal colonization in *Q. rubra* to be different from that in eucalyptus
(Tippett et al. 1983).

In susceptible eucalyptus a large arc of cambium was killed and some xylem discoloration was evident, resulting from what were termed 'aggressive' lesions (Tippett et al. 1985). In the resistant *Eucalyptus calophylla* Lindl., lesions killed the cambium at point of contact but were confined by periderm layers and then shed in a pattern typical of annual cankers. Phloem colonization in the three species of susceptible Mexican oaks resembles that in the susceptible *Eucalyptus marginata* Smith. In both groups, the lesion extension was marked by the phloem becoming discolored due to accumulation and oxidation of polyphenols and the deposition of starch which disappeared as the phloem tissues became necrotic and hydrolyzed. Within the Mexican oaks, the relatively smaller number of hyphae and secondary swellings within individual cankers of *P. cinnamomi* in *Q. salicifolia* as compared with *Q. glaucoides*, may reflect the greater resistance of that species evident in the field with natural infection.

An important commonality between disease expression in *E. marginata* and the Mexican oaks is extensive secondary phloem invasion, death of the cambium, and some degree of xylem discoloration. The visual extent of xylem discoloration in the three Mexican oaks is impressive and will be described in a later publication.

In the Mexican oaks, the rapidity of phloem colonization and concurrent cambium death in a tangential direction around the stem either directly cause subsequent tree death by girdling, or affect tree vigor to the extent that secondary pathogens and insects are able to colonize the secondary phloem, mechanically girdle the stem, and also cause relatively quick tree death (Tainter et al. 1999).

ACKNOWLEDGEMENTS

This research was supported in part by the McIntire Stennis Cooperative Forestry Program.

LITERATURE CITED

Brasier, C. M. 1996. *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. Ann. Sci. For. 53:347-358.

Crandall, G., G. Flippo, and M. M. Ryan. 1945. Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. Phytopathology 35: 162-180.

Erwin, D. C. and O. K. Ribeiro. 1996. Phytophthora Diseases Worldwide. APS Press, St.Paul, MN. 562 pp.

Gahan, P. B. 1984. Plant Histochemistry and Cytochemistry. Academic Press, London. 301 pp.

Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Inc., New York. 523 pp.

Jordan, A. P. and F. H. Tainter. 1996. The susceptibility of southern oaks to *Phytophthora cinnamomi*. Castanea 61:348-355.

Mircetich, S. M., R. N. Campbell, and M. E. Matheron. 1977. Phytophthora trunk canker of coast live oak and cork oak trees in California. Plant Dis. 61:66-70.

Pearce, R. B. 1986. Chlorantine fast green BLL as a stain for callose in oak phloem. Stain Tech. 61:47-50.

Robin, C. 1992a. La Maladie de l'encre du chene rouge *Quercus rubra* L. causee par *Phytophthora cinnamomi* Rands: Perspectives pour l'amelioration genetique de la resistance. Doctoral dissertation, No. 157, Universite de Bordeaux II. 140 pp.

Robin, C. 1992b. Trunk inoculations of *Phytophthora cinnamomi* in red oaks. Eur. J. For. Path. 22:157-165.

Robin, C., M. L. Desprez-Loustau, and C. Delatour. 1992a. Spatial and temporal enlargement of cankers of *Phytophthora cinnamomi* in red oak. Can. J. For. Res. 22:362-366.

Robin, C., M. L. Desprez-Loustau, and C. Delatour. 1992b. Factors influencing the enlargement of trunk cankers of *Phytophthora cinnamomi* in red oak. Can. J. For. Res. 22:367-374.

Robin, C., M. L. Desprez-Loustau and C. Delatour. 1998. First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. Ann. Sci. For. 55:869-883.

Gahan, P. B. 1984. Plant Histochemistry and Cytochemistry - An Introduction. AcademicPress, London. 301 pp.

Tainter, F. H., J. G. O'Brien, A. Hernandez, F. Orozco, and O. Rebolledo. 1999. *Phytophthora cinnamomi* as a cause of oak mortality in the state of Colima, Mexico. Plant Dis. 83: (In review).

Tippett, J. T., S. R. Shea, T. C. Hill, and B. L. Shearer. 1983. Development of lesions caused by *Phytophthora cinnamomi* in the secondary phloem of *Eucalyptus marginata*. Aust. J. Bot. 31:197-210.

Tippett, J. T., T. C. Hill, and B. L. Shearer. 1985. Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. Aust. J. Bot. 33:409-418.

Trockenbrodt, M. 1990. Survey and discussion of the terminology used in bark anatomy. IAWA Bull. 11:141-166.

Tuset, J. J., C. Hinarejos, J. L. Mira, and J. M. Cobos. 1996. Implicación de *Phytophthora cinnamomi* Rands en la enfermedad de la "seca" de encinos y alcornoques. Boletín de Sanidad Vegetal, Plagas 22:491-499.

Zentmyer, G. A. *Phytophthora cinnamomi* and the Diseases It Causes. Monogr. No. 10, American Phytopathological Society, St. Paul, Minnesota. 96 pp.

MANAGING THE RISKS OF PHYTOPHTHORA ROOT AND COLLAR ROT DURING BAUXITE MINING IN THE JARRAH FOREST OF WESTERN AUSTRALIA

Ian J. Colquhoun

Alcoa World Alumina Australia, PO Box 252, Applecross, WA 6253, Australia.

ABSTRACT

Control of *Phytophthora cinnamomi* in association with a large-scale bauxite mining operation in the jarrah (*Eucalyptus marginata* R. Br.) forests of Western Australia is an example of intensive disease management in native forest. It involves extensive use of disease mapping and hygiene measures to restrict the spread of the pathogen. Re-establishment of a jarrah forest on the rehabilitated mined area is a key environmental objective of the mining company. Monitoring procedures are in place to assess the effectiveness of the hygiene measures and audits occur regularly to ensure that the mine staff complies with the hygiene practices. The results indicate that the level of spread to adjacent uninfested forest is low – 0.003 ha of new infestation for every hectare cleared for mining.

INTRODUCTION

Alcoa World Alumina Australia operates open cut bauxite mines in the Darling Plateau, 55 km – 130 km south of Perth, Western Australia. Currently, the operations clear and rehabilitate 550 ha per year. The natural vegetation community of this region is tall open forest dominated by jarrah (*Eucalyptus marginata)* and marri (*Corymbia calophylla)* and is botanically diverse. Many of the plant species are susceptible to a disease called Phytophthora Root and Collar Rot (PRCR). This is caused by the introduced, root pathogen *Phytophthora cinnamomi*. It can kill jarrah trees and many understorey species, decrease botanical diversity and change species composition of native plant communities. Forest areas infested with *P. cinnamomi* are widespread but large areas of uninfested forest exist.

In 1990, Alcoa's Huntly mine was scheduled to move to a new mining locality where a high proportion of the forest was uninfested. Alcoa has a major environmental objective to minimise the spread of *P. cinnamomi* during its operations, so this presented a major environmental challenge.

Forest management authorities have established various regulations to minimise the spread of the pathogen by forest users. Essentially, these restrict access to some areas and provide for conditional access to the rest. As the pathogen is easily spread by water runoff and the transport of moist soil, access restrictions have mainly involved seasonal constraints. These allow most operations under dry soil conditions in summer months but prevent vehicular activity in wet soil conditions, mainly late autumn to early spring. Refining bauxite to alumina is a continuous operation that does not lend itself to seasonal variability in the supply of bauxite. The challenge for Alcoa's Mining Department was to develop a mining and environmental management system that allowed year-round access to the ore reserves, minimised the spread of *P. cinnamomi*, and was economical.

THE APPROACH

A multidisciplinary team composed of mine planners, field supervisors, environmental officers, production managers and a research scientist was established to develop the system to allow year-round access. The team systematically reviewed every stage of mining to establish:

- the risk of spreading the pathogen
- the effectiveness of potential control procedures
- the cost of the control procedures

Analysis of the mechanisms for, and the likelihood of, spreading the pathogen was based on the scientific understanding of the pathogen, its hosts, and the effects of the environment. It included examination of:

- the likely density of the pathogen spores in the material being disturbed during mining
- the likelihood of vehicles inadvertently transporting infested soil into uninfested areas
- the likelihood of mining causing water to drain from an infested site into uninfested forest.

The scientist contributed data on the likely presence of the pathogen while the field operators had the practical knowledge on transporting soil and altering drainage patterns. The other team members contributed to the selection of control procedures. Many of these involved changing mine schedules to exploit the seasons when the soil is dry and the risk of inadvertently spreading infested soil and water is very low. The mine planners helped determine if schedule changes were achievable.

'Off the shelf' control procedures were deemed unsuitable for some components of the mining process, and in some cases, new procedures had to be developed. Again, the scientific and operational knowledge of the team members was used to develop procedures that would minimise the likelihood of the spread of *P. cinnamomi*, but were practicable in the field.

CONTROL PROCEDURES

The team subsequently wrote a set of procedures for PRCR control. These were fully supported by the management of the company's Mining Department. They also had full support of the field supervisors and mine planners who felt strong 'ownership' of the procedures document. The procedures make use of the following strategic approach.

1.**Know where the pathogen is present** Reliable, up-to-date maps and field demarcation of diseased sites are the backbone of the control measures. Before commencing mining at the new locality at the Huntly mine, the presence of PRCR symptoms and screening of plants and soil were used to establish PRCR boundaries in the field. All the data on PRCR boundaries are stored on a Geographical Information System. This information is updated frequently to ensure that mine planners and environmental scientists have the best maps for their planning tasks.

2.**Intensively plan high-risk operations – schedule operations and locate mining infrastructure to minimise risk** Appropriate scheduling of operations can greatly decrease the risk of spreading *P. cinnamomi*. High-risk operations can be scheduled for the hot, dry months from November to May. For example, haul roads are usually

built during this dry soil period – this decreases the risk of inadvertently spreading infested soil on machinery and the risk of infested water draining into adjacent PRCR-free forest.
Other planning issues are also addressed to reduce the risks of spreading the pathogen; these include: the location of roads, the duration that a minepit is left active, the sequence of mining a large minepit and the location of stockpiles.

3.**Restrict vehicle movement from PRCR to PRCR-free areas** Success of the PRCR management strategy is reliant on the PRCR-free areas remaining free of *P. cinnamomi*. The 'unknown' presence of *P. cinnamomi* on a wet haul road has the potential to introduce *P. cinnamomi* to every PRCR-free area that vehicles visit. Controlling access to all PRCR-free areas is essential. This is achieved by blocking tracks so they cannot be used, using signs and bunting to limit access and inform users of access conditions, putting gates on all entry points to the mine, and constructing "bridges" across infested areas using gravel and rocks from uninfested sites.

4.**Clean vehicles before entering PRCR-free areas** Operations occur in both PRCR and PRCR-free sites so procedures need to be in place to minimise the risk of spreading infested soil across boundaries. Before any vehicle or mobile equipment moves from PRCR to PRCR-free sites, as much soil as possible needs to be removed. The most effective cleaning occurs in the workshop so scheduling is optimised to exploit workshop cleaning. Cleaning occurs at all stages of mining where vehicles are required to cross PRCR boundaries. Large trailer-mounted high-pressure water pumps are used in the field. Effective cleaning of vehicles in the field is difficult so the need for this task is reduced as much as possible by strategic planning.

5.**Don't mix infested and uninfested soils** The locations of boundaries between infested and uninfested areas are known and marked at all times during mining. In some cases a boundary needs to be moved further into the uninfested area for practical reasons, e.g., the PRCR area may be too small to enable a large mining vehicle to turn. However, this boundary change is marked in the field. Having obvious boundaries in the field means uninfested soil can be easily identified, enabling it to be moved and stored separately from the infested soil. All soil stockpiles are distinctively marked to show if they are infested or uninfested.

6.**Prevent water draining from infested to uninfested areas** Zoospores of *P. cinnamomi* are easily transported in water so surface water movement from infested to uninfested areas needs to be controlled. Surface water is not allowed to freely drain into the forest, irrespective of the PRCR status of the water or forest. Discharge is always controlled. In the rehabilitated minepits all surface water is directed back into the minepit, away from the forest. If an infested ore body site is located upslope of an uninfested site, then the duricrust in the lowest part of the boundary is disrupted by blasting or ripping with a bulldozer. The haul roads shed a lot of water. A system of drainage channels and high mounds prevents surface water flowing directly into the forest.

7.**Train all field staff and planners** PRCR control measures for each stage of mining and rehabilitation were documented during the risk assessment team meetings. All operators are trained in the control procedures relevant to their duties.

RESULTS

Government authorities also endorsed the Comprehensive PRCR Management System (as it became known) and constraints on the season of mining were removed for many of the stages of mining.

A detailed monitoring program was begun to assess the effectiveness of the PRCR management procedures. In 1996, forest classified as uninfested prior to mining was re-mapped for the presence of *P. cinnamomi*. Only 6.55 ha of recently infested forest was found adjacent to 1919 ha of land, which had been cleared for mining. If all the spread is attributed to the mining operations then this amounts to 0.003 ha of new infection for every hectare cleared for mining. If the pathogen moves downslope from these new infestations the area would increase to 28 ha; this equates to 0.014 hectares for every hectare cleared for mining. This is regarded as a tolerably low rate, though the objective of reducing it still further remains.

Major audits of the PRCR management procedures were undertaken in 1994 and 1997. These audits have assessed the mine's compliance with the documented procedures and the level of knowledge and understanding of the procedures. Opportunities to improve procedures were identified as part of these audits.

The procedures continue to be reviewed and revised. The revisions are based on:

- improved information on the mechanisms and/or likelihood of spreading the pathogen
- practical problems associated with implementing procedures arising from experience or prospective changes to operating conditions
- improvements in costing their implementation.

ACKNOWLEDGEMENTS

This paper is adapted from a case study presented in 'Best Practice Environmental Management in Mining Series - Environmental Risk Management', June 1999, published by Environment Australia, Canberra, ACT, Australia.

PHYTOPHTHORA ROOT AND COLLAR ROT IN REHABILITATED BAUXITE MINES AND THE ADJACENT *EUCALYPTUS MARGINATA* (JARRAH) FOREST OF WESTERN AUSTRALIA

G E. St. J. Hardy

School of Biology and Biotechnology, Division of Science and Engineering, Murdoch University, Murdoch 6150, Western Australia

SUMMARY

This study gives an overview of recent and current research activities that are being conducted in Western Australia on the biology, ecology and pathology of *Phytophthora cinnamomi* in rehabilitated bauxite mines and the adjacent jarrah (*Eucalyptus marginata*) forest. The work to date indicates that the biology of this pathogen does differ between rehabilitated mines and the adjacent jarrah forest.

INTRODUCTION

Western Australia is unique in its botanical diversity. There are over 7000 species of native vascular plants of which over 3000 are endemic (Keighery 1992). *Phytophthora cinnamomi* has a major impact on this diverse flora and directly effects over 2000 species from a diverse range of plant families (Wills 1993). Its indirect effects in terms of botanical impact through the loss of vertebrate and invertabrate pollinators, and loss of canopy and litter cover has not been measured. The pathogen was probably introduced into the state with the importation of nursery plants in particular fruit trees at the turn of the 20th century. The pathogen was then spread into the natural ecosystems principally by road building, forestry and other humankind activities. This spread has had a major impact in the jarrah forest, banksia woodlands and heathlands in the lower south-west of the state. The pathogen is an excellent example of an introduced pathogen with a wide host range causing considerable damage to a diverse community made of many susceptible plant species.

In Western Australia, the majority of past and current research on this pathogen has been conducted in the jarrah forest. This has been because *P. cinnamomi* has had a considerable impact on the ecology and management of the jarrah forest (Shearer and Tippett, 1989). The research was concentrated in the jarrah forest, since jarrah is an economically important hardwood species. However, more recently, research activities have also centered on the rehabilitation of bauxite mines. The jarrah forest in Western Australia is also the location of one of the largest and most productive bauxite mines in the world (Colquhoun and Hardy, 2000). The bauxite mining and alumina refining company, Alcoa Worldwide Alumina-Australia, supplies 22% of the world's alumina. In the jarrah forest, Alcoa mines and rehabilitates approximately 500 ha of forest annually. Although *P. cinnamomi* is estimated to only impact on 14% of the forest (Davison and Shearer 1990); many of Alcoa's ore bodies encompass areas of forest free of the pathogen. Since, *P. cinnamomi* is spread in soil and water the management of this pathogen between pathogen-free and pathogen-infested pits is daunting.

P. cinnamomi and the disease it causes in the non-mined forest

The jarrah forest occupies approximately 64,000 km^2 of the south-western corner of Western Australia (Abbott and Loneragan 1986). The forest grows on an ancient plateau composed of granite rocks intruded by dolerite dykes. The weathering of this rock has produced deep regolith profiles where the soil is usually about 0.5-1 m of gravelly sandy loam topsoil. Immediately below this soil layer is a concretionary layer of bauxite up to 5 m thick. Beneath this is a layer of up to 30 m of kaolinitic clay extending to bedrock. Bauxite mining occurs in those profiles where the alumina content is sufficiently high. These tend to occur in good quality forest (Abbot and Loneragan 1986).

The climate is typically mediterranean with cool wet winters and hot dry summers. The seasonal pattern of rainfall and temperature strongly influences temporal changes in the activity of *P. cinnamomi* in the jarrah forest. In addition, the soil architecture, its morphology, hydraulic properties, temperature and fertility are important factors that influence the life cycle of *P. cinnamomi* (Shearer and Tippett 1989). Jarrah forest soils are characteristically very permeable when moist, encouraging rapid infiltration of water through the soil profile. However, soil temperatures, soil fertility and soil water matric potentials and soil water content of the jarrah forest soils can be favourable for the activity of the pathogen (survival and dissemination) in autumn through to early summer in many areas. In addition, duricrust and clay layers can impede the vertical percolation of water resulting in near-surface transient perching with lateral flow and seepage of water below the soil surface in some upland areas (Kinal 1986). The importance of subsurface perching and lateral flow of water to the life cycle of *P. cinnamomi* cannot be overstressed. For example, it has been found at depths of 3 m below the soil surface (Shea *et al.* 1983; Kinal *et al.* 1987; Shearer and Shea 1987). Passive dispersal of the fungus downwards in root channels may be very important in distributing the fungus vertically within the soil profile. Water seepage from these upslope areas keep the gravel over clay soil profiles in the zone transitional between upland laterites and the headwaters of streams in shallow valleys moist well into the summer months (Shearer and Tippett 1989). Conditions are consequentially favorable for the survival of *P. cinnamomi* in these areas for most of the year.

There are approximately 600 species of vascular plants in the jarrah forest (Bell and Heddle, 1989), with an average of 58 species per 400 m^2 quadrat in the upland forest (Koch, J. pers. comm.). In the forest, *P. cinnamomi* has a patchy distribution with most of the valley floors being infested. In the western, high rainfall region of the forest the disease is present in many midslope and some upslope positions. The pathogen has been consistently associated with the death of understorey and overstorey species (Podger, 1972; McDougall, 1998).

P. cinnamomi and the disease it causes in rehabilitated bauxite mines

The mining process involves a defined sequence of events that results finally in a landscaped minesite seeded with a range of plant species similar to those of the adjacent forest . The process starts off with the removal of timber for wood products and firewood. The remaining timber is burnt. The top 5-10 cm of upper organic rich soil or topsoil is removed and immediately spread on to a rehabilitated minepit. This ensures minimal disturbance to seed bank and microbial biomass. The low organic gravel overburden is then removed to stockpiles adjacent to the future minepit or to a nearby minepit that has been mined. The duricrust is then shattered by explosives or bulldozer. This shattered ore is then excavated and transported to a primary crusher where the ore is broken into small

pieces and then transported to the alumina refinery. Once mined, the pit has had 3-5 m of ore removed, it is landscaped to fit the topography of the adjacent non-mined forest. The overburden from stockpiles is returned and then the topsoil. The rehabilitated minepit is then ripped with a winged tine to a depth of 1.2 m on the contour at 2 m spacing in a pattern that ensures no surface water from the pit enters adjacent forest. Ripping encourages the vertical infiltration of rainfall, decreases the risks of surface water ponding, minimizes surface water flow and provides vertical and horizontal cracks in the surface profile to enable roots to penetrate into the soil profile. Once ripped the pit is seeded with seed collected from adjacent forest. The process of mining and rehabilitation has been described in more detail by Colquhoun and Hardy (2000).

The mining process has therefore resulted in a substantial change in the forest. It has removed the duricrust layer and caprock. There has been a major disturbance of the organic rich topsoil and the age of plants is uniform. The disease triangle (Figure 1) outlines how the mining process has changed the host and environment relationships with *P. cinnamomi* and the diseases it causes.

In order to understand how *P. cinnamomi* was killing jarrah in pits, it was decided to sample young trees showing very early symptoms of stress. Symptoms included slight yellowing and purpling of the foliage. Unexpectedly, these trees has lesioned collar tissue at, or just above the soil line. At no time were roots necrotic in the absence of necrotic collars or lignotubers (Hardy *et al.* 1996). These results were in contrast to those from the jarrah forest which clearly show that it is the roots of jarrah that become infested (Shearer and Tippett 1989). It is for this reason we now consider *P. cinnamomi* as a root and collar rot pathogen (Colquhoun and Hardy, 2000). In the forest, it is believed that the pathogen most likely enters through the roots of primary structure or wounds and gradually moves up to lateral roots and the collars (Shearer and Tippett 1989).

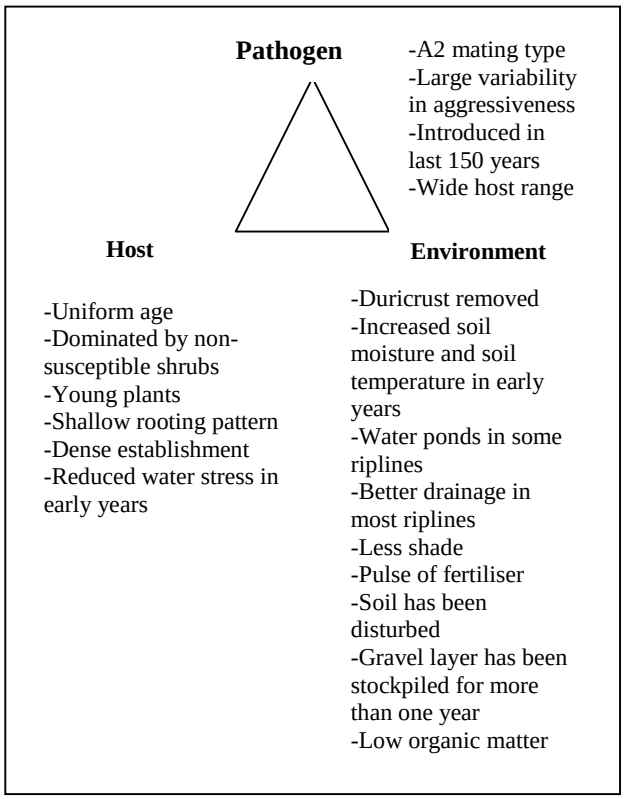


Figure 1. Disease triangle of *Phytophthora cinnamomi* in rehabilitated bauxite mines

Therefore, we (Hardy and Colquhoun, unpublished data) decided to test the null hypothesis that 'in rehabilitated bauxite mines, roots become infected and plants die as a result of the pathogen moving up the roots into the collars and girdle the stem'. A large trial was established in an uninfested minepit. In all, three hundred and sixty large bags which allowed water movement, but prevented large root growth through the bags were filled with minesite soil and buried in the minepit. Two six-month old jarrah seedlings were planted into the bags. At the time of planting three 19 X 600 mm hollow plastic tubes were placed at even spacing vertically into the bags. After 6 months, one seedling was removed from each bag. Approximately, 12 and 18 months after planting (the following spring and autumn) wooden plugs (20 mm x 12-18 mm dia.) colonised with *P. cinnamomi* were placed at different depths in the bags by removing the tubes and inserting the plugs. This ensured that minimal soil disturbance and root damage occurred. Once inoculated the bags were harvested 2, 4, and 8 weeks after inoculation or when symptoms were observed. At harvest, the bags were gently removed from the surrounding soil with a mechanical excavator and the soil around the trees was removed with care by hand to expose roots and plugs, respectively. The types of roots (fine, lateral or tap) which were in contact with the plugs, the presence and absence of lesions and the recovery of *P. cinnamomi* from the roots and plugs were recorded. At no time over 2 years were disease symptoms or the presence of lesions recorded. *P. cinnamomi* was never recovered from any plant material including those roots which were in contact with the inoculum plugs. This was despite *P. cinnamomi* being recovered in all cases from the inoculum plugs for the duration of the trial. Therefore, these observations confirmed our initial survey which indicated that in rehabilitated minepits roots do not readily become infected.

We therefore, instigated a study to determine if zoospores were able to infect and colonise jarrah through the periderm of non-wounded stems under conditions of temporary waterlogging. Trials were conducted in the glasshouse and field which mimicked temporary inundation or ponding of lower stems. This involved placing inoculum receptacles around the stems of trees into which motile zoospore suspensions of *P. cinnamomi* could be were placed. These studies (O'Gara 1999; O'Gara et al. 1996) clearly indicated that 100% of trees inoculated in this fashion became infected and that wounding was not required for the infection process to occur. These studies also showed that only short periods of ponding (2 days) was required for infection to occur. Another study showed that in the field these ponds were never hypoxic (Burgess *et al.*1999a).

In the 1980's a program was established by Alcoa, Murdoch University and CALM to select jarrah trees surviving in 'graveyard' sites. Seeds from these resistant clones were collected, propagated and screened for resistance against isolates of *P. cinnamomi* (Stukely and Crane, 1994). The most resistant seedlings were clonally propagated through tissue culture (McComb et al. 1990; 1992). Ninety-eight clones of jarrah that demonstrate resistance to *P. cinnamomi* in the glasshouse have been tissue cultured. Field trials have been established to check the field resistance of these clones. This involves planting clonal trees on a range of sites and inoculating the soil surrounding each plant with wooded plugs colonised with 4 isolates of *P. cinnamomi.* Since resistance to *P. cinnamomi* is a highly heritable trait (Stukely and Crane 1994), seed orchards have been established to produce plants that are cheaper than the average cost of US$4 a clonal plant.

However, despite the success of the clonal jarrah program we still had concerns about the long-term survival of these clones in PRCP sites. These concerns were raised from our observations that there is a large variation in the pathogenicity of isolates of *P. cinnamomi* with some isolates being significantly more pathogenic that the isolates which were originally used to screen for resistance. Our concerns were also increased by the observation that temporary ponding in certain rehabilitated pits in the presence of the pathogen resulted in stem not root infection. We also felt that temporary waterlogging and/or hypoxia might predispose the resistant clones to PRCR. We also wanted to determine whether older plants (6-7 years old) remained resistant to the pathogen, since all the earlier screenings had been conducted on approximately one-year-old plants. Finally, we wanted to confirm earlier observations that there was still a good

correlation between resistance in plants inoculated in the stems compared to root inoculation. Original selection of resistant clones had been based on underbark inoculation of stems in seedling jarrah that had been shown to compare favourably with root inoculation.

With our early field trial to determine how jarrah were becoming infected in minepits we had collected a large range of *P. cinnamomi* isolates from diseased and dying jarrah and marri trees (Hardy et al. 1996). Initial morphological studies indicated that isolates from marri and jarrah were slightly different from each other in oogonial and sporangial characteristics. However, this was not found to be the case although we did find that *P. cinnamomi* was able to produce multiple paragynous antheridia in addition to the amphigynous antheridium (Hüberli et al. 1997a; 1997b).

The pathogenicity of 84 isolates form marri and jarrah was then assessed in a number of trials. (i) The 84 isolates were screened on one year-old excised marri and jarrah stems over 3 days under controlled laboratory conditions. The results indicated that the isolates were equally pathogenic on marri or jarrah, regardless of their original host (Hüberli , Tommerup and Hardy, unpublished). However, the isolates did vary significantly in pathogenicity. (ii) All 84 isolates were then screened for pathogenicity in an evaporatively cooled glasshouse inoculation trial of a resistant jarrah clone (6 replicate plants per isolate). (iii) Ten isolates ranging in pathogenicity (4, 4 and 2 isolates highly, moderately and weakly pathogenic, respectively) were then screened on 2 resistant and 1 susceptible clone in climatically controlled phytotrons under a range of temperature regimes (15, 20, 25 and 32 °C) with plants either underbark inoculated or inoculated with zoospores. (iv) In the field, a range of 1 and 6 year old clonal plants of varying resistance (4 RR and 2 SS clones and seedlings) where underbark inoculated with one highly pathogenic and one weakly pathogenic isolate of *P. cinnamomi* selected from the previous trial. (v) Finally, we examined *in planta* the relationship between lesion development on roots and stems of resistant and susceptible clonal trees after underbark inoculation (Hüberli , unpublished).

Plants were either underbark inoculated or inoculated with zoospores using the methodology of O'Gara et al. (1996). Briefly, plants were underbark inoculated with *P. cinnamomi* colonised Miracloth (Calobiochem, USA) discs (5 mm^2 in glasshouse studies or 5 cm^2 in field studies), whilst motile zoospores were placed into plastic receptacles on the collars of plants. There was a large variability in the pathogenicity of the 84 isolates when excised stems of jarrah were inoculated and harvested 3-4 days later. This observation was repeated when the single clonal line of 18 month-old jarrah was underbark inoculated in stems *in situ*. However, there was not a good correlation in pathogenicity between isolates inoculated into excised stems and those inoculated *in planta* with zoospores or colonised Miracloth discs. There was, however, a good correlation between zoospore inoculation and underbark inoculation. In the glasshouse inoculation trial, the most pathogenic isolate killed all trees within 5 weeks. In contrast, the least pathogenic isolate failed to kill any plants during the 26 week trial period. In the temperature controlled phytotron trial the two resistant clones died as rapidly as the susceptible clone at 28 °C, whilst at 20 °C the susceptible clone died significantly faster than the resistant clones (Hüberli et al. 1998). In the 1 and 6 year-old field trials, one resistant clone was more susceptible than the susceptible clones, whilst seedling trees were more susceptible than the *P. cinnamomi* susceptible clones. However, no differences were observed in pathogenicity between the two isolates. To date there does not appear to be a difference in susceptibility between the 1 year old and 6 year old clonal plants. Finally, a good relationship was found between lesion development in the stems and roots of the jarrah plants. This was heartening as it continues to justify the use of stems to screen for resistant jarrah plants.

Our work has also shown that there is a large variation in the pathogenicity between isolates of *P. cinnamomi* (O'Gara *et al.* 1997; Hüberli *et al.* 2000). It appears that host-environment interactions influence the pathogen as to whether it will express itself as a biotroph or a necrotroph. We have also shown that measuring lesion lengths as a means of determining pathogenicity is inappropriate. Often an isolate under a given set of conditions (for example, temperature and host water status) can colonise the host extensively with only a very small lesion apparent. Another isolate under the same set of conditions can produce large lesions and little colonisation of tissue beyond the lesion. The former isolate in the past might have been considered less pathogenic than the latter. Yet under field conditions be the more aggressive of the two. This observation has management implications. For example, the screening process used to select resistant jarrah for clonal production has only used a few isolates. These isolates are intermediate in their pathogenicity, as a consequence the selection process has not been rigorous enough. At present we are trying to elucidate how the host and the environment interact with a range of isolates of *P. cinnamomi* to influence the expression of the pathogen as a necrotroph or biotroph. It is apparent that isolates of *P. cinnamomi* are extremely 'plastic' in their ability to express themselves as pathogens. This plasticity varies considerably with changes in the host and the environment, and it is this characteristic that probably helps account for the pathogen having such a wide host range across the lower south-west of Western Australia and elsewhere. Essentially, conditions under which one isolate is pathogenic another might not be pathogenic, however, as conditions change their ability to act as pathogens might change. Tommerup et al (2000) discuss the genetics and inheritance of these traits and their implications.

An aeroponics system was developed which allowed the roots of clonal or seedling plants to be readily subjected to hypoxia (2 mg O_2 l^{-1}) or anoxia. The system allows for the roots and stems to be easily accessible for inoculation with zoospores, non-destructive monitoring and harvest for enzyme and histological studies (Burgess and Hardy, 1996; Burgess et al. 1997; 1998). When plants were inoculated in the stems using the inoculum receptacle technique of O'Gara et al. (1996), colonisation by the pathogen extended further up the stems in a resistant clone than in a susceptible clone subjected to hypoxia rather than normal oxygen levels (Burgess et al. 1999b). This result was surprising as a much higher concentration of soluble phenolics leached into the inoculum receptacles from the resistant compared to the susceptible clones. Neither the duration of root hypoxia nor the timing of root hypoxia in relation to the ponding affected the final extent of stem colonisation by *P. cinnamomi*. If root hypoxia was imposed before inoculation the plants were stessed and the pathogen entered the stem rapidly; if hypoxia was imposed after ponding and inoculation, then the pathogen, which was present on the stem, was able to take advantage of the induced stress and penetrate rapidly

The activity of enzymes involved in the phenylpropanoid pathway (PAL, 4-Cl, CAD) were monitored in plants that were subjected to hypoxia or normal oxygen conditions in the absence or presence of the pathogen (Burgess et al. 1999c). Root hypoxia induced activity of these enzymes and their products in stems of the plants. Colonisation by *P. cinnamomi* further increased the activity of these enzymes. Actual levels of enzyme activity were higher in plants exposed to hypoxia, however, the relative increase in enzyme activity in response to the pathogen was greater in control plants grown under normal conditions. Peroxidase induction appeared to reflect tissue damage rather that plant defence. Overall, plants subjected to hypoxia were less able to switch on rapid defence responses against the pathogen.

Another study that also used the aeroponics system, examined the effects of varying periods of hypoxia (0, 2, 5, 11 or 29 days) on the roots of a resistant *E. marginata* clone before being inoculated with zoospores of *P. cinnamomi.* A similar set of roots was inoculated 3 days after the hypoxia treatments. All hypoxia treatments reduced root extension. However, 6 days after the hypoxia treatments, root extension had returned to normal for roots that had been exposed to 5 days of hypoxia, while for roots exposed to 11 or 29 days, extension was half the normal rate. Exposure to hypoxia for 5, 11 or 29 days was shown to reduce cell division, but not cell expansion. In the case of roots exposed to 3 days of hypoxia, the apical meristem appeared

normal at the end of the treatment, but 3 days after the return to normal oxygen conditions many of the apical meristems had died. Thus, *E. marginata* roots have an acclimatization period to hypoxia of between 2 and 5 days, after which they can tolerate hypoxia for extended periods. (Burgess et al. 1999c). Root extension ceased when a lesion developed. A hypoxic pretreatment effected lesion development posthypoxia by reducing the number of roots that became infected and any lesions that developed were significantly ($P<0.05$) smaller than those that developed under normal oxygen conditions. Six days after the resumption of normal oxygen conditions, lesion development in roots exposed to 5 days of hypoxia had returned to normal, while for roots that had been exposed to 2, 11 or 29 days of hypoxia, the lesions that developed were still significantly smaller. Therefore, root tips of jarrah exposed to hypoxia and then returned to normal oxygen conditions were more resistant to invasion by *P. cinnamomi* than roots grown under normal oxygen conditions. The study suggested that *P. cinnamomi* was only able to enter roots and infect through actively growing apices.

Current Research Activities

At present, we have a number of other studies in place. One study is determining the effects of drought on disease development after infection. Previous studies have shown that once bark moisture drops, *E. marginata* is able to contain lesion development (Tippett and Hill 1983; Tippet et al. 1987 ; Bunny et al. 1995). There is some concern that if the south-west of Western Australia starts to experience summer rainfall events, a predicted result of the greenhouse effect, then we will experience more deaths of jarrah. The study involves subjecting 2 year old clonal jarrah resistant or susceptible to *P. cinnamomi* to continuous watering, withholding water over summer (drought), or mimicking a summer rainfall event after inoculation with *P. cinnamomi* in spring. Plants are being harvested monthly over 2 years and rated for disease status. If continuous watering or summer rainfall events do increase deaths in jarrah on rehabilitated mines, it will be necessary to develop strategies to reduce this impact.

Another study is examining the long-term survival of *P. cinnamomi* in rehabilitated mines and adjacent jarrah forest. It is looking at saprophytic ability, potential exogenous and endogenous dormancy of chlamydospores and the effects of environmental factors on survival. Outcomes of this trial will be helpful in reducing the spread of *P. cinnamomi* in stockpiled topsoil or during the movement of soil and vehicles during the mining process.

Monitoring of rehabilitated bauxite mines for plant deaths caused by *P. cinnamomi* has indicated that deaths are less prevalent in areas where *Acacia* species are abundant. In the jarrah forest it has been observed that understorey dominated by *Acacia* species significantly changes the soil environment to one with a greater suppressive effect on the population levels of the pathogen (Shearer and Tippett, 1987). Therefore, we are examining the impact of a range of *Acacia* species on disease impact, sporangial production and zoospore release and survival of *P. cinnamomi* in rehabilitated minesite soils in the pits and in glasshouse studies.

The impact of *P. cinnamomi* is a major concern to the management of natural plant communities and rehabilitated communities during and after mining in Western Australia. This concern arises from the fact that this introduced pathogen has a wide host range which includes jarrah, the dominant tree species of the jarrah forest and many other understory species that make up key structural components of the forest. In order to effectively manage all the structural components of the vegetation in rehabilitated mines it is important to understand the dynamics of the pathogen and ascertain how this differs from the adjacent forest. This knowledge will assist managers to effectively reduce the impacts of *P. cinnamomi* in natural plant communities.

ACKNOWLEDGEMENTS

I would like to thank Emer O'Gara, Keith McDougall, Daniel Hüberli, Sarah Collins and Anne Lucus whose work has been mentioned in this paper, and Ian Colquhoun, Inez Tommerup and Bryan Shearer for their support and input over the years.

REFERENCES

Abbott, I., and Loneragan, O. W. (1986). Ecology of jarrah (*Eucalyptus marginata*) in the northern jarrah forest of Western Australia. Department of Conservation and Land Management, Perth, Australia. Bulletin No. 1.

Bell, D. T., and Heddle, E. M. (1989). Floristics, morphology and vegetation diversity. Pages 53-66 in: The Jarrah Forest. A Complex Mediterranean ecosystem. B. Dell, J. J. Havel and N. Malajczuk eds., Kluwer Academic Publishers, The Netherlands.

Bunny, F. J., Crombie, D. S. and Williams, M. R. (1995). Growth of lesions of *Phytophthora cinnamomi* in stems and roots of jarrah (*Eucalyptus marginata*) in relation to rainfall and stand density in mediterranean forest of Western Australia. *Canadian Journal of Forest Research* **25,** 961-969.

Burgess, T., and Hardy, G. E. St. J. (1996). Effect of low oxygen on peroxidase activity in roots of clonal *Eucalyptus marginata* infected with *Phytophthora cinnamomi*. Phytopathology 86:S102 (Abstract).

Burgess, T., McComb, J. A., and Hardy, G. St. J. (1997). Responses of *Eucalyptus marginata* to simulated waterlogging and *Phytophthora cinnamomi* infection. Acta Hortic. **447**, 401-406.

Burgess, T., McComb, J., Hardy, G. (1998). The use of aeroponic chambers to study the influence of low oxygen levels on root and lesion development of *Eucalyptus marginata* Donn. Ex Smith infected with *Phytophthora cinnamomi* Rands. Plant Dis. 82:368-373.

Burgess, T., Collins, S., Hardy, G. E. St. J., Colquhoun, I. J., and McComb, J. A. (1999a). A survey of the frequency, duration and oxygen content of surface and sub-surface water in rehabilitated mined areas at Huntly mine. Alcoa World Alumina Australia Research Bulletin No. 29, Applecross, Australia.

Burgess, T., Hardy, G.E.St.J., McComb, J.A., and Colquhoun, I. (1999b). Effects of hypoxia on root morphology and lesion development in *Eucalyptus marginata* infected with *Phytophthora cinnamomi. Plant Pathology* **48,** 786-796.

Burgess, T., McComb, J.A., Colquhoun, I. and Hardy, G.E.St.J. (1999c). Increased susceptibility of *Eucalyptus marginata* to stem infection by *Phytophthora cinnamomi* resulting from root hypoxia. *Plant Pathology* **48,** 797-806.

Colquhoun, I. J. and Hardy, G. E. St J. (2000). Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the *Eucalyptus marginata* (jarrah) forest of Western Australia *Plant Disease* **84,**

Davison, E. M., and Shearer B. L. (1990). *Phytophthora* spp. in indigenous forests in Australia and New Zealand. N.Z. J. Forest Science **19**; 277-289.

Hardy, G. E. St. J., Colquhoun, I. J., and Nielsen, P. (1996). The early development of dieback disease in *Eucalyptus marginata* and *Eucalyptus calophylla* growing in rehabilitated bauxite mined areas. Plant Path. **45**; 944-955.

Hardy, G. E. St J., Colquhoun, I. J. and Shearer, B. L. (2000). This publication

Hüberli, D., Hardy, G. E. St J. and Tommerup, I. C. (1997a). Morphological and physiological characteristics of two *Phytophthora cinnamomi* populations *Eleventh Biennial Australasian Plant Pathology Society Conference* Perth. p. 307.

Hüberli, D., Tommerup, I. C. and Hardy, G. E. St J. (1997b). The role of paragynous and amphigynous antheridia in sexual reproduction of *Phytophthora cinnamomi*. *Mycological Research* **101,** 1383-1388.

Hüberli, D., Tommerup, I. C., Hardy, G. E. St J. and Colquhoun, I. J. (1998). Temperature changes resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi. Seventh International Conference of Plant Pathology*, Edinburgh, Scotland. p. 2.5.4.

Hüberli, D., Tommerup, I. C. and Hardy, G. E. St J. (2000). False-negative isolations or absence of lesions may cause misdiagnosis of *Phytophthora cinnamomi* diseased plants *Australasian Plant Pathology* (in press)

Keighery, G. (1992). The impact of *Phytophthora* species on rare plants. Pages 29-36 in: Dieback – What is the future? M.J. Freeman, R. Hart, and M. Ryall, eds. Northern Sandplain Dieback Working Party, Perth, Australia.

Kinal, J. (1986). Perching and throughflow in a lateritic profile in relation to the impact of *Phytophthora cinnamomi* in the northern jarrah forest. Honours thesis, Murdoch University, Western Australia.

Kinal, J., Fairman, R. G. and Shearer, B. L. (1987). Perching and the throughflow in relation to the impact of *Phytophthora cinnamomi* in the jarrah forest. Abstract 20. Proceedings of the Australasian Plant Pathology Society Sixth National Conference. University of Adelaide, South Australia.

McDougall, K. L. (1996). Vegetation patterns in the northern jarrah forest of Western Australia in relation to dieback history and the current distribution of *Phytophthora cinnamomi*. Murdoch University, **1996 McComb, J.A., Bennett, I.J., Cahill, D., Stukely, M., and Crane, C.** (1992). Selection, propagation, field and laboratory testing of jarrah (*Eucalyptus marginata*) resistant to dieback (*Phytophthora cinnamomi*). IUFRO/AFOCEL conference on 'Mass production technology for genetically improved fast growing tree species'. Bordeaux, France. **2;** 451-452.

McComb, J. A, Bennett, I., Stukely, M and Crane, C. E. (1990). Selection and propagation of jarrah for dieback resistance. *Combined Proceedings of the International Plant Propagators' Society* **40,** 86-90.

O'Gara, E. (1999). Infection and disease of *Eucalyptus marginata* (jarrah), caused by *Phytophthora cinnamomi* in rehabilitated bauxite mines in the south-west of Western Australia. PhD thesis, Murdoch University, Murdoch, WA, Australia.

O'Gara, E., Hardy, G. E. ST. J., and McComb, J. A. (1996). The ability of *Phytophthora cinnamomi* to infect through unwounded and wounded periderm tissue of *Eucalyptus marginata*. Plant Path. **45**; 955-963.

O'Gara, E., McComb, J. A., Colquhoun, I. J. and Hardy, G. E. St. J. (1997). The infection of non-wounde and wounded periderm tissue at the lower stem of *Eucalyptus marginata* by zoospores of *Phytophthora cinnamomi*, in a rehabilitated bauxite mine. *Australasian Plant Pathology* **26,** 135-141.

Podger, F. D. (1972). *Phytophthora cinnamomi*, a cause of lethal disease in indigenous plant communities in Western Australia. *Phytopathology* **62,** 972-981.

Shea, S. R., Shearer, B. L., Tippett, J. T. and Deegan, P. M. (1983). Distribution, reproduction and movement of *Phytophthora cinnamomi* on sites highly conducive to jarrah dieback in south western Australia. *Plant Disease* **67,** 970-973.

Shearer, B. L., and Shea, S. R. (1987). Variation in seasonal population fluctuations of *Phytophthora cinnamomi* within and between infected *Eucalyptus marginata* sites of south western Aust. For. Ecol. Man. **21**; 209-230.

Shearer, B. L., and Tippett J. T. (1989). Jarrah Dieback: The Dynamics and Management of *Phytophthora cinnamomi* in the jarrah (*Eucalyptus marginata*) Forests of South-Western Australia. Department of Conservation and Land Management, Perth, Australia. Research Bulletin No 3.

Shearer, B. L. (1990). Dieback of native plant communities caused by *Phytophthora* species - a major factor affecting land use in south-western Australia. *Land and Water Research News***,** 15-26.

Stukely, M. and Crane, C. E. (1994). Genetically based resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*. *Phytopathology* **84,** 650-656.

Tippett, J. T., Crombie, D. S. and Hill, T. C. (1987). Effect of phloem water relations on the growth of *Phytophthora cinnamomi* in *Eucalyptus marginata. Phytopathology* **77,** 246-250 .

Tippett, J. T. and Hill, T. C. (1983). The relationship between bark moisture and invasion of *Eucalyptus marginata* by *Phytophthora cinnamomi*. *Australasian Plant Pathology* **12,** 40-41.

Tommerup, I. C., Dobrowolski, M.P., Hüberli, D., O'Brien, P.A., Hardy, G. E. St. J. and Shearer, B.L. (2000). Risks and threats due to genetic variation in *Phytophthora cinnamomi* for disease management in natural vegetation ecosystems. This proceedings.

Wills, R. T. (1993). The ecological impact of *Phytophthora cinnamomi* in the Stirling Range National Park, Western Australia. *Australian Journal of Ecology* **18**, 145-159.

Zentmyer, G. A. (1980). *Phytophthora cinnamomi* and the diseases it causes. *American Phytopathological Society Monograph* **No. 10**, St. Paul MN, 96 pp.

PHOSPHITE AND ITS POTENTIAL TO CONTROL *P. CINNAMOMI* IN NATURAL PLANT COMMUNITIES AND ADJACENT REHABILITATED MINESITES IN WESTERN AUSTRALIA

G E. St. J. Hardy

School of Biology and Biotechnology, Division of Science and Engineering, Murdoch University, Murdoch 6150, Western Australia

SUMMARY

To date the majority of work on the systemic fungicide phosphite (phosphonate) to control *Phytophthora* diseases has been conducted on horticultural crops. There is a paucity of work on the control of *Phytophthora* root and collar rots in natural plant communities. This paper gives an overview of studies conducted in Western Australia which examine the potential of using phosphite in plant communities rich in diversity and heavily impacted by *Phytophthora cinnamomi.* Details are given on possible beneficial and detrimental effects of using phosphite in natural plant communities to control *P. cinnamomi.*

INTRODUCTION

Phytophthora cinnamomi is a widespread and devastating plant pathogen in the south-west of Western Australia. It effects horticulture, mining, forestry and natural plant communities (Colquhoun, 2000; Hardy, 2000; Tommerup et al. 2000). Spot infections (< 1ha) in the *Eucalyptus marginata* (jarrah) forest or banksia woodlands and heathlands to be mined (alumina or mineral sand mines) cause operational problems for mining companies. In addition, new spot infections in the forest or heathlands caused by mining and other users are a threat to adjacent uninfested forest and heathlands. These 'spots' may be as small as one or two individual plants and do offer managers the opportunity to use systemic fungicides to minimise the risk of spreading the fungus, and to conserve trees and understory plants in the infested areas. The development of a method to contain or eradicate *P. cinnamomi* in such sites will help mining companies and managers of natural plant communities (National Parks, State Forests or Reserve Lands) meet their objectives of minimising the spread of the pathogen and to help financially reduce costs associated with hygiene.

The phosphite fungicides control many plant diseases caused by *Phytophthora*, even at concentrations *in planta* that only partially inhibit pathogen growth *in vitro* (Guest and Bompeix, 1984; Guest and Grant, 1991.). They are unique among fungicides in that they are translocated in both the xylem and the phloem (Ouimette and Coffey, 1989). In the phloem, phosphite is trapped and therefore translocated through the plant in association with photoassimilates in a source-sink relationship (Saindrenan et al, 1988, Ouimette and Coffey, 1990; Guest and Grant, 1991). Photoassimilates and therefore phosphite concentrations are thought to be higher in regions of the plant undergoing rapid growth, such as the roots and shoots (Whiley et al. 1995). The phosphite concentration in plant tissues is directly related to its application rate (Smillie et al. 1989). Phosphite treatment induces a strong and rapid defense response in the challenged plant (Guest and Bompeix, 1990). These defense responses stop pathogen spread in a large number of hosts. Phosphite exhibits a complex mode of action, acting directly on the pathogen and indirectly in stimulating host defence responses to ultimately inhibit pathogen growth (Guest and Grant, 1991). Phosphite has also been shown to inhibit sporulation of *Phytophthora* spp. at low concentrations (Farih, et al, 1981). Elicitors and chemicals such as phosphite are known to activate the phenolpropanoid pathway, although phosphite only stimulated host defences, including the phenylpropanoid pathway, after pathogen challenge (Saindrenan et al. 1988; Nemestothy and Guest, 1990). If resistance of plants to *Phytophthora* spp. is increased and the pathogen in the phosphite treated tissue cannot reproduce, then potentially the pathogen could be eradicated from the treated areas.

Research activities in Western Australia

In Western Australia, early work conducted by the Department of Conservation and Land Management showed that phosphite could control *Phytophthora* spp. in *Banksia grandis*, *E. marginata* and rare and endangered *Banksia* spp. (Komorek and Shearer 1997; Shearer and Fairman, 1997). They have applied the fungicide as trunk injection, foliar spray to run-off, and from aircraft as ultra-low-volume or mist sprays. Although these studies looked promising, they had only been conducted on a limited number of native plant species mainly from the Proteaceae and on *E. marginata.* Therefore, we initiated a number of studies to examine the potential of phosphite to control *P. cinnamomi* in native plant communities in and adjacent to mining. These studies aimed to determine:

- rates of phosphite that could be safely used in native plant communities
- if phosphite could prevent multiple deaths in a range of native plant species,
- could minimise the spread of *P. cinnamomi* from infested into uninfested areas,
- the persistence of phosphite in plant tissues and its long-term ability to control *P. cinnamomi,*
- if phosphite could prevent the sporulation and release of zoospores of *P. cinnamomi* from contained lesions,
- if phosphite adversely influences the reproductive fitness of annual and perennial plant species,
- if phosphite is detrimental to beneficial mycorrhizal associations, and
- differences between foliar application to run-off and foliar application as a mist (low volume application) in the uptake of phosphite in plant tissues.

If phosphite is found to be effective and safe to use the outcomes of these studies will provide us with a set of practicable and economic procedures for the application of phosphite in natural plant communities and rehabilitated minesites. Such information would include details of application rates, frequency of application and season of application.

Rates of application

Our research shows that phosphite as a foliar spray to run-off can safely be applied to natural plant communities at 5 g L^{-1} phosphite with 2.5 g L^{-1} synetrol oil (Organic Crop Protectants Pty. Ltd. NSW, Australia) as a sticking agent and that this rate controls *P. cinnamomi* in plant tissues for at least 6 months. Higher rates of phosphite (10 g and 20 g L^{-1}) result in phytotoxicity symptoms in a range of plant species, causing defoliation and death of some plant species. In contrast, as a mist application, phosphite is routinely applied at 24 kg ha^{-1} and controls *P. cinnamomi* for at least 2 years (Komerek et al. 1997). Since, the two methods of application (spray to run-off and mist or low-volume spray from aircraft) are being used, it was

decided to make a comparison of phosphite uptake between the two types of application. Eight month old *Eucalyptus calophylla* (marri) grown in an evaporatively cooled glasshouse were sprayed (i) to run-off with 2.5, 5 and 10 g L^{-1} phosphite, (ii) misted with 100, 200 and 400 g L^{-1} phosphite or (iii) as a 10 g L^{-1} phosphite soil drench. The phospite concentrations in plant tissues (root tips, shoot tips, mature roots, fully expanded leaves and mature leaves) were determined by High Performance Ion Chromatography (HPIC)(Roos et al. 1999), 7 days after the spray treatments. Phosphite concentrations were higher in the root or shoot apices than in other more mature parts of the plant. The highest concentrations were recorded in root tips of the soil-drenched plants. When the different foliar treatments were compared in the shoot apices, spray to run-off at 5 g L^{-1} gave a comparable concentration to the 100 g L^{-1} mist treatment, whilst a 10 g L^{-1} phosphite spray to run-off was comparable to 200 or 400 g L^{-1} phosphite mist treatment. A comparison of root apices revealed that spray to run-off at 5 and 10 g L^{-1} gave comparable concentrations to a 100 or 200 g L^{-1} mist treatment. All treatments except the 2.5 g L^{-1}, 5 g L^{-1} and soil drench (10 g L^{-1} phosphite) caused some phytotoxicity to the foliage.

The uptake and subsequent tissue (lower stem, lignotuber, tap root and lateral roots) distribution of phosphite in *E. marginata* growing in a rehabilitated minesite 7 days after being treated with 0, 5 or 10 g phosphite L^{-1} to run-off showed that significantly more phosphite was taken up by plants treated with 10 than 5 g L^{-1} phosphite to run-off (Pilbeam, unpublished). At 5 and 10 g L^{-1} the lower stems contained more phosphite (209 and 423 ug g^{-1} dry wt, respectively) than the other tissues. The lower stems contained more phosphite than the other plant tissues.

There are large differences in the uptake of phosphite and phytotoxicity in plants grown in the glasshouse and those growing naturally in natural plant communities. For example, after spray application of phosphite the concentrations of phosphite in glasshouse grown plants are much higher than those recorded in plants growing in the wild. Wilkinson et al (2000c) found that jarrah grown in the glasshouse and treated with phosphite contained 8 times more phosphite than jarrah in a rehabilitated minesite. The higher rates could be due to the higher relative humidity in the glasshouse compared to the rehabilitated mine sites. High humidity is thought to accelerate sorption of herbicides and pesticides through the stomata and cuticle by slowing the drying time of the spray droplet. (Fairbanks et al. (2000) found the levels of phosphite in glasshouse grown *Eucalyptus calophylla* 7 days after a 10g L^{-1} phosphite treatment to run-off to reach 3561 and 2550 µg g^{-1} in root tips and shoot tips, respectively. Also in the glasshouse, plants appear not to be so sensitive to the phytotoxic effects of phosphite as they are in the field.

Control of *Phytophthora cinnamomi*

Our research has shown that phosphite can contain the spread of *P. cinnamomi* in many native plant species from a range of susceptible families (Pilbeam et al, 2000a, b; Wilkinson et al, 1999; Barrett, unpublished). The pathogen disappears from the "walled off" lesions over time, but the rate at which it disappears depends on the isolate and the host. There appears to be a large variation in the survival and aggressiveness between isolates (Hardy, unpublished). However, when plants are inoculated 6-to-18 months after phosphite treatment, the ability of the fungicide to contain the pathogen is reduced. And in some plant species, 6 months after phosphite treatment, lesion development is slow but not halted in the stems of inoculated plants (Pilbeam et al, 2000b; Wilkinson et al, unpublished). This indicates that the pathogen is not being contained but rather slowed down in its ability to colonise host tissue, as a consequence it is likely that phosphite will need to be sprayed every 1-to-2 years.

In glasshouse studies (Wilkinson et al, 1997) showed that phosphite, when applied as a foliar spray, did not prevent *P. cinnamomi* sporulating from diseased tissue. Zoospores from this tissue were able to infect *Pimelea ferruginea* cotyledons, indicating that zoospores are capable of causing disease, this was despite the pathogen being effectively contained within the plant. This study was repeated in a rehabilitated minesite on 1-2 year old jarrah (Wilkinson et al, 1999). The stems of the jarrah were underbark inoculated and lesions were allowed to develop for approximately 7 days. The plants were then treated with phosphite as a foliar application, buckets were then attached to the stems below the lesions and the inoculated area of the stem was flooded. *Pimelea ferruginea* cotyledons were placed in the water and plated regularly onto a *Phytophthora* selective medium. A fine mesh was placed around the stems to ensure the leaves did not touch the stems and become infected through mycelial contact. In addition, aliquots of water were collected regularly and plated onto the *Phytophthora* selective medium. Once again, infected and phosphite treated plants were able to produce zoospores. Therefore, the treatment of infested sites may prevent death of plants but not necessarily prevent the spread of inoculum into non-infested areas. This observation also raises questions about the use of phosphite in container nurseries, where the pathogen may be controlled but not killed, and once plants are planted out the pathogen can be disseminated.

Effects on ectomycorrhizal fungi

Preliminary work on ectomycorrhizal and endomycorrhizal fungi indicates that when phosphite is applied at recommended rates (5 g L^{-1} foliar application to run-off) it has no detrimental effects *in vitro* or *in planta* on these symbiotic associations (Howard et al, 1999). In these studies a range of *Pisolithus tintorius, Scleroderma* spp., *Descolea* sp. and *Laccaria laccata* isolates were screened *in vitro* and *in planta* for sensitivity to phosphite. *In vitro*, growth of the isolates was stimulated by concentrations of phosphite that inhibits isolates of *P. cinnamomi*. *In planta*, phosphite application had no effect on ectomycorrhizal formation but did stimulate a four-fold increase in arbuscular mycorrhiza (AM) colonisation. However, at 10 g L^{-1} phosphite did significantly decrease infection by *Descolea* (Howard et al. 2000). This is the first study to examine the effect of phosphite on ectomycorrhizal fungi. However, there are conflicting results on the effects of phospite on AM in annual species. For example, Jabahi-Hare and Kendrick (1987) observed an increase in AM in leek treated with phosphite, whereas Seymour et al. (1994) and Sukarno et al. (1996) found phosphite to decrease AM in maize and onion, respectively. In addition, spore germination and root infection were also not adversely affected by phosphite (Howard, unpublished data). Therefore, although only a few isolates from a small number of species were tested, it appears that phosphite used at recommended rates will not be detrimental to ectomycorrhizal fungi in natural plant communities.

Phytotoxicity symptoms and plant uptake of phosphite

Phytotoxicity is a major problem associated with the application of phosphite. There is a fine balance between rates of phosphite applied, phytotoxicity symptoms and the control of *P. cinnamomi*. In general, as the rates of phosphite applied increase so do the phosphite concentrations in plant tissues. However, above 5 g L^{-1} (spray to run-off) or 36 kg ha^{-1} (mist or low volume application) phosphite phytotoxicity symptoms increase substantially in a large range of species from different genera and families. These high rates can result in plant deaths, reduced growth, growth abnormalities and reduced reproductive capacity. There is also a large variation in uptake of phosphite and phytotoxicity symptoms between plant species and within individuals of a plant species. For example, Pilbeam et al. (2000) found that after the foliar application of phosphite to run-off, the foliage of naturally growing *Adenanthos barbiger* and *Daviesia decurrens* had mean phosphite concentrations of 80 and 871 µg g^{-1} dry weight, respectively. In another study, also in a natural plant community, five weeks after phosphite application between 36 kg ha^{-1} to 144 kg ha^{-1}, foliar phosphite concentrations varied from 1400-4500 ppm, 73-185 ppm, 124-402 ppm, 481-1055 ppm and 672-590 ppm for *Jacksonia spinosa*, *Adenanthos cuneatus*, *Melaleuca thymoides*, *Lysinema ciliatum* and *Banksia coccinea*, respectively (Barrett, in preparation). All of these species were

closely associated with each other and indicate the large differences between plant species in their uptake of phosphite under a given set of conditions. These species also varied in their sensitivity to phosphite as indicated by phytotoxicity symptoms. *J. spinosa* and *L. ciliatum* expressed the highest phytoxicity ratings, whilst *B. coccinea* and *A. cuneatus* expressed the lowest phytotoxicity ratings. Overall, phytotoxicity increased with increasing levels of phosphite measured in the tissues. This again suggests that there are differences in the uptake of phosphite in these species. For example, *J. spinosa* is characterised by having stems that are in the form of green photosynthetic cladodes which may take up and retain higher phosphite concentrations than woodier stem material (Barrett, *pers. com.*). In addition, they do not shed leaves as a response to phytotoxicity, unlike many of the other species tested, which might also account for the high levels of phosphite measured *in planta*. Eleven macro- and micro-morphological leaf characteristics were assessed to determine which affected the phytotoxicity ratings. Growth form, leaf size, leaf orientation, position of veins relative to the leaf surface and mode of regeneration post fire (re-seeders or re-sprouters) did not influence phytotoxicity ratings (Barrett, in preparation). Whilst, plant height, leaf shape, leaf hairs, the distribution and position of stomata relative to leaf surface and the presence of oil glands significantly influenced phytotoxicity ratings. Phytotoxicity ratings were significantly higher in species with oil glands present and significantly lower in species which had stomata restricted to the lower surface and species with leaf hairs (Barrett, in preparation). In addition, the taller the plant species the greater the likelihood that phytotoxicity ratings would be high. The influence of stomatal characteristics on phytotoxicity ratings suggests that they may be important in phosphite uptake. The presence of leaf hairs also suggests that phosphite uptake is reduced by preventing effective spray contact, and droplet spread. Features such as cuticle thickness and epidermal cell size still need to be assessed for their effects on phosphite uptake and phytotoxicity (Barrett, *pers. com.*). There are large micro- and macromorphological differences of leaf characteristics between species within a genus that would account for the variation in phosphite uptake and effects of phytotoxicity. Growth abnormalities can also occur in a range of species from different families after phosphite treatment, these include 'little leaf' (Barrett, unpublished) and fasciation (Hardy, unpublished). Finally, in some species where phytotoxicity symptoms have been observed the incidence of aerial cankers can increase (Hardy, unpublished; Barrett, unpublished).

Effects on plant reproductive process

Pollen tube development and seed viability are definitely affected in the short-term by treatment with phosphite at recommended rates as foliar applications to run-off or as ultra-low volume foliar application (Fairbanks et al, 1998; Fairbanks et al, 1999). Phosphite influences plant species differently, depending upon their life cycle and when they flower in relation to the season of spraying. For example, phosphite at 2.5, 5 and 10 g L^{-1} (recommended rate is 5 g L^{-1}) and above significantly reduced *Dryandra sessilis* (perennial species) pollen fertility when the plants were sprayed in autumn and winter, with pollen germination being influenced up to one year after spraying. Pollen fertility was not affected after a spring spray possibly due to the time duration between spraying and when the plant flowered. Seed germination was not effected. Similar observations were observed for other perennial species (Fairbanks, unpublished). In an annual species, *Pterocheata paniculata,* it was shown that phosphite had no effect on plants sprayed in the vegetative stage, but when sprayed at flower initiation there was a reduction in pollen germination at 2.5 g L^{-1} and above. Seed germination was reduced by 5 g L^{-1}. Overall, phosphite has been found to affect annuals much more than perennials. In all species, pollen fertility was affected by phosphite concentrations lower than those recommended. It appears that in perennials despite pollen germination being affected seed germination was not. In contrast, in the annual species studied, seed germination is detrimentally influenced. This affect is enhanced in self-fertilising species (Fairbanks, unpublished).

In another study, also conducted in natural plant communities, phosphite influenced flower production and fruit production when applied before flowering and during flowering. Fruit production was significantly inhibited in a number of plant species after low volume phosphite applications at 36, 72 and 144 kg ha^{-1}. At 144 kg ha^{-1} inhibition was occasionally 100% (Barrett, unpublished). However, the long-term effects still need to be determined, especially on annual species. Flowering tended to be less affected than fruiting and often abortions of immature fruit were observed. In a number of species seed germination was markedly reduced by phosphite treatment and this reduction varied between species. Seed germination in some species was adversely affected by all phosphite rates, whilst in others it was only affected at 144 kg phosphite ha^{-1} (Barrett, unpublished). Phytotoxicity ratings did not in general correlate directly with effects on flowering and fruiting. In some species phytotoxicity ratings were low, yet phosphite had a marked effect on fruiting.

Stimulation of biochemical defence mechanisms

Although phosphite has been shown to be effective in the control of *P. cinnamomi* in *E. marginata* (jarrah), the biochemical mechanisms behind phosphite protection are poorly understood. Using an aeroponics system (Burgess et al, 1998) jarrah clones resistant to *P. cinnamomi* were treated with foliar applications of phosphite (0 and 5 g L^{-1}). The roots were then inoculated with zoospores of *P. cinnamomi* at 4 days before and 0, 2, 5, 8 and 14 days after phosphite application. Root segments were then analysed for the activity of selected host defence enzymes (4-coumarate coenzyme A ligase [4-Cl], cinnamyl alcohol dehydrogenase [CAD] and the concentration of soluble phenolics and phosphite. Lesions were most effectively reduced when the phosphite concentrations were the highest within roots between 8-14 days. During this time, the levels of host defence enzymes remained relatively unchanged. Lesions were also effectively restricted when phosphite concentrations within the roots were lowest (between days 2 and 5). However, a significant increase in host defence enzymes was associated with this decrease in lesion development in the absence of high phosphite tissue concentrations. We concluded that the control of the pathogen by phosphite is determined by phosphite concentration at the host-pathogen interface. When phosphite concentrations within the roots are low, phosphite interacts with the pathogen at the site of ingress to stimulate host defence enzymes. Whilst at high phosphite concentrations, phosphite acts directly on the pathogen to inhibit its growth before it is able to establish an association with the host. At this time, host defences remain unchanged (Jackson et al, 2000). There is still a need to examine how phosphite stimulates plant defences at the biochemical level.

Timing of phosphite application

Timing of phosphite application does not appear to influence the effectiveness of disease control. We found similar results between plants sprayed in spring and autumn. Generally, phosphite is sprayed in autumn in Western Australia since most plants are not flowering at this time, which should reduce any detrimental effects to reproduction at this time. However, we did find that if plants were drought-stressed, uptake of phosphite was less effective than in non-stressed plants. Therefore, it is appropriate to apply phosphite when the plant is not dormant or drought stressed as the chemical is not taken up effectively under these conditions.

Differences between glasshouse and 'field' trials

There are large differences in the uptake of phosphite and phytotoxicity in plants grown in the glasshouse and those growing naturally in the wild. For example, after spray application of phosphite the concentrations of phosphite in glasshouse grown plants are much higher than those recorded in plants growing in the wild. Wilkinson et al (2000c) found that jarrah grown in the glasshouse and treated with phosphite contained 8 times more phosphite than jarrah

in a rehabilitated minesite. The higher rates could be due to the higher relative humidity in the glasshouse compared to the rehabilitated mine sites. High humidity is thought to accelerate sorption of herbicides and pesticides through the stomata and cuticle by slowing the drying time of the spray droplet. (Fairbanks et al. (2000) found the levels of phosphite in glasshouse grown *Eucalyptus calophylla* 7 days after a $10gL^{-1}$ phosphite treatment to run-off to reach 3561 and 2550 $\mu g\ g^{-1}$ in root tips and shoot tips, respectively. Also in the glasshouse, plants appear not to be so sensitive to the phytotoxic effects of phosphite as they are in the field.

Phosphite resistant *Phytophthora cinnamomi* isolates

Recently we have found that there is evidence of *P. cinnamomi* resistance to phosphite treated plants among isolates from native vegetation which have not been exposed previously to phosphite (Wilkinson *et al*. 1999b; Hüberli *et al.* 2000). This observation is of concern especially since large areas of vegetation in Western Australia are being and will be sprayed at regular intervals in the future. Regular spraying will provide a selection pressure for these more phosphite resistant isolates and could pose additional problems to managers in the future. There is also some evidence that these more phosphite 'tolerant' isolates are more pathogenic than the less 'tolerant' isolates. Therefore, more research is required to examine these observations in detail. In addition, there is not a good correlation between phosphite tolerance *in vitro* and that *in planta.* Seventy-one isolates of *P. cinnamomi* (68 from Western Australia) were tested for sensitivity to phosphite on agar. Isolates could be divided into sensitive (9% of isolates), intermediate (82% of isolates) and tolerant (9% of isolates) groups. Sensitivity varied greatly between isolates with EC50 values ranging from 4 to 148 µg phosphite/mL (Wilkinson et al. 2000a). Selected isolates that were tolerant to phosphite *in vitro* were not tolerant to phosphite *in planta* (Wilkinson et al. 1999; Wilkinson et al. 2000c). Therefore, in order to screen for phosphite tolerant isolates of *P. cinnamomi* it appears to be a requirement that screening is conducted *in planta.* In addition, *P. cinnamomi* can be isolated from plants that have effectively stopped its colonisation and walled it off and it can be isolated from months to years after being contained (Ali and Guest, 1989; Shearer, *pers. com.*; Pilbeam, 2000b). The levels of phosphite *in planta* are often higher than those in agar on which the isolate would be inhibited. For example, Wilkinson et al. (2000c) found phosphite concentrations detected in stems of *Banksia hookeriana* and *E. marginata* in a glasshouse trial to be 40 and 14 times higher, respectively, than the highest levels used *in vitro*. However, the growth inhibition of the isolates *in planta* were less than those *in vitro*. Therefore, if phosphite directly inhibits growth *in planta* when it is present at high levels then it would be expected that the 12 *P. cinnamomi* isolates used in this study would have been more inhibited *in planta* than *in vitro*, and this was not the case Wilkinson et al. (2000c). One isolate was found to be less inhibited than 5 other isolates when inoculated into phosphite treated jarrah in the field. This isolate was also the most tolerant of the 5 isolates to phosphite *in vitro*. However, another isolate found to be tolerant to phosphite *in vitro* was the most inhibited in plants on the minesite.

In conclusion, there appears to be no correlation between phosphite sensitivity *in vitro* and *in planta.*

THE FUTURE

It will be necessary to determine why the effectiveness of foliar applications of phosphite appear to be less effective than trunk injections. Shearer and Fairman (1997) have found that trunk injections can effectively contain the pathogen for longer than 5 years. In contrast, our results indicate that approximately 6 months after foliar application, the affects of phosphite are disappearing in some plant communities and that these applications will need to be repeated approximately every 2 years.

It will be beneficial to screen more surfactants and sticking agents at different concentrations with phosphite to see if phosphite uptake into plants can be increased without causing increased phytotoxicity.

More work needs to be conducted on the biochemistry of how phosphite activates plant defence mechanisms and these should be compared between *P. cinnamomi* tolerant and susceptible species. For example, in *E. marginata* (jarrah) the activation of defence mechanisms is much less pronounced than in *B. grandis* after phosphite application (Shearer, *pers comm.*). An understanding of how phosphite stimulates plant defence mechanisms may allow us to improve these mechanisms through other means.

The continued poor reproductive performance 12 –17 months post-phosphite treatment is of some concern. This is exacerbated by our observation that in order to control *P. cinnamomi* it is likely to be necessary to apply phosphite as a foliar application at least every 2 years. Therefore, it will be necessary to determine if regular spraying will exacerbate the effects on reproduction and how this will influence seed banks of perennial and annual species. The question of seed bank viability is important, since regular burns of the natural plant communities are made approximately every 7-10 years. However, it could be argued that *P. cinnamomi* is having a detrimental on plant communities where it is present and without phosphite these species (especially rare and endangered species) will be lost permanently. At least with phosphite germplasm material can be maintained for the future. Despite this we need to better understand the long-term impacts on plant reproduction with the continued use of phosphite.

It will be beneficial to examine host-environment-pathogen interactions in more detail after phosphite treatment, with particular emphasis on its uptake, persistence and effectiveness. There is some evidence that temperature, plant water status and nutritional status all influence the effectiveness of controlling *P. cinnamomi* in its hosts.

In conclusion, phosphite provides us with a very effective and cheap method of reducing the impact of diseases caused by *P. cinnamomi* in natural plant communities and rehabilitated minesites, and currently, it is really the only tool we have. It is, however, important not to rely on this chemical indefinitely and to continue research activities on finding other control strategies for this devastating plant pathogen.

ACKNOWLEDGEMENTS

I acknowledge the contributions made by the studies of Sarah Barrett, Janet Box, Meredith Fairbanks, Kay Howard, Daniel Hüberli, Ros Pilbeam, Kim Tynan, and Carla Wilkinson. Many thanks to Ian Colquhoun and Alcoa World Alumina, Bryan Shearer and Inez Tommerup for their continued support and collaboration.

REFERENCES

Burgess, T., McComb, J., Hardy, G. (1998). The use of aeroponic chambers to study the influence of low oxygen levels on root and lesion development of *Eucalyptus marginata* Donn. Ex Smith infected with *Phytophthora cinnamomi* Rands. *Plant Disease* **82,** 368-373.

Colquhoun, I. J. and Hardy, G. E. St J. (2000). Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the *Eucalyptus marginata* (jarrah) forest of Western Australia *Plant Disease* **84,** 116-127.

Colquhoun, I. J. (2000). This publication.

Fairbanks M. M., Hardy G. E. St. J., and McComb J. A. (1998). The effect of the fungicide phosphite on pollen germination, pollen fertility and seed germination. In: Proceedings of the XVth International Congress on Sexual Plant Reproduction, Wageningen (Abstract).

Fairbanks, M. M., Hardy, G. E. St. J., and McComb, J. A. (1999). Does phosphite affect pollination and seed viability in native plants? Abstract 57. Proceedings of the Ecological Society of Australia, Inc. Annual Conference, Fremantle, Western Australia.

Farih, A., Tsao, P., and Menge, J. (1981). Fungitoxic activity of efosite aluminium on growth, sporulation and germination of *Phytophthora parasitica* and *P. citrophthora*. Phytopathology 71:934-936.

Guest, D. I., and Bompeix, G. (1984). Fosetyl-Al as a tool in understanding the resistant response in plants. *Phytophthora Newsletter* **12**, 62-69.

Guest, D. I., and Bompeix, G. (1990). The complex mode of action of phosphonates. *Aust. Plant Path*. **19,** 113-115.

Guest, D., and Grant, B. 1991. The complex action of phosphonates as antifungal agents. *Biol. Rev*. **66**, 159-187.

Hardy, G. E. St. J. (2000**).** Managing the risks of Phytophthora root and collar rot during bauxite mining in the *Eucalyptus marginata* (jarrah) forest of Western Australia This publication.

Jabahi-Hare, S. H. and Kendrick, W. B. (1987). Response of an endomycorrhizal fungus in *Allium porrum* L. to different concentrations of the systemic fungicides metalaxyl (Ridomil) and fosetyl-Al (Aliette). *Soil Biology and Biochemistry* **19,** 95-99.

Jackson, T. J., Burgess. T., Colquhoun, I. J., and Hardy G. E. St. J. (2000). Biochemical effects of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. *Plant Pathology* **49,** 147-154.

Komorek, B. M., Shearer, B. L. Smith, B. and Fairman, R. (1997). The control of *Phytophthora* in native plant communities. Final Report to the Threatened Species and Communities Unit, Environment Australia. Department of Conservation and Land Management, Como, Western Australia.

Komorek, B. M., and Shearer, B. L. (1997). Phosphonate offers a practical method for the control of *Phytophthora cinnamomi* in native plant communities. In: Proceedings of the Australasian Plant Pathology Society Eleventh National Conference, Perth, Western Australia (Abstract).

Ouimette, D. G., and Coffey, M. D. (1989). Comparative antifungal activity of four phosphonate compounds against isolates of nine *Phytophthora* species. *Phytopathology* **79**, 761-767.

Ouimette, D. G., and Coffey, M. D. (1990). Symplastic entry and phloem translocation of phosphonate. *Pesticide Biochemistry and Physiology* **38,** 18-25.

Pilbeam, R. A., Colquhoun, I. J., Shearer, B., and Hardy, G. E. St. J. (2000). Phosphite concentration: Its effect on phytotoxicity symptoms and colonisation by *Phytophthora cinnamomi* in three understorey species *of Eucalyptus marginata* forest. *Aust. Plant Path*. (in press).

Pilbeam, R. A., Colquhoun, I. J., Shearer, B., and Hardy, G. E. St. J. (2000). The extension of *Phytophthora cinnamomi* in *Eucalyptus marginata* stems inoculated at different times after treatment with three concentrations of phosphite. *Australasian Plant Pathology* (in preparation).

Howard, K., Dell, B., and Hardy, G. E. St. J. (1999). Phosphite has no effect on ectomycorrhizal formation or persistence. Abstract 322 in: Proceedings of the Australasian Plant Pathology Society Twelfth National Conference, Canberra, Australian Capital Territory, Australia.

Howard, K., Dell, B., and Hardy, G. (2000). Phosphite and mycorrhizal formation in seedlings of three Australia Myrtaceae. *Australian Journal of Botany* (in press).

Hüberli, D., Tommerup, I. C. and Hardy, G. E. St J. (2000b). How variable are morphological and pathological phenotypes among *Phytophthora cinnamomi* isolates from two localised populations in Western Australia (to be submitted to *Mycological Research*).

Roos, G. H. P., Loane, C., Dell, B., and Hardy, G. E. StJ. (1999). Facile high performance ion chromatographic analysis of phosphite and phosphate in plant samples. *Communications in Soil Science and Plant Analysis.* **30**, 2323-2329.

Saindrenan, P., Barchietto, T., Avelino, J. and Bombeix, G. (1988). Effects of phosphite on phytoalexin accumulation in leaves of cowpea infected with *Phytopthora cryptogea. Physiological and Molecular Plant Pathology* **32,** 425-35.

Seymour, N. P., Thompson, J. P., and Fiske, M. L. (1994). Phytotoxicity of fosetyl-Al and phosphonic acid to maize during production of vesicular-arbuscular mycorrhizal inoculum. *Plant Disease* **78,** 441-446.

Shearer B. L., and Fairman R. G. (1997). Foliar application of phosphite delays and reduces the rate of mortality of three *Banksia* species in communities infested with *Phytophthora cinnamomi*. In: Proceedings of the Australasian Plant Pathology Society Eleventh National Conference, Perth, Western Australia (Abstract).

Smillie, R. H. , Grant, B. R. and Guest, D. (1989). The mode of action of phosphite: evidence for both direct and indirect modes of action on three *Phytophthora* spp. in plants. *Phytopathology* **79,** 921-926.

Sukarno, N., Smith, F. A., Smith, S. E. and Scott, E. S. (1996). The effects of fungicides on vesicular-arbuscular mycorrhizal symbiosis. II. The effects on area of interface and efficiency of P uptake and transfer to plant. *New Phytologist* **132,** 583-592.

Sukarno, N., Simth, F. A., Scott, E. S., Jones, G. P., and Smith, S. E. (1998). The effects of fungicides on vesicular-arbuscular mycorrhizal symbiosis. III. The influence of VA mycorrhiza on phytotoxic effects following application of fosetyl-Al and phosphonate. *New Phytologist* **139,** 321-330.

Tommerup, I. C., Dobrowolski, M.P., Hüberli, D., O'Brien, P.A., Hardy, G. E. St. J. and Shearer, B.L. (2000). Risks and threats due to genetic variation in *Phytophthora cinnamomi* for disease management in natural vegetation ecosystems. This publication.

Whiley, A. W., Hargreaves, P .A., Pegg, K. G., Doogan, V. J. Ruddle, L. J., Saranah, J. B. and Langdon, P. W. (1995). Changing sink strengths influence translocation of phophonate in avocado (*Percea americana* Mill.) tree. *Australian Journal of Agricultural Research* **46,** 1079-1090.

Wilkinson, C., Shearer, B., Holmes, J., Tynan, K., Dell, B., McComb, J., Maroudas, J., and Hardy, G. E. St. J. (1999). A comparison of the *in vitro* and *in planta* responses of *Phytophthora cinnamomi* isolates to phosphite. Abstract 231 in: Proceedings of the Australasian Plant Pathology Society Twelfth National Conference, Canberra, Australian Capital Territory, Australia.

Wilkinson, C., Hardy, G. E. St. J., and Shearer, B. L. (1997) The effect of phosphite (phosphonate) on *Phytophthora cinnamomi* zoospore production *in planta.* In: Proceedings of the Australasian Plant Pathology Society Eleventh National Conference, Perth, Western Australia (Abstract).

Wilkinson, C., Shearer, B., Holmes, J., Tynan, K., Dell, B., McComb, J., Maroudas, J., and Hardy, G. E. St. J. (1999). Does phosphite prevent zoospore production from *Phytophthora cinnamomi*-colonised plants in the field? Abstract 230 in: Proceedings of the Australasian Plant Pathology Society Twelfth National Conference, Canberra, Australian Capital Territory, Australia.

Wilkinson, C, Shearer, B. L., Jackson, T. J. and Hardy, G. E. St. J. (2000a). Variation in sensitivity of Western Australian isolates of *Phytophthora cinnamomi* to phosphite *in vitro. Australasian Plant Pathology* (in press).

Wilkinson, C., Shearer, B., Holmes, J., Colquhoun, I. , Tynan, Dell, B., and Hardy, G. E. St. J. (2000b). The effect of phosphite on *Phytophthora cinnamomi* zoospore production *in planta*. *Plant Disease* (submitted).

Wilkinson, C. J. , Holmes, J. M., Dell, B., Tynan, K. M., McComb, J. A., Colquhoun, I. J., and Hardy, G. E. St J. (2000c). Sensitivity of *Phytophthora cinnamomi* isolates to phosphite *in planta*. *Plant Disease* (submitted).

CONSERVATION STRATEGY FOR PORT-ORFORD-CEDAR

Donald L. Rose[1], Laura Chapman[2], Mike Martisch[2]

[1]Port-Orford-cedar manager, USDA Forest Service, Central Point, OR 97502, USA
[2]Forest Planning, USDA Forest Service, Eureka, CA, 95501-3834, USA

Port-Orford-cedar root disease (*Phytophthora lateralis*) is a methodical, pervasive killer of Port-Orford-cedar (*Chamaecyparis lawsoniana*). The spread of this disease can lull managers into inaction because the spread often is not spectacular and attention grabbing. Action is essential, however, to maintain Port-Orford-cedar (POC) as an ecological and economic component of our forests.

A conservation strategy to insure the continuity of Port-Orford-cedar in a variety of habitats and locations involves a complex mixture of approaches and a commitment from managers and users of forest lands where Port-Orford-cedar resides. Learning from the past and analyzing the spread of *Phytophthora lateralis* through research and historical case studies can give us clues to a direction for the future. Understanding the variety of environments where POC lives, the biology and epidemiology of the disease organism, and the limits and appropriate uses of genetic manipulation and management all play a part in the overall strategy.

General components of the overall strategy should include:

- Identify levels of risk at various scales - landscape, watershed, local projects, trails, and roads. Include consideration for all the factors affecting risk.
- Utilize all the management techniques available where appropriate, monitor their effectiveness, and adapt our strategy as needed. Develop new control methods being used in other countries.
- Continue to work toward genetic improvement and maintain gene banks.
- Educate the public and adjacent landowners to acquire a commitment from all visitors to the forest and neighbors. Coordinate control strategies between various landowners and agencies.
- Utilize research and administrative study to answer questions as they arise.

Identifying Levels of Risk

Describing risk across a landscape requires a knowledge of the factors which affect risk. Table 1 displays the main factors which have been identified which increase the level of risk in areas where POC resides. The factors listed are only those which managers have some control over. There are many other factors such as animal vectors, soil moisture, or land ownership patterns which are beyond the control of managers. It is best to concentrate our efforts on those factors which we can control.

Analyzing risk at a variety of scales can help prioritize where management treatments can be implemented first. As the scale of analysis becomes larger, the specificity of management actions increase greatly. Individual roads and trails would be the most specific level of risk analysis. It is important to consider human values when looking at individual projects. Individual stands or roads may rise in importance depending on the circumstance.

The following section of this paper describes a process which was used to describe risk at the rangewide scale for POC on National Forest lands in Oregon and California.

Analysis of Relative Risk at the Landscape Level

The most current maps available of Port-Orford-cedar stands were used to determine relative risk to Port-Orford-cedar stands from the two primary vectors of *Phytophthora*, roads and streams. Several protection classes were identified for the mapped Port-Orford-cedar stands utilizing topographic position, road and infection proximity, and potential or completed measures to prevent the spread of the disease as factors. The protection classes which were mapped included:

1. Port-Orford-cedar stands which are in roadless or formally designated protected areas such as wilderness and Research Natural areas, with no roads within 500 feet of any part of the stand.
2. Port-Orford-cedar stands which are not in roadless or wilderness, but have no roads within 500 feet of any part of the stand.
3. Port-Orford-cedar stands protected by a gate or barrier.
4. POC stands in roadless or wilderness areas which have a road upslope outside of the non-roaded area.
5. POC stands where a mitigation measure could be implemented that would further reduce risk of infection.
6. POC stands downhill from roads that have been sanitized of POC.
7. POC stands in riparian zones where a *Phytophthora* infection source is present upstream.
8. POC stands where infested areas are directly adjacent to the stand. These stands have some risk due to animal or human transport upslope.

TABLE 1 Ranking of manageable physical, biological, human, and road related factors on relative dispersal of spores and spore density.

Factors affecting exposure and spore density	Risk	Control	Rank
Biological factors			
Proximity to stream or water or flood -plains	H	M	5
POC density, extent, justaposition	M	H	5
Density or cover of other hosts	M	H	5
Adjacent infection	H	H	6
Recent dead (density and proximity)	H	H	6
Seral stage	L	H	4
Road related vectors and factors			
Road density	H	M	5
Road surface	H	H	6
Proximity to road	H	M	5
Culverts	H	H	6
Ditches	H	H	6
Traffic density	H	M	5
Traffic type	M	H	5
Off-road vehicles traffic	H	M	5
Trails (same as roads)	M	H	5
Human projects and activities			
Harvest frequency	M	H	5
Harvest method	H	H	6

Two different maps were used to conduct the analysis with differing levels of accuracy between them. The analysis in California used current plant association mapping based on plots and field verification completed in 1998. This mapping was an intensive effort and is considered more accurate than the map used in Oregon, which is a combination of field observances, stand examinations, aerial photo interpretation, and formal surveys. Mapped stands of healthy Port-Orford-cedar were not available for BLM and private lands at the time of this analysis. Figure 1 displays an example of relative risk at the landscape scale. The visible dead trees were infected from a road crossing the stream at the upper end of these dead trees. Port-Orford-cedar trees upstream are uninfected, however there is another road above those trees. The road above the uninfected trees is recommended for closure and would show up as recommended

mitigation in the analysis. Uninfected Port-Orford-cedar downstream would be labeled as in-channel risk due to infection above. Port-Orford-cedar directly upslope and at least 500 feet away from any road, but not within the riparian zone, would be lower risk.

FIGURE 1 Infected Port-Orford-cedar in the landscape displaying concepts or relative risk

Table 2 shows that nearly 60% of the acres with POC stands mapped in California and approximately 43% in Oregon are in a relatively low risk location in the landscape due to their distance away from existing roads or protection status behind closed gates during the wet season. Less than 10% of the mapped area is or will soon be infected (Figure 2). Figure 3 shows an example of the results of the mapping for the National Forest lands in Oregon.

The nature of the distribution of Port-Orford-cedar is very different between California and Oregon. Port-Orford-cedar in California is relegated to riparian habitat more frequently and is not found on upland sites as much as in Oregon. Therefore mapping of the relative risk is probably more accurate and predictable in California than Oregon. Stands in Oregon that are in upland areas may show up in the table as at risk when in fact, these stands may be fairly low risk if they are not directly adjacent to roads and water sources. Infested stands also typically do not suffer 100% mortality. Trees on microsites and away from stream channels do survive even within areas shown as infected. Therefore, the number of trees infected overall is something less than 9%.

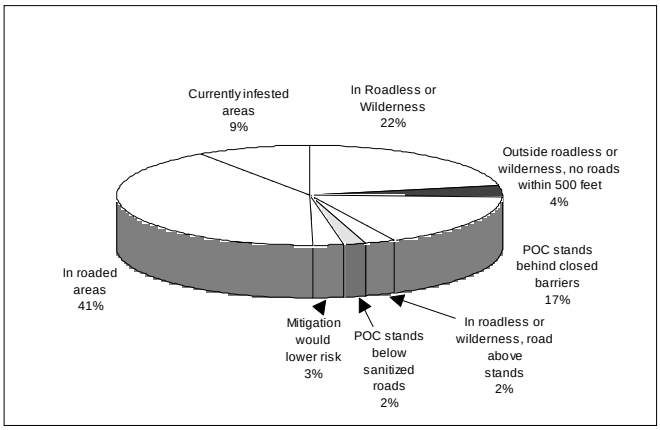


FIGURE 2 Relative risk summary for Port-Orford-cedar on National Forest lands in Oregon and California.

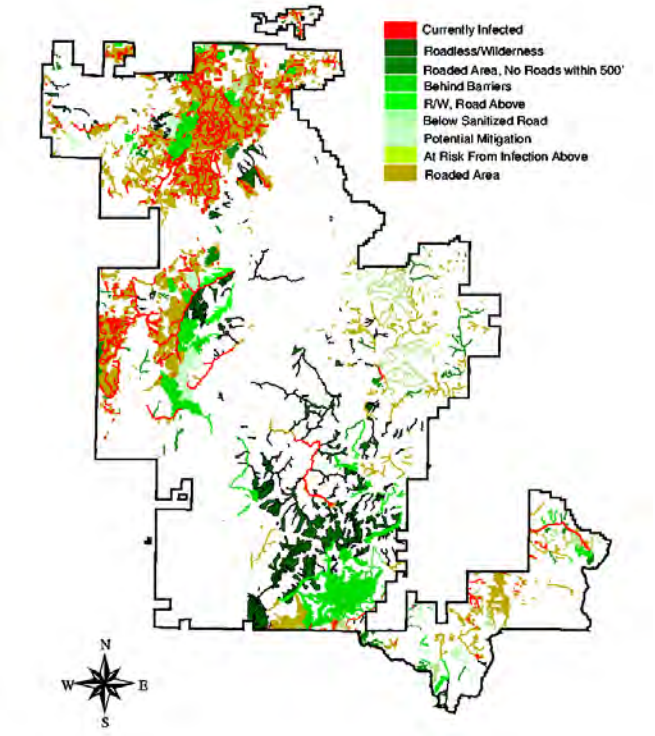


FIGURE 3 Relative risk map of Port-Orford-cedar stands on Siskiyou National Forest, Oregon, during summer, 1999.

The analysis of adjacency showed that a large majority of the stands that have some level of risk reduction are not adjacent to infested areas.

There are some cautions to keep in mind with this analysis.

Port-Orford-cedar stands in wilderness or roadless areas are not free of risk of infection by *Phythophthora*. They are relatively much lower risk than areas with roads.

Port-Orford-cedar stands protected by closed gates and barriers are not always effective due to gate vandalism, breach of closures, and some occurrence of wet periods during the dry season when the roads are open.

The scale at which the mapping was done (1:63,560) is relatively gross and is only useful for planning at the landscape level. Additional analysis of this type needs to be carried out at the watershed scale and project level in order to get an accurate picture of how much Port-Orford-cedar is likely to remain uninfected on the landscape and how much is already infected.

The mapping of relative risk needs to be expanded to include BLM and private lands within the natural range of Port-Orford-cedar, which is estimated to be about 50% of the total Port-Orford-cedar. The relative risk mapping should be used in conjunction with existing land management plans and operational plans to determine whether areas of high value are located in areas of low risk or land allocations where disturbance is not likely to occur. Recommendations on Port-Orford-cedar areas to conserve for assurance of future viability should take into account these relative risk ratings.

Utilize a Multitude of Management Techniques

There are a number of treatments which have been tried in southern Oregon or northern California. Many of these have proven to be very successful at preventing spread of the disease. These include:

Exclusion of humans from POC areas.
Exclusion during the wet season only.
Restricting project activities to dry season.
Sanitation of POC from roadsides.
Equipment and vehicle washing.
Road design and maintenance.
Water and rock selection and use.
Use of Clorox as preventive agent.
Planting of POC on low risk sites.
Exclusion of POC from high risk sites.
Prescribed fire.
Trail and trailhead relocation.
Developing and planting resistant POC.
Regulating non-timber forest users.
Public education programs.
Restricting order of operations on projects.

In addition to these management strategies, specific measures need to take place to insure that representative Port-Orford-cedar ecosystems remain in functioning condition throughout the range of POC.

A Proposed Conservation Strategy

1. Where we have plant associations mapped, manage a representative amount of each mapped plant association that has Port-Orford-cedar within each watershed in a relatively low risk condition. Try to manage more than one stand, where possible, for each plant association within each 5th field watershed in an area identified as low risk.
2. Insure that representative stands from ultramafic and non-ultramafic soils are in low risk areas. Maintenance of the distribution of plant associations within each watershed should provide for adequate representation of soils, however, we should check to verify this variation in parent soil types is represented since it is an important factor in genetic variation and seed zoning.
3. Rare or unique plant associations are likely representative of unique genes. These plant associations may be important to provide extra protection. Look at each rare association on a case by case basis to decide on appropriate actions. Examples of areas where extra efforts at protection may be needed include the Horse Mountain and Shelley Creek areas on Six Rivers NF, some populations within the upper Sacramento and Trinity Rivers, and healthy stands within the northern part of the range of POC between Port Orford and Coos Bay.
4. Where we don't have plant associations mapped, we may need to look at the distribution of Port-Orford-cedar and the location of low risk stands on a watershed by watershed basis (in some areas, live Port-Orford-cedar is yet to be mapped and some further data collection is necessary). We need to identify low risk populations in each 5th field watershed that represent each elevation band that Port-Orford-cedar is found within that watershed. Suggested elevation bands to represent are 0-1500 feet, 1500-3000 feet, 3000-3750 feet, 3750-4500 feet, 4500-5250 feet, 5250-6000 feet, 6000-6500 feet, and above 6500 feet.
5. Look at the likelihood of disturbance of the stands within the context of land allocations within the Northwest Forest Plan.
6. Work together as neighbors within watersheds with as many landowners as possible and have a consolidated effort at conservation. Work with watershed councils, user groups, industrial and non-industrial private landowners, and the general public to gain further acceptance and support of root disease prevention practices.
7. Concentrate efforts where the most gain can be made with the least input of money and time. Areas that are heavily used by people that are potential sources of spore dispersal should get more attention than isolated areas which have less traffic.
8. The allowance for bough cutting on Federal lands has caused disease spread to occur in the past. Additional measures are needed to limit the potential for spread while providing adequate amounts of boughs for the greenery market. Some suggestions include:
    a. Limit bough cutting to the dry season only.
    b. Restrict bough cutting to identified bough harvest areas such as planted areas under powerlines.
    c. Encourage production of boughs on private lands to provide enough boughs to meet demand.
    d. Determine the level of demand and timing of need for the boughs.
9. Continue development of disease resistant POC stock. Outplant genetically improved stock into microsites that are low risk in infected areas or in uninfected upland sites first.
10. Maintain adequate amounts of dead and down POC along streams. There may be opportunities, however, to salvage dead POC where a surplus of dead material is present. Involve wildlife and fisheries personnel to determine adequate amounts of riparian and stream structure needed.
11. Develop and utilize maps of clean rock and water sources. Use Clorox at a concentration of 1 gallon/1000 gallons of water in infested water if it is needed for use in dust abatement, fire control, road construction, or equipment cleaning.
12. Continue research and administrative study to answer questions about the disease, the hosts, and the ecological impacts of their interaction

TABLE 2 Protection status, infection status, and relative risk of infection of mapped Port-Orford-cedar stands on National Forest lands in southern Oregon and northern California.

Infection and Protection Status of mapped POC areas	Siskiyou NF Acres	California NF Acres	Total NF Acres	Siskiyou % of total POC	California % of total POC	Total % of total POC
Uninfected areas within wilderness or roadless areas and no infection nearby	36,636	11,036	47,672	16.1	29.0	17.9
Uninfected areas within wilderness or roadless areas with infected water source below stands	8,394	2,170	10,564	3.7	5.7	4.0
Uninfected areas at least 500 feet away from any roads outside of roadless or wilderness areas, no infection nearby	5,172	825	5,997	2.3	2.2	2.3
Uninfected areas at least 500 feet away from any roads outside of roadless or wilderness areas with infected water source below stands	3,321	790	4,111	1.5	2.1	1.5
Uninfected areas behind closed gates in roaded areas and no infection source below stands	26,911	7,153	34,064	11.8	18.8	12.8
Uninfected areas behind closed gates in roaded areas and infected water source below stands	10,964	762	11,726	4.8	2.0	4.4
Uninfected areas in roaded areas, roadsides have been sanitized and no infection source below stand	4,169	0	4,169	1.8	0	1.6
Uninfected areas in roaded areas, roadsides have been sanitized and infection source below stand	2,176	0	2,176	1.0	0	0.8
Uninfected areas with roads less than 500 feet above POC stands in wilderness or roadless and no infection below stands	1,225	1,360	2,585	0.5	3.6	1.0
Uninfected areas with roads less than 500 feet above POC stands in wilderness or roadless and infection source below stands	1,246	1,009	2,255	0.5	2.7	0.8
Uninfected areas in roaded areas with no protection measures in place, but if protection measure implemented, risk would be lowered [1]	14,981	2,073	17,054	6.6	5.5	6.4
Uninfected areas in roaded areas with no protection measures in place.	99,558	9,221	108,779	43.6	24.3	40.9
Uninfected areas in-channel and downstream from stands infected upstreams (very high risk).	70	180	250	<0.1	0.5	0.1
Currently Infected	21,429	3,510	24,939	9.4	9.2	9.4
Total Port-Orford-cedar	228,138	38,016	266,154	[2]		

1-Down stream infection status not identified in this category

2-Percentage do not total 100% due to overlap between identified mitigation category and relative risk categories

SCREENING AND BREEDING PROGRAM FOR GENETIC RESISTANCE TO *PHYTOPHTHORA LATERALIS* IN PORT-ORFORD-CEDAR (*CHAMAECYPARIS LAWSONIANA*): EARLY RESULTS

Richard A. Sniezko[1], and Everett M. Hansen[2]

[1]USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR 97424, USA
[2]Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR 97331, USA

SUMMARY

One part of an integrated strategy to manage Port-Orford-cedar in the presence of the exotic pathogen *Phytophthora lateralis* is to develop genetically resistant trees to use in restoration and reforestation. Based on earlier work at Oregon State University that demonstrated the presence of a small number of trees with genetic resistance to *P. lateralis*, the USDA Forest Service and USDI Bureau of Land Management have begun a program to evaluate a large number trees for resistance and to breed for increased resistance.

A relatively small number of field selections had been made prior to 1997. From 1997 to 1999, over 7000 trees that appeared to be resistant in the forest to *P. lateralis* have been selected for further evaluation at Oregon State University using a branch lesion test. Field selections have been made over much of the natural range of Port-Orford-cedar. The branch lesion test allows for rapid screening of many selections, and is used at this stage principally to identify the trees with the highest resistance to the root disease. Initial results indicate that there is only a low frequency of resistance in natural populations, and that most surviving trees are escapes. The top rated trees from branch lesion testing are vegetatively propagated at Dorena Genetic Resource Center, and included in a breeding orchard and/or undergo further evaluation for resistance.

Field validation of selections has begun. Early results from plantings in 1989 and 1993 indicate that only a few current selections have relatively strong resistance, and that breeding will be needed to enhance the resistance of the field selections. Results from a 1989 planting at the Oregon State University Botany Farm indicate that resistant clones can survive at least 10 years, although some ramets of these clones died in the first few years.

Port-Orford-cedar lends itself well to cone and seed production at a young age (less than four years old) and this would allow the potential of rapid increase in resistance. A recurrent breeding cycle of selection, breeding, and evaluation for further resistance could proceed much more rapidly than with most other conifers. Control crosses among some of the pre-1997 selections have already yielded seed, and the progeny are currently under evaluation.

Field selections will be separated into groups (breeding zones) based on information relating to adaptability of Port-Orford-cedar gathered in other trials. Information on underlying mechanisms of resistance is lacking, and such information would be beneficial in maximizing progress in developing Port-Orford-cedar resistant to *P. lateralis*. Once a population of resistant parents is developed, seed could be produced relatively rapidly in containerized seed orchards, or rooted cuttings could be utilized to meet reforestation needs.

INTRODUCTION

Early reports of infection of Port-Orford-cedar (POC) with *Phytophthora lateralis* indicated that all tested ornamental varieties of POC, and some varieties of *Chamaecyparis obtusa* were susceptible, while *Chamaecyparis pisifera* varieties showed resistance (Tucker and Milbrath 1942). In early tests for resistance using thousands of cuttings from hundreds of trees that were phenotypically resistant in natural stands, all cuttings died, indicating that resistance is very low and/or that the inoculation level was too high to allow expression of resistance among the clones tested (Roth et al 1972, Roth 1985, Zobel et al 1985). The first demonstration of resistance in POC was published in 1989 (Hansen et al 1989).

In 1988, a review of the potential threat of the exotic disease *P. lateralis* led to a series of recommendations, including the need to understand more about the genetic variation that exists within POC and the extent of genetic resistance to *P. lateralis* root rot (unpublished 1988 USDA Forest Service Region 5/Region 6 Action Plan for the Management of Port-Orford-cedar). Since 1988 a range of activities has led to a better understanding of the genetic variation within POC and *P. lateralis*, and the relative level of genetic resistance to *P. lateralis* that is present within POC. There is limited information of resistance of forest trees to species of *Phytophthora*, but significant variation in genetic resistance to *P. cinnamomi* has been found within several species (Butcher et al 1984, Stukely and Crane 1994)

Up to the mid-1980's, occasional POC trees had been found that survived infection or showed delayed death, but no attempts to breed for resistance or hybridize with resistant Alaska-cedar (*C. nootkatensis*) or Asiatic *Chamaecyparis* species had been attempted (Roth et al 1987). A few POC survivors that have lived for an extended period of time in the presence of *P. lateralis* were noted in the cold frames near the Oregon State University (OSU) greenhouses and at the OSU Botany farm, and were thought to represent some type of "slow dying" resistance (Roth 1985). With little hope of finding immune individuals within the species, work began in the early 1980's on fine tuning an inoculation system to allow susceptible and relatively tolerant individuals to be distinguished. Screening of randomly selected seedlots in 1996 from much of the range of POC, utilizing two screening techniques, indicated that there was high individual tree heritability for the root dip test, and relatively low heritability for the stem lesion test (unpublished data).

This paper will present an overview of branch screening results from 1989, 1990, 1997, 1998 (excludes 1995 and 1996 screening), and the earliest field tests, established in 1989 at the OSU Botany Farm, and in 1993 on two sites on the Siskiyou National Forest (excludes a failed 1996 planting, and the 1998 and 1999 plantings)

SCREENING POC TREES FOR RESISTANCE

Starting in 1989, the USDA Forest Service began selecting candidate POC trees in natural stands to evaluate for resistance to *P. lateralis* (USDI Bureau of Land Management selections began in 1994). In 1996, a range-wide sample of seedling families was evaluated for disease resistance to examine potential geographic trends in resistance. Operational screening of candidate trees commenced in 1997. From 1989 to 1998, over 7000 healthy trees in natural stands from throughout much of the range of POC have been screened using a branch inoculation technique. At this point, it is anticipated that the final large scale screening of candidate trees from natural stands will take place in 1999. Based upon results of these screenings and field validation data, base populations for future breeding work will be established.

Once a candidate parent tree (or clone) was selected, branches from the tree, or seedling offspring from the candidate trees (1996 only) were sent to OSU for screening. The screening process has been modified somewhat since 1989 and 1990 when large branches were collected and an incision made in the branch which was then inoculated with *P. lateralis* (wound inoculation technique). Generally, the procedure since 1994 is to send six to 10 small branch tips to OSU, and these branch tips are dipped in a zoospore suspension of *P. lateralis*. When seedlings are used for testing, notably in 1996, either a stem dip technique (immersing the bottom two centimeters of a cut portion of the seedling in a zoospore suspension) or a root dip technique (immersing the bottom two centimeters of the container containing the seedling roots in a zoospore suspension) has been utilized.

Several weeks after branch dip inoculation, the length of the lesion growth is determined (in mm), with small lesion length denoting greater relative resistance. Beginning in 1993, there has generally been both a high resistance checklot (usually clone OSU-CF1) and a low resistance checklot (usually clone OSU-CON1 for 1997 and subsequent years) included as each group of clones was evaluated. Due to the large number of candidate trees screened in 1997 and 1998, the screening was done in many groups (or "runs") spread throughout much of the year. The checklots were used to help determine how many clones from each screening group to vegetatively propagate for the clone bank, breeding orchard, or to evaluate further. Many of the candidate trees were selected in localities showing mortality from *P. lateralis* infection.

Results from 1989 and 1990 branch inoculation screening

Through support provided by the Siskiyou National Forest, the initial screening of 193 parent trees from Forest Service land took place in 1989 and 1990 utilizing the branch wound inoculation technique. Screening included trees from Siskiyou and Six Rivers National Forests and parent trees tested were selected from areas where other POC had died from *P. lateralis*. Generally, the range of lesion lengths varied widely among these 193 selected trees, with some tested trees having small lesion scores comparable to the best trees previously tested, while other trees appeared to have little resistance based upon this technique. Rooted cuttings or seedling offspring were available for some of these clones in 1998 at the U.S. Forest Service's Dorena breeding orchard and/or at BLM's Tyrell preservation orchard. Control crosses among some of these clones began in 1996.

Results from the 1997 and 1998 branch dip screening

Over 6500 parents were screened in 1997 and 1998 using the branch dip inoculation technique. The six branches for each parent selected were divided into two blocks, and randomized. With this operational technique, branch ends about a 0.3 meter long are collected from parents, the cut ends are trimmed and immersed in a zoospore suspension for 24 hours, then the branches are stuck in moist vermiculite for three weeks before evaluating for lesion length (Hansen 1996). The number of parents screened at any inoculation varied, and inoculations proceeded through much of the year. Because of the ease and speed of applying the branch dip technique, this technique was used to operationally screen large numbers of candidate trees from disease areas in 1997 and 1998, and will also be used in 1999.

For screening in 1997, 1998, and 1999, approximately 10% of the candidates are being selected based upon stem lesion scores, and will be included in the breeding orchard. In general, if a tested parent had a branch lesion <20mm, or less than the OSU-CF1 checklot, then it was selected for propagation and further testing. Some additional branch test selections were added, usually those that were best in a particular inoculation group (if none met the other two criteria).

Initial evidence suggests there is only a weak correlation between branch lesion scores and scores from the root dip method or field results. Many of the candidate trees selected based upon branch lesion scores may also undergo additional screening using rooted cuttings to validate their resistance before inclusion in future breeding. Results from branch lesion screening in 1997 and 1998 showed that the high resistant checklot, clone OSU-CF1, had a smaller lesion length (often considerably smaller) than the mean of the clones in each run for 70 of the 71 runs, the only exception being in a Run 5 in 1998 where OSU-CF1 had an atypically high lesion length. Parent trees differed significantly in lesion length for most of the 45 runs in 1997 and for all 22 runs in 1998. The lesion length of the best candidate tree in each run was often slightly less than for OSU-CF1. In these runs, lesion length for the low resistant checklot, clone OSU-CON1, was usually much larger than for the run mean, but often was less than the candidate tree with the largest lesion length.

There was a wide range in lesion lengths among the clones (parent trees) screened in 1997 and 1998 (see Figure 1 for distribution of clonal means for 1998 branch lesion screening). Within a run, there was generally wide variation in branch lesion means among the clones in 1997 and 1998, with some outstanding clones for both high and low lesion length. From preliminary examination of the data collected in 1997 and 1998, no obvious geographic trend is notable for relative branch lesion length.

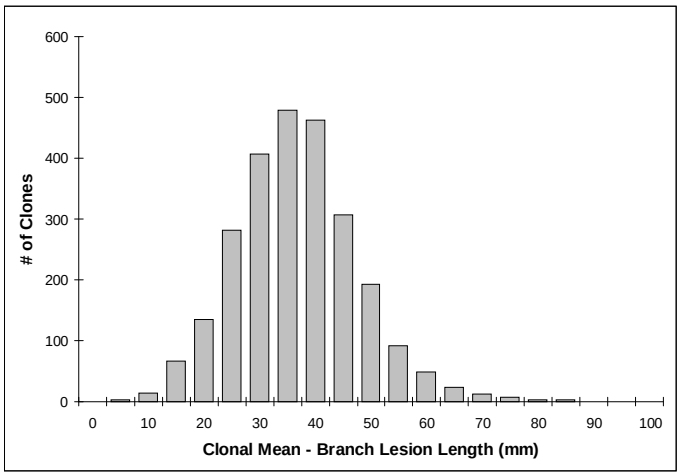


FIGURE 1. 1998 POC Branch dip inoculation – Distribution of clonal means for branch lesion length,

Over 7000 parent trees have been screened for *P. lateralis* resistance since 1989. Locations of most of the candidate trees screened from 1989 to present are known, and this may be useful for projects in the future. The trees may serve as monitors to track additional POC related mortality over its natural range.

In several screenings with different methods over the years, several OSU clones (notably OSU-CF1), and clone 510015 (from Gasquet Ranger District on the Six Rivers National Forest) have consistently been best or near the top for measures of resistance. It appears that there are relatively few clones (perhaps 1 - 2%) that repeatedly stand out in all screening tests. The remainder of the clones may have resistance, but it may be more subtle and not apparent under heavy inoculum loads. Initial indications are that there is little genetic variability in the isolates of the exotic fungus *P. lateralis* present in the Pacific Northwest; if the geographic origin of *P. lateralis* is determined at a later date, it may be necessary to test the most resistant POC against other isolates. At this time, there is no direct evidence on how many resistance mechanisms may be present, nor on the nature of the resistance to *P. lateralis*. If funding is available in 2000, the investigations will begin on the nature of any resistance mechanisms.

FIELD PLANTINGS AND VALIDATION OF GREENHOUSE SCREENING

Greenhouse screening techniques developed at OSU, such as the branch dip and root dip techniques are methods to survey many candidate trees quickly for an indication of relative resistance. The branch dip technique is quicker than the root dip technique (which requires gathering seed or rooting cuttings from the parent trees) and has been used to for most of the POC operational screening program to make selections for the breeding orchards. However, it is possible that some resistance mechanisms may be by-passed in a branch inoculation that would be present in a root test. Little is known about how the greenhouse tests relate to resistance in the field, including relative susceptibility of different clones in the field, as well as how much longer the more resistant clones would thrive under field conditions. Field plantings were established in 1993, 1996, 1998, and 1999 by the Forest Service and BLM to work with OSU to validate the screening methods, to make selections for breeding, and as demonstration plantings.

1989 Botany Farm Planting

A small planting of rooted cuttings and open-pollinated seedlings from resistant and susceptible parents was planted at the OSU Botany Farm in spring 1989. Eighteen to 25 cuttings (or seedlings) were planted, and distributed over four replications. Most mortality occurred within the first year of planting (Figure 2). Several resistant clones, including CF1 and CF2, although suffering mortality of some ramets, continue to show moderate to high survival after 10 years (Figure 2), while most susceptible clones have suffered 100% mortality (usually within the first two years). It is encouraging that several resistance selections seem to do vary well on at least one site for as long as ten years. Future trials may investigate cause of early mortality in some ramets of the most resistant clones.

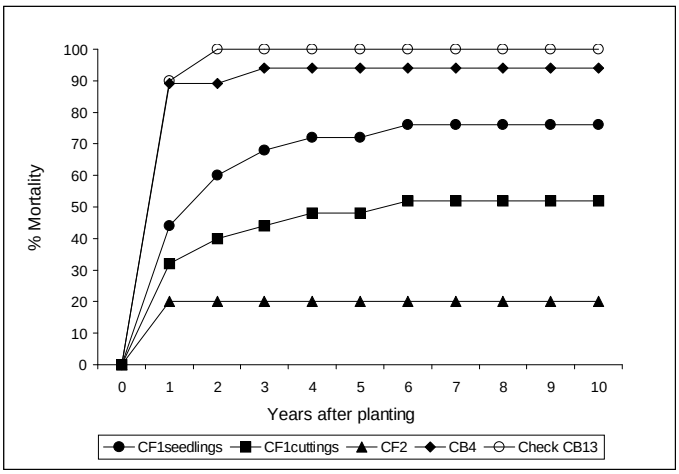


FIGURE 2. *Phytophthora lateralis* mortality over 10-years of four POC clones and one seedling family in 1989 OSU Botany Farm planting.

1993 Quosatana and Flannigan Plantings

The April 1993 plantings utilized one-year old seedlings (sown in May 1992) for 28 open-pollinated families. Seedlings were planted in southwest Oregon at two sites known to have *P. lateralis* present (Quosatana on the Gold Beach Ranger District and Flannigan on the Powers Ranger District, both on the Siskiyou National Forest). Twenty-seven of these 28 families were seedling offspring of 27 of the 193 clones screened via branch inoculation at OSU in 1989 and 1990 (the only parents with seed available). Sixteen replications with each family represented by one tree per replication (except for a few families where fewer seedlings were available) were planted at each site. Each replication centered around POC trees that had been previously killed by *P. lateralis.* Assessment of these plantings involved recording presence of trees dead from *P. lateralis*.

Survival in April 1997 was 18% at Quosatana and 29% at Flannigan. Comparison of family means at the two sites showed some parents such as 510015 with relatively good survival at both sites (44% at Q, 56% at F), but some inconsistencies among other parents (Figure 3). Correlation (using family means) between the two sites varies with age of assessment. At least through the 1997 assessment, seedling survival for family 510015 seems to be fairly evenly spread between plots with moderate and high mortality, suggesting that the resistance may hold up even under areas of high disease incidence. To-date, clone 510015 has shown among the highest resistance in all tests, whether tested as a clone or as a seedling family, and whether tested with a stem inoculation, root inoculation, or in the field. Fifty percent mortality at the sites occurred within one year of outplanting, and over 65% within 19 months of planting. There was relatively little additional mortality at the two sites between 1995 and 1997. Only a few families have 100% mortality, at even one site. Further time will be needed to see if some of the survivors are escapes due to micro-environmental factors.

A remaining question that this early planting will help elucidate is how long will the best families from natural stands continue to show survival, and what percentage of the trees in these families survive. The correlation of the family means (using the survival as of 1997) for these sites with the results from the 1989/1990 branch lesion test was 0.42 and 0.27 for Flannigan and Quosatana. The correlation changed over time, and has actually decreased slightly in recent years as the mortality at the field sites increased. The large amount of early mortality that occurred at these field sites bodes well for further establishment of any other trials at close spacing which makes it easier to find suitable field sites, and to reuse the same site over time.

While the high early mortality in the various field tests is cause for discouragement, the differentiation among families or clones in early survival is encouraging, especially with the potential of relatively rapid breeding cycles for POC. Inclusion of the more resistant families in the breeding populations will hopefully allow us to continue to increase the resistance within POC. Several short-term tests involving root infection are also under investigation, including planting seedlings in an infected raised bed.

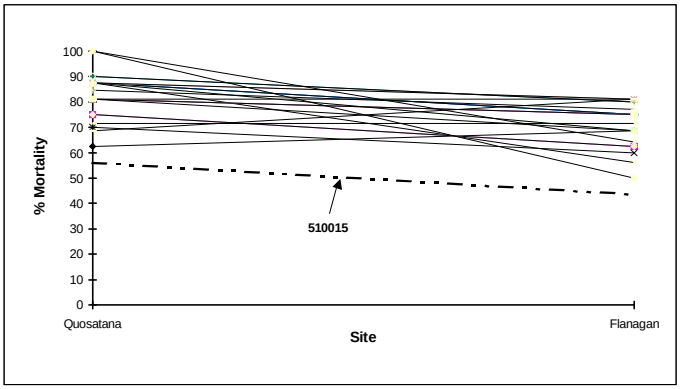


FIGURE 3. Percent mortality through April 1997 (49 months after planting) for families planted at two sites.

BREEDING

Because the expected resistance from screening parent clones from natural stands was expected to be relatively low and/or the frequency of resistant parents also low, the Forest Service and BLM began planning to do breeding work to attempt to increase resistance, utilizing the more resistant selections from the operational screening program. Based on work done elsewhere on flower induction in POC, the Forest Service began to try methods of flower induction in POC in the early 1990's that would work under containerized seed orchard conditions present at the Forest Service's Dorena Genetic Resource Center, near Cottage Grove, Oregon. With the relative ease of flower induction at an early age (by three or four years) in this species, the next step was to fine tune pollination techniques for this

species to work on small, young trees in pots at Dorena. Several years of pollinations and seed yields indicate that for the most part, it should be feasible to obtain seed from most crosses.

Currently, the goal is to produce rooted cuttings at Dorena GRC for all parent trees selected as "resistant" after undergoing artificial inoculation in the branch screening program from natural selections. Once rooted these clones are placed into a potted breeding orchard at Dorena. If there are enough cuttings, the clones are also disease tested with a second method (starting in 1999) and are also established in a preservation orchard at BLM's Tyrrell Seed Orchard (starting in late 1998). Once the majority of the selections have been made (after the 1999 screenings are completed) further discussions will be to prioritize breeding zones for breeding. Breeding zones will be based on genetic variation found in height and other traits (Kitzmiller and Sniezko, in press).

Control crosses have been underway for several years, but until recently relatively few parents have been available. Many more parents will be available in the next few years as the rooted cuttings from the 1997 screenings become large enough to flower. A breeding plan will be devised that takes into account available information on resistance and breeding zones and incorporates budget needs.

ACKNOWLEDGMENTS

Many individuals from the U.S. Forest Service, BLM, and OSU have contributed significantly to the various phases of this resistance program. A special thanks to D. Goheen, K. Marshall, K. Casavan, W. Sutton, A. Bower, L. Elliott, J. Linn, J. Hamlin, J. Kitzmiller, C. Frank, R. Stevens and D. Rose.

LITERATURE

Butcher, T.B., Stukely, M.J.C., and Chester, G.W. 1984. Genetic Variation in Resistance of *Pinus radiata* to *Phytophthora cinnamomi*. Forestry Ecology and Management (8) 197-220.

Hansen, E.M., Hamm, P.B., and Roth, L.F. 1989. Testing Port-Orford-cedar for resistance to Phytophthora. Plant Disease 73(10): 791-794.

Hansen, E.M. 1996. Testing Port-Orford cedar for resistance to *Phytophthora lateralis*. (Unpublished final Report to USDA Forest Service, Region 6).

Kitzmiller, J.H. and Sniezko, R.A. 1999 (in press). Range-wide genetic variation in Port-Orford-cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.): I. Early height growth at coastal and inland nurseries. Journal of Sustainable Forestry.

Roth, L.E. 1985. Inoculation Methods for Quantitatively evaluating the response of Port Orford-cedar trees to *Phytophthora lateralis*. Unpublished report. 17 p.

Roth, L.E., Bynum, H.H., and Nelson, E. E. 1972. Phytophthora root rot of Port-Orford-cedar. USDA Forest Service Forest pest leaflet 131. 7 p.

Roth, L.E., Harvey, R.D. Jr., and Kliejunas, J.T. 1987. Port-Orford-cedar root disease. 1987. USDA Forest Service R6 FPM-PR-294-87.

Stukely, M.J. and Crane, C.E. 1994. Genetically Based Resistance of *Eucalyptus marginata* to *Phytophthora cinnamomi*. Phytopathology (84) 650-656.

Tucker, C.M. and Milbrath, J.A. 1942. Root Rot of *Chamacyparis* caused by a species of *Phytophthora*. Mycologia 34: 94-103.

Zobel, D.B., Roth, L.F., Hawk, G.M. 1985. Ecology, pathology, and management of Port-Orford-cedar (*Chamaecyparis lawsoniana)*. Gen. Tech. Rep. PNW-184. Portland, OR. USDA Forest Service. Pacific Northwest Forest and Range Experiment Station. 161 p.

SPREAD OF *PHYTOPHTHORA* SPP. IN OAK WOODS OF SOUTHERN ITALY

M. C. Bianco, T. de Gioia, N. Luisi and P. Lerario

Dipartimento di Biologia e Patologia vegetale, Università degli Studi, Bari, Italy.

ABSTRACT

Phytophthora genus is a primary widespread soilborne root pathogen, associated with mortality and decline of several oak woods in Europe and Mediterranean regions as well as in North America.

A survey, meant to estimate the possible occurrence and distribution of *Phytophthora* spp. in Southern Italy was carried out in 7 declining pure or mixed oak woods, chosen among the most representative in the area under study.

Detection of *Phytophthora* spp. in soil samples, collected in spring and autumn at the base of healthy and declining oaks, was performed by "baiting" technique and using selective agar media. Specific identification was based on morphological characteristics of the colonies grown in Petri dishes on different media and on microscopic observations of hyphae, sporangia and oogonia.

Phytophthora species occurred in all investigated stands, with a percentage varying from 30 to 58% of sampled trees; the identified species were the following: *P. cactorum* (Leb. e Cohn) Schröeter, *P. cambivora* (Petri) Buisman, *P. cinnamomi* Rands, *P. citricola* Sawada

INTRODUCTION

Since the early 1980's, a severe oak decline has been reported in different European and Mediterranean regions, although other Countries the onset of decline is thought to have started much earlier (Delatour, 1983; Donaubauer, 1987; Leontovyc and Crapek, 1987; Oleksin and Pryzbyl, 1987; Siwecki and Liese, 1991; Luisi *et al.*, 1993). In Spain and Portugal the oak species involved in the decline are primarily the evergreen *Quercus ilex* L. and *Q. suber* L. (this last also reported in Tunisia and Morocco) and, to a lesser extent, *Q. faginea* Lam., *Q. pyrenaica* Willd. and *Q. rotundifolia* Lam. (Brasier *et al.*, 1993a, b). In France and Germany, *Q. robur* L., *Q. petraea* (Matt.) Liebl. and *Q. rubra* L. are also involved in the decline (Jung *et al.*, 1996; Robin *et al.*, 1998) and in Italy mainly the deciduous *Q. cerris* L., *Q. frainetto* Ten. and *Q. pubescens* Willd. (Raddi, 1992; Sicoli *et al.*, 1998).

Chlorosis, dieback of branches and part of the crown, microphylly, defoliation, emission of epicormic shoots, tarry exudations and necrotic rootlets are some of the symptoms of this complex and chronic syndrome, recognized as a multifactor disease, with predisposing factors (e. g. unsuitable site, adverse climatic conditions, atmospheric pollution, root competition), inciting factors (e. g. drought, frost, snow, insect defoliation) and contributing factors (e. g. improper management, over-pasturing, secondary pathogens) (Manion, 1981; Siwecki and Liese, 1991; Luisi *et al.*, 1993; Sicoli *et al.*, 1998).

Among the fungal parasites involved, *Phytophthora* spp., primary widespread soilborne root pathogens in many Authors' opinion, could play an important role in oak decline (Brasier, 1993; Blaschke *et al.*, 1995; Jung and Blaschke, 1996; Jung *et al.*, 1996).

Owing to the lack of researches regarding the pathosystem *Quercus-Phytophthora* in Italy, the aim of this study was to assess the occurrence of *Phytophthora* spp. in declining oak woods of Southern Italy in view of establishing, by further investigations, their possible involvement in the decline.

MATERIALS AND METHODS

This research was carried out in 7 declining pure or mixed oak woods, selected among the most representative oak forests in Southern Italy, located in Deliceto (Foggia), Gravina in Puglia (Bari) and Manduria (Taranto) in Apulia, Brindisi di Montagna (Potenza) and Ferrandina (Matera) in Basilicata, Campana (Cosenza) in Calabria and Corleone (Palermo) in Sicily (Fig. 1).

In each stand, a representative plot of 600 m^2 was established, and different parameters were determined for each of them (Table 1). Declining status was assessed according to the European System (Anonymous, 1997).

FIGURE 1. Geographical distribution of woods investigated for *Phytophthora* occurrence.

Four highly declining and 4 apparently healthy (if any) or slightly declining plants were chosen for each plot (Table 2). In mixed woods, the plants selected belonged to the dominant species. Soil samples, consisting of 4-8 sub-samples, were collected at a distance of about 1 m from the base of each plant in spring and autumn 1998 and in spring 1999.

TABLE 2. Declining degree of the 8 sample-trees in each oak wood investigated in Southern Italy.

Region	Wood locations	Sample-trees							
		Declining				Healthy (if any)			
		I	II	III	IV	V	VI	VII	VIII
Apulia	Deliceto (Foggia)	3	1	3	2	0	0	0	0
	Gravina in P. (Bari)	3	3	3	2	0	0	0	0
	Manduria (Taranto)	3	2	3	2	1	1	1	1
Basilicata	Brindisi di M. (Potenza)	3	2	1	1	0	0	0	0
	Ferrandina (Matera)	2	1	3	1	0	0	0	0
Calabria	Campana (Cosenza)	2	3	2	2	1	1	1	1
Sicily	Corleone (Palermo)	2	2	3	2	1	0	0	1

Detection of *Phytophthora* species from soil flooded by water (200 ml per sample in 500 ml of distilled water) was performed by "baiting" technique, using *Q. robur* L. leaflets as baits (Tsao, 1983; Jung *et al.*, 1996). As a control, the "baiting" test was applied also to a sterile soil sample artificially inoculated with *P. cinnamomi* Rands. Leaflets showing black spots or turning brownish or blackish were collected and small fragments of necrosed tissue were plated on PARPNH selective medium (Tsao, 1983) and incubated at about 22 °C in the dark. All PARPNH plates were examined daily under a dissection microscope; developing *Phytophthora* colonies were transferred to and maintained on multivitamin agar medium.

Tests, aimed at the specific identification of *Phytophthora* spp., were carried out by comparing the characteristics of the colonies grown at about 22 °C in Petri dishes on different media (Potato-Dextrose-Agar, Multivitamin-Agar, Malt-Extract-Agar and Corn-Meal-Agar) and the microscopic features of hyphae, sporangia and oogonia with descriptions of the species reported by Waterhouse and Waterstone (1966a, b), Waterhouse (1970) and Stamps *et al*. (1990), and by DNA analysis (Tooley *et al*., 1996; Cooke and Duncan, 1997; Schubert *et al.*, 1998).

Production of sporangia and oogonia was stimulated by flooding a small square fragment (about 0.25 cm^2) of colonized medium, in a suspension of sterile soil in distilled water 1:5 ratio (w:v) for 1-3 days at about 22 °C.

DNA analysis was effected by means of the polymerase chain reaction (PCR). Total genomic DNA was extracted according to the TES/CTAB/chloroform-isopropanol method described by Möller *et al.* (1992) or using a nucleon DNA extraction kit, according to the manufacturer's instructions (Dynal A. S., Oslo, Norway). The Internal Transcribed Spacer region (ITS) of the ribosomal RNA gene was amplified by the primers ITS1 and ITS4 (White *et al.*, 1990).

The PCR reaction mixture included: 2.5 units Taq polymerase (Roche Diagnostics, Germany), the buffer supplied with the enzyme, 200 µM each dNTP (Roche Diagnostics, Germany) and 0.5 µM each primer (Tib Molbiol S.r.l., Italy) in a volume of 25 µl. Ten ng of DNA were added as template. PCR reaction was carried out in a 'Gene Cycler™ ' model (Biorad Laboratories, California, USA) thermal cycler programmed for an initial denaturation at 95 °C for 1 min, followed by 30 cycles of 94 °C for 15 s, 55 °C for 15 s and 72 °C for 15 s, followed by a 6 min extension at 72 °C to ensure a double-stranded amplicon. A negative control with all reaction reagents and distilled sterile water replacing DNA template was included in each set of reactions, in order to assess the occurrence of contaminants in reagents and/or the reaction mixtures.

Aliquots of the amplified DNA were digested with the endonuclease *Msp I* (Roche Diagnostics, Germany) according to the manufacturer's instructions. In particular, 14 µL of the amplified regions were digested for 1-2 hours at 37 °C with 2-4 enzyme units and the buffer supplied with the enzyme, in a volume of 20 µl.

Both the amplified DNA and the restriction fragments were electrophoresed in agarose (Biorad Laboratories) gel in TBE [89 mM Tris, 89 mM boric acid and 2 mM EDTA (pH 8)] buffer system, using 2% agarose and 100 V for about 2 hours in the first case, and 4% agarose and 150 V for about 4 hours in the second one. The products were visualized under UV illumination after being stained in an ethidium bromide solution (Sambrook *et al*., 1989). The sizes of the amplified products and the digestion fragments were determined by comparing their migration distances to those of 1 Kb and 100 bp molecular markers (Roche Diagnostics), respectively, using a "Quantity One - version 4" software (Biorad Laborarories).

RESULTS AND DISCUSSION

The observation of growth patterns of the colonies, the presence of restrictions at the base of the hyphae, the hyphal swellings and the shape of sporangia (Fig. 2) allowed the isolates obtained to be identified as *Phytophthora* species. In all investigated stands *Phytophthora* spp. occurred in a percentage varying from about 30 to 58% (Table 3), sometimes from samples collected at the base of apparently healthy plants, too.

TABLE 3. Number of Phytophthora spp. Isolates obtained from soil collected near the 8 sample-trees in each oak wood investigated in Southern Italy.

Region	Wood location	Period of sampling		
		Spring 1998	Autumn 1998	Spring 1999
Apulia	Deliceto (Foggia)	3	4	7
	Gravina in P. (Bari)	3	4	6
	Manduria (Taranto)	2	3	4
Basilicata	Brindisi di M. (Potenza)	0	2	5
	Ferrandina (Matera)	3	4	6
Calabria	Campana (Cosenza)	4	2	3
Sicily	Corleone (Palermo)	3	1	3

On the basis of the observed macro and microscopic characteristics of fungal colonies and structures, some of the isolates collected were identified as: *P. cactorum* (Leb. and Cohn) Schröeter, *P. cambivora* (Petri) Buisman, *P. cinnamomi* Rands and *P. citricola* Sawada.

The most widespread species was *P. citricola*, which had non-deciduous, obpyriform, semipapillate sporangia and oogonia with paragynous antheridia. Hyphal swellings often occurred on the sporangiophores. The isolates of *P. cactorum* instead, formed ovoid, papillate, caducous sporangia with a prominent papilla. Hyphal swellings were observed on the sporangiophores. The isolates of *P. cambivora* produced large, ovoid, nonpapillate sporangia, simple and unbranched hyphae and hyphal swellings. Finally, the isolates of *P. cinnamomi* formed ovoid, rounded at the base, nonpapillate and noncaducous sporangia and a coralloid-type mycelium.

Molecular methods used in this study confirmed that these isolates belonged to the identified *Phytophthora* species. In fact, the size of the amplified ITS region was about 900 bp and the *Msp I* restriction pattern was unique and peculiar for each species. In *P. cambivora* the sum of all band's sizes was larger than expected, due to the occurrence of a polymorphism within the recognition site of the enzyme, as reported by Cooke and Duncan (1997) (Fig. 3, Table 4).

The species identified in the present study are common to those found in declining oak woods of Europe, in particular in Portugal (Brasier *et al*., 1993a), Spain (Brasier *et al*. 1993b), France (Robin *et al*., 1998) and Germany (Jung *et al*., 1996). On the basis of their high pathogenicity on *Q. ilex, Q. robur, Q. suber., Q. cerris* L. and *Q. petraea*, assessed in various tests, *P. cinnamomi* and *P. cambivora* were indicated by numerous Authors as "primary" agents in the decline of pure and mixed oak woods in such Countries (Brasier, 1993; Brasier *et al*., 1993a, b; Robin *et al*., 1998). Moreover, Brasier *et al*., (1993b) assumed that *P. cinnamomi* can be associated to the fast mortality of oaks occurring in some Regions of Italy and also in Tunisia and Morocco. *Phytophthora citricola* and *P. cactorum*, instead, resulted variously pathogenic on *Quercus* spp. (Mircetich *et al*., 1977), so that their role in the decline is still not clear. It is known, however, that the aggressiveness of *Phytophthora* species is strongly influenced by environmental conditions and other biotic factors, surely different in the stands here surveyed with respect to those examined by the aforesaid Authors, which, moreover, assessed the pathogenicity of *Phytophthora* spp. only on a limited number of *Quercus* species. The occurrence of *Phytophthora* spp. in all the investigated oak woods let us think that they could play an important role in oak decline also in Southern Italy. The result of the pathogenicity tests still in progress on various oak species, with the study of the environmental characteristics of the woods, will concur to better clarify such a role.

TABLE 4. *Msp I* restriction fragments of the amplified ITS region of rDNA of *Phytophthora* species.

Species	Fragment sizes (bp)
P. cinnamomi	383, 223, 165, 144
P. cactorum	375, 224, 185, 110
P. citricola	335, 291, 220
P. cambivora	395, 301, 225, 168, 146

TABLE 1. Main characteristics of oak woods of Southern Italy investigated for *Phytophthora* occurrence.

Region	Wood location	Altitude (m a.s.l.)	Exposure	Slope (%)	Bedrock	Oak species *	Stand from	Age (years)	Density**	Stand height (m)	Declining status***
Apulia	Deliceto (Foggia)	600	W/N-W	17	sand-clay	***Q. cerris,*** *Q. pubescens*	stump	20	19.2	8	0.5
	Gravina in P. (Bari)	400	N	10	sand	*Q. cerris,* ***Q. frainetto,*** *Q. pubescens*	stump	31	17.6	13	1.5
	Manduria (Taranto)	86	S-W	5	limestone	***Q. ilex***	seed	40	15.9	15	0.4
Basilicata	Brindisi di M. (Potenza)	700	N-E	40	clay	***Q. pubescens*** *Q. cerris*	seed	30	17.2	8	0.3
	Ferrandina (Matera)	550	-	0	sand-clay	***Q. frainetto,*** *Q. cerris*	seed	32	19	13	0.2
Calabria	Campana (Cosenza)	790	N	35	sand	***Q. frainetto,*** *Q. cerris*	seed	38	20.4	18	1.6
Sicily	Corleone (Palermo)	600	N-E	25	sand	***Q. cerris***	seed	56	16.9	25	1.4

* Dominant species is reported in boldface ** Expressed as basal area/ha ***Expressed in a 0-4 scale

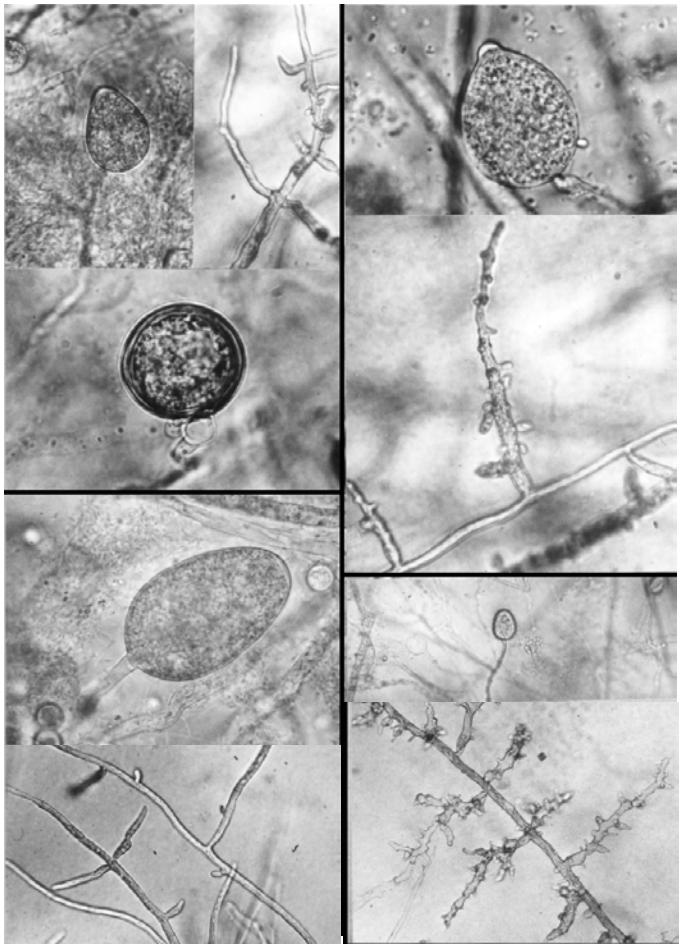


FIGURE 2. Structures of *Phytophthora* spp.: sporangium and hyphae (x 130) and oogonium (x 300) of *P. citricola* (top, left); . sporangium (x 300) and hyphae (x 130) of *P. cactorum* (top, right); sporangium (x 300) and hyphae (x 80) of *P. cambivora* (bottom, left); sporangium (x 30) and hyphae (x 80) of *P. cinnamomi* (bottom, right).

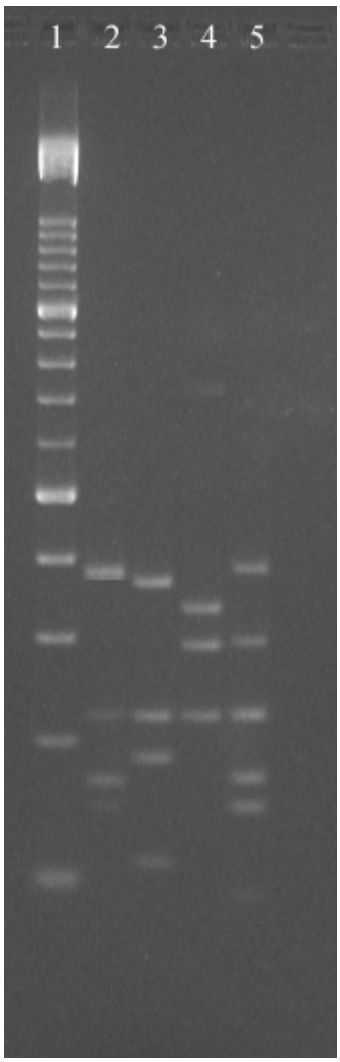


FIGURE 3. Ethidium bromide stained agarose gel (4%) of *Msp I* digestion products of the ITS region of *Phytophthora* spp. Lane 1: 100 bp molecular marker; lane 2: *P. cinnamomi;* lane 3: *P. cactorum;* lane 4: *P. citricola* and lane 5: *P. cambivora.*

ACKNOWLEDGEMENTS

This study was carried out with the financial support from the Commission of the European Comunities, Agriculture and Fisheries (FAIR) specific RTD program, CT 97-3926, "Long term dynamics of oak ecosystems: assessment of the role of root pathogens and environmental constraints as interacting decline inducing factors" (PATHOAK).

REFERENCES

Anonymous, 1997. Manual on methods and criteria for harmonised sampling, assessment, monitoring and analysis of the effects of air

pollution on forests. United Nation Economic Commission for Europe, Internat. Co-operative Program. 4th edition.

Blaschke H., T. Jung, E. Paoletti, F. Bussotti, 1995. First report of *Phytophthora* on roots of declining *Quercus ilex* in Italy. Giornale Botanico Italiano, 129-132.

Brasier C. M., 1993. *Phytophthora cinnamomi* as a contributory factor in European oak declines. In: Recent Advances in Studies on Oak Decline (N. Luisi, P. Lerario, A. Vannini, Eds), Dipartimento di Patologia vegetale, Università, Bari, Italy, 49-58.

Brasier C. M., A. C. Moreira, J. F. P. Ferraz, S. Kirk, 1993a - High mortality of cork oak in Portugal associated with *Phytophthora cinnamomi*. In: Recent Advances in Studies on Oak Decline (N. Luisi, P. Lerario, A. Vannini, Eds), Dipartimento di Patologia vegetale, Università, Bari, Italia, 461-462.

Brasier C. M., F. Robredo, J. F. P. Ferraz, 1993b. Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. Plant Pathology, 42, 140-145.

Cooke D. E. L., J. M. Duncan, 1997. Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of the ribosomal RNA gene repeat. Mycol. Res., 101 (6), 667-677.

Delatour C., 1983. Les Dèpèrissement du Chêne en Europe. Revue forestière française, 4, 265-282.

Donaubauer E., 1987. Auftreten von Krankheiten und Schädlingen der Eiche und ihr Bezug zum Eichensterben. Osterreichische Forstzeitung, 3, 46-48.

Jung T., H. Blaschke, 1996. *Phytophthora* root rot in declining forest trees. Phyton (Horn, Austria), 36, 95-101.

Jung T., H. Blaschke, P. Neumann, 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. Eur. J. For. Path., 26, 253-272.

Leontovyc R., M. Crapek, 1987. Eichenwelken in der Slowakei. Osterreichische Forstzeitung, 3, 51-52.

Luisi. N., P. Lerario, A. Vannini (Eds), 1993. Recent Advances in Studies on Oak Decline. Proc. Int. Cong., 13-18 September 1992, Selva di Fasano (Brindisi), Italy, 541 pp.

Manion P. D., 1981. Tree disease concepts. NJ: Prentice-Hall, 399 pp.

Mircetich S. M., R. N. Campbell, M. E. Matheron, 1977. *Phytophthora* trunk canker of coast live oak and cork oak trees in California. Plant Disease Reporter, 61, 66-70.

Möller E. M., G. Bahnweg, H. Sandermann, H. H. Geiger, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Research, 20 (22), 6115-6116.

Oleksin J., K.Przybyl, 1987. Oak decline in the Soviet Union: scale and hypotheses. Eur. J. F. Path., 17, 321-336.

Raddi P. (Ed), 1992. Mediterranean forest tree decline in Italy. Consiglio Nazionale delle Ricerche (CNR), 64 pp.

Robin C., M. L. Desprez-Loustau, G. Capron, C. Delatour, 1998. First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. Annales des Sciences Forestières, 55 (8), 869-883.

Sambrook J., E. F. Fritsch, T. Maniatis, 1989. Molecular cloning. A laboratory manual. Second edition. Cold Spring Harbor Laboratory Press.

Schubert R., G. Bahenweg, J. Nechwatal, T. Jung, D. E. L. Cooke, J. M. Duncan, G. Müller-Starck, C. Langebartels, H. Sandermann Jr, W. Oßwald, 1998. Detection and quantification of *Phytophthora* species which are associated with root-rot diseases in European deciduous forests by species-specific polymerase chain reaction. Eur. J. For. Path., 27, 1-19.

Sicoli G., T. de Gioia, N. Luisi, P. Lerario, 1998. Multiple factors associated with oak decline in Southern Italy. Phytopath. medit., 37, 1-8.

Siwecki R., W. Liese (Eds), 1991. Oak decline in Europe. Proc. Int. Symp., 15-18 May 1990, Kornik, Poland, 360pp.

Stamps D. J., G. M. Waterhouse, F. J. Newhook, G. S. Hall, 1990. Revised tabular key to the species of *Phytophthora*. Mycological Papers, 162, 1-28.

Tooley P. W., M. M. Carras, K. F. Falkenstein, 1996. Relationships among group IV *Phytophthora* species inferred by restriction analysis of the ITS2 region. J. Phytopathology, 144, 363-369.

Tsao P. H., 1983. Factors affecting isolation and quantitation of *Phytophthora* from soil. In: *Phytophthora*. Its biology, taxonomy, ecology and pathology. Ed. by Erwin D. C., Bartnicky-Garcia S., Tsao P. H. St Paul MN: The American Phytopathological Society, 219-236.

Waterhouse G. M., J. M. Waterstone, 1966a. *Phytophthora cactorum*. C.M.I. Descriptions of pathogenic fungi and bacteria, no. 111. Kew, Surrey: CMI.

Waterhouse G. M., J. M. Waterstone, 1966b. *Phytophthora citricola*. C.M.I. Descriptions of pathogenic fungi and bacteria, no. 114. Kew, Surrey: CMI.

Waterhouse G. M., 1970. The genus *Phytophthora* de Bary. Mycological Papers no. 122. Kew, Surrey: CMI.

White T. J., T. Burns, S. Lee, J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., D. H. Gelfand, J. J. Sninsky and T. J. White (Eds), PCR Protocols, 315-322, Academic Press, San Diego, CA, USA.

SCREENING PORT-ORFORD-CEDAR FOR RESISTANCE TO PHYTOPHTHORA LATERALIS: RESULTS FROM 7000+ TREES USING A BRANCH LESION TEST

Bower, A. D.[1], Casavan, K.[2], Frank, C.[3], Goheen, D.[5], Hansen, E.[4], Marshall, K.[5], Sniezko, R.[1], Sutton, W[4].

[1] USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR
[2] USDA Bureau of Land Management, Roseburg District Office, Roseburg, OR
[3] USDA Forest Service, North Zone Genetic Resource Unit, Yreka, CA
[4] Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR
[5] USDA Forest Service, SW Oregon Forest Insect and Disease Service Center, Central Point, OR

ABSTRACT

As part the process of developing an operational program breeding for genetic resistance to a root disease of Port-Orford-cedar (*Chamaecyparis lawsoniana*) caused by *Phytophthora lateralis*, an inter-agency effort has been undertaken to select and screen phenotypically resistant trees throughout the range of Port-Orford-cedar. The USDA Forest Service, USDI Bureau of Land Management, and Oregon State University (OSU) have selected over 7000 trees across south-west Oregon and northern California, predominantly on federal lands. Generally, these selections were healthy trees in areas where *P. lateralis* was known to be present. Screening began at OSU in 1989, and different techniques were developed and used through 1996. Operational screening of field selections began in 1997, and through 1998, over 6700 clones were screened using the branch dip method. This method had previously been found to identify at least the most resistant clones. Lesion length was measured on 6 branches per clone, and these measurements were compared with a resistant and a susceptible control tree. Relatively few of the clones screened in 1997 and 1998 had branch lesion means smaller than the resistant control. Approximately the top 10% of each run was selected to be included in the breeding program. Over 1000 clones are currently in the breeding program, for the purposes of retesting, crossing, and archiving these genotypes. The screening effort is continuing in 1999 with many areas of private land within the range of Port-Orford-cedar being targeted for selection and testing, with an ultimate goal of screening a total of 9000 trees.

INTRODUCTION

P. lateralis has been found in pockets across the natural range of Port-Orford-cedar, with young trees being killed in a matter of months, and even large older trees dying within a few years of infection. A small percentage of individuals however have shown marked resistance to the pathogen, and consistently perform well in testing. An operational program to find, breed, and propagate individuals expressing resistance is currently under way cooperatively with the USDA Forest Service, USDI Bureau of Land Management, and Oregon State University (OSU).

Beginning in 1989, healthy trees growing in areas with a known presence of *P. lateralis* were tested for resistance. From 1989 through 1996, approximately 700 individuals were tested, using various methods developed at OSU. A fast and effective method of artificial inoculation with *P. lateralis* was developed, and beginning in 1997, the operational screening program was begun. In 1997 and 1998, nearly 7000 individuals were selected in the field, and screened at OSU. The selection and screening is continuing in 1999, with a goal of screening a total of 9000 individuals.

MATERIALS AND METHODS

- Beginning in early spring, branches from selected trees are sent to OSU and screened in "runs" as they are received from the field.
- The cut ends of 6 branches approximately 1 foot long from each individual, separated into 2 blocks of 3 branches each, are exposed to inoculum for 24 hours.
- After inoculation, the branches are stuck in tubes filled with vermiculite, and watered once a day.
- After 21 days, the bark and cambium are scraped away, revealing any lesion, and the length of the lesion is measured.
- As a basis for comparison, a highly resistant clone (CF1) and a susceptible clone (CON1) are tested in each run.

RESULTS

(results from 1997 and 1998 are similar; only 1998 results are shown)

- In each run, there is a wide range in mean lesion length among clones (figs. 1 and 2).
- CF1 mean is usually the smallest or one of the smallest in the run (fig. 1).
- CON1 mean is usually higher than the run mean (fig. 1).
- Despite a wide range in clonal means in each run, in almost all runs, significant clonal differences were detected.
- The difference between the checklots (CON1 - CF1) also varies by run, but to a lesser degree, and there is no trend with time.
- Less than 5% of the clones screened had mean lesion lengths smaller than CF1.
- Overall mean lesion length of all non-control trees is almost identical between 1997 (32.97 mm) and 1998 (32.89 mm) (table 1).
- Approximately 10% of the clones with the smallest mean lesion scores (table 2) were selected for inclusion in resistance breeding program.

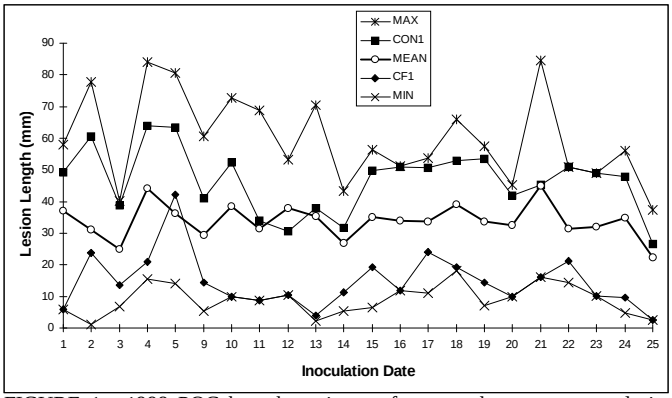


FIGURE 1. 1998 POC branch testing performance by run – mean lesion lengths for top and bottom family per run relative to the two controls and the run mean.

.

TABLE 1. Total yearly selections of Port-Orford cedar for disease resistance screening.

	Number of Selections Tested						Number of Trees Selected for Program					
	1989[b]	1990[b]	1995	1996	1997	1998	1989	1990	1995	1996	1997*	1998
Medford – BLM			190	117	1074	17			20	30	99	3
Roseburg – BLM					263	187					30	14
Coos Bay – BLM				15	461	877				10	45	117
Siskiyou – USFS	24	115[c]		66	2148	1266	24	113		28	203	122
California – USFS	15	41		112	276	205	15	41		61	20	13
Private land – OR				10						6	13	
Private land – CA				24								
TOTAL	39	154	190	344[a]	4222	2552	39	154	20	148	397	269
GRAND TOTAL						**7501**						**1027**

* Includes 17 selections that were dead, cut, or missing when re-visited.
[a] 27 families previously tested in 1989-90.
[b] Original selections from heavily infected areas.
[c] 2 families selected and tested in 1989.

TABLE 2. POC disease resistance screening site mean lesion scores.

		1997			1998		
		n	Lesion Length[a]	CF1 lesion	n	Lesion Length[a]	CF1 lesion
Coos Bay							
	Coos Bay	461	38.95	18.65	885	32.51	11.9
Siskiyou							
	Gold Beach R.D.	511	32.83	24.26	386	33.94	17.08
	Power's R.D.	1517	35.08	15.77	799	37.44	15.77
	Illinois Valley R.D.	120	23.24	10.25	55	32.98	10.17
	Gallice R.D.				26	31.79	10.17
sub-total		2148			1266		
Medford							
	Grants Pass BLM	736	30.81	13.76			
	Medford BLM	338	35.25	19.7	17	28.58	10.17
sub-total		1074					
Roseburg							
	Roseburg BLM	263	31.57	12.01	179	30.88	17.29
California	(watershed)						
	Klamath River	65	34.59	11.55	129	35.03	35.82[b]
	Sacramento River	96	36.21	10.21	38	36.81	11.83
	Smith River – middle	29	33.62	17.17	15	35.62	11.83
	Smith River – north	12	30.06	17.17			
	Smith River	3	26.94	17.17	5	30.02	11.83
	Smith River – south	13	37.98	17.17	18	30.42	11.83
	Trinity River	58	34.49	12.44			
sub-total		276			205		
OVERALL		4222	32.97	15.52	2552	32.89	12.72

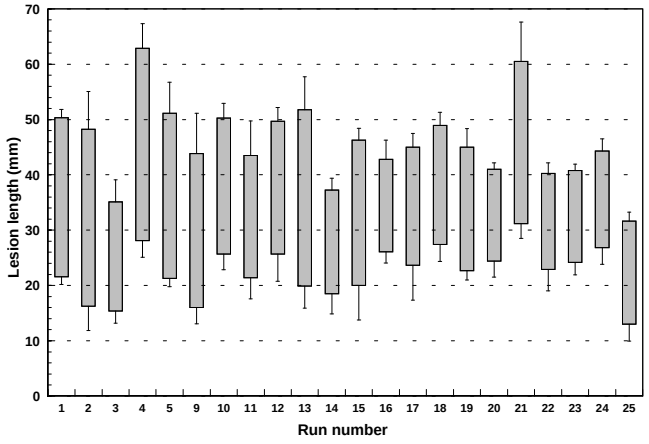


FIGURE 2. Clonal variation in branch lesion length within and between 22 runs in 1998.

The selection of Port-Orford-cedar to date has covered the natural range very well on federal lands. Selection and testing is continuing in 1999, focusing on adjacent private lands, in order to obtain complete sample from across the landscape.

ACKNOWLEDGEMENTS

Frank Betlejewski, Mary Brennan, Jay Esperance, Ed Hall, Jim Hamlin, Debi Kroeger, Joe Linn, Bill Powers, Rod Stevens, Nick Vagle, USDA Forest Service Region 5 North Zone Genetic Resource Unit, USDA Forest Service Forest Health and Protection Special Technology Development Project, and all of the USDA Forest Service and USDI Bureau of Land Management personnel involved in collection and screening of the branches, and management of the data.

A COMPARISON OF AFLP DIVERSITY IN PHYTOPHTHORA QUERCINA AND PHYTOPHTHORA CITRICOLA POPULATIONS

David E. Cooke[1], Thomas Jung[2], Naomi A. Williams[1], Roland Schubert[3], Wolfgang Osswald[2] and James M. Duncan[1]

[1]Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Faculty of Forest Sciences
[2]Section of Forest Pathology, Ludwig Maximilians University of Munich, Am Hochanger 13, D-85354 Freising, Germany
[3]Section of Forest Genetics, Ludwig Maximilians University of Munich, Am Hochanger 13, D-85354 Freising, Germany

ABSTRACT

New methods of molecular analysis are allowing a more detailed insight into the population biology of many *Phytophthora* species in forestry, agriculture and natural plant communities. An Amplified Fragment Length Polymorphism (AFLP) annuluses of seventy two isolates of *Phytophthora quercina*, a pathogen of *Quercus* species and, to date, found only in Europe, and thirty four isolates of *Phytophthora citricola*, a broad host range species of world-wide distribution, has been carried out. We have related the genomic DNA fingerprints to site, host species and season within each *Phytophthora* species and compared the extent and significance of the molecular diversity between the two species. This is helping us to understand the genetic structure of these natural pathogen populations thus addressing the important issues of the nature, origins and spread of disease outbreaks, the extent and maintenance of pathogen diversity and an assessment of the possibilities for resistance breeding and control.

PATHOGENICITY OF PHYTOPHTHORA SPECIES ON OAKS

Claude Delatour [1], Marie-Laure Desprez-Loustau [2], Cécile Robin [2]

1 INRA de Nancy, F-54280 Seichamps, France
2 INRA de Bordeaux, URSV, BP 81, F-33883 Villenave-d'Ornon, France

INTRODUCTION

Current research in Europe aims at clarify the potential involvement of the *Phytophthora* group in oak decline. A project is supported by the European Commission in which we participate (PATHOAK). A variety of oak forests were surveyed in France, with or without presence of oak decline. First results confirmed that quite numerous species of *Phytophthora* could be present in the soil of oak forest (Hansen and Delatour, 1999), in agreement with previous reports from Germany and elsewhere (Jung *et al*., 1997). The species most frequently baited in northern France from soil or the related substrates were *Phytophthora citricola, P. quercina, P. gonapodyides* and *Phytophthora* "Type 6", a undescribed homothallic species. *P. cinnamomi* is also widespread in the south-west and the mediterranean oak forests of France (Lévy, 1995 ; Robin *et al*., 1998).

P. cinnamomi excepted, pathogenicity of these *Phytophthora* species was seldom tested on oaks. In forest conditions, *P. cinnamomi* was associated with severe dieback of evergreen oaks in Iberia (Brasier, 1992). An association was also suggested between the common European oak decline and other *Phytophthora* species, especially *P. quercina* (Jung *et al*., 1997).

The aim of the research was to test pathogenicity of the *Phytophthora* species most frequently present in the French oak forest. First information is additionnally given on roots and health status of oaks in the soil of which these *Phytophthora* species were detected.

METHODS

Pathogenicity Test

Oak seedlings 2 y.o. (*Quercus robur, Q. petraea*, and *Q. rubra*) were potted in autumn 1998, at Nancy (experiment 1) or at Bordeaux (experiment 2). The seedlings were inoculated by adding infected millet seeds to the substrate and temporary flooded in Spring 1999. Isolates of 5 *Phytophthora* species were used, mainly from French oak forests (TABLE 1). Plants were monitored for growth (height, diameter) and for symptoms during 3 months. In July-August 1999 the roots were examined for abundance and damage (visual rating, dry weight, taproot necroses). Data were analyzed according to the general linear models procedure (SAS), Dunnett's T test was used for comparisons at the 0.05 level. Reisolation was attempted from fine roots and taproots of all the seedlings (by plating on selective medium, CARPBHy) and from some substrates (baiting).

In Forest

Three plots were investigated in broad-leaved mixed oak forests, Amance (Meurthe-et-Moselle ; age : 75 years ; soil pH=4.8 ; no obvious decline present), Mersuay (Haute-Saône ; 150 y.o., pH=4.9 ; decline present), and Filain (Hte-Saône ; 95 y.o. ; pH=5.1 ; decline present). In each plot 8 neighbouring trees (*Quercus robur*) were selected, 4 healthy and 4 with crown symptoms of decline. Soil samples were collected below each tree, the small roots included. Soils were baited for *Phytophthora*. Among the roots collected, medium sized roots of oak were sorted (1-5 mm of diam.), length was measured and the alive fine roots (diam.≤ 1 mm) present along them were counted.

RESULTS

Disorders

Flooding damage. The first flooding induced direct wilting or mortality of some plants, especially in *Quercus rubra* (40%) compared to *Q. petraea* (7%) and *Q. robur* (0 and 12%). It did not depend on the *Phytophthora* species inoculated.

Alien infections. Three species of *Phytophthora*, as a contaminant, were isolated from the plants at the end of the experiments.

P. cinnamomi was detected in few plants in both experiment (4 in exp.1, and 7 in exp.2).

P. cambivora was in 7 plants of the exp. 1, mainly on *Q. petraea* (7%).

P. quercina was present in 13 out of the 16 treatments not inoculated with it in the exp. 1, including the two oak species, the overall rate of infection was 31%.

All these defective plants were discarded from the further analysis. So, the mean number of plants per treatment dropped from 10 to 7.5 (range 2-10).

TABLE 1. Pathogenicity test parameters.

	Experiment 1 (Nancy)		**Experiment 2 (Bordeaux)**	
Site	glasshouse		plastic greenhouse	
Temperature, light	natural (substrate : ≤ 18°C)		natural	
Substrate	sand:peat (2:1) + fertilizer		sand:perlite:'peat' (1:1:1) + fertilizer	
	not disinfected		'peat' disinfected	
	pH : 5.2 to 6.1		pH : 6.1	
	containers : 5 L (grouped by modalities)		containers : 2.5 L (randomized)	
Host plants (2-3 y.o.)	*Quercus robur; Q. petraea*		***Quercus robur; Q. rubra***	
Isolates (*Phytophthora*)	*P. cinnamomi*	P.382, from UK	*P. cinnamomi*	P.382, from UK
	P. quercina	QUE.67, from Germany	*P. quercina*	QUE.67, from Germany
		QUE.2AU1: QUE.EV4, from France		QUE.EV4, from France
	P. citricola	CIT.2AE5; CIT.AB2, from France	*P. citricola*	CIT.585, from France
	P. gonapodyides	GON.AB4; GON.2AE1, from France	*P. gonapodyides*	GON.AB4, from France
	Phytophthora sp. 'type 6'	AL.2AU2; AL.AY, from France		
Replicates (No.)	10		10	
Inoculation	12 April 99 (flushing)		30 April 99	
	Infected millet seeds added to the soil		Infected millet seeds added to the soil	
	40 mL/pot		20 mL/pot	
Flooding (48H)	2 floodings (12 Apr.; 12 May, 99)		1 flooding (30 Apr. 99)	
Exp. duration	3 months		3 months	

TABLE 2. Re-isolation of the *Phytophthora* species inoculated

Phytophthora species	Oak species							
	Quercus petraea		***Quercus robur***				***Quercus rubra***	
	Experiment 1		Experiment 1		Experiment 2		Experiment 2	
	trees[(1)] (no.)	infected (%)	trees[(1)] (no.)	infected (%)	trees[(1)] (no.)	infected (%)	trees[(1)] (no.)	infected (%)
P. cinnamomi	8	100	10	100	9	89	9	78
P. quercina	28	46	28	50	15	0	15	0
P. citricola	16	19	14	14	8	25	8	13
P. gonapodyides	17	0	11	9	4	0	7	14
P. 'type 6'	13	8	13	0	-	-	-	-

(1) number of seedlings available

Re-isolation of the inoculated Phytophthora species (TABLE 2)

P. cinnamomi was easily detected in the inoculated plants, and in the substrate when done.

P. quercina was detected in exp.1 only, where half the inoculated plants were positive ; it was also detected in some inoculated substrates.

The other *Phytophthora* species inoculated, *P. citricola*, *P. gonapodyides*, and *Phytophthora* 'type 6' were detected in few cases, from the seedlings or from the substrate.

Impact of inoculation on the host plants

General

All the parameters measured were very variable.

Symptoms. Above collar symptom typical of *Phytophthora* infection did not develop during the experiments.

Tree growth. No impact of inoculation was detected on height growth and on diameter growth during the experiments (results not shown).

Damage to the lateral roots

Dead fine roots were present as a rule. In all the treatments visual rating was similar (exp. 2), except in those inoculated with *P. cinnamomi* in which trees tended to be twice damaged on average (FIGURE 1).

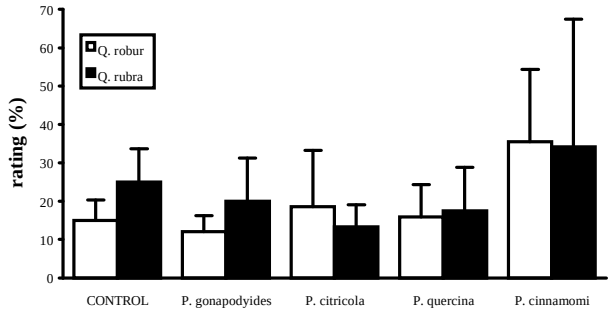


FIGURE 1. Dead fine roots in *Quercus robur* and *Q. rubra* (3 y.o.) rated 3 months after soil inoculation with *Phytophthora* spp. (exp. 2) [Error bars : s.d.]

Dry weight of the whole lateral root systems, were significantly different between the treatments. In exp.1, significant differences originated from the *Phytophthora* species, not from the isolates or the oak species or the interactions. Seedlings inoculated with *P. cinnamomi* had total roots dry weight lower than those of only three other treatments (*P. gonapodyides*, *Phytophthora* 'type 6', and *P. quercina*. Results not shown).

Dry weight of the medium sized roots (diam ≥ 2 mm) specifically considered in exp. 1 showed more accurate differences. The differences originated more clearly from the *Phytophthora* species. Only the seedlings inoculated with *P. cinnamomi* had medium sized roots significantly lighter than those of all the other treatments, controls included (FIGURE 2). An interaction was detected between oak species and isolates. That means that the isolates of a given species of *Phytophthora* could behave differently on *Q. robur* or *Q. petraea*.

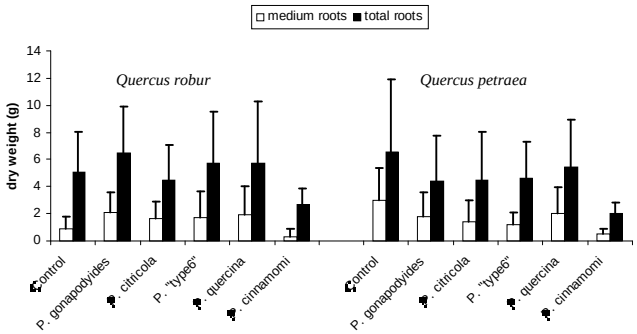


FIGURE 2. Dry weight of the lateral roots of Quercus robur and Q. petraea (3y.o.) 3 months after soil inoculation with *Phytophthora* spp. (exp 1)

Damage to the taproot

Bark necroses were present on the taproot of some seedlings. It consisted in blackish patches, round or elongated.

Damage to the cambium were also present, whatever the size they were always limited by the healing reaction.

Length of the lesions depended only on the *Phytophthora* species, not on the isolates or on the oak species (exp. 1). Only trees inoculated with *P. cinnamomi* harbored significantly longer cambium lesions compared to the other species (FIGURE 3).

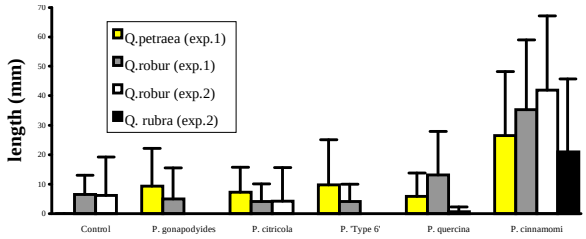


FIGURE 3. Damage to the taproot of oaks (3 y.o.) 3 months aftre inoculation with *Phytophthora* spp. (mean total length of necroses per taproot)

Special considertion of *Phytophthora quercina*

As *P. quercina* was frequently present in a variety of treatments in exp. 1, we compared trees infected and not infected by *P. quercina*, and distinguished between those inoculated or not inoculated with it. All the trees infected by another species were discarded.

Lateral roots dry weight was variable, not obviously different between the four groups of trees, even when considering the medium sized and fine roots separately in both *Q. robur* and *Q. petraea* (results not shown).

Cambium damage to the taproot was different between infected and uninfected trees in *Q. robur* only :

lesions in the infected trees were more numerous than in the uninfected trees (3.3 lesions per taproot, and 0.8 respectively),

length of the lesions to the cambium showed a similar situation, it averaged about 20 mm in infected trees (FIGURE 4).

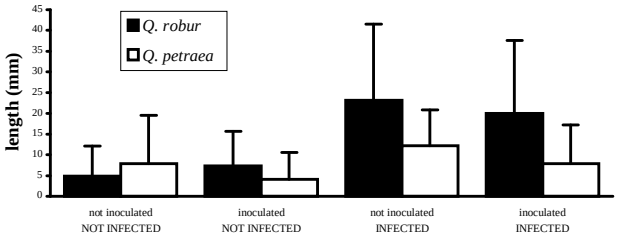


FIGURE 4. Necroses to the taproot of oaks (3 y.o.) infected or not infected by *Phytophthora quercina* (exp. 1)

Comparatively, the number of lesions induced by *P. cinnamomi* was similar (3.1 per taproot), but the length was larger (30-40 mm, FIGURE 3).

Information from the forest plots

The *Phytophthora* species detected in the three plots are shown in TABLE 3. There was no obvious difference between healthy oaks and declining oaks as regards presence / absence of *Phytophthora* in the soil of the 3 plots investigated.

Root parameters were not significantly different between groups of trees whatever the groups considered (FIGURES 5 and 6).

Results are provisional because of the small number of oaks investigated at present.

TABLE 3. Phytophthora species detected in the 3 forest plots investigated for root damage.

Plots	*Phytophthora* species (No. of trees)			
	quercina	*citricola*	*gonapodyides*	*P."type 6"*
Amance	2	0	1	1
Mersuay	1	0	0	1
Filain	3	2	0	1

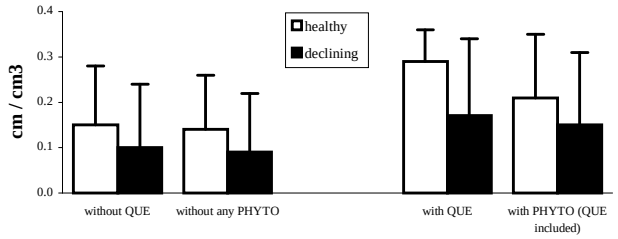


FIGURE 5. Abundance of medium sized roots in the soil below oaks in forest, considering presence of *Phytophthora quercina* or *P. spp*.

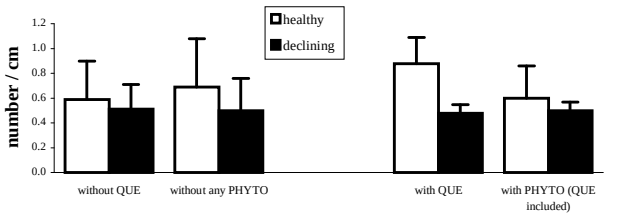


FIGURE 6. Frequency of fine roots on medium sized roots below oaks in forest, considering presence of *Phytophthora quercina* or *P. spp.*

CONCLUSIONS

- *P. cinnamomi* is the only species which caused significant root damage in oak seedlings in the pathogenicity test, compared to *P. gonapodyides*, *P. citricola*, and *P. quercina*.
- *P. quercina* could be prevalent in oak seedlings grown in commercial nursery conditions. It was associated with bark damage to the taproot.
- No obvious relation was delineated between visual evidence of health status of trees and presence of *Phytophthora* in soil in both seedlings inoculated (3 months test) and oaks in forest.

ACKNOWLEDGEMENTS

This study is being carried out with the financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT97-3926, " Long term dynamics of oak ecosystems : assessment of the role of root pathogens and environmental constraints as interacting decline inducing factors " (PATHOAK). It is the sole responsibility of its publishers and in no way represents the views of the Commission or its services or their future policy in this area.

Thanks to Benoit Marçais who performed statistics (exp. 1), to Souhaila Fetni, Mathieu Ruillet and Gilberte Guinot for collecting a number of data, and to Olivier Caël and Gilles Capron for management of the susceptibility tests.

REFERENCES

Brasier, CM., 1992- Oak tree mortality in Iberia. Nature London 360, 539.

Hansen, EH., Delatour, C., 1999- *Phytophthora* species in oak forests of North-East France. Annals of Forest Science, in press.

Jung,T., Blaschke, H., Neumann, P., 1996- Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. European Journal of Forest Pathology 26, 253-272.

Levy, A., 1995- L'encre du chêne rouge d'Amérique : répartition en France, facteurs stationnels dans le piémont des Pyrénées Occidentales. Cahiers du DSF 1, 1-41.

Robin, C., Desprez-Loustau, ML., Capron, G., Delatour, C., 1998- First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. Annales des Sciences Forestières 55, 869-883.

CONE AND SEED PRODUCTION IN A PORT-ORFORD-CEDAR CONTAINERIZED ORCHARD

Leslie Elliott and Richard A. Sniezko

USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR

INTRODUCTION

With cultural management, Port-Orford-cedar can produce seed at a young age. This provides a unique opportunity to breed for increased resistance to *Phytophthora lateralis*, and to produce seed for reforestation in a relatively short period of time. In 1992, the USDA Forest Service initiated a Port-Orford-cedar breeding arboretum at Dorena Genetic Resource Center, near Cottage Grove, Oregon. Trees from this arboretum have been used to refine techniques for control pollinations and to develop a prototype containerized seed orchard.

MATERIALS AND METHODS

Composition: The prototype containerized seed orchard (CSO) was established in February 1997 using 23 clones from the breeding arboretum at Dorena. The clones were USDA Forest Service and Oregon State University selections for *Phytophthora lateralis* resistance made prior to 1991. Rooted cuttings or grafts of these clones had been established in the breeding arboretum from 1992 to 1994. The CSO consisted of 90 trees in four randomized replications, with one ramet per clone per replication (two clones were not represented in Rep 4). The CSO was used to examine cultural techniques for enhancing seed production. Trees in the breeding arboretum were used for control pollinations. Both the CSO and breeding arboretum were located in unheated greenhouses.

Flower Induction: In 1996, all trees in the prototype CSO and the breeding arboretum were treated with a gibberellic acid (GA_3) to induce flowering in spring 1997. Only two replications of the CSO were treated with GA_3 in 1997 to induce flowering in spring 1998. GA_3 treatment consisted of spraying trees on a weekly basis, beginning in June, for six weeks at a rate of 200 mg/liter until foliage saturation.

Pollination: Pollen shed and seed cone receptivity occur from February through April at Dorena. In 1997, wind-pollination was utilized for all four replications of the prototype CSO; in addition, supplemental pollen was applied to two of the four replications (approximately 8 cc's of pollen) three times over a ten-day period. In 1998, only wind pollination was utilized in all four replications of the CSO.

For controlled crosses, pollination of the receptive seed cones was done using small paint brushes. For most crosses, fresh pollen collected one or more days beforehand was utilized.

Cone Collection: Cone collection occurred in September of the same year as pollination (1997 and 1998). Cones were collected and seed extracted by tree for the CSO, and by control cross for the arboretum. Total number of cones collected and seed extracted was estimated by counting 100 cones and 100 seed per tree (or per cross), and utilizing the ratio of weight of 100 cones (or seed) to the total weight. Percent filled seed was determined using x-rays to examine 100 seed per tree from the CSO, and 100 seed per cross for the breeding arboretum.

RESULTS

- GA_3 treatment dramatically increased pollen cone and seed cone production. 98% of the cones and filled seed in 1998 came from the 44 GA_3 treated trees (versus 46 non-treated) (Table 1).
- Although only 44 of the 90 trees in the CSO were treated with GA_3 the second year, cone yields in 1998 were greater than in 1997 (28,740 versus 20,559), and number of filled seed was only slightly less (154,237 versus 177,866).
- Over all 23 clones, filled seed per cone averaged 8.6 in 1997 and 5.4 in 1998. Percentage filled seed was nearly identical for the two years (61.6% versus 61/7%).
- The top five producing clones yielded 60% of the filled seed in 1997, the top six in 1998 yielded 64% of the filled seed; interestingly however, none of the top yielding clones were common in the two years (Figure 1).
- Application of GA_3 in 1996 produced cones in 1997, but had little or no residual effect on the 1998 cone yield.
- Control pollinations varied in success, with average filled seed yields of 5.2 per cone for 1997 and 1998, and a mean of 43.6% filled seed (Table 2).

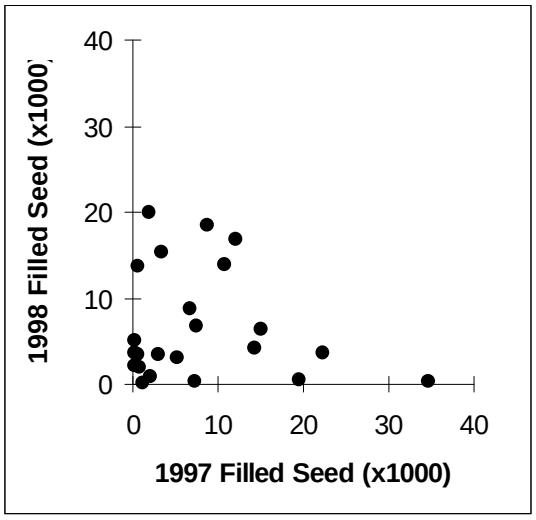


FIGURE 1. Clonal variation over two years in filled seed yield.

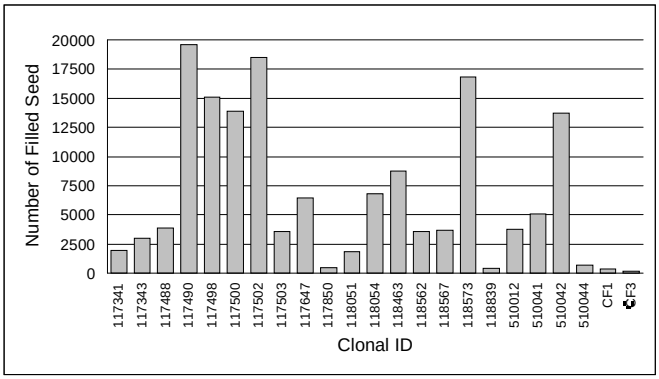


FIGURE 2. Clonal differences in 1998 seed yields with GA3 treatments

TABLE 2. Mean Cone and seed yields from full-sib crosses, self-pollinations, open-pollinations, and unpollinated controls in 1997 and 1998.

	1997				1998			
	Full-sib cross	Self pollination	Open pollination	Unpollinated Controls	Full-sib cross	Self pollination	Open-pollination	Unpollinated Controls
No. of Trees/Cross	73	14	13	23	68	11	3	12
No. of flowers bagged	50.6	59.2	na	57.2	93.8	99.8	na	118.6
No. of ConeS Harvested	23.1	29.6	na	1.5	28.8	31.3	1026.7	6.3
Ratio of Cone Yield/Flowers Pollinated	0.45	0.50	na	0.02	0.32	0.32	na	0.07
Filled Seed per Cone	5.5	3.0	na	0.0	4.9	3.2	0.1	0.0
Filled Seed %	44.3%	23.5%	54.8%	0.0%	42.9%	20.1%	25.7%	0.0%

TABLE 1. Filled Seed Yield in CSO in 1998

Rep	**Treatment**	Filled Seed Yield
1	none	477
2	GA_3	78,071
3	none	1784
4	GA_3	73,905

- Self-pollinations averaged 3.1 filled seed per cone, and 21.8% filled seed in 1997 and 1998. Bagged, unpollinated seed cones yielded only a few mature cones and no filled seed in 1997 and 1998 (Table 2).

- A seed insect (Port-Orford-cedar midge, *Janetiella siskiyou*) was found in many of the 1998 cones. Further investigation is needed to determine how to mitigate potential loss of seed from this insect.

- Supplemental mass pollination increased total number of filled seed in 1997, but not the percentage of filled seed (Figure 3).

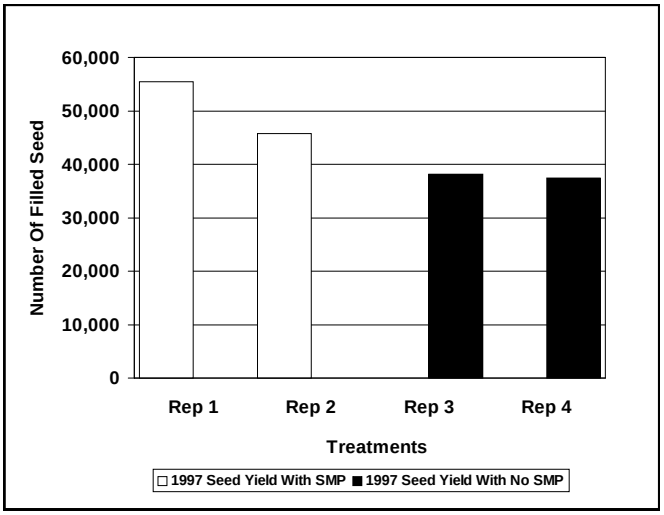


FIGURE 3. Prototype containerized seed orchard 1997 seed yields with supplemental mass pollination.

SUMMARY

Using GA_3 to induce flowering, good cone and seed yields should be possible at a young age in Port-Orford cedar. Confirmation of similar yields with young trees from seeds (versus rooted cuttings and grafts of older material) is needed.

Control crosses are feasible at a young age for most selections. Some clones may require several years of pollinations to obtain adequate seed yields for resistance evaluation.

ACKNOWLEDGEMENTS

Branch cuttings and scion wood for the trees in the clonal orchard used for this work were collected by USDA Forest Service personnel. Culture and maintenance of the trees was done by staff and technicians at Dorena Genetic Resource Center. Jerry Berdeen was responsible for cone processing, seed extraction, and determinations of filled seed percentage. Partial funding for this project was through the USDA Forest Service Forest Health and Protection Special Technology Development Project. A special thanks also goes to Mary Brennan, Umpqua National Forest, for the computer and artistic skills in putting together this poster in a short timeframe.

PORT-ORFORD-CEDAR ROOT DISEASE IN SOUTHWESTERN OREGON AND NORTHWESTERN CALIFORNIA

D. Goheen[1], P. Angwin[2], R. Sniezko[3], K. Marshall[1]

[1]USDA Forest Service, Southwest Oregon Forest Insect and Disease Service Center, 2606 Old Stage Road, Central Point, OR 97502, USA
[2]USDA Forest Service, Northern California Shared Service Area, 2400 Washington Avenue, Redding, CA 96001, USA
[3]USDA Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, OR 97424, USA

SUMMARY

Port-Orford-cedar (*Chamaecyparis lawsoniana*) is affected by an introduced pathogen *Phytophthora lateralis*, cause of Port-Orford-cedar root disease. The pathogen was first reported killing ornamental Port-Orford-cedar nursery stock around Seattle, Washington in 1923, and was detected in the host's native range in 1952. It has since spread into many areas occupied by Port-Orford-cedar in southwestern Oregon and northwestern California. Origin of the pathogen is unknown. *P. lateralis* is well adapted for active spread in water and passive, long distance transport in soil. High risk areas for infection are stream courses, drainages, or low lying areas down slope from already-present infection centers or below roads or trails where new inoculum can be introduced by earth movement in road construction, road maintenance, logging, traffic flow on forest roads, or by animals. *P. lateralis* is an extremely virulent pathogen. Once established on microsites with characteristics favorable for its spread, the pathogen kills hosts rapidly. To date, management of Port-Orford-cedar root disease has involved excluding the pathogen from areas where it does not yet occur and minimizing spread in already infested areas. Components of such programs have been road closures, timing access into stands with Port-Orford-cedar during dry weather, washing equipment that is being moved from infested to uninfested areas, roadside sanitation treatments, special care in road building and maintenance operations, and featuring Port-Orford-cedar on sites unfavorable for the pathogen (upslope situations, convex slopes, well-drained microsites, away from roads and streams). Recent discovery of Port-Orford-cedars with a degree of resistance to *P. lateralis* offers promise of another management approach for the future. Screening of Port-Orford-cedar for resistance is in progress across the range of the species, and a breeding program to enhance resistance is in the developmental phase.

PORT-ORFORD-CEDAR ROOT DISEASE

Port-Orford-cedar (*Chamaecyparis lawsoniana*) is affected by *Phytophthora lateralis*, an extremely virulent, introduced root pathogen. The pathogen was first reported killing Port-Orford-cedar stock in ornamental nurseries in Seattle, Washington in 1923 (Hunt 1959, Zobel et al. 1985); in the 1930s and 40s, it was found in the Willamette Valley of Oregon killing Port-Orford-cedar landscape trees (Tucker and Milbrath 1942, Zobel et al. 1985); and it was reported at Coos Bay, Oregon first causing tree mortality in the native range of Port-Orford-cedar in 1952 (Roth et al. 1957). Subsequently, it has spread through much of the cedar's rather small natural range in southwestern Oregon and northwestern California.

Except for *P. lateralis*, Port-Orford-cedar has few significant enemies. Cedar bark beetles (*Phloeosinus* spp., especially *P. sequoiae*) and amethyst cedar borers (*Semanotus amethystinus*), infest some trees, but usually only trees of very much reduced vigor. They rarely kill trees by themselves, but commonly administer the *coup de grace* to severely stressed cedars, especially those infected by *P. lateralis*. Black bears (*Ursus americanus*) can be locally damaging to Port-Orford-cedar when they peel bark and feed on the cambium of trees in early spring. When feeding is extensive, trees may be completely girdled and killed. Port-Orford-cedars, especially those occurring on drier sites, may succumb to drought damage during periods of protracted dry weather. Drought may also predispose cedars to attack by bark beetles or woodborers. By and large, Port-Orford-cedar is a remarkably decay resistant species. Several decay fungi, including *Phellinus pini* and *Heterobasidion annosum* have been found on Port-Orford-cedar, but are uncommon and appear to have little impact.

The origin of *P. lateralis* is unknown. Many investigators believe that it is an Asian species based on the high level of resistance to the pathogen exhibited by Asian *Chamaecyparis* species (Tucker and Milbrath 1942, Zobel et al. 1985). However, the pathogen has not actually been found in Asia. Europe has been suggested as another possible point of origin of the pathogen (Erwin and Ribeiro 1996), but evidence for an introduction from that source is not substantial. Hansen et al. (1999) confirmed the identity of *P. lateralis* isolated from container-grown Port-Orford-cedar seedlings in France but strongly believed that its presence there resulted from a recent introduction from North America rather than natural occurrence of the pathogen in Europe. Another theory concerning the source of *P. lateralis* is that the pathogen may have originated from some location in North America outside of the rather small area where Port-Orford-cedar is native. Roth (unpublished) postulated that *P. lateralis* might be an uncommon, relatively benign endemic on yellow-cedar (*C. nootkatensis*) that infected highly susceptible ornamental Port-Orford-cedars when they were planted in the range of the former species in British Columbia and then spread south in Port-Orford-cedar landscape plantings. *P. lateralis* has never been found on yellow-cedar in the wild but is capable of occasionally infecting that species when it is inoculated in the laboratory (Torgeson et al. 1954) or planted together with Port-Orford-cedars on an infested site (Hansen, Roth, and McWilliams unpublished).

P. lateralis appears to be quite host specific. Besides Port-Orford-cedar, only Pacific yew (*Taxus brevifolia*) has been found infected in the wild (DeNitto and Kliejunas 1991, Kliejunas 1994). Pacific yew is much less susceptible to the pathogen than Port-Orford-cedar, and evidence indicates that it mainly becomes infected when in close association with substantial numbers of already-infected cedars (Murray and Hansen 1997).

P. lateralis is highly adapted for spread in water. Zoospores can swim for several hours in standing water and can be carried considerable distances in flowing water. Zoospores are attracted by host root exudates and will follow an increasing gradient of chemical concentration until they contact living root tissue, encyst, germinate, penetrate the root, and initiate infection. Infection occurs primarily through the unsuberized growing tips of small rootlets that occur in the duff or at shallow depths in soil. Port-Orford-cedars produce a multitude of fine rootlets in these strata (Gordon and Roth 1976, Zobel et al. 1985). Sporangial development and zoospore production of *P. lateralis* are favored by cool, moist conditions and are optimal at temperatures between 10° and 20° C (Trione 1974). Under favorable cool, wet conditions, *P. lateralis* populations can amplify rapidly in areas where hosts are numerous because of the rapid and continuing production of flagellate zoospores.

P. lateralis is capable of surviving for considerable periods of time when conditions are unfavorable for zoospore spread and infection. When suboptimal moisture and temperature conditions develop, the pathogen forms laterally arranged chlamydospores on hyphae in infected roots. Ostrofsky et al. (1977) showed that *P. lateralis* populations detected by baiting around infected trees decreased substantially when unfavorable warm, dry conditions typical of summer months in the range of Port-Orford-cedar occurred. However, the pathogen did survive at a reduced level as chlamydospores in organic matter, especially in small roots on infected trees and fragments of roots in the surrounding soil. Hansen and Hamm (1996) demonstrated that the pathogen can survive in infected roots and fragments for at least seven years. *P. lateralis* chlamydospores are incapable of direct movement, but their structure provides protection during passive movement in infected roots or organic material in soil and mud. Oospores also can act as resting spores and may be transported from one place to another in infected root tissues. When environmental conditions become favorable, resting spores germinate, forming zoospore-containing sporangia.

Long distance spread of *P. lateralis* results from moving infected seedlings and, especially, infested soil into previously disease-free sites. Humans have been the main vectors of the pathogen. Major spread has occurred via earth movement in road construction, road maintenance, mining, logging, and traffic flow on forest roads (Roth et al. 1957, Roth et al. 1972, Kliejunas 1994). In general, the pathogen has not spread into areas where physical barriers or lack of access have prevented human activity. Movement of the pathogen in soil clinging to the feet of elk, cattle, and humans also is known to occur but on a much more localized basis than that associated with vehicles (Harvey et al. 1985, Kliejunas 1994, Kliejunas and Adams 1980, Roth et al. 1972). Spread of *P. lateralis* occurs primarily in the late fall, winter, and early spring when the cool, moist environmental conditions favorable for the pathogen prevail. Little or no spread occurs in the hot, dry summer months.

Once introduced to a new area, *P. lateralis* spreads in water downslope from the roads and trails where it is initially established. Inoculum often builds to high levels in concentrations of hosts growing on disturbed areas close to road edges, thus increasing the likelihood of downhill spread. In virtually all cases, infection of Port-Orford-cedar by the pathogen occurs in areas where obvious avenues for water-borne zoospore dispersal exist. Infection is dependent on the presence of free water in the immediate vicinity of susceptible tree roots. High risk areas for infection include stream courses, drainages, low lying areas downslope from existing infection centers, and areas below roads and trails where new inoculum could be introduced in the future. Topography has a considerable influence on spread. Steep slopes dissected by drainages quickly channel zoospore-infested water into streams. Cross slope spread is restricted. On broad slopes or flat areas, infested water may spread out over larger areas and move more slowly. Because they are easily flooded, concave areas with Port-Orford-cedar are very vulnerable to damage. Convex slopes, on the other hand, have limited vulnerability. Port-Orford-cedar growing on sites or microsites that are unfavorable for spread of the pathogen often escape infection, even in areas where infected trees are nearby. Tree to tree spread of *P. lateralis* via mycelial growth across root contacts does occur (Gordon 1974) but is considered to be much less significant in the epidemiology of the pathogen than spread by zoospores in free water.

Port-Orford-cedar root disease is identified in the field by the rapid death of individual hosts, by the fact that only Port-Orford-cedar and rarely Pacific yew are affected, by the characteristic distribution of the disease in sites favorable for the water-borne spread of the pathogen, and by the distinctive symptoms that *P. lateralis* causes on infected cedars (Zobel et al. 1985). Crowns of infected trees first fade slightly or appear somewhat wilted. They subsequently change color from their normal green or blue green to yellowish gold, bronze, reddish brown, and finally dull brown. Symptoms manifest themselves especially rapidly and tree death occurs quickly in seedlings and saplings during periods when warm, dry weather develops after infection. With such trees, the entire progression of symptoms may occur within a few weeks. Large Port-Orford-cedars die much more slowly, declining over periods of one to four years. Signs of infection in Port-Orford-cedar roots include loss of luster of root tips, water-soaking of rootlets, and death and decay of roots. Bark on main roots may darken or turn somewhat purplish. The mycelium of the pathogen grows in the inner bark and cambium of the host, colonizing and killing much of the root system, and ultimately girdling the main stem in the lower bole. In live Port-Orford-cedar exhibiting crown symptoms, a cinnamon-colored stain that abuts abruptly against healthy, cream-colored inner bark is apparent at or above the root collar. This stain, which can be followed down into the roots, is considered diagnostic of infection by *P. lateralis*. Once a Port-Orford-cedar dies, the inner bark of the entire bole turns brown, and it is no longer possible to use presence of staining as an identification tool.

Port-Orford-cedar root disease centers consist of variable-sized groups of dead and dying trees. Port-Orford-cedar is a prolific seed producer, and new regeneration of the host often becomes established in infection centers. This regeneration usually becomes infected in turn, resulting in chronic disease expression. Because of its ability to reproduce at an early age and produce large numbers of seeds, and because many trees that occur on sites with characteristics unfavorable for spread of *P. lateralis* completely escape infection, Port-Orford-cedar has not been eliminated by *P. lateralis* in any significant portions of its range. Nonetheless, *P. lateralis* has caused very substantial amounts of mortality on individual infested sites and has greatly influenced stand structure by killing larger trees and preventing smaller trees from attaining large size. The disease can greatly influence the ecological roles of Port-Orford-cedar, particularly in streamside areas where conditions are most favorable for spread of the pathogen.

CURRENT MANAGEMENT

In the first two and a half decades after the introduction of *P. lateralis* into the native range of Port-Orford-cedar, few if any attempts were made to manage the disease it caused. The spectacular virulence of the exotic pathogen and the speed with which it spread along roads and streams as well as the obvious tie between spread and then-practiced timber harvesting techniques led to statements such as "There appears to be no hope of raising another crop of Port-Orford-cedar under existing conditions of disease and land use." and "(Production of Port-Orford-cedar) will likely decline and ultimately drop to nearly nothing as the remaining merchantable trees die or are harvested (Roth et al. 1972)." It was felt by many that with the pathogen established, active management of Port-Orford-cedar as a timber species was not worthwhile. Emphasis was placed on extensive salvage of large disease-killed cedars, and the prevailing attitude among forest managers toward Port-Orford-cedar was one of considerable pessimism.

Attitudes about managing Port-Orford-cedar root disease changed dramatically in the 1980s and 90s. Forest managers on Federal Lands administered by the United States Department of Agriculture Forest Service and the United States Department of the Interior Bureau of Land Management, are now involved in an active program to minimize detrimental impacts of the root disease. There is a strong commitement to do whatever is possible to maintain and, where feasible, enhance both the ecological and economic viability of Port-Orford-cedar. Management directions for the Port-Orford-cedar program are incorporated in agency land management planning documents. The goals of the program are to integrate strategies for Port-Orford-cedar management into environmental analyses and project planning for all areas that support the species on Federal lands; to manage Port-Orford-cedar as an appropriate component of forest stands in the suitable plant associations; and to use the best practices identified from experience and research to prevent or reduce the spread of Port-Orford-cedar root disease.

Port-Orford-cedar root disease management strategies involve planned combinations of treatments that together reduce probability of disease spread and intensification as much as possible across a landscape. Treatments considered for use in a strategy are aimed at reducing the risk of introducing the pathogen into new areas or at managing a component of Port-Orford-cedar in areas that are already infested. A number of disease management techniques have been recommended and used (Betlejewski 1994, Filip et al. 1994, Hadfield et al. 1986, Hansen and Hamm 1996, Hansen and Lewis 1997, Harvey et al. 1985, Jimerson 1994, Kliejunas 1994, Kliejunas and Adams 1980, Neilsen 1997, Roth et al. 1957, Roth et al. 1972, Roth et al. 1987, Tainter and Baker 1996, Thies and Goheen 1999, USDA Forest Service 1983, Zobel 1990, Zobel et al. 1985). These include:

1) Exclusion

The exclusion approach involves protecting Port-Orford-cedar by completely preventing entry of vehicles, the main carriers of the disease organism, into areas where the disease is not yet present. Under this approach, no new roads are built into selected uninfested areas, and existing roads are permanently closed in selected areas where roads do occur but the disease has not yet been introduced. Road closures are done in such ways that vehicles cannot broach them or detour around them. "Tank traps," large berms, or rock piles are strategically located at sites where it is impossible to bypass them. Alternatively, roads are completely obliterated with their culverts removed and their beds destroyed. To be successful, exclusion must be practiced in a location that realistically can be protected. Exclusion is best used where an entire drainage or at least the upper portion of such a feature can be treated as a protection unit.

2) Temporary Road Closures

Like exclusion, the temporary road closure approach seeks to protect Port-Orford-cedar by preventing vehicles from carrying propagules of the disease organism into uninfested areas. It differs from total exclusion by allowing controlled road use in vulnerable areas during times when conditions are unfavorable for establishment and spread of the pathogen. Roads are closed during the cool, wet season of the year, typically from Oct. 1 to June 1. In addition, special closures are applied during particularly wet periods at other times of the year. Roads are closed with locked gates, guardrails, or other movable barriers, and closures are located in areas where they cannot be bypassed. Temporary closures require considerable attention to ensure that they are indeed in place when they need to be (during wet periods at any time of year) and that they are not broached. Since the roads are still present beyond the closures, uninformed, thoughtless, or uncaring people sometimes find ways around the closures at times of the year when they should not or actually force open or destroy gates or other structures to gain access. Placement and strength of barriers are important considerations in use of temporary closures, as is constant vigilance.

3) 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. 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 of vehicles using the road. Since zoospores, the propagules of *P. lateralis* that would 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. The key feature of any sanitation treatment 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 general recommendation now given by the Forest Service is to treat all Port-Orford-cedars in a buffer zone extending 8.5m above the road or to the top of the cutbank. Below the road, recommended treatment width is 8.5m to 17m with the greater distances applied where streams or drainages cross the road or where amount of road fill is particularly substantial, resulting in especially steep slopes. Sanitation treatments need to be repeated periodically to ensure that roadside buffers are not reinvaded by Port-Orford-cedar regeneration. The preferred approach is to monitor treated areas and retreat them whenever Port-Orford-cedar seedlings 15 cm in height or greater become evident.

4) Vehicle Washing

This approach involves thoroughly cleaning vehicles and equipment to remove adhering soil or plant debris before driving them into areas where healthy Port-Orford-cedars occur or moving them from *P. lateralis* infested to uninfested areas within the forest. When vehicle washing is used as part of a Port-Orford-cedar root disease management strategy, location and design of washing stations are extremely important. Stations should be located in areas where run-off water has no chance of entering adjacent streams or drainages or of threatening nearby Port-Orford-cedars. Furthermore, stations must be designed so that vehicles that have been washed are not likely to be recontaminated by passing through wash water that contains *P. lateralis* propagules on their way out of the station.

5) Appropriate Operations Planning, Scheduling, And Execution

This technique involves separating operations in disease-free locations in both space and time from work in diseased stands. Forest management projects in stands with Port-Orford-cedars, especially in uninfested areas, are performed when conditions are unfavorable for pathogen spread and survival. Projects are preferentially scheduled and completed in the warm, dry months. Operations are usually limited to some time within the period of June 1 through October 1. Operations may be allowed outside of the normal season if especially dry conditions prevail, but such exceptions are carefully regulated. Operations are discontinued when wet conditions develop, even during the stated operating season. Repeated entries onto vulnerable microsites are avoided, and work is scheduled to proceed from healthy to infested sites, not the other way around. Equipment is not allowed to operate from a contaminated area into a clean one nor is equipment allowed to move from a clean area into a contaminated area and return. Whenever possible, access to project areas is planned along routes with the least occurrence of infested sites. Where timber harvesting operations are being done in stands with Port-Orford-cedars or where streams run into stands with Port-Orford-cedars below harvesting units, systems that minimize amount of soil movement, especially across slope movement, are preferred. Skyline systems or helicopter logging systems are used, and tractor logging systems are avoided. Where possible, all root disease prevention/management activities are coordinated with adjacent landowners (private and other agencies).

6) Integrating Disease Treatments In Road Design, Engineering, and Maintenance

Minimizing the risk of *P. lateralis* spread is an important consideration in designing and building new roads and in maintaining or improving existing roads in areas with Port-Orford-cedars. For new construction, routing decisions are made with knowledge of where Port-Orford-cedar concentrations occur. When possible, new roads or spurs are not located above concentrations of Port-Orford-cedar. Instead, they are situated below such concentrations or on the opposite sides of ridges. Culverts and waterbars are designed to direct water quickly into existing well-defined water channels away from areas where Port-Orford-cedars exist. Road beds are insloped, and, in some cases, site specific berms are used on the outside edges

of roads to prevent downslope flow of water. Road building and maintenance is restricted as much as possible to the dry season and only clean equipment is used. Movement of soil and debris from one place to another in construction or maintenance is minimized, and casting gravel from roads with infected trees along them into drainage ditches, streams, or over road berms during road maintenance is avoided. During construction or road upgrades, clean rock is used for road surfacing (quarry rock from certified clean rock pits is preferred over river rock). When possible, pavement or uninfested rock is added to raise roadbeds that pass through infested sites. Stream crossings on new roads are designed to keep vehicles out of contact with water and primitive roads that cannot be closed are upgraded so that fords and puddles are eliminated. Care is taken in moving soil and other material when endhauling, repairing flood damage, or removing slides, especially in or near infested areas.

7) Water Source Selection and Treatment

Under this technique, water sources on Federal lands are inventoried and those that are infested by *P. lateralis* are identified. Subsequently, when water is needed for fire fighting or dust abatement, uninfested water sources are used if possible. Where no clean water sources exist and water must be taken from a probably infested source, it is treated with Clorox before use. In areas where water sources have not been inventoried, Clorox is used as a matter of course. Adding chlorine bleach to *P. lateralis*-infested water will kill many propagules of the pathogen. Murray, McWilliams, and Hansen (unpublished) demonstrated that complete mortality of *P. lateralis* zoospores occurred after 60 minutes in 100 ppm chlorine bleach, and complete mortality of chlamydospores occurred after 30 minutes in 5000 ppm chlorine bleach. Clorox has recently been registered for use in treating water for firefighting or dust abatement to decrease probability of *P. lateralis* spread.

8) Featuring Port-Orford-cedar in Areas Unfavorable for the Pathogen

This approach involves preferentially managing Port-Orford-cedars on sites where conditions make it likely that hosts will escape infection by *P. lateralis* even if the pathogen has already been established nearby or may be introduced nearby in the future. Cedars are featured above and away from roads, uphill from creeks, on ridgetops, and on well-drained sites. Maintaining existing Port-Orford-cedars on low vulnerability sites as well as actually developing "cedar production areas" by planting and actively managing Port-Orford-cedars on sites with such characteristics are both approaches being used.

9) Special Direct Management in Currently Infested Areas

This approach involves managing some component of Port-Orford-cedar in stands occurring on already infested sites by situating or maintaining cedars in locations where they individually have a low likelihood of becoming infected and by spacing cedars widely enough that probability of root to root spread as well as spread via water is reduced. Cedars are favored in plantings and thinnings on microsites that are unfavorable for the pathogen (particularly mounds and other high places) and are not managed on microsites especially favorable for infection (close to and below roads, in or very close to streams or drainage ditches, and in lowlying wet areas). Cedars are planted or retained in thinnings in mixed species stands at wide spacing (8.5m or more between individual trees or in small groups of 10 to 20 trees 33m apart).

10) Species Manipulation

This approach involves favoring other tree species that are appropriate for local sites over Port-Orford-cedars in management. It is especially applicable on sites where *P. lateralis* is already established or in sites that are particularly favorable for future establishment of the pathogen such as wet areas, stream sides, or concave slopes below roads. Other tree species can occupy sites where Port-Orford-cedars are no longer able to and will themselves face no risk of being damaged by the root disease. However, no other species can command the same economic value as Port-Orford-cedar or consistently fulfill the same ecological roles. Some hardwoods, western red cedar (*Thuja plicata*) and yellow-cedar may be able to play similar ecological roles in riparian zones in some portions of Port-Orford-cedar's range and blister rust-resistant western white pine (*Pinus monticola*) and sugar pine (*P. lambertiana*) may ultimately provide large tree components in some stands on ultramafic soils. By and large, however, Federal managers are interested in maintaining Port-Orford-cedar itself in as many appropriate areas as possible.

11) Regulating Non-timber Uses

A number of special use activities including Port-Orford-cedar bough collecting, mushroom picking, salal gathering, grazing, and mining are engaged in on Federal forest lands and have potential to influence spread of *P. lateralis*. Several of the activities involve extensive vehicle travel. Some, if not controlled, can involve vehicle movement from infested to uninfested areas. And some, especially bough collecting and mushroom hunting, are preferentially engaged in at times of the year when the cool, wet conditions most favorable for spread of the pathogen prevail. Port-Orford-cedar bough collecting, of course, is concentrated in areas where cedars occur, and there is considerable anecdotal evidence associating bough collecting with spread of *P. lateralis*. Concerns about vectoring of *P. lateralis* with special use activities are similar to those associated with forest management projects, but special use activities are much harder to control. Where possible, managers attempt to regulate issuance of permits, specify where activities can be done, regulate the sequence of operations, and determine the appropriate timing of activities with the objectives of limiting Port-Orford-cedar root disease spread. Efforts are also made to inform permittees about the disease and convince them of the need to cooperate with disease management recommendations. Recreationists, including hikers, mountain bike riders, horseback riders, hunters, off road vehicle users, and campers also have considerable potential to spread *P. lateralis*. They are even harder to monitor and regulate than special use permittees. Recreationists can be controlled to some degree by closures and blockades, and organized, Federally sanctioned activities can have specific, enforceable rules aimed at decreasing risk of disease spread. However, most recreationists are probably best approached through education.

12) Educational Efforts

Humans are responsible for most spread of *P. lateralis*, yet a surprising number of forest workers as well as recreationists have no idea of the significance of the pathogen and may inadvertently aid its spread due to lack of knowledge and understanding regarding the issue. Some know a little about Port-Orford-cedar root disease but do not fully appreciate the implications of their own activities in vectoring the disease organism. Federal agencies are heavily involved in efforts to disseminate information on the biology and ecology of *P. lateralis* with emphasis on how the pathogen spreads and how spread can be prevented. Presentations at training sessions, workshops, and symposia as well as newspaper articles, television interviews, pamphlets, journal articles, displays at public functions, classroom teaching materials, and information signs at BLM offices, Ranger Stations, visitor information centers, and in campgrounds, along forest roads, and at trail heads are used.

DISEASE RESISTANCE

The most intriguing potential long term Port-Orford-cedar root disease management option is the possibility of developing genetic resistance to *P. lateralis* in Port-Orford-cedar populations. Hansen et al. (1989) demonstrated that some Port-Orford-cedars exhibit a degree of resistance to *P. lateralis*. Although no immune trees have been found, some trees are less readily infected by the pathogen and some survive longer than others when infected. Mechanisms of resistance are unknown but appear to be heritable.

The Forest Service and Bureau of Land Management in cooperation with Oregon State University have embarked on a program to identify resistance mechanisms, screen a large sample of candidate trees from across the range of Port-Orford-cedar, conserve resistant families, and develop a breeding program to enhance resistance.

Over 8,000 phenotypically resistant candidate trees selected in the forest have been screened using Oregon State University's branch lesion test. Cuttings are in the process of being rooted from the 961 best performers, a conservation orchard has been started at the Bureau of Land Management's Tyrrell Seed Orchard, and a containerized seed orchard for breeding has been developed at the Dorena Genetic Resource Center. Screening and field testing is continuing and breeding work has started. Fortunately, Port-Orford-cedar is very amenable to breeding efforts. Clones are relatively easy to root from cuttings, and flowering can be induced on most trees at very early ages (as little as two years old). Outplantings of the first trees identified as exhibiting a degree of resistance show that a substantial number can survive for at least 10 years in constant presence of the pathogen on infested sites that have characteristics particularly favorable for *P. lateralis* spread and infection.

The Federal agencies believe that results of the resistance effort so far are very encouraging. However, there is no guarantee that usable resistance will result. Resistance as a disease management technique cannot be realistically evaluated until the development effort has progressed further. If Port-Orford-cedars with a usable level of resistance to *P. lateralis* are ultimately developed, careful consideration and planning to determine how they should best be deployed in the field will be required. It has been determined that *P. lateralis* is genetically quite simple (Mills et al. 1991, Winton, McWilliams, and Hansen unpublished); this is consistent with the idea that there was probably a single introduction of the pathogen. If the genetic variation of *P. lateralis* is minimal, this should increase the likelihood that if resistant cedars can be found or developed through a breeding program, the resistance should hold up over time.

LITERATURE CITED

Betlejewski, F. 1994. Port-Orford-cedar management guidelines. U.S. Department of the Interior Bureau of Land Management. 32 p.

DeNitto, G., and J.T. Kliejunas. 1991. First report of *Phytophthora lateralis* on Pacific yew. (Abstract) Plant Disease 75:968.

Erwin, D.C., and O.K. Ribeiro. 1996. *Phytophthora* diseases worldwide. American Phytopathological Society, Saint Paul, Minnesota. 562 p.

Filip, G.M., A. Kanaskie, and A. Campbell III. 1995. Forest disease ecology and management in Oregon. Oregon State University Extension Service. 60 p.

Gordon, D.E. 1974. The importance of root grafting in the spread of Phytophthora root rot in animmature stand of Port-Orford-cedar. M.S. Thesis. Oregon State University, Corvallis. 116 p.

Gordon, D.E. , and L.F. Roth. 1976. Root grafting in Port-Orford-cedar- an infection route for root rot. Forest Science 22:276-278.

Hadfield, J.S., D.J. Goheen, G.M. Filip, C.L. Schmitt, and R.D. Harvey. 1986. Root diseases in Oregon and Washington conifers. U.S. Department of Agriculture Forest Service, Pacific Northwest Region. R6-FPM-250-86. 27 p.

Hansen, E.M., P.B. Hamm, and L.F. Roth. 1989. Testing Port-Orford-cedar for resistance to *Phytophthora*. Plant Disease 73:791-794.

Hansen, E.M., and P.B. Hamm. 1996. Survival of *Phytophthora lateralis* in infected roots of Port-Orford-cedar. Plant Disease 80:1075-1078.

Hansen, E.M., and K.J. Lewis. 1997. Compendium of conifer diseases. American Phytopathological Society. 101 p.

Hansen, E.M., J.-C. Streito, and C. Delatour. 1999. First confirmation of *Phytophthora lateralis* in Europe. Plant Disease 83:587.

Harvey, R.D., J.H. Hadfield, and M. Greenup. 1985. Port-Orford-cedar root rot on the Siskiyou National Forest in Oregon. USDA Forest Service, Pacific Northwest Region. 17 p.

Hunt, J. 1959. *Phytophthora lateralis* on Port-Orford-cedar. USDA Forest Service, Pacific Northwest Forest and Range Experiment Station. Research Note 172. 6 p.

Jimerson, T.A. 1994. A field guide to Port-Orford-cedar plant associations in Northwest California.U.S. Department of Agriculture Forest Service, Pacific Southwest Region.
R5-ECOL-TP-002. 109 p.

Kliejunas, J.T. 1994. Port-Orford-cedar root disease. Fremontia 22:3-11.

Kliejunas, J.T., and D.H. Adams. 1980. An evaluation of *Phytophthora* root rot of Port-Orford-cedar in California. USDA Forest Service, Region 5 Forest Pest Management Report No. 80-1. 16 p.

Mills, S.D., H. Foster, and M.D. Coffey. 1991. Taxonomic structure of *Phytophthora cryptogea* and *P. drechsleri* based on isozyme and mitochondrial DNA analyses. Mycological Research 95:31-48.

Murray, M.S., and E.M. Hansen. 1997. Susceptibility of Pacific yew to *Phytophthora lateralis*. Plant Disease 81:1400-1404.

Nielsen, J. 1997. Port-Orford-cedar--a reasonable risk for reforestation (under specific conditions). Northwest Woodlands 13: 22-23.

Ostrofsky, W.D., R.G. Pratt, and L.F. Roth. 1977. Detection of *Phytophthora lateralis* in soil organic matter and factors that affect its survival. Phytopathology 67:79-84.

Roth, L.F., H.H. Bynum, and E.E. Nelson. 1972. *Phytophthora* root rot of Port-Orford-cedar. USDA Forest Service, Forest Pest Leaflet 131. 7 p.

Roth, L.F., R.D. Harvey, and J.T. Kliejunas. 1987. Port-Orford-cedar root disease. U.S. Department of Agriculture Forest Service, Pacific Northwest Region. R6 FPM-PR-294-87. 11 p.

Roth, L.F., E.J. Trione, and W.H. Ruhmann. 1957. *Phytophthora* induced root rot of native Port-Orford-cedar. Journal of Forestry. 55:294-298.

Tainter, F.H. and F.A. Baker. 1996. Principals of forest pathology. John Wiley and Sons, Inc. 805 p.

Thies, W.G., and E.M. Goheen. 1999. Major forest diseases of the Oregon Coast Range and their management. Summary of the COPE Project. in press.

Torgeson, D.C., R.A. Young, and J.A. Milbrath. 1954. *Phytophthora* root rot diseases ofLawson cypress and other ornamentals. Oregon State College, Agricultural Experiment Station. Bulletin 537. 18 p.

Trione, E.J. 1974. Sporulation and germination of *Phytophthora lateralis*. Phytopathology 64:1531-1533.

Tucker, C.M., and J.A. Milbrath. 1942. Root rot of *Chamaecypari~~s~~* caused by a species of*Phytophthora*. Mycologia. 34:94-103.

U.S. Department of Agriculture Forest Service. 1983. Forest disease management notes. Pacific Northwest Region. GPO 1983 695-726. 52 p.

Zobel, D.B. 1990. *Chamaecyparus lawsoniana* (A. Murr.) Parl. Port-Orford-cedar. In R.M. Burns and B.H. Honkala Technical Coordinators. Silvics of North America. Volume 1, Conifers.U.S. Department of Agriculture Forest Service. Agricultural Handbook 654. p. 88-96.

Zobel, D.B., L.F. Roth, and G.M. Hawk. 1985. Ecology, pathology, and management of Port-Orford-cedar (*Chamaecypari~~s~~ lawsoniana*). USDA Forest Service Pacific Northwest Forest and Range Experiment Station. General Technical Report PNW-184. 161 p.

EXPERIENCES WITH DIFFERENT BAIT TESTS AND SEROLOGICAL METHODS TO DETECT *PHYTOPHTHORA* SPP.

Renate Hahn, Karin Themann, Sabine Werres *

Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Horticulture, Messeweg 11/12, D - 38104 Braunschweig
*corresponding author

INTRODUCTION

Detecting the presence of *Phytophthora* species in samples from diseased trees, tree seedlings, nursery soil and irrigation water, forest plantations and natural woodlands presents special problems. For routine screening, modification or development of methods is required in order to deal with large numbers of voluminous samples. In addition, such methods should not be influenced by sample quality, which can vary greatly, particularly with water samples. Three bait tests and two serological methods for the detection of *Phytophthora* spp. in root and water samples have been investigated over recent years, and results indicating their range and sensitivity of detection are presented.

MATERIALS AND METHODS

Three bait tests and two serological methods were used. The apple test was used according to CAMPBELL (1949). The lupine seedling test was prepared according to CHEE and NEWHOOK (1965). And the Rhododendron leaf test was made according to WERRES *et al*. (1997) and THEMANN and WERRES (1997). For detection of *Phytophthora* spp. with serological methods the DAS-ELISA (Double-antibody-sandwich Enzyme-linked Immunosorbent-Assay) was used with water and sediment samples (THEMANN, unpublished data) and the DIBA (Dot Immunobinding Assay) with root samples (HAHN and WERRES, 1997). For ELISA two *Phytophthora* specific polyclonal antisera, one (557) produced against *P. cactorum* 9/88/92 and the other one (556) produced against *P. cinnamomi* 8/88/92 were used. With DIBA only the antiserum 557 was chosen.

To test whether the different methods can detect *Phytophthora* spp. *in vitro* and *in vivo* experiments were prepared. For the *in vitro* experiments the *Phytophthora* isolates were cultivated on carrot piece agar. For detecting the fungal like microorganisms in samples from nurseries roots were taken from commercially produced plants ready for sale and from water recirculation systems in nurseries which collect the surplus water for irrigation.

RESULTS

Detection of *Phytophthora* species *in vitro*

Range of *Phytophthora* species detected

A wide range of important *Phytophthora* species gave positive results with the three detection methods (table 1). Only the *in vitro* experiments with *P. erythroseptica* were not satisfactory with the Rhododendron leaf test and were negative with the DIBA. Furthermore the Rhododendron leaf test gave negative results with both varieties of *P. fragariae*. The DIBA did not show a clear halo around the spot with *in vitro* culture of *P. richardiae*.

Sensitivity towards zoospores

First results with the isolates *P. cactorum* 9/88/92 and *P. cinnamomi* 8/88/92 indicate that the minimum detection limit depends on the method and the *Phytophthora* species. The lupine seedling and the Rhododendron leaf test detected one zoospore/ml of *P. cinnamomi* 8/88/92. For this isolate the apple test was less sensitive (1000 zoospores/ml). But all three bait tests could detect zoospores of *P. cactorum* 9/88/92 at a minimum level of 100 zoospores per ml. With ELISA, the sensitivity towards small amounts of zoospores was highly influenced by the antiserum. For *P. cactorum* zoospores the minimum detection limit with ELISA and the antiserum produced against this isolate was 10 zoospores/ml. That means ELISA was more sensitive than all three bait tests. But for *P. cinnamomi* the DAS-ELISA with the antiserum against this isolate was extremely unsensitive (50,000 zoospores/ml).

TABLE 1: Detection of *Phytophthora* species with different methods *in vitro*

Phytophthora species	Bait test with Rhododendron leaves	DAS-ELISA With antisera 556/557	DIBA with antiserum 556
P. cactorum	X	X	***X***
P. cambivora	X	---	X
P. cinnamomi	X	X	X
P. citricola	X	X	X
P. cryptogea	X	X	X
P. drechsleri	X	X	X
P. erythroseptica	(X)	X	Ø
P. fragariae var. *fragariae*	Ø	X	X
P. fragariae var. rubi	Ø	---	X
P. gonapodyides	X	X	X
cf. *P. gonapodyides*	X	---	X
P. katsurae	*X*	---	---
P. megasperma	X	X	X
P. nicotianae	X	X	X
P. palmivora	X	X	X
P. richardiae	X	X	(X)
P. syringae	X	---	---
P. vignae	X	X	X
'alder *Phytophthora*'	X	---	---

DAS-ELISA = Double-antibody-sandwich Enzyme-linked Immunosorbent-Assay
DIBA= Dot Immunobinding Assay
antisera:*Phytophthora* specific polyclonal antisera produced against *P. cinnamomi* 8/88/92 (556) or *P. cactorum* 9/88/92 (557)
X = detection successful Ø = detection not successful
--- = not under investigation

TABLE 2: Detection of *Phytophthora* spp. in roots of commercially produced *Chamaecyparis lawsoniana* 'Columnaris'

Nursery	Plant no	Symptoms on the plants		*Phytophthora* species trapped with the Rhododendron leaf test	DIBA with antiserum 556
		discoloration and wilting of the needles	black roots/lack of feeder root		
A	1-3 [1)]	Ø	Ø	**P. cinnamomi**	+
	4 [1)]	Ø	X	*P. cinnamomi*	+
B	1 [3)]	Ø	X	*P. citricola, P. spp.*	+
	2-5 [3)]	Ø	X	*P. cryptogea*	+
C	1-3 [1)]	X	Ø	**P. cryptogea**	+
D	1 [2)]	X	X	*P. cryptogea*	+
	2 [2)]	X	X	*P. cryptogea, P. citricola*	+
E	1-3 [1)]	X	X	*P. citricola*	+

Ø = no symptoms visible, X = symptoms visible, + = detection positive
container size: 1) = 3 liter, 2) = 5 liter, 3) = rooted cuttings

Table 3: Number of water and sediment samples with *Phytophthora* spp.

Nursery	Number of samples [1)]	Samples (%) with positive Phytophthora spp. Detection				
		Baiting test with			DAS-ELISA with antiserum	
		Apples	Lupine seedlings	Rhododendron leaves	556	557
1	24	4.2	4.2	8.3	20.8	4.2
2	31	22.5	32.3	71.0	38.7	16.1
3	51	15.6	13.7	43.1	25.4	9.8
4	22	27.3	22.7	68.1	40.9	13.6

[1)] the samples were taken over one year from different parts of the recirculation systems

Detection of *Phytophthora* spp. *in vivo*

Detection in roots of commercially produced *Chamaecyparis lawsoniana* 'Columnaris'

With the Rhododendron leaf test and with the Dot Immunobinding Assay *Phytophthora* spp. could be detected in healthy looking but latently infected plants (table 2). Root constituents present in the naturally dark roots of older Lawson cypress plants and in discoloured roots had no inhibitory effect on the detection success nor of the serological method or of the Rhododendron leaf test.

Detection in samples from water recirculation systems in nurseries

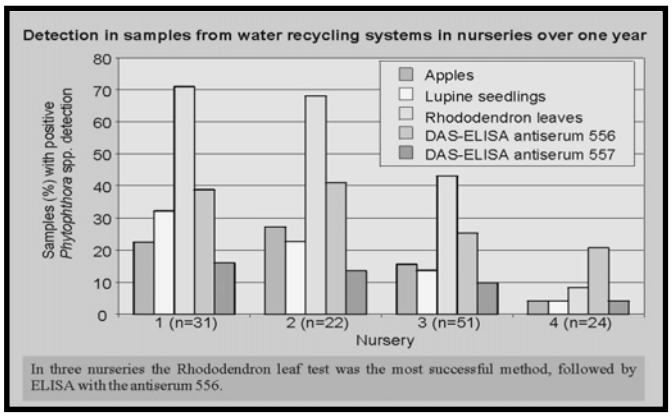
In three of the four nurseries where water and sediment samples were taken the Rhododendron leaf test was the most successful method followed by ELISA with the antiserum 556 (Table 3).

The range of *Phytophthora* species detected in water samples from ponds where the surplus water was collected was greatest with the Rhododendron leaf test (Table 4).

Table 4: Range of *Phytophthora* species detected in the water samples from recirculation systems *)

Baiting test with		
Apples	Lupine seedlings	Rhododendron leaves
P. cactorum	*P. citricola*	***P. citricola***
P. citricola	cf. *P. lateralis*	*P. cambivora*
P. citrophthora	cf. *P. cryptogea*	cf. *P. cambivora*
cf. *P. gonapodyides*	cf. *P. drechsler*	*P. citrophthora*
cf. *P. lateralis*	cf. *P. gonapodyides*	***P. cryptogea***
cf. *P. palmivora*	cf. *P. undulata*	cf. *P. cryptogea*
		P. drechsleri
		cf. *P. drechsleri*
		cf. *P. gonapodyides*
		cf. *P. lateralis*
		cf. *P. palmivora*
		cf. *P. richardiae*
		P. syringae
		cf. *P. undulata*
		Phytophthora spp.

*) The samples (n = 59) were taken over one year from ponds in four different nurseries



CONCLUSION and DISCUSSION

- For screening, a combination of a serological method and the Rhododendron leaf test appears to be optimal. With the Rhododendron leaf test, living *Phytophthora* propagules can be detected. With the serological methods, damaged as well as living propagules yield positive results.
- With the Rhododendron leaf test, large quantities of one root or water sample can be examined, making it highly probable that low numbers of *Phytophthora* propagules will be detected.
- With DIBA results are available within 1 day, with ELISA within 2 days. The Rhododendron leaf test gives results within 3 to 10 days.

REFERENCES

Campbell, W.A. 1949. A method of isolating *Phytophthora cinnamomi* directly from soil Plant Disease Reporter **33**, 134-135

Chee, K.H. and Newhook, F.J. 1965. Improved methods for use in studies on *Phytophthora cinnamomi* and and other *Phytophthora* species. New Zealand Journal of Agricultural Research **8**, 88-95

Themann, K. and Werres, S. (1997): *In vitro* comparison of different diagnostic methods for the detection of *Phytophthora* species in water. *Proceedings of the 9th International Congress on Soilless Culture, St. Helier, Jersey 12.-19. April 1996, ISOSC, Wageningen*, 535-550.

Hahn, R. and Werres; S. (1997): Development of a Dot Immunobinding Assay to detect *Phytophthora* spp. in naturally dark-rooted woody plants. *Journal of Applied Biology* **130**, 453-466.

Werres: S., Hahn, R. und Themann (1997): Application of different techniques to detect *Phytophthora* spp. in roots of commercial produced *Chamaecyparis lawsoniana. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **104**(5), 474-482.

Themann K. und Werres S. (1998): Verwendung von Rhododendronblättern zum Nachweis von *Phytophthora*-Arten in Wurzel- und Bodenproben. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **50**(2), 37-45.

http://www.bba.de/phytoph/diagn_r.htm or
http://www.bba.de/phytoph/diagnose.htm

MANAGING PORT-ORFORD CEDAR AND THE INTRODUCED PATHOGEN, *PHYTOPHTHORA LATERALIS*

Everett M. Hansen[1], Donald J. Goheen[2], Erik S. Jules[3], Barbara Ullian[4], Wendy Sutton[1]

[1]Botany and Plant Pathology, Oregon State University
[2]USDA Forest Service, SW Oregon Forest Insect and Disease Technical Center
[3]Department of Environmental Studies, University of California Santa Cruz
[4]Conservation Director, Siskiyou Regional Education Project

We don't know where *Phytophthora lateralis* came from, but since the 1920s it has been killing Port Orford-cedar (POC) trees in the Pacific Northwest. Today the pathogen has spread throughout the native range of its host with dramatic ecological and economic consequences, and losses continue. Now renewed energy, new research, and environmental vigilance offer hope for reversing the decline of a valuable tree.

THE HOSTS

Chamaecyparis lawsoniana, Port-Orford cedar or Lawson's cypress, is the largest member of the Cypress family. It is found in the wild only in a limited area of southwest Oregon and northwest California. In the southern part of its range, POC is usually found primarily along streams and areas with year-round seepage. It often grows within the active stream channel. In the north, POC commonly grows mixed with other conifers, in upland as well as riparian areas.

POC tolerates high concentrations of heavy metals in the ultramafic soils scattered throughout the region. It is commonly found in association with many rare plant species, such as Darlingtonia.

THE PATHOGEN

Phytophthora ("plant killer") is a genus of plant pathogenic Oomycetes. These are water molds, with swimming zoospores that emerge from sporangia and thick-walled resting spores, the oospores and chlamydospores .

The disease was first reported in 1923 near Seattle in nurseries growing POC for the ornamental trade. It is now found killing ornamental POC throughout the Pacific Northwest. Trees are colonized rapidly, with the advancing margin of red-brown necrotic phloem extending 50 cm or so above ground.

Phytophthora lateralis was first reported on Pacific yew (T. brevifolia) in 1991. While yew is a host for P. lateralis, it is much less susceptible than POC. A limited field survey found yew killed by Phytophthora only where it was growing along streams in close association with dead and dying POC. In a streamside survey, 46% of the cedar was dead, compared to 10% of the yew.

The situation along streams is especially critical. Essentially all POC growing with their roots in contact with normal winter high water flows are killed within a few years of introduction of the pathogen to the stream.

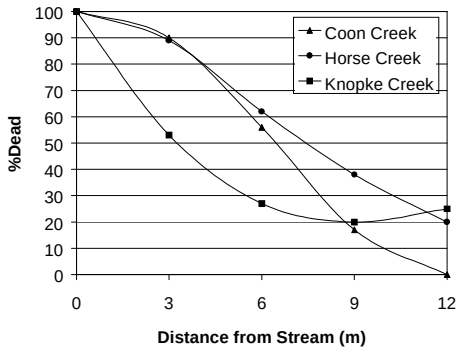


Figure 1. Port-Orford-cedar mortality (%) at intervals along transects perpendicular to streams on the Gasquet District, Six Rivers National Forest, CA.

Four 1/2 mile stretches of infested forest road first surveyed 7 years before were recently resurveyed. Cedars are still present along the roads in all areas, but mortality continues and inoculum from these roadside trees has spread further along the roads as well as downslope.

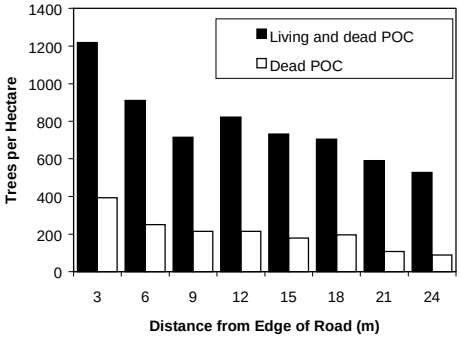


Figure 2. Abundance and mortality of Port-Orford-cedar down slope from 4 infested roads in southwest Oregon.

SPREAD, INTENSIFICATION, AND IMPACTS

In 1952 dead cedars were first seen in the lowland forests of southwest Oregon. Spread of P. lateralis into the mountains followed accelerated road building and timber harvest activity in the 1960s and 1970s.

The road system in cedar country is largely infested and provides the principle pathway for disease spread. POC regenerates prolifically in disturbed soil, and is especially abundant, and vulnerable, immediately adjacent to roads.

In a 1993 survey of 3 infested streams, mortality of larger POC growing within 3 m of the streams ranged from 65% to 92%.

DISEASE MANAGEMENT

STOP THE SPREAD
- ROAD CLOSURES
- VEHICLE WASHING

REDUCE INOCULUM
- ROADSIDE SANITATION

BRING BACK CEDAR
- SILVICULTURE
- RESISTANCE

The first substantial, coordinated, and sustained initiative to protect POC was instigated in 1985, not by the Forest Service but by the environmental community. Their challenge led to a "POC Action Plan," now incorporated into the cedar management program of the BLM and Forest Service. A number of techniques are currently being used by the Federal agencies to minimize spread and intensification of cedar root disease.

Most of the disease management effort to date has been directed at road management. Year-round road closures provide the greatest protection from the spread of the disease. Wet season closures, enforced by gates, are used in other areas, in order to preserve motorized access.

In some areas official vehicles and maintenance and harvesting equipment are routinely washed before passing between infested and uninfested areas.

Sanitation aims to reduce the probability of spread and intensification by reducing inoculum loads along roads. Sanitation is designed to lower the chances for inoculum increase, by cutting the most vulnerable cedar trees adjacent to the roads.

RESISTANCE TO PHYTOPHTHORA LATERALIS

Since the demonstration of heritable resistance to P. lateralis in 1989, the POC resistance program has slowly been gaining momentum. Richard Sniezko from the Dorena Tree Improvement Center calculated family mean resistance heritabilities. They were surprisingly high, 0.21 and 0.91 for stem and root resistance tests, respectively. The family correlation between tests was low, suggesting the possibility of independently inherited resistance mechanisms.

The earliest replicated outplanting test of trees selected at Oregon State University for resistance to P. lateralis is now 10 years old. Mortality was high for all families in the first year after planting, but after that the rate of disease increase for resistant and susceptible families diverged.

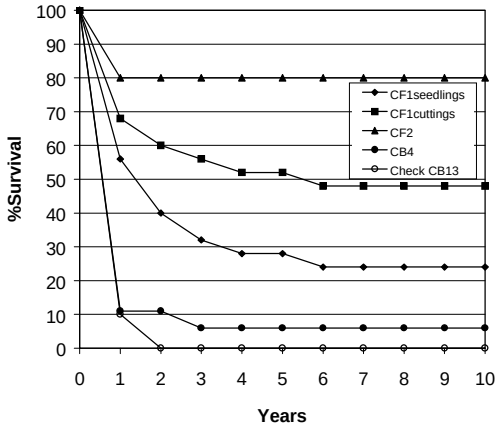


Figure 3. Ten year survival of seedlings (or cuttings) of 4 parent POC trees on an infested site at the OSU botany farm. CF1 and CF2 are resistant trees, and CB4 and CB13 are susceptible

Surviving trees from several of these families were tested for resistance with a stem dip test. The cut ends of branches removed from each tree were immersed in a zoospore suspension, and the subsequent rate of growth of the pathogen up the branch was measured. Families with better survival rates had lower stem dip lesion scores.

Resistance to P. lateralis offers the best hope yet of reestablishing POC in areas where the pathogen is already established. It must be emphasized, however, that the resistance program alone offers no protection for surviving stands of POC. Efforts to halt transport of the pathogen into uninfested watersheds and to generally reduce inoculum pressure must be redoubled and sustained.

PHYTOPHTHORA IN A FRENCH OAK FOREST

E. Hansen [1] and C. Delatour [2]

1. Botany and Plant Pathology, Oregon State University, Corvallis OR USA
2. Pathologie forestière, INRA, Nancy, France

INTRODUCTION

In support of a larger European Union project investigating *Phytophthora* and oak decline, (PATHOAK) we are exploring the local distribution and seasonal abundance of the species present in an intensively managed French oak forest.

The Forêt d'Amance, on the Lorrain Plateau near Nancy in NE France, covers about 1200 ha and is comprised primarily of *Quercus petraea* and *Q. robur*. Topography is gentle and most streams and drainage channels flow only during periods of heavy rain. Soils have a high clay content. The area has been managed for forest products for hundreds of years, and periodic cuttings continue. Despite repeated disturbances from harvest, defoliation, roads, and war, Amance forest is healthy. Portions of the forest are periodically defoliated by insects, and individual trees are affected by *Collybia fusipes* and other pathogens. Overall, however, growth is good and symptoms of decline are absent.

METHODS

Soil samples were collected 1 meter from the base of trees, then flooded and baited. Baits were floated in streams and standing water in the forest. In addition, forest litter (primarily fallen leaves) was collected from streams and the soil surface and baited.

Three types of baits have been used extensively :

- *Chamaecyparis lawsoniana* foliage (Lawson's cypress or Port Orford cedar);
- very young leaflets of *Quercus robur*;
- young *Q. robur* root tips from germinating acorns.

After three days, baits were plated on *Phytophthora* selective media (usually corn meal agar with 200 mg ampicilin, 10 mg rifampicin, 15 mg benomyl, 10 mg pimaricin and 50 mg hymexazol per liter).

Isolates were grouped by growth pattern and morphology into species "types". Specific names are tentative pending molecular confirmation.

RESULTS

Phytophthora species at Amance

A diverse and abundant *Phytophthora* community is present in Amance Forest, including at least 8 species. (TABLE 1 *Phytophthora* was recovered from water and from leaves and soil in water (or where water had been) throughout the forest, from soil in low-lying sites that are wet through the winter, and from scattered upland sites. Only in a small forest nursery was it causing obvious disease. (TABLE 2, Figure 1)

One site (31.15) was sampled in more detail. This parcel of forest is low-lying, and drained by several shallow ditches. Water is standing in the ditches and low spots throughout the winter. The frequency of crown dieback was not noticeably different from adjacent upland areas.

Phytophthora (and *Pythium*) was regularly and abundantly recovered from water and from soil around trees. Four species were present, including *P. quercina*. Trees growing a few meters away, on slightly higher ground, had no *Phytophthora*, and *Pythium* was very infrequent.

TABLE 1. *Phytophthora* species from Amance Forest

Species group	oogonia	sporangia	notes
Phytophthora gonapodyides	none	non-papillate, often nested proliferation	regular, dense colony margin
Phytophthora citricola ?	30-35 µ, sometimes enveloped in hyphae	semi-papillate, often irregular	
Phytophthora quercina	present but often slow, irregular shapes	papillate, often irregular shape	very slow growth, with distinctive hyphal branching
Phytophthora "type 6"	about 40 µ, with tapered base and thick oospore wall	non-papillate, ovoid/pyriform	very fluffy, aerial colonies
Phytophthora "type 7" ?	40-50 µ, apleurotic	non-papillate, l/w ratio 1.8, ellipsoid/oval	
Phytophthora "type df"	heterothallic, A1	non-papillate, ovoid/pyriform	
Phytophthora "type 11.13"	none	papillate or semi-papillate, ovoid/pyriform	regular, very dense colony margin
Phytophthora (or Pythium) undulatum	none	elliptical, l/w ratio >2	very fast growth, with chlamydospores

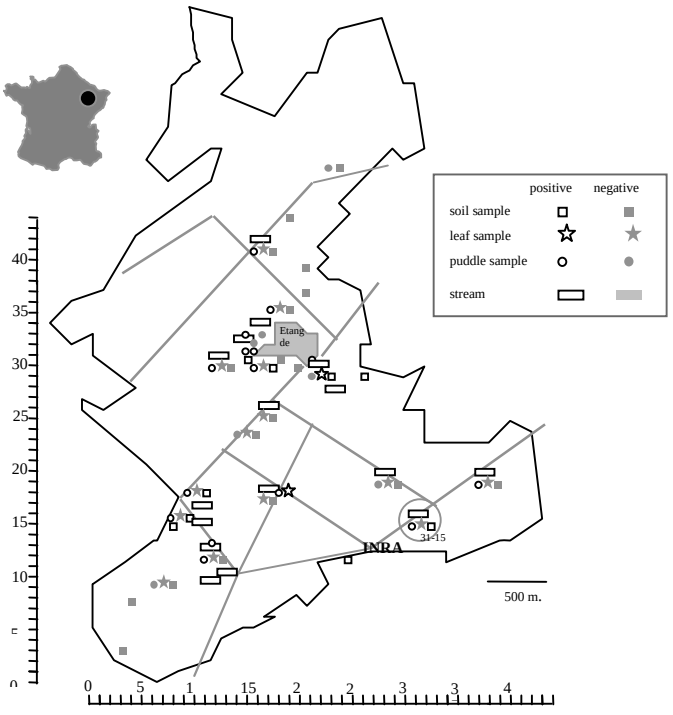


FIGURE 1. Forêt d'Amance *Phytophthora* sampling.

TABLE 2. Occurence of *Phytophthora* species in Amance Forest

SPECIES	LOCATION	SUBSTRATE	FREQUENCY
P. gonapodyides	throughout	water, leaves, saturated soil	abundant
P. citricola ?	23.29 stream & lake, 31.15	water, soil/roots	several samples
P. quercina	scattered	soil/roots	several samples
P. "type 6"	31.15, 16.31	soil/roots, water	locally abundant
P. "type 7"	16.31	water	several samples at 1 time
P. "type df"	nursery	soil and roots	abundant at 1 location
P. "type 11.13"	11.13 stream	water	recovered once
P. (or Pythium) undulatum	throughout?	water	isolated twice, but probably more common

***Phytophthora gonapodyides* at Amance**

Phytophthora gonapodyides is apparently resident in all of the streams that originate on the forest, and in most places where water accumulates after heavy rain. 26 collections have been made at different times of year, from 15 separate streams and all were positive; 15 of 22 ephemeral puddles yielded *Phytophthora*. *P. gonapodyides* is readily baited directly from the water and from submerged leaf litter and occasionally from fallen leaves on the ground but never from the soil immediately beneath those leaf samples (TABLE 3). *P. gonapodyides* is occasionally recovered from soil samples collected near the water line in stream courses and where soil is saturated through the winter. *P. gonapodyides* is seemingly abundant in the water of this forest. Nearly every bait was colonized in sampling conducted from October to July. (TABLE 4)

***Phytophthora quercina* at Amance**

P. quercina is very slow growing and is difficult to isolate if *Pythium* and other *Phytophthora* species are abundant. Oak leaflets are the most efficient bait.

P. quercina is present on all sites appropriately sampled. At each location it was recovered from scattered trees.

P. quercina was not closely associated with symptoms of crown decline.(TABLE 5)

TABLE 3. Grid sampling for *Phytophthora gonapodyides* by baiting from soil, leaf litter on soil, leaf litter in streams, and leaf litter in standing water, January 1998.

Grid Point	Soil	Leaves on Soil	Leaves in Stream	Leaves in Standing Water
11.13	-	+	+	/
11.15	-	-	+	+
13.09	-	-	+	+
13.29	-	-	+	+
15.29	/	/	/	+
16.31	/	/	+	+
17.23	-	-	/	-
17.29	-	-	/	+
17.41	-	-	/	+
19.17	-	-	+	/
19.25	-	-	+	/
19.35	-	-	/	+
23.29	-	+	+	-
31.15	-	-	+	+
36.18	-	-	+	+

TABLE 4. Seasonal recovery of *Phytophthora gonapodyides* from streams at Amance Forest
(+ = *Phytophthora* recovered, / = not sampled).

Site	1997	1998						
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
13.9	dry	/	/	+	/	/	/	+	+
16.31	dry	/	+	+	/	/	/	+	+
23.29	+	+	+	+	/	/	/	+	+
31.15	dry	/	/	+	+	+	+	dry	dry
19.17	dry	/	+	+	/	/	/	+	+

CONCLUSIONS

- A diverse and abundant *Phytophthora* population is present in this healthy oak forest.
- *Phytophthora* species are not especially associated with symptomatic or declining trees.
- *Phytophthora gonapodyides* is ubiquitous in streams and ephemeral pools of water, and is capable of saprophytic existence.
- *Phytophthora quercina* is present in soil around some oak trees, on both wet and upland sites

TABLE 5. *Phytophthora quercina* at Forêt d'Amance

Site	Tree no.	Sampling date (1998)	Diam (cm)	Crown Condition(1)	*C. fusipes* incidence (2)	*P. quercina*	*Phytophthora spp*	*Pythium*
31-15	AL=BU	April/May	48	0	/	-	type 6	+
31-15	AZ=BZ	April/May	49	0	/	-	+	++
31-15	AX	April	49	0	/	✚	type 6, gonap.	?
31-15	AY	April	75	2	/	-	type 6	?
31-15	BV	May	56	1	/	-	citricola	++
31-15	BW	May	92	1	/	-	-	+
31-15	BX	May	58	0	/	-	-	(+)
31-15	BY(qs)	May	59	0	/	-	-	(+)
31-15	CA	May	54	4	/	-	-	(+)
31-15	CB	May	57	1	/	-	+	(+)
31-15	CC	May	61	1	/	-	-	-
31-15	CD	May	73	1	/	-	?	-
31-15	CE(qs)	May	37	0	/	-	-	?
31-15	DN	June	83	1	/	-	-	-
31-15	DO	June	50	1	/	✚	+	++
31-15	DP	June	43	0	/	-	-	-
31-15	DQ	June	50	0	/	-	type 7	-
31-15	DR	June	51	2	/	-	citricola	++
17-29	DV(116)	June	38	1	0.5	✚	-	-
17-29	DX(114)	June	24	1	1.1	✚	-	++
17-29	DY(9)	June	30	1	2.9	-	citricola	+
17-29	DZ(115)	June	25	1	0.0	✚	-	-
17-29	EA(10)	June	46	1	0.0	-	-	-
27-29	DW(20)	June	38	1	2.8	✚	-	-
27-29	EB(21)	June	44	3	1.3	-	-	-
11-15	EN(118)	July	34	1	0.0	-	-	-
11-15	EO(117)	July	36	2	3.0	✚	-	-
9-13	EP(63)	July	41	3	(+)	✚	-	(+)
9-13	EQ(64)	July	41	1	(-)	-	?	+
11-13	ER(62.1)	July	61	0	/	-	-	-
11-13	ES(58)	July	53	2	2.3	-	-	-
11-13	ET(57)	July	69	2	1.0	-	-	-
11-13	EU(60)	July	70	1	/	✚	-	-

(1) Crown condition :
0 = healthy ;
1 = few fine twigs dead ;
2 = dead branches present in upper part of canopy (<50%) ;
3 = dead branches present (> 50%) ;
4 = tree nearly dead or dead.

(2) *Collybia fusipes* incidence : the root infection index of a tree takes into account (i) root rating (0 to 4) and (ii) root diameter for all individual major roots. Trees with a rating 0-0.5 are «not damaged», 0.5-2 = lightly infected, 2-4 = heavily infected

PORT-ORFORD-CEDAR: EXTENT, VALUES, THREATS AND DIVERSITY

Thomas M. Jimerson

USDA Forest Service, Pacific Southwest Region, Six Rivers National Forest, 1330 Bayshore Way, Eureka, California 95501

DISTRIBUTION

Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) is a member of the Cypress Family, *Cupressaceae*, and the largest in size of its genus, *Chamaecyparis*. Other genera of the family include Incense cedar (*Libocedrus*), arborvitae (*Thuja*), cypress (*Cupressus*) and juniper (*Juniperus*). There are six other species of *Chamaecyparis* found throughout the world, *Chamaecyparis pisifera* and *Chamaecyparis obtusa* of Japan, *Chamaecyparis formosensis* and *Chamaecyparis taiwanensis* of Taiwan, *Chamaecyparis nootkatensis* of northwest North America and *Chamaecyparis thyoides* of the eastern seaboard of the United States. These species and their narrow ranges are what remains of a genus that was found widespread during the Tertiary, distributed throughout much of central and southern Europe, western Asia and western North America (Zobel et. al., 1985). Fossil locations in western North America date back as far as 50 million years ago (Edwards, 1983). Today, Port-Orford-cedar is found only from coastal central Oregon to northwest California, primarily in the Coast Ranges, Siskiyou and Klamath Mountains with a small disjunct population in the Scott Mountains. The range of Port-Orford-cedar spans a north/south axis of 280 km and an east/west axis of 145 km and covers an estimated 176,000 hectares.

ENVIRONMENT

Although Port-Orford-cedar has a narrow geographic distribution, it occupies many different environments from sea level to 1950 meters elevation at its most interior locations. It is found on all aspects, but primarily those with northerly exposures or topographic shading. Landforms include glacial basins, streamsides, terraces and mountain side-slopes from lower to upper 1/3 positions. Soils are derived from a variety of parent materials, including sandstone, schist, phyllite, granite, diorite, gabbro, serpentinite, peridotite and volcanic rocks. The soils are primarily Entisols, Inceptisols, Alfisols and Ultisols included in the mesic and frigid temperature regimes and udic and xeric moisture regimes. Port-Orford-cedar also shows adaptability to a wide range of summer evapo-transpiration stress, from very high humidities along the coast to very low summer humidities inland. This great ecological amplitude of Port-Orford-cedar is believed to reflect a geographic concentration of genetically based characteristics that had developed in a larger geographic range which included parts of Idaho, Montana, California, Oregon and as far as east as Nebraska (Edwards, 1983).

SOCIAL VALUES

The social values of Port-Orford-cedar are many and represent a history of use dating back to aboriginal North Americans. Native American tribes such as the Hoopa and Karuk of northwest California revere the wood and use it in construction of ceremonial dance pits, sweat houses and the living home. Its value was recognized early by European settlers and by 1857, Port-Orford-cedar was the highest priced and most useful lumber in San Francisco (Zobel et. al., 1985). Some of the properties of the wood which make it noteworthy are its precise machineability, decay resistance, resistance to chemical corrosion and aromatic quality. Undoubtedly, its greatest commercial value exists with the log export industry. The Japanese have paid as high as $5000.00 per thousand board feet due to the similarity of Port-Orford-cedar to Hinoki cypress, a highly valued tree of Japan. Japanese mills custom cut the Port-Orford-cedar for special uses such as household shrines, door and window frames and sushi counters. In the United States it has been used in the manufacture of venetian blinds, battery cell separators, arrows, fences and sill plates. Because of its exceptional beauty, it was also recognized early as a tree of horticultural value. Due to its great genetic variability, the nursery trade in Europe and the United States has developed over 200 cultivars.

ROOT DISEASE

Up until the early 1950s, natural stands of Port-Orford-cedar had few serious pests (Roth et. al., 1987). Then a root disease, *Phytophthora lateralis*, appeared from unknown sources, although the nursery trade is highly suspect. The fatal root disease has now spread throughout its native range except for the populations in the Trinity River watersheds. The disease spreads by motile aquatic zoospores, nonmotile soil-borne chlamydospores and root grafting. To date there is little known genetic resistance, although, there is active research in this field, but with some promise to date.

The potential effects of this disease to biodiversity are many. Foremost is the loss or significant decline of a major shade tolerant tree species found in many plant communities, in particular those found on serpentine soils and riparian habitats. Preliminary indications in areas decimated by the disease point toward both changes in species composition and stand structure that could lead to degradation of riparian habitat. This is particularly important in Port-Orford-cedar riparian areas identified as critical habitat for the Coho salmon. The threats related to the root disease and its affect on Port-Orford-cedar has prompted the Forest Service and Bureau of Land Management to undertake the development of a conservation strategy for the species.

ECOLOGY

Various ecological roles of Port-0rford-cedar have been well summarized by Zobel et. al.(1985). Port-Orford-cedar helps improve soil fertility by incorporating calcium into the soil at a much higher rate than members of the *Pinaceae*. This ability is especially significant on serpentine soils where the low calcium to magnesium ratio is not favorable to the growth of many tree species. By ameliorating the effect of high magnesium in these soils, Port-Orford-cedar may be aiding the establishment of other conifer species.

Wildlife use of Port-Orford-cedar snags appears not as high as pines or Douglas-fir (Jimerson 1992), but this is likely partially offset by the longevity of the snags. Port-Orford-cedar logs persist for a very long period due to their resistance to decay and large size. They provide structural diversity and long-term habitat in riparian areas where they are particularly important to fish, reptiles and amphibians.

The thick, fibrous bark and resistance to decay following injury to the cambium combine to give Port-Orford-cedar special value in fire-disturbed ecosystems. Trees that have burned all the way through the bole with two "legs" of cambium left, have persisted for decades in an other-wise healthy appearing state. This has special significance for natural regeneration of sensitive riparian environments following fire.

The stabilizing effects and habitat contributions of large woody material and root mass in stream channels has often been described as the primary geomorphic control for soil movement. Loss of Port-Orford-cedar in a riparian ecosystem could lead to degradation of the stream channel over time.

PLANT SPECIES AND COMMUNITY DIVERSITY

Port-Orford-cedar plant associations are key elements of the biodiversity of Southwest Oregon and Northwest California. Its plant communities display among the richest plant species diversity of all forest types in the region (Jimerson and Creasy 1991). The diversity within Port-Orford-cedar stands is exemplified by the high number of species found by layer in association with it (Table 1). In the overstory tree layer alone 29 species were identified. The shrub layer included 93 species and the forb layer had an amazing 446 species. The tree and shrub layers were considered indicator species of environment change. Ecological classifications that describe these indicator species and the environmental gradients they represent can be used in the development of a conservation strategy for the species. These include the classifications produced by Atzet et. al. (1996), Jimerson (1994), Jimerson et. al. (1995 and 1996) and Jimerson et. al. (In Press). These classifications in conjunction with genotypic variability analysis by plant association (Millar et al. 1991) will greatly aid in the identification of Port-Orford-cedar genotypes and environments necessary for the continued existence of the species. In addition, these classifications can serve as blueprints for restoring Port-Orford-cedar plant associations decimated by the root disease.

TABLE 1. Number of species by layer found on Port-Orford-cedar plots in Oregon and California (N=1076).

Layer	Number of Species
Overstory	29
Understory	32
Shrubs	93
Forbs	446
Grasses	44

Port-Orford-cedar is found in association with a wide range of species with differing ecological requirements. These species change in conjunction with the portion of Port-Orford-cedar's range in which they are found and their position along the wide environment gradients. For instance, on low elevation sites in the northwest portion, Port-Orford-cedar is found in association with western hemlock (*Tsuga heterophylla*), in the southwest with coast redwood (*Sequoia sempervirens*) and tanoak (*Lithocarpus densiflora*), in the central portion Douglas-fir (*Pseudotsuga menziesii*), at higher elevations in the eastern portion of its range, white fir (*Abies concolor*), western white pine (*Pinus monticola*), red fir (*Abies magnifica* var. *shastensis*) and mountain hemlock (*Tsuga mertensiana*). This wide array of tree species with differing ecological requirements contributes to the high diversity of Port-Orford-cedar plant associations. Port-Orford-cedar has been noted as a component of more than ninety-three plant associations in Oregon and California (Atzet et. al. 1996, Jimerson 1994, Jimerson et. al. 1995 and 1996 and Jimerson et. al. In Press).

Direct gradient analysis was performed on the tree and shrub indicator species using canonical correspondence analysis [CANOCO] (Ter Braak 1988, Jongman et al. 1995). This technique constrains the ordination of the main matrix (species cover) by a multiple regression on environment variables contained in the secondary matrix. CANOCO was used as the primary tool to define the environment gradients that best explained the variability of Port-Orford-cedar communities (McCune and Mefford 1995).

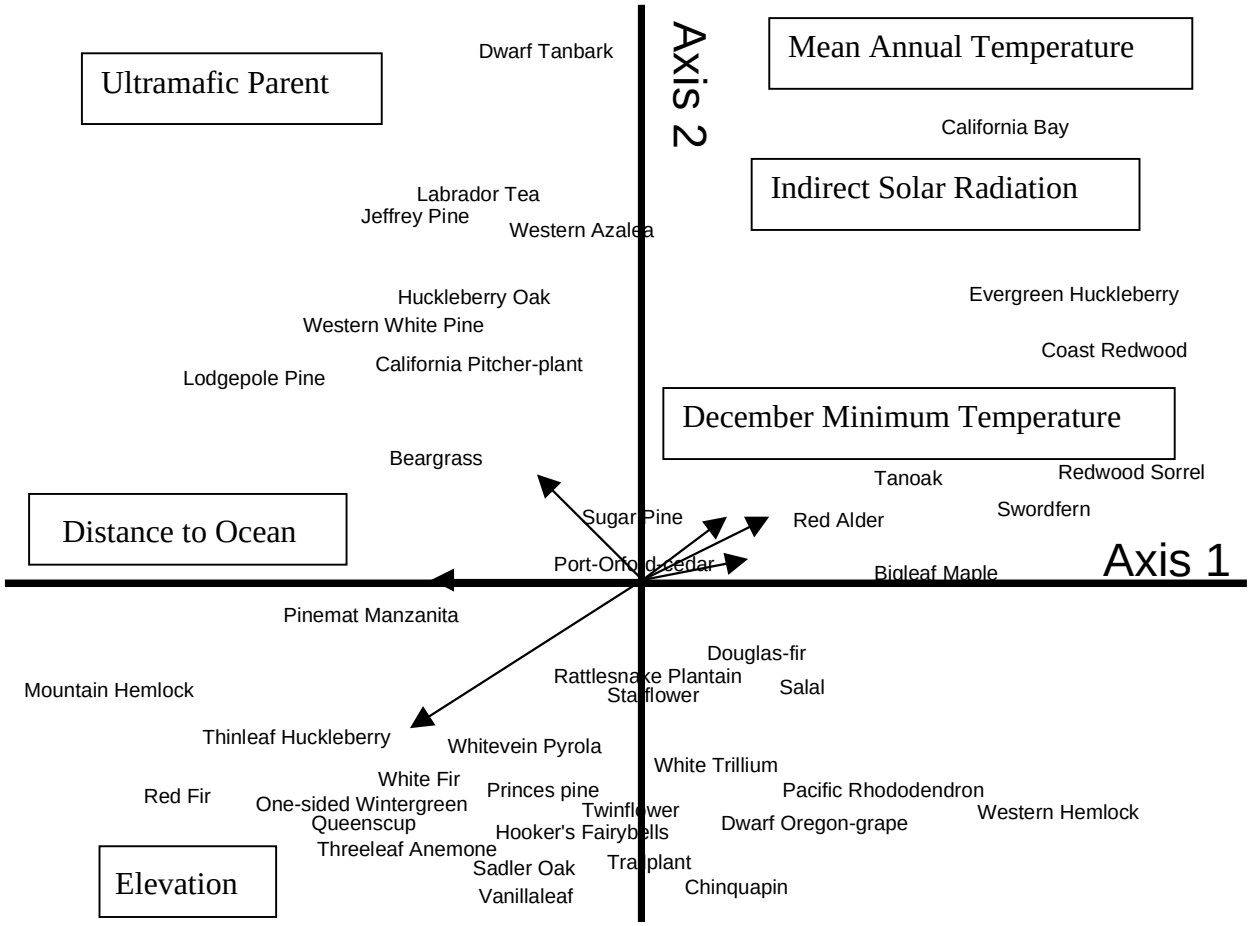


FIGURE 1. Rangewide Canonical Correspondence Analysis (CCA) species ordination (axis 1 and 2) of Port-Orford-cedar stands. Highly correlated variables are shown in boxes, length of arrows indicates the strength of the correlation.

This high species diversity is typified by the wide ecological gradients in which Port-Orford-cedar and its associated tree species were found (figs. 1). The first and most prominent gradient (axis 1) was most highly correlated with elevation (r = -.93). This is evidenced by the location of mountain hemlock a high elevation species (X = 1684 m) on the far left hand side of the graph and coast redwood a low elevation species (X = 353 m) on the right side of the graph. These same two species best demonstrate the high correlation of distance to the Ocean (r = -.54) with axis 1. Mountain hemlock is found in association with Port-Orford-cedar on the farthest inland sites in the Sacramento River drainage (X = 109 km), while coast redwood is found on coastal sites near the ocean (X = 19.5 km). Other variables correlated with axis 1 are December minimum temperature (r = .69), mean annual temperature (r = .61)and indirect solar radiation (r = .49). Axis 2 was most highly correlated with ultramafic parent rock (r = .83). The influence of ultramafic parent rock is clearly visible here, based on the appearance of dwarf tanbark, Labrador-tea, Jeffrey pine and huckleberry oak (ultramafic indicator species) along the positive portion of axis 2. The mixture of species found in the positive portion of axis 2 point toward warm summer temperatures and cool winter ones.

Figure 1 demonstrates the wide environment gradients included within Port-Orford-cedar communities that are assumed based on the work of Millar et. al. (1991) to represent genetic diversity. The species depicted in figure 1 help to define the major environment gradients used to describe vegetation series and subseries. Species found in the shrub and forb layers help define the major and minor gradients and are used in the plant association classifications. In order for a conservation strategy to be successful, this variation in Port-Orford-cedar communities and their environments need to be represented.

CONCLUSIONS

The limited distribution, wide environment gradients, high genetic diversity, high social values, importance to wildlife, high species and community diversity point towards the need for a conservation strategy designed to maintain Port-Orford-cedar as a continuing element of our biodiversity. Such a strategy needs to be applied on a rangewide basis and incorporate all the factors described earlier. In particular, it must include areas protected from the disease that represent the biological and genetic diversity of the species that are well distributed throughout its range and are arrayed along the wide environment gradients described earlier.

LITERATURE CITED

Atzet, T.; D.E. White; L.A. McCrimmon; P.A. Martinez; P. Reid Fong; V.D. Randall. 1996. Field Guide To The Forested Plant Associations Of Southwest Oregon. R6-NR-ECOL-TP-17-96, USDA For. Serv.. Pacific Northwest Region, Portland, OR. 353 p.

Edwards, S.W. 1983. Cenozoic history of Alaskan and Port Orford *Chamaecyparis* cedars. University of California, Berkeley, CA. Ph.D. dissertation, 271 p.

Jimerson, T.M. and R.M. Creasy. 1991. Variation in Port-Orford-cedar plant communitiesalong environmental gradients in Northwest California. pp. 122-133, In: Proceedings of the Symposium on Biodiversity of Northwestern California. R.R. Harris, D.C. Erman, H.M. Kerner, Tech. Coords. University of California, Berkeley, CA. 308 p.

Jimerson, T.M. 1992. Uses of sugar pine by cavity nesting birds. pp. 45-50, In: Sugar Pine Status, Values, and Roles in Ecosystems. Proceedings of a symposium March 30-April 1, 1992, University of California, Davis. B.H. Kinloch, M. Marosy, M.E. Huddleston, Editors. University of California, Davis, CA. 225 p.

Jimerson, T.M. and S. L. Daniel. 1994. A field guide to Port-Orford-cedar plant associationsin Northwest California. R5-ECOL-TP-002, USDA For. Serv.. Pacific Southwest Region, San Francisco, CA. 154 p.

Jimerson, T.M.; L.D. Hoover: E. A. McGee; G. DeNitto; R.M. Creasy; S. L. Daniel. 1995. A field guide to serpentine plant associations and sensitive plants in Northwest California. R5-ECOL-TP-006, USDA For. Serv. Pacific Southwest Region, San Francisco, CA. 338 p.

Jimerson, T.M.; E. A. McGee; D.W. Jones; R.J. Svilich; E. Hotalen; G. DeNitto;T. Laurent; J.D.Tenpas; M.E. Smith; K. Heffner-McClellan; S. L. Daniel. 1996.A field guide to the tanoak and the Douglas-fir plant associations in Northwest California. R5-ECOL-TP-009, USDA For. Serv. Pacific Southwest Region, San Francisco, CA. 546 p.

Jimerson, T.M.; E. A. McGee; G. DeNitto (In Press). Supplement to; A field guide to Port-Orford-cedar plant associations in Northwest California: Port-Orford-cedar plant associations in the Trinity and Sacramento River drainages.

Jongman, R.H.G.; C.J.F. Ter Braak, and O.F.R. Van Tongeren. 1995. Data Analysis inCommunity and Landscape Ecology. Cambridge University Press, New York. 299 p.

McCune, B., and M.J. Mefford. 1995. PC-ORD. Multivariate Analysis of Ecological Data, Version 2.0. MJM Software Design. Gleneden Beach, Oregon, USA.

Millar, C.I.; D.A. Delaney; R.D. Westfall; T. Atzet; M. Greenup; T.M. Jimerson. 1991. Ecological factors as indicators of genetic diversity in Port-Orford-cedar: Applications to genetic conservation. Internal competitive grants program. Progress Report 1: July 1, 1991. USDA For. Serv. Pacific Southwest Forest and Range Experiment Station, Berkeley, CA. 3 p.

Roth, L.F.; R.D. Harvey; J.T. Kliejunas. 1987. Port-Orford-Cedar Root disease. USDA For. Serv. Res. Pacific Northwest Region, R6 FPM PR 010 91. 11 p.

Ter Braak, C.J.F. 1988. CANOCO - a FORTRAN program for Canonical CommunityOrdination. Microcomputer Power, Ithaca, New York, USA.

Zobel, D.B. L.F. Roth, and G.L. Hawk. 1985. Ecology, pathology and managementof Port-Orford-Cedar (*Chamaecyparis lawsoniana*). USDA For. Serv. Gen. Tech. Rep. PNW-184. Pac. Northwest For. and Range Expt. Stn. Portland, OR. 161 p.

INK DISEASE OF THE NORTHERN RED OAK CAUSED BY *PHYTOPHTHORA CINNAMOMI* : DISTRIBUTION IN FRANCE, SITE FACTORS IN THE PIEDMONT OF THE WESTERN PYRÉNÉES

by
André LÉVY, Département de la Santé des Forêts, F – 33150 CENON

Fig. 1 : Ink disease on red oak

Fig 3 : Cross section of a diseased red oak

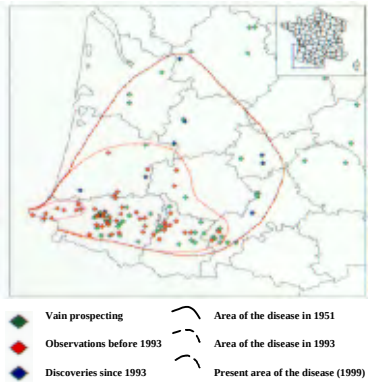


Fig. 4: Communes in South West of France where occurrences of ink disease of red oak have been looked for

INTRODUCTION

At the end of the 19th century in western Pyrénées, the ink disease of chestnut-tree and oidium of pyrenean oak were a major cause of forest destruction. Part of these area was replanted with the red oak (*Quercus rubra*).

The disease becomes first visible on the stump and then spreads upwards the bole. The typical symptoms are swelling cankers out of which a black liquid flows (Fig. 1). The cross cut displays scars with excrescences including old or active cankered bark (Fig. 3). Finally the produced butt log is useless.

In France the ink disease of red oak was first described in 1948 and its cause identified as *Phytophthora cinnamomi* in 1952. A few cases of this disease are observed on pedonculate oak. The disease seems to be unknown so far beyond southern Europe and has been never related to any red oak decline. However, *P. cinnamomi* may be an active pathogen in some declines of old cork oak and holm oak, both showing similar symptoms (Robin C. et al, Ann. Sci. For.55 (1998) 869-883).

In the south of Europe nearly all chestnut-trees decayed between 1840 and 1930. This was caused by *P. cambivora* (Italy 1917), and also by *P. cinnamomi* (Spain 1947) as described only in 1917 and 1922 respectively. Hence, until the fifties the ink disease of red oak and ink disease of chestnut-tree were frequently confused.

THE SURVEY

The French Forest Health Department organised a survey from 1990 to 1993 in the piedmont of the western Pyrénées, where the ink disease hasalready been observed since the end of the 19th century. (Lévy A. Cahier du DSF 1 (1995) 1-41). **124 stands of red oak were assessed at different altitudes, exposures and topographic situations**.

The ink disease was rated using the following variables:

DS (diseased stand) = % presence – absence in the stands

DT (diseased trees) = % diseased trees in the stand

IS (Intensity in the stand) = % average of damage in the stand using the following scale : 0 = no ink disease found, 20 = disease visible up to 1m, 60 = disease visible up to 2 m, 100 = disease visible above 2 m height. This scale was chosen to estimate roughly the percentage of unusable volume of the butt log.

IT (Intensity on diseased trees) : 100*IS/DT (only in diseased stands).

We assume that :

- the variable presence - absence (DS) is correlated to certain site factors being responsible for the introduction and contamination,
- the variable diseased trees (DT) is correlated to certain site factors being responsible for the dissemination of the pathogen inside the stand,
- the variable intensity on diseased trees (IT) is correlated to certain site factors being responsible for the development of the pathogen in the trees when already established.

Area of ink disease

Fig. 4 shows areas of the disease in 1951 and in 1993 based on observations in restricted domains, without checking presence of the disease outside the limits . In 1993, at the end of the survey, every red oaks stand older than 30 in the south west of France was visited. Some of them could be considered free of disease (blue and green signs). As a few of them were founds diseased between 1993 and 1999 (bleu signs) **we assume that, during last 6 years, area of ink disease of red oak has increased.**

Age

Fig. 6 – 7 show that, **the older the stand, the more important the damage is.** For that reason, the values of the variables were normalised to the age class of 30-60 years.

RESULTS

Averages

Fig. 5 shows average values of each variable :
- **70% of the stands are diseased** (DS)
- the average of diseased trees in the stands is 6.8% (DT), the maximum reaching 41%,
- **unusable volume of butt log is 3%** (IS), **the maximum can reach 41%**.

Altitude and exposure

At the altitude from 0 to 400m, 74% stands are diseased but from 400 to 440m only 50% stands and **none above 440m**. Fig. 8 shows that NNW does not prevent the contamination of stands but does limit it to less then half in number of diseased trees and in symptoms intensity. On the other hand, SSE exposures are favourable to the disease within each term.
This leads one to think of the **repressive effect of cold**. **(**Marçais B. et al. Ann Sci For (1996) 53, 369-382).

Coolness

This is a subjective variable of assessment for the forest environment based on ecological indicators of vegetation and geomorphology. Soil humidity is often an important factor, however others factors (soil texture, exposure, ...) may also play a major part. The scale used is : "dry", "fresh" and "humid".
"Dry" sites are more often contaminated than "fresh"/"humid" (DT = 83 vs. 67). In addition they have more diseased trees (DT = 10.2 vs. 8.8). However there is no difference in disease intensity on trees.
Dry sites seem to by especially prone to the introduction of the disease into the stand and dissemination in the stand.

Chestnut-tree presence

The presence or absence of chestnut-trees is not an hint of the ink disease of red oak. However, in the Pays Basque region, where all stands of red oak are contaminated, the presence of chestnut-tree is correlated to a higher disease level of DT = 18 %, IS = 8.4 % and IT = 54 %. Since the chestnut-trees are indicators of relatively dry sites, we assume that dry conditions are more prone to the establishment of the ink disease.

Topography – soil texture

Talwegs and crests / tophills are particularly prone to ink disease. We assume that, due to a water stress, roots are damaged on the one hand by hydromorphism in talwegs and, on the other hand, by water shortage in crests / tophills. (Marçais B. et al. Eur. J. For. Path. 23 (1993) 295-305). Talwegs are found to be the most productive sites and, on the contrary, crests / tophills are the less productive sites (34 and 24 m at 60 years).
The other sites can be filed in two groups : flat sites (shelf, plateau, plain) and slopes (half and bottom). All flat sites are less diseased than slopes in term of presence – absence and intensity on trees. They can also be respectively filed according to the textures of soils (from silty - clay to silty - gravelly-stony). We found in both cases that sites of pure silty soils are more diseased then sites with silty - stony to silty -sandy soils.
Sites with porous soils (silt with sand, gravel and stone) are less productive than average (23m in flat sites and 24m in slopes vs. 26m at 60 years) Cf.fig.10-11.

CONCLUSION

Finally it seems that, in South West of France, ink disease of red oak cannot spreads far beyond the present area. In this area damages can be limited by avoiding sites prone to ink disease such as crests and hilltops or pure silt soils and by giving preference to north or north-east exposure and upper altitudes. Nevertheless, one will have difficult choices to do for example in talwegs which have good productivity but lots of unusable butt logs if the rotation age is too high…
An other result of this survey was the unexpected high number of red oaks with damage caused by *Collybia fusipes* root disease. This pathogen could be the next preoccupation with red oak...

Fig. 6 :

age	15 - 30	30 – 60	60 – 90
DS (%)	54	70	78
DT (%)	2.8	*6.8	8.7
IS (%)	1.0	*3.0	3.6
IT (%)	35	40	46

Fig. 7 :

stage	pole	young	mature
DS (%)	58	62	79
DT (%)	4.2	4.1	*8.9
IS (%)	1.4	1.9	*3.9
IT (%)	31	42	42

Fig. 8 :

exposure	NNW	ENE	Flat	SSE	WSW
DS (%)	69	57	60	*96	80
DT (%)	2.2	*5.7	5.6	*11	12
IS (%)	0.88	0.38	2.6	*4.6	4.4
IT (%)	26	34	26	36	28

Fig. 10 : **texture**

of slope site	silt and clay	pure silt	silt and gravel and stone
DS (%)	65	100	80
IT (%)	34	40	24
f (m)	28	27	23

f = site index (height at 60 years)

Fig. 11 : **texture**

of flat site	silt and clay	pure silt	silt and gravel and stone
DS (%)	52	75	10
IT (%)	20	30	6
f (m)	26	25	24

* = highly significant

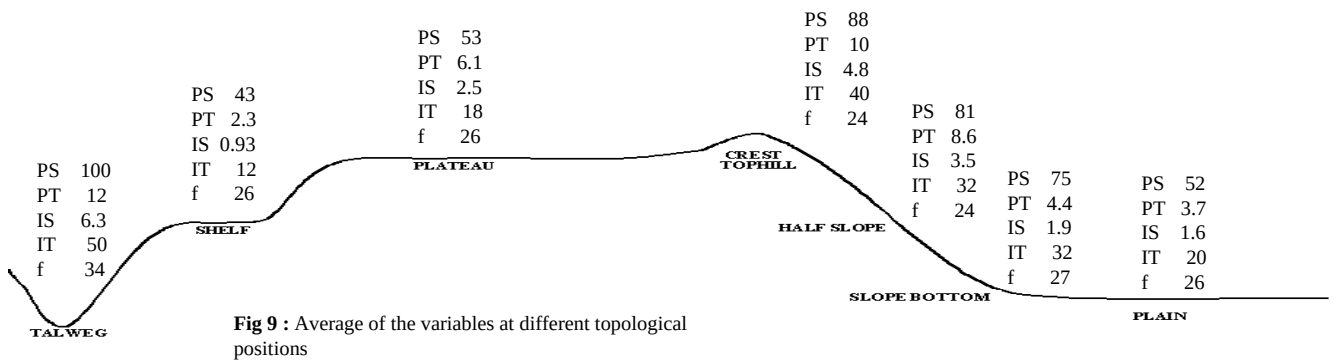


Fig 9 : Average of the variables at different topological positions

Mapping ink disease hazard for oaks in France

Marçais, B. *, Bergot, M. +, Perarnaud, V. +, Levy, A. ++ and Desprez-Loustau, M.L. *

* INRA, + Météo-France, ++ Département de Santé des Forêts

- **Trunk canker induced on oaks by *Phytophthora cinnamomi***
- Described for the first time on *Quercus rubra* in the Basque area in 1951, but up to now, present only in the south-west of France on *Q. rubra* and *Q. robur* and on the Mediterranean coast on *Q. suber*

(Levy, A., 1995, Cahiers du D.S.F. n° 1, D.E.R.F; Robin, C., Desprez-Loustau, M.L., Capron, G., Delatour, C., 1998, Ann. Sci. For., 55, 869-883)

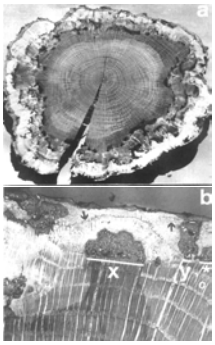


Fig 1. Feature of a *P. cinnamomi* infected red oak trunk.
(a) Disk.
(b) Detail view of an included lesion.
Lesion of width X developed in year O whereas lesion of width Y developed the next year (*)

Cankers development was followed by dendro-chronological method on 20 trees from 4 sites

⇒ **Many cankers healed after 1985, year with an especially cold winter**

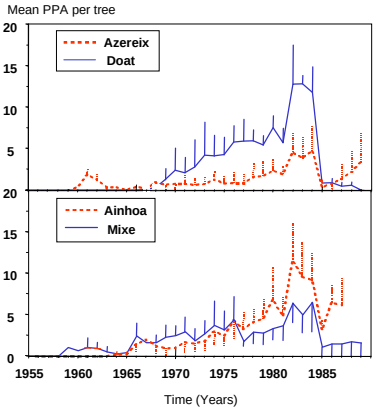


Fig 2. Evolution of the ink disease on *Quercus rubra* trees: mean annual Percentage of Perimeter Attacked (PPA) per tree in 4 sites of South-West France

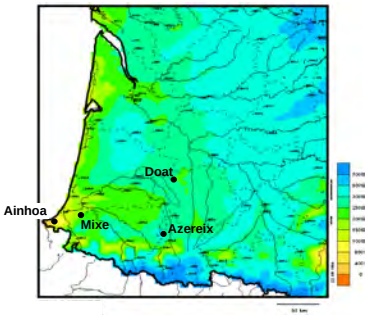


Fig 3. Frost Indices of the 1984-1985 winter in South-Western France - Interpolation based on topography from the 57 meteorological stations by the Aurelhy method.

Frost Index = cumulated degree-hours under 0° C in the winter (November to March)

Even where the disease is present, **Winter frost = main factor** limiting canker extension

⇒
- A model was built to estimate the survival of *P. cinnamomi* in the bark lesion during winter
- Compute degree-hours under 0°C in the cambium from air temperature and derive from it a *Phytophthora* Survival Index (Marçais, B., Dupuis, F., Desprez-Loustau, M.L., 1996, Ann. Sci. For. 53, 369-382)

Comparison of *Phytophthora* Survival Index with the evolution of cankers on infected trees shows that with Survival Index lower than

- 0.5, cankers tend to heal and
- 0.05, *P. cinnamomi* is eliminated from the canker, i.e. for Frost Index higher than 2000

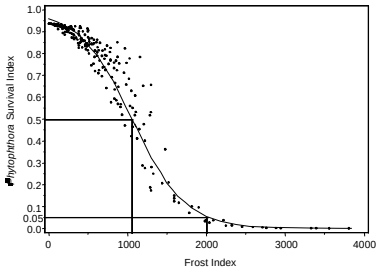


Fig 4. Relationship between the *Phytophthora* Survival Index and the Frost Index in 10 sites of South-West France.

Importance of the threshold at a Frost Index of 2000

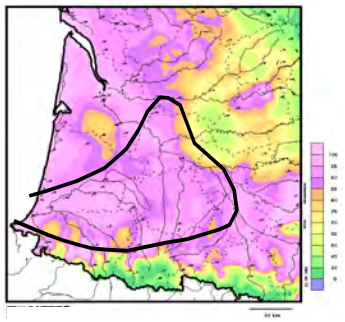


Fig 5. Frequency of occurrence of annual Frost Index values lower than 2000 in South-Western France - Interpolation from the 57 meteorological stations by the Aurelhy method.

Frequency of Frost Index lower than 2000 and ninetieth percentile of the Frost Index were mapped

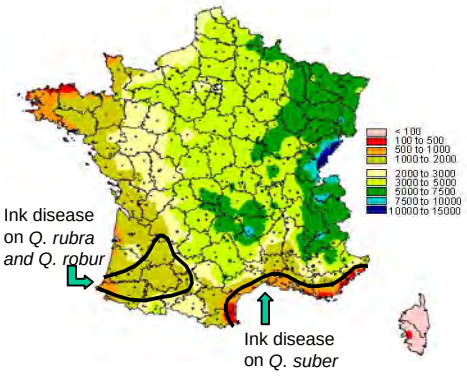


Fig 6. Ninetieth percentile of annual Frost Index in France Interpolation based on the inverse distance weighted method applied on 503 meteorological stations.

The present extension of the ink disease of oak in France corresponds to area where Frost Index is seldom over 2000. However, a much wider zone is favourable to *P. cinnamomi* winter survival. Therefore, the disease could develop in some areas presently free of the disease like Brittany

PRELIMINARY RESULTS OF EFFECTIVENESS MONTORING OF PORT-ORFORD CEDAR ROADSIDE SANITATION TREATMENTS IN SOUTHWEST OREGON

Katrina Marshall and Donald J. Goheen

USDA Forest Service, Southwest Oregon Forest Insect and Disease Service Center, Central Point, Oregon 97502, USA

INTRODUCTION

Roadside sanitation treatments are widely recommended and used in Southwest Oregon as one component of Port-Orford-cedar root disease management strategies (Hansen 1993, Kliejunas 1993, Roth et al. 1987, Zobel et al. 1985). The objective of roadside sanitation is to create a zone along treated roads where there are no live Port-Orford-cedar roots. This is believed to eliminate or reduce the amount of inoculum available to be transported out of infested areas by vehicle traffic and reduce the probability of introducing the pathogen into uninfested areas. The treatment consists of killing all Port-Orford-cedar in a zone extending to 8.5 meters above the road (or to the top of the cut bank) and 8.5 to 17 meters below the road. Periodic retreatment is necessary to remove Port-Orford-cedar regeneration from the sanitized area

Until recently, monitoring the effectiveness of roadside sanitation treatments was limited to visually assessing stands adjacent to the sanitized zone for the presence or absence of new Port-Orford-cedar mortality. However, such observations cannot really determine whether roadside sanitation treatments reduce the incidence and movement of the pathogen. Since sanitation treatments are costly and controversial, forest managers and the public need quantitative, sample-based measures of treatment effectiveness to make decisions about the desirability of using the treatment.

METHODS

In 1997, we began monitoring four sites with a systematic sampling procedure using small, tubed Port-Orford-cedar seedlings as baits. The baits were outplanted in ten transects along a 0.4 to 0.8 kilometer segment of road at each site. Ten baits were used in each transect. Transects were located where introduction or movement of inoculum was likely (existing dead Port-Orford-cedar, stream crossings, swampy areas, pullouts, etc) and also at random points along the road. The baits were removed from the tubes and planted perpendicular to the road, on both sides of the road, beginning at the edge of the road and then periodically along the transect and into the adjacent stand beyond the boundary of the sanitized area. They were also planted in the roadside ditches above and below the intersection with each transect. At stream crossings with water present, seedlings were left in their tubes and secured in the channel with metal stakes. The location of the baits were mapped so the transects could be resampled 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. Baits were left in the streams for two weeks, then retrieved and incubated in the tubes for four weeks. Planted baits were left on the site for six weeks. After six weeks all the baits were examined for evidence of infection by *P. lateralis*.

As of 1999, we have monitored 12 different sites (including the original four). One site is infested but has not been sanitized, one was sanitized but is not infested and the other ten are infested and have been sanitized. Once transects are installed, we repeat the procedure with the baits in the same locations at approximately the same time each year. We hope to monitor each site for at least five years.

PRELIMINARY RESULTS AND CONCLUSIONS

There has been an overall decrease in the number of infected bait trees beginning in the third year after the sanitation treatment (Figure 1). Prior to treatment (year zero), an average of 24% of bait trees were infected. Five years after treatment, an average of 6% of bait trees were infected. In three years of monitoring at the infested site that has not been treated, the level of infection in the bait trees has remained between 14 and 22 percent. We believe that the reduction of inoculum observed in areas that were infested prior to sanitation treatment suggests that treatments in such areas are indeed worthwhile.

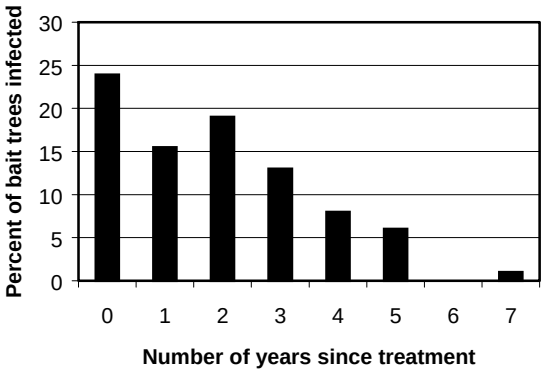


FIGURE 1. Average percentage of Port-Orford-cedar bait trees infected in sampled areas where roadside sanitation treatments were done.

Within transects, the location of infected baits has varied greatly from year to year. It is probably affected by the highly variable weather conditions during spring in southwest Oregon. This affects soil moisture and temperature and the amount and temperature of water in streams and ditches, all factors that would affect the activity of the pathogen. In general, we have found the greatest number of infected baits in the roadside ditches. This suggests that these 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*. Relatively few infected baits have been found near the outer edges of the sanitized areas.

In general, we have also retrieved fewer infected bait trees from streams than we expected. Putting the seedlings in the stream with the tubes still in place may make it more difficult for infection to occur, or the high velocity of the water in many of the streams may make it unlikely for infection to occur during the short duration of the trial.

One shortcoming of this procedure so far is the difficulty and uncertainty of monitoring success of sanitation treatments in uninfested areas. If we don't find infection on any baits in transects installed along roads where there was no disease present before sanitation treatments, it isn't possible to tell whether inoculum has not been introduced or whether our baits were not located in the right places to intercept it.

LITERATURE CITED

Hansen, Everett M. 1993. Roadside surveys for Port-Orford-cedar root disease on the Powers Ranger District, Siskiyou National Forest. USDA Forest Service, Pacific Northwest Forest and Range Experiment Station. PNW-93-0414. 17 p.

Kliejunas, John T. 1993. Port-Orford-cedar root disease. Fremontia 22: 3-11.

Roth, Lewis F., R.D. Harvey and J.T. Kliejunas. 1987. Port-Orford-cedar rot disease. USDA Forest Service. Pacific Northwest Region. R6-FPM-PR-294-87.

Zobel, Donald B., L.F. Roth and G.M. Hawk. 1985. Ecology, pathology and management of Port-Orford-cedar (*Chamaecyparis lawsoniana*). USDA Forest Service, Pacific Northwest Forest and Range Experiment Station. GTR-PNW-184. 161 p.

APPLICATION OF A HIGHLY SPECIFIC AND SENSITIVE MOLECULAR METHOD TO IDENTIFY *PHYTOPHTHORA CINNAMOMI* IN CORK OAK ROOTS AND FOREST SOILS

A.C.Moreira[1], M.G.Horta[2], A.C.Coelho[3], D. Neves[2] & A.Cravador[2]

[1]Estação Agronómica Nacional, Quinta do Marquês, 2780 Oeiras, Portugal.
[2]Universidade do Algarve, Unidade de Ciências e Tecnologias Agrárias, Campus de Gambelas, 8000-810 Faro, Portugal.
[3]Universidade do Algarve, Escola Superior de Educação, Campus da Penha, 8000 Faro.

INTRODUCTION

From the beginning of this century the mortality of cork oak trees has been reported by several authors who, nevertheless, disagree about its causes. Recently, some authors reported a close association between *P. cinnamomi* and cork oak trees with symptoms of decline disease.

In order to improve the reliability of the detection of this pathogen we have recently developed a highly specific, sensitive and reliable method based on the identification of a PCR-amplified target DNA using mycelium from pure cultured *P. cinnamomi.*

The application of this method to detect the fungus directly from roots and infested soil is hampered by the inhibition of PCR by contaminants usually present in soil and roots.

We have applied various protocols to prevent inhibition. All involve several steps of DNA purification. In this paper we describe an optimised method to isolate DNA from *P. cinnamomi* present in roots and soils. This DNA can be consistently used as substrate to the application of an improved PCR method in which the amplification is linked with a colorimetric sandwich capture hybridisation assay.

MATERIALS AND METHODS

Fungal culture method

The fungal isolate of *P. cinnamomi* (XIV-4R) used in this study was isolated from roots of a cork oak tree located in S. Brás de Alportel, Algarve, Portugal. Fungal mycelium was grown in V8 liquid medium incubated at 25ºC in the dark for 4 days for soil infestation. Liquid medium V8 was prepared by the addition of 5 g $CaCO_3$ to 354 ml of V8 juice (Campbell Soup Company). The mixture was shaken for 15 min, then centrifuged for 20 min at 3800 *g*. The supernatant was collected and diluted four times with distilled water. For plant tissue colonisation the fungus was grown in potato dextrose agar (PDA) and incubated at 25ºC in the dark for 5 days.

Detection of *P. cinnamomi* in plant tissues

Artificially infected plant tissue

Three approaches were followed

1. Roots of seedlings of cork oak 1 month old, grown on vermiculite were infected with slices of agar culture placed directly over the roots during 48 hours, in the dark at room temperature. The slices were removed after that period, the roots remained for 2 more days at room temperature and then were frozen at minus 80ºC.
2. Roots samples from seedlings grown during one month under controlled conditions on artificially infested soils were removed from the soil and washed with water, externally disinfected with 1% sodium hypochlorite and abundantly washed with sterilised water. One half of these samples were frozen at minus 20ºC ready for DNA extraction. Another half was placed in isolation selective medium (malt 5%, agar 2%, rifampicine 10 ppm, pimarycine 10 ppm, ampiciline 250 ppm, benomyl 15 ppm, hymexazol 50 ppm, distilled water 1000ml) to confirm *P.cinnamomi* infection.
3. Green apples (Granny smith) and young cork and holm oak leaves can be used as baits to capture *P. cinnamomi* from infected roots and soils. Apples were infected directly either with mycelium of *P. cinnamomi* or with artificially infected roots. Two holes were made per fruit with a cork borer, previously disinfected in 70% ethanol. The holes were filled in either with mycelium or with roots and then sealed with tape. Fruits were left for 10 days in a moisture chamber at 24-25ºC. After that period and as soon as necroses started to appear, some pieces of necrosed tissue were collected, placed on selective medium and incubated at 25ºC in the dark for colony development of *P. cinnamomi.* Some other samples collected from this region were frozen at minus 20ºC to extract DNA.

Naturally infected tissue

Two approaches were followed:

1. Samples from fine roots collected from naturally infected cork oak forest trees exhibiting decline symptoms were treated as described above for the root samples from artificially infested soil.
2. Young cork and holm oak leaves were used as baits to capture the fungus from samples of natural infested soil. Fragments of young leaves were placed on the water surface of a soil suspension during 48h at room temperature. The leaves were then removed, washed in sterile water and blotted dry. Some pieces were frozen at minus 20ºC for DNA extraction and others were surface sterilised in 1% sodium hypochlorite for 1 min, rinsed in sterile water and placed on selective medium. Plates were observed for *P. cinnamomi* development after incubation at 25ºC in the dark.

   DNA was isolated from about 100-120 mg fresh wt. of roots, and from 120-200 mg fresh wt. of apple tissue and leaves.

DNA extraction and purification from plant tissues

Lysis methods

a) Tissues (120 –150 mg) from leaves, roots or apples were reduced to small pieces placed on 1 ml of extraction buffer (Tris, 50 mM; EDTA, 20 mM, pH 8; NaCl, 100 mM; PVPP, 1% W/V) and boiled for 10 or 15 min.

b) Roots were ground in liquid nitrogen, transferred to the described above extraction buffer and then boiled for 10 min or 15 min.

Extraction and purification methods

The boiled or ground tissues were centrifuged (3800 *g*) for 5 min., and the supernatant containing DNA was collected. The pellet was resuspended in 500 µl of extraction buffer and centrifuged for more 5 min. This procedure was repeated twice. DNA was precipitated with ethanol and centrifuged at 12000 *g*, for 15 min, at 0ºC. The dried pellet was resuspended in 200 µl TE buffer. The DNA solution was purified through two types of columns namely Sephadex G-200 (Pharmacia) and Elutip-d (Schleicher & Schuell). The Sephadex columns were packed into 5 ml syringes and equilibrated with TE buffer (pH 8). The procedure for the Elutip-d columns was done as specified by the manufacturer.

The purified DNA was at the end precipitated at 16000 *g* with ethanol and resuspended in 40 µl of pure water.

Detection of *P. cinnamomi* in soil

Soils characterisation

Four different soil samples were used to evaluate the efficiency of DNA extraction. **NCs** designate a non-cultured soil for at least 30 years and **Cs** designates a cultured soil. These soil types originate

from different fields of Estação Agronómica Nacional (Lisbon, Portugal) and are usually used on greenhouse experiments. **NIs 1** is a naturally infested soil collected in Corgas Bravas (S.Brás de Alportel, Algarve, Portugal) and **NIs 2** is a naturally infested soil collected in Ameixeira (S.Brás de Alportel, Portugal). *P.cinnamomi* was isolated, by current methods from both NIs 1 and NIs 2 soils. Soil properties are summarised in table 1.

TABLE 1. Physical and chemical characteristics of soil samples used in the present study

Soil	Amount (%)			Organic C	Nitrogen	pH in
	Sand	Silt	Clay	content (%)	content (%)	water
NCs	30,8	21,5	47,7	3,9	-	7,7
Cs	88,0	3,4	8,6	3,1	-	6,4
NIs 1	-	-	-	1,9	-	-
NIs 2	48,5	29,4	22,1	5,2	0,17	5,5

Artificially infested soils

The 4 days old mycelium grown in V8 liquid medium was washed in sterile water and excess moisture was withdrawn by contact with absorbent paper. Soil samples (1 g) were thoroughly mixed with 100 mg, 10 mg, 1 mg or 0,1 mg of the prepared mycelium. Negative controls consisted in non-infested soil samples and positive controls in DNA purified from mycelium suspensions applying the protocol for extraction and purification from soil.

Naturally infested soils

Samples from naturally infested soils were either analysed without any previous treatment, or were previously incubated on water for 24, 48 or 72 h, at room temperature (ca 25ºC) and indirect sunlight. The incubation water was also analysed.

DNA extraction and purification from soil

The infested soil was sonicated with a titanium tapered microtip (∅=3mm) in 3 ml of iced water at 2/10 maximum power (300 W, Vibracell) for 5 min at 70% of active cycles. Overheating of the sample was avoided by cooling it in ice (Degrange & Bardin, 1995). The bulk soil and gross cell debris were removed by centrifugation (2500 *g*, 4ºC for 5 min) and the supernatant containing DNA was saved. The pellet was submitted to two washes with 2 ml of water to remove the remaining DNA. The supernatants were pooled and the DNA concentrated by centrifugation (15000 *g*, 15 min, 4ºC).

The resulting pellet was resuspended in 1 ml of methanol (50% v/v) and transferred to a 1.5 ml reaction tube cooled on ice. 50 µl of a 20% (w/v) $CaCl_2$ aqueous solution and 1% (v/v) β-mercaptoethanol were added to the samples. This mixture was made homogenous by vortexing for 10 min at 4ºC and then centrifuged at 16000 *g* for 10 min at 4ºC. The methanol/$CaCl_2$ extraction was repeated once, followed by a final extraction without $CaCl_2$ addition prior to DNA solubilization.

400 µl of benzyl chloride and 500 µl of extraction buffer [100 mM Tris-HCl; 20 mM EDTA; 1,4 M NaCl; 2% (w/v) CTAB (hexadecyltrimethylammonium bromide), pH 8 to which 1% (v/v) β-mercaptoethanol and 2% (w/v) PVPP (polyvinylpolipirrolidone) were added before use)] were added to the pellet cooled on ice. The mixture was vortexed for 10 min at 4ºC. Then 300 µl of chloroform (stored at minus 20ºC) and 100 µl *Nucleon PhytoPure* resin (Amersham) were added, and the resulting mixture was vortexed another 5 min at 4ºC and centrifuged for 10 min, at 4ºC, at 1500 *g*. The supernatant was transferred to a new tube containing 300 µl chloroform (stored at minus 20ºC) and 100 µl *Nucleon PhytoPure* resin.

The mixture was stirred for 5 min at 4ºC and centrifuged as before at 1500 *g*. 400 µl of the supernatant were carefully removed to a new tube and 200 µl of isopropanol were added and mixed by tilting. The sample was left for 10 min on ice, centrifuged for 10 min at 4ºC and 16000 *g* and the supernatant was poured away. The pellet was washed for 5 min at room temperature with 1 ml of 70% ethanol/0,1 M sodium acetate, 1 ml 100% ethanol and finally 1 ml chloroform.

The pellets were dried for 30 min at room temperature and the DNA was dissolved in 100 µl TE buffer (pH 8). (Bahnweg *et al.*,1998)

PCR

Amplification was carried out in a final volume of 50 µl containing 0,4 µM of each primer, 0,2 mM of each deoxynucleotide triphosphate (dATP, dGTP, dTTP and dCTP)(Boehringer Mannheim GmbH, Germany), reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1,5 mM $MgCl_2$, pH 8,3), Taq DNA polymerase (2.5 U) (Boehringer Mannheim), 10 µl sample DNA. Water was used as negative control.

After initial denaturing of the samples for 3 min at 94ºC, reactions were run for 35 cycles consisting of 1 min at 94ºC for denaturing, 1 min at 62ºC for annealing and 30 s at 72ºC for extension. In the last cycle, the extension step proceeded for 7 min at 72ºC. Cycling was performed on a thermocycler Biometra.

Colorimetric hybridisation assay

The PCR mixture (10 µl) was treated with 40 µl of 0.25 M NaOH for 10 min. Simultaneously oligonucleotide-coated plates were incubated at room temperature with 250 µl/well of a Tris-buffered solution (0.1 M Tris-HCl, 1 M NaCl, 2 mM $MgCl_2$, 0,05 % Triton X-100, pH 7.5), containing 5 % non-fat dried milk. The solution was eliminated after 10 min..Four pmoles of the detection probe were mixed with the hybridisation buffer composed of Tris-buffered solution, containing 0.2 M acetic acid and 2,5 % non-fat dried milk. 50 µl of this solution and 50 µl of chemically denatured PCR product were applied into a well of a 96-well plate, carrying the covalently linked capture oligonucleotide probe. This procedure was always made in duplicate. After 90 min. of incubation at 37ºC under slight agitation the wells were washed five times with Tris-buffered solution, containing 5 % non-fat dried milk. For detection, 100 µl of streptavidin-horseradish peroxidase (DAKO, Copenhagen, Denmark), diluted 1000 fold in the Tris-buffered solution containing 3 % of Bovine Serum Albumin (Sigma), were added to each well and plates were incubated at room temperature for 30 min. Then, the plates were washed five times with 250 µl of Tris-buffered solution and incubated for 15 min. in the dark with 200 µl of 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma). The reaction was stopped by the addition of 100 µl of a solution 10 % sulphuric acid and the optical density (OD) at 450 nm was then measured in a Novapath microplate reader (Biorad, Richmond, USA). The PCR product concerning the blank PCR (sample without DNA) was tested in five wells in each hybridisation assay. The cut-off value was defined as four standard deviations above the mean of the blank PCR mixtures.

RESULTS

Detection of *P. cinnamomi* in plant tissues

Boiling pieces of the tissues for 10 min in pure water, centrifuging and collecting the supernatant for analysis gave unsatisfactory results. So different techniques were tried for the isolation of genomic DNA, suitable for PCR, from plant tissues. The method described in M & M to extract and purify DNA from roots, leaves and apple tissues yields DNA that can be used for the PCR assay and allows detection of *P. cinnamomi*. This purification method is based on the fractionation of the DNA extract through chromatography columns.

With extract of apple tissues the use of a Sephadex G-200 column (Table 1) was enough to purify the DNA and give positive results (Fig 1). In contrast, for roots and leaves a supplementary purification step of the DNA solution through an Elutip-d column was necessary. DNA extracted from artificially and naturally infected roots had to be diluted in pure water 10 or 100 fold to allow amplification.

Other methods, not described on materials and methods, were used, but the results were not consistent or reproducible.

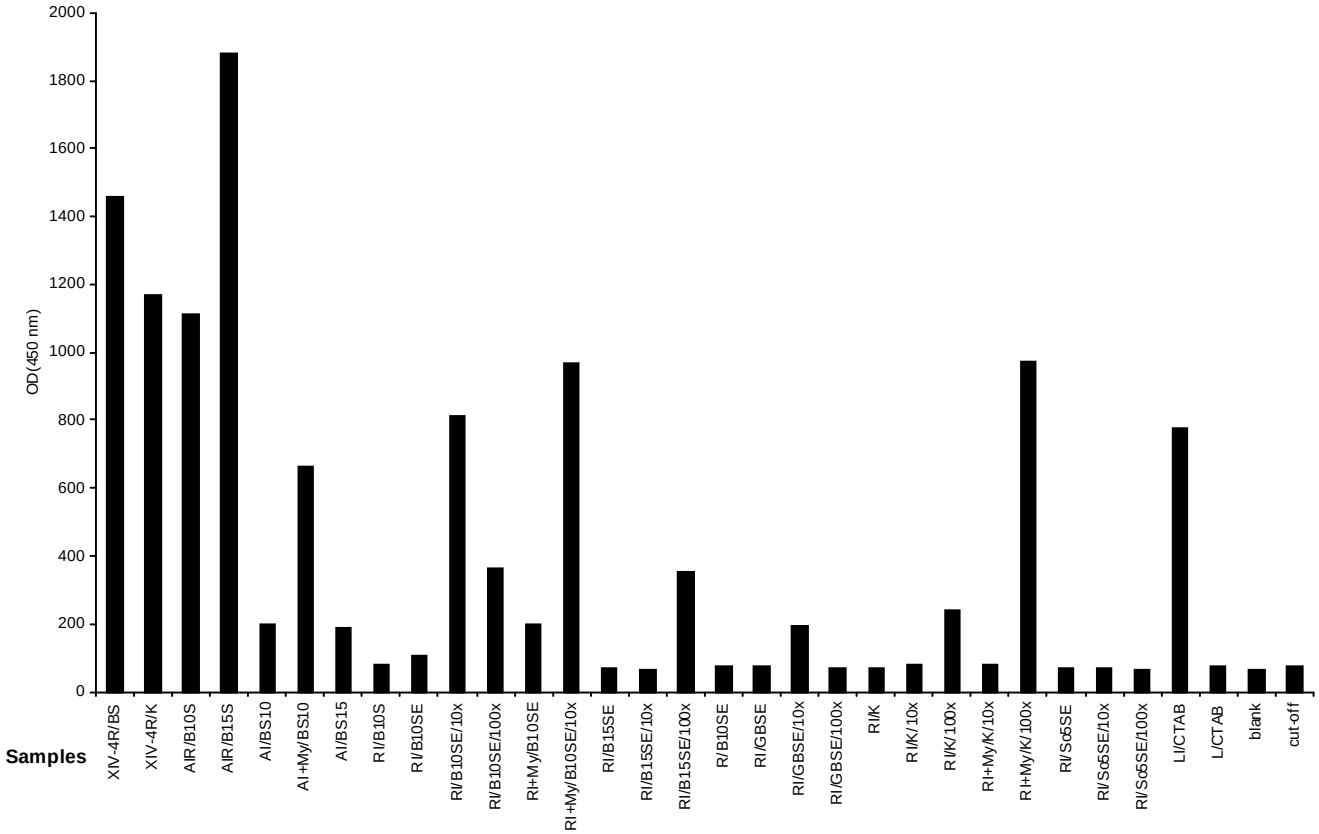


FIGURE 1. Analysis of *P. cinnamomi* in plant tissues (cork oak roots, leaves and apple tissues) using the colorimetric hybridisation assay described in M. & M. (Coelho *et al*., 1997). The reference x10 and x100 in the samples corresponds to 10-fold and 100-fold dilutions of the resulting DNA extracted with the different methods before being submitted to PCR. Blank: negative control of PCR reaction. The cut-off value was defined as four standard deviations above the mean for the blank PCR mixtures.

Sample designation:

M-XIV-4R/BS, **M**ycelium of *P. cinnamomi* (XIV-4R), isolated from roots of cork oak tree located in S. Brás de Alportel, Algarve, Portugal, **B**oiled +**S**ephadex G-200 column;

AIR/B10S, **I**nfected **a**pple tissues (apples were used as baits to capture *P.cinnamomi* (XIV-4R) from artificially infected roots), **B**oiled for **10** min and purified on a **S**ephadexG-200 column;

AI/B10S, **I**nfected **a**pple tissues (apples were infected with mycelium of *P. cinnamomi* (XIV-4R), **B**oiled for 10min and purified on a **S**ephadexG-200 column;

AI+Mi/B10S, **I**nfected **a**pple tissues (apples were infected with mycelium of *P. cinnamomi* (XIV-4R)) + **my**celium, **B**oiled for 10min and purified on a **S**ephadexG-200 column;

RI/B10SE, artificially **i**nfected **r**oots (infected with mycelium (XIV-4R)), **B**oiled for 10 min. and purified on a **S**ephadex G-200 columns and on a **E**lutip-d column;

RI+Mi/B10SE; Artificially **i**nfected **r**oots (infected with mycelium (XIV-4R))+ Mycelium, **B**oiled 15 min and purified on a **S**ephadex G-200 column and on a **E**lutip-d column;

RI/GBSE; Artificially **i**nfected **r**oots (infected with mycelium (XIV-4R)), **G**round, **B**oiled and purified on a **S**ephadex G-200 column and on a **E**lutip-d column;

RIN/B10SE; Naturally **i**nfected **r**oots (roots infected in a soil artificially infested), **B**oiled for 10 min and purified on a **S**ephadex G-200 column and on a **E**lutip-d column;

RIF/B10SE; Naturally **i**nfected **r**oots (roots samples from naturally infected trees located in an infested site, Ameixeira, S. Brás de Alportel, Algarve, Portugal, **B**oiled for 10 min and purified on a **S**ephadex G-200 column and on a **E**lutip-d column;

R/B10SE, Non infected **R**oots, **B**oiled for 10 min and purified on a **S**ephadex G-200 column and on **E**lutip-d column;

LI/B10SE, **I**nfected cork oak **l**eaves (cork oak leaves used as baits to capture *P. cinnamomi* from natural infested soil), **B**oiled for 10min and purified on a **S**ephadex G-200 column and on a **E**lutip-d column.

Detection of *P. cinnamomi* in soil

The obtention of DNA free from PCR inhibitors involved several purification steps described in M. & M., adapted from Bahnweg *et al.*(1998). Briefly, cell wall lysis was performed by direct sonication of soil, DNA isolation was carried out through centrifugation and washing steps, followed by organic solvent extraction and polysaccharide removal by *PhytoPure* resin treatment in the cold and finally solvent washing.

This procedure was applied to artificially and to naturally infested soils.

In order to assess the sensitivity of this method to detect DNA extracted from soils, decreasing levels of infestation were simulated by mixing either non-cultured or cultured soil and the pathogen. We were able to detect DNA purified from 1 g of each type of soil infested with 0.1mg of mycelium as a lower limit (Fig. 2).

DNA purified from both type of soils by chromatographic methods, using Sephadex G-200 and Elutip-d columns, could also be amplified and detected. However, the level of detection was considerably higher requiring down to 100 mg of mycelium in 1 g of soil (data not shown).

PCR amplification and the colorimetric detection were negative when the DNA sample was obtained by sonication of the fungus in the soil without any purification step (data not shown).

The analysis of naturally infested soils did not allow detection of the fungus by any of these methods.

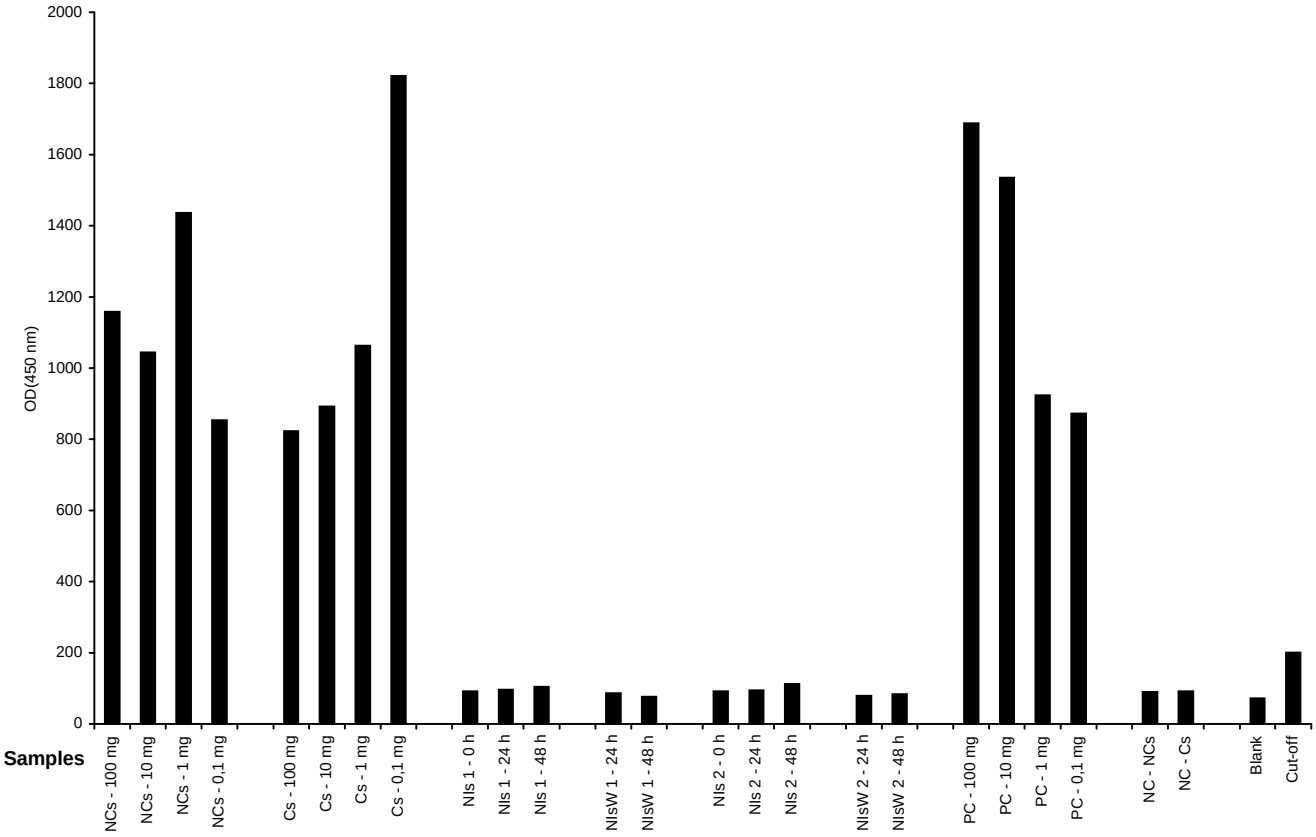


FIGURE 2. Specific detection of *P. cinnamomi* in soil samples using a colorimetric hybridisation assay (Coelho *et al.*, 1997). Sample description is shown below. Weighs refer to mycelium in artificially infested soil samples. Times relate to incubation periods of the naturally infested soils. The cut-off value was defined as four standard deviations above the mean for the blank PCR mixtures.

Sample designation:

NCs = Non Cultured soil; Cs = Cultured soil;
NIs 1 = Naturally Infested soil 1 NIs 2 = Naturally Infested soil 2;
NIsW 1 = Naturally Infested soil Water 1[1] NIsW 2 = Naturally Infested soil Water 2 [1];
NC-NCs = Negative Control of Non Cultured soil NC-Cs = Negative Control of Cultured soil
PC = Positive Control

[1] "Naturally infested soil water" is the collected liquid after a certain period of sample incubation in naturally infested soil.

DISCUSSION

The application of the PCR based method for the identification of *P. cinnamomi* to its detection in roots and soil is not straightforward.

To be efficient and reliable the method should allow direct detection in small samples of roots or soil without the need for isolation intermediate steps such as the use of baits.

The inhibition of the PCR by contaminants present in soil and roots is well documented. Humic acids in soil and phenolic compounds in roots are the more frequently reported inhibitors. Several reports claim success in the application of PCR to DNA isolated from micro-organisms present in soil by various techniques that get rid off contaminants. Clearly the type of soil and the nature of the micro-organisms and of the tissue are determinant.

We have applied and adapted several methods to isolate and purify DNA from *P. cinnamomi* present in cork oak roots and in soil. The use of baits, namely apples facilitates DNA purification, but it is an indirect method of detection that depends on the efficiency of the bait to selectively capture the fungus.

Direct detection in roots, either artificially or naturally infected always required two chromatographic purification steps, but showed to be reproducible.

Direct detection from soil strongly depends on its origin and hence on its composition. We have artificially infested two types of soil, having a similar organic content but very different composition of sand, silt and clay: cultured soil used in greenhouse experiments and a non-cultured soil. We were able to detect DNA from *P. cinnamomi* in an artificially mixture of mycelium and each of these soils, prepared in a proportion of one hundred nanograms and more of mycelium to one gram of soil.

The detection of the fungus in naturally infested forest soil was until now negative, reflecting the difficulty of eliminating polymerase inhibitors from this soil.

We are working out procedures to further improve purity and yield of DNA isolated from soils and hence to increase the sensitivity of our PCR assay applied to detection of *P. cinnamomi* in forest soils.

ACKNOWLEDGEMENTS

We acknowledge Fundação para a Ciência e Tecnologia (FCT) (PRAXISXXI 3/3.2/FLOR/2112/95, INTERREG 14/REG II/6/96) and NATO (SfS PO-CORKOAKS II) for financial support. ACM and ACC thank Fundação Luso Americana para o Desenvolvimento and Fundação Calouste Gulbenkian for their travelling grants and MGH thanks FCT for their PRAXISXXI research grant (BIC J/17089/98).

REFERENCES

Coelho, A.C.; Cravador, A.; Bollen, A. Ferraz, J.F.P.; Moreira,A.C.; Fauconnier, A. & Godfroid, E. Highly specific and sensitive non-radioactive molecular identification of *Phytophthora cinnamomi*. *Mycological Research*, **101** (12): 1499-1507 (1997).

Degrange, V. & Bardin, R. Detection and counting of *Nitrobacter* populations in soil by PCR. *Applied and Environmental Microbiology*, **61**, 2093-2098 (1995).

Bahnweg, G.; Schulze, S.; Moller, E.M.; Rosenbrock, H.; Langebartels, C. & Sandermann, H. Jr. DNA isolation from recalcitrant materials such as tree roots, bark, and forest soil for the detection of fungal pathogens by polymerase chain reaction. *Analytical Biochemistry*, **262**, 79-82 (1998).

THE INVOLVEMENT OF PHYTOPHTHORA CINNAMOMI IN CORK AND HOLM OAK DECLINE IN PORTUGAL

A.C. Moreira (1) , J. F. P. Ferraz (2) & J. Clegg (3)

(1) Estação Agronómica Nacional, Quinta do Marquês, 2780 Oeiras, Portugal
(2) Universidade do Algarve, Unidade de Ciências e Tecnologias Agrárias, Campus de Gambelas,8000-810 Faro, Portugal.
(3) St.Mary's , Strawberry Hill, Twickenham,TW1 4SX, U.K

ABSTRACT

The decline of cork and holm oak tree populations in Portugal has been attributed to infection by Phytophthora cinnamomi. The results of surveys carried out over the last four years for the presence of P. cinnamomi in soils and in roots of Quercus suber and Q. rotundifolia, showed that the fungus is widespread from the north to the south of the country. The most severely affected plantations are found under a variety of soil conditions in the southern Alentejo and Algarve regions and are especially associated with poor, shallow soils or poorly drained soils. These areas are subjected to severe drought, which can last for more than six months, during very hot summers.

Results from tests on the pathogenicity of P. cinnamomi to these two species, under stress conditions, indicate that the holm oak tree is very susceptible to the fungus, while cork oak trees must be subject to stress conditions before symptoms are expressed. The observed susceptibility of cork oak trees associated with drought conditions, led us to believe that factors such as type of soil and soil water content, may be involved in the decline of this species and they may interact with P. cinnamomi infection.

INTRODUCTION

Quercus suber L. is a very widespread tree in the central and southern parts of Portugal and is an important source of the commercial raw material, cork.

From the beginning of this century there are references describing unexplained deaths of cork oak trees. The deaths appeared independently of the age of the tree. Some references report the decline of the cork oak tree and assigned it to insects and pathogenic fungi in the aerial part of the trees, and other biotic and abiotic factors. The same kind of symptoms have been observed recently on Q. rotundifolia Lam., a very common tree in inland areas of Alentejo and Algarve regions. Affected areas are more frequent in the central and southern regions of Portugal, though some oak plantations in the northern parts of the country reveal symptoms of decline. For example, in the Algarve, affected trees may be found all over the region, with 90% of the trees being affected in some cases, especially on south facing slopes. However, field observations of diseased trees did not suggest a clear pattern of disease distribution. On the basis of recent studies some authors (Brasier, et.al.,1993a, Brasier et.al., 1993b, Moreira et.al., 1993) proposed that Phytophthora cinnamomi Rands was a very important contributory factor in the rapid decline of cork oak trees.

Southern regions of Portugal have a Mediterranean type of climate, characterised by short wet winters and long dry seasons culminating in hot summers. In some years the area can be subjected to cyclic droughts. In general, the soils in the affected areas we surveyed are thin, poor in nutrients and in some places poorly drained. Many are subjected to periodical tilling practices to control shrubby vegetation.

Studies on pathogenicity indicated that holm oak plants are very susceptible to P. cinnamomi infection, while cork oak plants are more tolerant displaying a less extensive root colonisation than in holm oak (Moreira et.al.,1997). To show disease symptoms cork oak plants must be predisposed to infection, as has been confirmed in some other species. For example lower resistance to P. cinnamomi infection of Q. rubra during and after water stress was observed by Marçais et.al., (1993), and the highest mortality of "jarrah" was mostly detected on sites with impeded drainage following exceptionally heavy rainfall (Podger et.al.,1965).

This present study aims to observe the behaviour of these two forest species growing in different soil types and under different soil water contents, with and without P. cinnamomi. Our goal is to contribute to a better understanding of how the abiotic factors described above interact with P. cinnamomi, predisposing cork and holm oak plants to the decline disease in Portugal.

MATERIAL AND METHODS

Experiment 1

A mixture of 20% soil, 80% sand and 10% compost was infested with colonised seeds of Panicum milliaceum L.. The seeds were colonised by P. cinnamomi within two weeks. The soil was a very light mixture of air dry clay soil and washed sand, we used 60g of colonised seeds for each pot, and the infestation was done at the same time as the mixture was placed into the pots. Cork and holm oak seedlings, six months old, were planted in the infested mixture. Control plants were prepared in the same way except the inoculum of P. cinnamomi was substituted by sterilised seeds of P. milliaceum. Plants were kept in the greenhouse with temperatures ranging 24 - 32ºC for one month to restart growth. After this period, with temperature between 15-26ºC, plants were subjected to two watering treatments: (1) soil water content at saturation point for 5 days followed by 13 days without any irrigation; (2) normal irrigation, pots were irrigated every three days, according to their needs. Every three months, 15 plants from each treatment (flooding/non flooding ; infested/uninfested), were selected at random, and were removed from the pots. The plants were scored for mortality and taproot infection (by tap root necrosis). The root biomass in dry weight was also assessed. The objective of this experiment was to determine the effect of two soil water contents on the susceptibility of Q. suber and Q. rotundifolia to infection by P. cinnamomi over time.

Experiment 2

In this experiment six month old plants of Q. suber and Q. rotundifolia were subjected to flooding episodes (as described in the Experiment 1), during the four following months. After that period treatments were imposed. Treatments included: "infestation" by P. cinnamomi; "soil type" (clay soil and sandy soil); and "watering treatments" mimicking by weight the water content at field capacity and near drought. Each plant had three variables (infested/non infested; clay soil/sandy soil ; and water content at field capacity/near drought). Eight plants per species were studied per treatment.

The inoculation of the plants was performed by soil infestation (as in the Experiment 1). Plants were kept in the greenhouse during 11 months, with the temperature between 15-26ºC. All the plants were

harvested at the end of the experiment, and we studied the same parameters as we did for the Experiment 1. We observed the responses of both species to the interactions of the three variables.

RESULTS

Experiment 1

Tested plants of Q. suber, under flooding conditions, showed symptoms of decline only at the end of the experiment, i.e., 9 months after planting. Table 1 indicates that root biomass of tested plants is significantly lower than the controls only at the end of the experiment. Taproot necrosis with almost total loss of rootlets was observed on 95% of the infected plants. These results confirm that disease development on cork oak plants is slow despite the favourable conditions for root infection. In fact, periodical flooding was severe enough to induce taproot necrosis on 25% of the controls. In contrast, tested plants growing under normal irrigation did not show any visible symptoms of decline during the experiment. Also, these plants showed development of new root biomass compared with the controls. This reaction has been observed in others species and can be explained by the plants response to infection by P. cinnamomi. The response normally involves the induction of many new roots, similar to the response to root pruning.

The tested holm oak plants grown under both watering regimens (Table 1) showed a significant decrease in root biomass over time compared to the control plants. Three months after planting 80% of the tested plants had taproot necrosis. At the end of the experiment all the plants had deep necrosis and a high root loss. Dead incidence after 9 months of infestation was 25% and 16.7% under flooding and non flooding conditions, respectively. These results clearly demonstrate the high susceptibility of Q. rotundifolia to infection by P. cinnamomi, even when plants are grown under normal irrigation and in a light soil mixture.

Experiment 2

Root development (expressed as dry weight of cork oak plants in Table 2), was significantly lower on control plants subjected to the water content near drought conditions. More than 60% of tested plants (Table 2) showed taproot necrosis in all of the treatments. The percentage of taproot infection and the number of plants with taproot necrosis were higher under field capacity water conditions. Control plants growing under drought conditions exhibited taproot necrosis: 62.5% and 14.3%, on clay soil and on light soil, respectively (Table 2). The drought conditions induced a reduced shoot development on control and tested plants with smaller plants, thicker and darker green leaves than were found on plants subjected to the the other water treatment. These results indicate that drought conditions can affect the good development of cork oak plants, particularly on heavy soils. As in previous experiments, these results confirm that a high level of water in the soil is a more influential predisposing factor to P. cinnamomi infection than drought conditions.

Holm oak tested plants showed higher severity values than control plants regardless of the treatments used. In this Experiment there were no dead plants (Table 2) but the whole root system was severely affected in a high number of plants. Taproot infection and the number of plants with taproot necrosis (> 87.5%) shown on Table 2, was higher on tested plants irrespective of the soil type. However, root biomass of tested plants did not show significant differences in any of the treatments, but under field capacity water conditions the root biomass had a high decrease in comparison with drought conditions. These results confirm the previous experiments with flooding and non flooding treatments. On the control plants the lowest root biomass values were observed on plants growing under drought conditions and these were not significantly different from those obtained with the same treatment on tested plants. The behaviour of tested and control plants of Q. rotundifolia growing in soil under field capacity water content, supports the previous results with flooding and non flooding treatments. Also it was shown that drought conditions imposed a significant decrease on root biomass on tested plants and non tested plants. A low number of control plants with taproot necrosis were also observed on both types of soil.

Table 1. Effect of two watering regimes on the severity of cork and holm oak plants growing in soil artificially infested by P. cinnamomi.

Watering regimens	Months	Cork oak plants						Holm oak plants					
		Tested			Control			Tested			Control		
		[a]root biomass	%pl. tp.n.	% dead	[a]root biomass	%pl. tp.n.	% dead	[a]root biomass	%pl. tp.n.	% dead	[a]root biomass	%pl. tp.n.	% dead
Flooding	3	3.7 bcde*	0.0	0.0	3.5 cdefg	0.0	0.0	2.6 h	80	0.0	2.9 fgh	0.0	0.0
	6	3.8 abcde	0.0	0.0	3.1 efgh	0.0	0.0	2.6 h	90	0.0	4.3 ab	0.0	0.0
	9	2.7 h	95	0.0	3.6 bcdef	25	0.0	2.6 h	100	25	4.1 abc	35	0.0
Normal irrigation	3	3.9 abcd	0.0	0.0	3.9 abcde	0.0	0.0	2.6 h	50	6.7	3.8 abcde	0.0	0.0
	6	3.9 abcd	0.0	0.0	3.3 defgh	0.0	0.0	2.8 h	70	0.0	3.9 abcd	0.0	0.0
	9	4.4 a	0.0	0.0	3.6 bcdef	0.0	0.0	2.9 fgh	100	16.7	3.8 abcde	0.0	0.0

a-dryweight; tp.n.-taproot necrosis;*-number followed by the same letters are not significantly different at the 5% level.

Table 2. Influence of two watering regimes crossed with two soil types on severity of cork and holm oak plants growing in artificially infested soil by P. cinnamomi.

Treatments	Cork oak plants						Holm oak plants					
	Tested			Control			Tested			Control		
	[a]Root biomass	%pl tp.n.	% dead pl.	Root biomass	%pl tp.n.	% dead pl.	Root biomass	%pl tp.n.	% dead pl.	Root biomass	%pl tp.n.	% dead pl.
FC/clay soil	10.9 b*	75.0	0.0	21.1 a	0.0	0.0	4.7 e	100	0.0	10.3 c	14.3	0.0
D/clay soil	10.7 b	62.5	0.0	13.1 b	62.5	0.0	7.6 cde	100	0.0	7.8 cde	14.3	0.0
FC/light soil	12.4 b	100	0.0	20.7 a	0.0	0.0	4.3 e	100	0.0	10.4 c	25.0	0.0
D/light soil	12.2 b	75.0	0.0	14.7 b	14.3	0.0	5.6 de	87.5	0.0	8.8 cd	0.0	0.0

FC- field capacity; D-drought; a- dry weight ; b- tp.n- taproot necrosis; *- numbers followed by the same letters are not significantly different at the 5% level.

DISCUSSION

These results confirm the previous studies on susceptibility to P. cinnamomi in Q.suber and Q. rotundifolia reported by Tuset et al. (1996) and Moreira & Ferraz (1997). Plants from both species had reduced root systems when colonised by P. cinnamomi. Root infection by P. cinnamomi can cause the same effect as drought, by causing a decrease in water absorption capacity through the loss of fine roots. In Eucalyptus marginata and avocado, P. cinnamomi infection induces water stress lowering the xylem water potential (Dawson & Weste, 1984; Sterne, et.al.,1977). The effect of root loss and the decrease in water absorption capacity (unpublished results) was clearly observed in our results with Q. rotundifolia seedlings; root and shoot biomass decreased significantly in all the watering treatments (flooding and non flooding conditions) on both experiments. However, P. cinnamomi infection of plants subjected to non flooding conditions was not as rapid as was found in plants under flooding conditions. There is a synergistic effect of flooding/infection by P. cinnamomi on the severity of the disease. Soil saturation conditions facilitate the dispersion of spores of the pathogen and the invasion roots. P. cinnamomi zoospores are stimulated and grow in anaerobic roots that produce ethanol (Pessarekli,1994). In the presence of free water this fungus quickly increases its population by a repetitive life cycle, and consequently has the capacity to cause multiple infections on the host roots. Q. suber is a more tolerant species to P. cinnamomi infection than Q. rotundifolia. There was no mortality and the tested plants not submitted to flooding did not show severe root symptoms during the 9 months of the Experiment 1. It was observed that P. cinnamomi infection induced the production of many new roots, similar to a root pruning effect. This could be the result of a low soil water content due to the characteristics of the light soil mixture in which the plants were growing and consequently a low level of P. cinnamomi inoculum. The tested cork oak plants were severely infected when subject to a continuous high soil water content. This high level of soil water favours the inoculum build up of P. cinnamomi (Tables 1 and 2). This is very evident in the results of Experiment 2 where the cork oak plants show a high decrease on root biomass and a high number of plants exhibit taproot necrosis on the clay soil compared to the plants in the same water treatment on the light soil mixture.

Q. suber showed a significant decrease in root biomass, a high loss of rootlets and taproot necrosis in control plants submitted to drought conditions, particularly on the clay soil. That would indicate some susceptibility to drought. Although, Q. suber is considered a xerophyte species, Carvalho (1992) observed the presence of high quantity of suberine in cork oak leaves compared with the concentration of this compound in other Mediterranean species. This result suggests also some susceptibility of this species to drought. On tested plants the increase of the number of necrotic taproots on both species under drought conditions was not evident and the necrotic extensions were lower (unpublished results), confirming the results of Smith & Marks (1986). Under field capacity water conditions a quicker progression of root infection was observed on tested plants.

However, in our experimental conditions the cork oak seedlings can survive during a long period with a high loss of fine roots, without showing aerial shoot symptoms. This result was also reported by Robin et.al. (1998). Our results verify, particularly for the cork oak plants, that soil type and water relations can influence P. cinnamomi root infection. The plants with the root system colonised by P. cinnamomi are more vulnerable to drought and flooding than healthy plants. In Portugal, not a great deal is understood with regard to the behaviour of cork oak plants infected with P. cinnamomi , which are subject to stressful environmental conditions. The tolerance to infection of the cork oak plants in our native conditions must be studied. Episodic flooding conditions are only observed in some sites during winter season but drought is the most common condition observed, particularly in the southern regions which are often associated with poor and shallow soils. These soil characteristics are also associated with jarrah forest decline sites in Western Australia (Marks & Smith, 1991). According to Weste & Marks (1987) soil characteristics act in various ways to promote or suppress P. cinnamomi. Soil pore size, drainage capacity, topography, depth and microbial populations are all factors that interfere with growth, reproduction and inoculum potential of P. cinnamomi.

The results obtained in this study suggest that the effects of water relations and the soil type can enhance P. cinnamomi infection on cork oak seedlings. In conclusion, the interaction between soil environmental factors such as water relations and soil characteristics and P. cinnamomi infection contribute strongly to the decline disease of cork and holm oak trees in Portugal.

ACKNOWLEDGEMENTS

We acknowledge NATO (SfS PO-CORKOAKS II) for financial support. ACM thanks Fundação Luso Americana para o Desenvolvimento and Fundação Calouste Gulbenkian for her travelling grants.

REFERENCES

Brasier C.M.,Robredo F., Ferraz J.F.P.,1993(a). Evidence of Phytophthora cinnamomi involvement in Iberian oak decline. Plant Pathology 42, 140-145.

Brasier C.M., Moreira, A. C., Ferraz J.F.P.,& Kirk, S., 1993(b). High mortality of cork oak in portugal associated with Phytophthora cinnamomi.In: Recents advances in studies on cork oak decline (N.Liugi, A.Vanini, eds.) 461-462.

Carvalho J.S. 1992. Sobre a susceptibilidade da Quercus suber à secura. 2º Encontro sobre os montados de sobro e azinho, 345-351 pp., 4-5 Junho, Évora.

Dawson P., Weste G. 1984. Impact of root infection by Phytophthora cinnamomi on the water relations of two Eucalyptus species that differ in susceptibility. Phytopathology 74: 486-490.

Marçais B., Dupuis F., & Desprez-Loustau M.L.,1993. Influence of water stress on susceptibility of red oak (Quercus rubra) to Phytophthora cinnamomi. European J. For.Path. 23: 295-305.

Marks G.C. & Smith I.W. 1991. The Cinnamom fungus in Victorian Forests. Lands and Forests Bulletin nº31.

Moreira A.C., Caetano P., Correia S., Brasier C.M. & Ferraz J.F.P., 1993. Phytophthora cinnamomi associated with cork oak decline in southern Portugal. 6th International Congress of Plant Pathology, 28 July-6 August , Montréal-Canada.

Moreira A.C., Ferraz J. F. P., Clegg J.M., 1997. Susceptibility of Quercus suber and Q. rotundifolia to infection to Phytophthora cinnamomi. Procceedings 10th Congress Mediterranean Phytopathology. Julho, Montpellier-France.

Podger F.D., Doepel R.F. & Zentmeyer G.A. 1965. Association of Phytophthora cinnamomi with a disease of Eucalyptus Marginata forest in Western Australia. Plant Disease Reporter, 49:943-947.

Pessarekli M. 1994. Handbook of Plant and Crops Stress.

Robin C., Desprez-Loustou M-L ,Capron G. & Delatour C. 1998. First record of Phytophthora cinnamomi on cork and holm oaks in France and evidence of pathogenicity . Ann. Sci. For. 55: 869-883.

Shearer B. 1990. Dieback of Native Plant Communities caused by Phytophthora cinnamomi-A Major Factor affecting Land use in South-Western Australia. Land and Water Research News.Issue nº5 :15-26.

Shearer B.L. & Dillon M.1996. Susceptibility of plant species in Banksia woodlands on the swan Coastal Plain, Western australia, to infection by Phytphthora cinnamomi.Aust.J.Bot. 44: 433-445.

Smith I.W. & Marks,G.C., 1986. Effect of moisture stress in Eucalyptus sieberi on growth of lesions caused by Phytophthora cinnamomi.Aust. For. Res. 16: 273-279.

Sterne R.E., Zentmyer G.Z. & Kaufmann M.R. 1977. The effect of matric and osmotic potencial of soil on Phytophthora root disease of Persea indica . Phytopathology 67: 1491-1494.

Tuset J.J., Hinarejos J.L., Mira J.L. & Cobos J.M. (1996). Implication of Phytophthora cinnamomi Rands en la enfermedad de la seca de encinas y alcornoques. Bol. San. Veg. Plagas 22 : 491-499.

West G. & Marks G.C.,1987. The Biology of Phytophthora cinnamomi in Australasian forests. Ann. Rev. Phytopathol. 25: 207-229.

Distribution and survival of *Phytophthora cinnamomi* in a comparative plantation of three chestnut provenances.

Cécile Robin, Gilles Capron, Xavier Capdevielle.

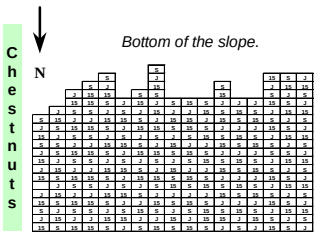
Unité de Recherches Santé Végétale, INRA Bordeaux, BP 81, 33 883 VILLENAVE D'ORNON – France

In the Basque country (France), the ink disease, caused by *Phytophthora cinnamomi*, induces severe losses in European chestnut (*Castanea sativa* Mill.) plantations.
The symptom is a quick and severe dieback (A), sometimes preceded by cortical lesion which can girdle the trunk (B).
To face this epidemic, Asiatic chestnuts (*C. mollissima* and *C. crenata*) have been introduced. These resistant species served as genitors to create interspecific hybrids. The latter have shown high levels of resistance, however this is not always sufficient to restrict the lesions in roots. Moreover, clonal hybrids, used as rootstocks in orchards, can not always be planted for afforestation.

Our aim is to study the disease development in a chestnut plantation and to compare the performances of different genotypes in the presence of *P. cinnamomi*.

Three provenances of chestnut were used in this experiment:
- *C. sativa* trees (**symbol S)**
- a progeny obtained from an open pollination of clonal hybrid trees CA 15 (**symbol 15)**. This clone, which is an INRA obtention, is the result of a cross between *C. sativa* and *C. crenata*
- a progeny obtained from an open pollination of hybrid trees ((*C. crenata* x *C. sativa)x C. sativa*), known in the Basque country as "Japanese" chestnuts (**symbol J)** and commercialised by a local nursery.

They were planted in an infected plot in the Basque country in Marsh 1995. This plot is surrounded old chestnut trees apparently healthy and by red oaks (showing symptoms caused by *P. cinnamomi*).



Localisation of the chestnut trees in the experimental plot (each cell in the map is a tree).

General view of the experimental plot (from the bottom of the slope), 4 months after plantation (June 1995).

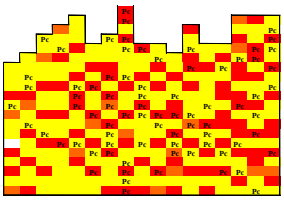
MONOTORING OF *P. cinnamomi* IN SOIL

Detection of *Phytophthora* sp. was performed in April 1995 and June 1999 under each still alive tree.

MONITORING OF DISEASE DEVELOPMENT

Every year, symptoms and growth were noted.

Healthy tree Symptomatic tree Dead tree



Detection of *P. cinnamomi* in soil in 1995, and health status of trees in 1997.

In 1995, 26% of soil samples provided *P. cinnamomi* isolates. The 3 provenances were subjected to a similar inoculum: 31.5 % of the positive soil samples were removed under S trees, 26% under 15 trees, and 31.5 % under J trees.

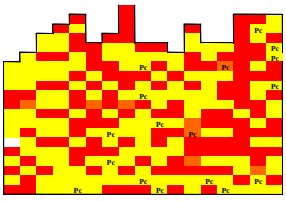
The pattern of *P. cinnamomi* distribution was analysed by ordinary run analysis (Madden *et al.* 1981):

U = number of runs (= successions of one or more infected soil samples),
E(U) expected number of runs, S(U) standard deviation of U

Test of non-randomness of infected soil samples sequences

Zu= [U+ 0.5 – E(U)] / s(U)

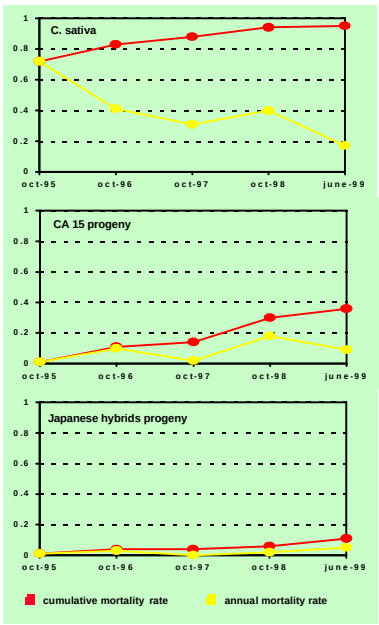
If –Zu> 1.64 , P<0.05 and clustering of infected soil is significant



Detection of *P. cinnamomi* in soil and health status of trees in 1999.

In 1995, Zu= -1.98 showing an aggregated pattern of the pathogen, which develops in foci.

2 years after plantation, half of these trees (mostly « S » trees) were *Phytophthora* infected and dead.
4 years after plantation, 13 % of soil samples removed (18 out 142) were infected.
In 12 of these soils *P. cinnamomi* had not been isolated in 1995 and were removed from the most infected zones of the plot or from its top.
This shows that *P. cinnamomi* was still present and is spreading from the foci into previously uninfected trees.
By contrast, 20 soil samples (2 samples under "S" trees, 6 under "15" trees and 12 under "J" trees) were positive in 1995 and negative in 1999. It is likely that *P. cinnamomi* survival was lower under tolerant trees than under the very susceptible *C. sativa* trees.



Time course of the mortality rates for the three chestnut provenances.

4 years after plantation, the mortality rate was superior to *P. cinnamomi* soil detection rate for «S » and « 15 » trees. However mortality rate of this hybrid provenance was inferior to 40%. The « J » trees had the lowest mortality rate. These F2 hybrids, which have a high level of resistance, could significantly increase chestnut production in plots contaminated by *P. cinnamomi*.

Monitoring of this plantation will continue in the next few years….

DETECTION OF *PHYTOPHTHORA QUERCINA* AND *PHYTOPHTHORA CITRICOLA* IN FIELD SOIL SAMPLES AND IN INFECTED BAITING LEAVES WITH PCR AND SPECIFIC PRIMERS

A. Schlenzig[1], J. Nechwatal[1], T. Jung[1], D. Cooke[2], J. Duncan[2] and W. F. Oßwald[1]

[1]Forest Botany, Section Phytopathology, LMU-Munich Am Hochanger 13, 85354 Freising, Germany
[2]Scottish Crop Research Institute, Invergowrie Dundee DD2 5DA, UK

ABSTRACT

In a field study at 27 sites in Germany, Switzerland, Hungary, Italy and Slovenia, Jung et al. (1996) isolated several Phytophthora species, including *P. citricola, P. cactorum, P. cambivora, P. gonapodyides* and *P. undulata* from the rhizosphere soil and from roots of declining oaks. The most frequently isolated Oomycete turned out to be a new species and was described recently as *Phytophthora quercina* (Jung et al. 1998). In our poster we describe the modification and application of a floating technique of Timmer et al. (1993) to detect the root rot fungus *Phytophthora quercina* in field soil samples collected under declining oak trees with semi-nested PCR and specific primers. The characteristic 820 bp DNA fragment of *Phytophthora quercina* was amplified in each water sample with semi-nested PCR, when the whole floating water was used. However, not all organic debris samples were contaminated with *Phytophthora quercina*. The exsistance of *P. quercina* was confirmed for all collected soil samples in parallel with baiting experiments using *Quercus robur* leaflets. It was also possible to detect *P. quercina and P. citricola* in infected baiting leaflets with PCR in combination with the specific primers. Cleaning the DNA samples with the Wizard-Kit led to an increase of signal intensity. Negative water controls as well as DNA from non-infected and *Pythium*-infected leaflets did not reveal any signals.

LITERATURE SITED

Jung T., Blaschke H. and Neumann P. (1996) Isolation, identification and pathogenicity of Phytophthora species from declining oak stands. Eur. J. For. Path. 26: 253-272

Jung T., Cooke D.E.L., Blaschke H., Duncan J. and Oßwald W. (1998) *Phytophthora quercina* sp. *nov*., a new species causing root rot of European oak species. Mycol. Res. in press

Timmer L.W., Menge J.A. and Miller S.A. (1993) Comparison of ELISA techniques and standard isolation methods for Phytophthora detection in Cirus orchards in Florida and California. Plant Disease 77:791-796

GENETIC RESISTANCE OF PORT-ORFORD-CEDAR (*CHAMAECYPARIS LAWSONIANA*) TO *PHYTOPHTHORA LATERALIS*: RESULTS FROM EARLY FIELD TRIALS

Richard A. Sniezko[1], Everett Hansen[2], Andrew Bower[1], Don Goheen[3], Katy Marshall[3], Kirk Casavan[4], and Wendy Sutton[2]

[1] USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR 97424 USA
[2] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331 USA
[3] USDA Forest Service, Southwest Oregon Forest Insect and Disease Service Center, Central Point, Oregon 97502, USA
[4] USDI Bureau of Land Management, Roseburg, OR 97470 USA

ABSTRACT

Field trials of Port-Orford-cedar (*Chamaecyparis lawsoniana*) (POC) established by the USDA Forest (USFS), USDI Bureau of Land Management (BLM), and Oregon State University (OSU) at two sites in 1993 and three sites in 1998 provide early information on genetic resistance to a POC root pathogen, *Phytophthora lateralis*. This information adds to the results of an older small planting on the OSU Botany Farm. Family 510015 was the top survivor among the 28 open-pollinated families planted in 1993, with a mean survival of 50% versus a mean of 23% for all families through 1997. Family 510015 was also among the top performers in root lesion and branch lesion tests following artificial inoculations. In the 1998 plantings, crosses involving clones 510015 or CF1, or crosses involving progeny of 510015 were rated among the best for lesion score. CF1 had previously shown good survival in the older clonal planting on the OSU Botany farm where all susceptible clones have died. At this point only a few parents show relatively strong resistance, but screening of thousands of phenotypically resistant parents is underway. The underlying mechanisms of resistance are not known at this point.

INTRODUCTION

Port-Orford-cedar is an important forest tree species in southwestern Oregon and northwestern California. It also is susceptible to the root pathogen *Phytophthora lateralis*, which was first found in Port-Orford-cedar forests around 1952 (having been identified in horticultural nurseries as early as 1923). The spread of this exotic pathogen throughout the natural range continues each year, with trees often dying within a year of visible symptoms. Loss of these trees will alter some ecosystems in which this species plays an important role. The USDA Forest Service (USFS), USDI Bureau of Land Management (BLM), and Oregon State University (OSU) have been involved in a program to stop the spread of the disease, and to try to re-establish resistant Port-Orford-cedar in areas were heavy mortality has occurred. Genetic resistance to *P. lateralis* is one management tool that could play an important role, especially in restoration of Port-Orford-cedar to infected ecosystems. Current evidence indicates that the frequency of resistant trees in natural populations is very low. Several field plantings have been established from 1989 to 1998 to determine whether resistant genotypes identified in greenhouse screening trials or phenotypic selections from areas of high disease mortality also showed short-term or long-term resistance in the field.

MATERIAL AND METHODS:

1) 1989 planting: Resistant and non-resistant rooted cuttings and seedlings were planted at the OSU Botany Farm. This planting included rooted cuttings from six clones (18 - 25 cuttings per clone, distributed over four blocks), and open-pollinated seedlings from one of these clones (20 seedlings). This planting was evaluated yearly for mortality from *P. lateralis*. In spring 1999, branches from surviving trees were inoculated at Oregon State University to evaluate relative resistance using one of the short-term screening methods developed (the branch dip technique involves immersing six small branches per tree in a solution containing *P. lateralis* zoospores, and scoring branches for lesion length three weeks afterward).

2) 1993 planting: 28 open-pollinated seedling families (one-year old seedlings) were planted at two sites on the Siskiyou National Forest in Oregon. Seedlings were distributed over 16 blocks per site, with each family represented once per block (some families did not have enough seedlings for all blocks). Mortality has generally been scored once or twice a year since establishment. Twenty-seven of the parent clones of these families had been screened in 1989/1990 for resistance using a branch inoculation technique.

3) 1998 planting: 107 families (including four control crosses, six open-pollinated checklots, and 97 trees from throughout much of the range of POC that were not phenotypically selected for resistance) were established at three sites. Four to seven blocks (with four to six seedlings per family per block) were planted at each site. One of the sites includes four raised beds at OSU that have been inoculated with *P. lateralis*; the other two sites had Port-Orford-cedar naturally present that had been killed by *P. lateralis*. Four of the five blocks at Quosatana, and five of the seven blocks at Camas Valley were evaluated for relative amount of lesion present (lesion score) and percent of trees infected, by removing seedlings from soil several months after planting and examining the root system.

RESULTS

1) 1989 Botany Farm Planting:

- Mortality in the three susceptible clones was early and was nearly 100%, while the three resistant clones had 45 - 80% survival (Table 1); the one resistant seedling family showed 24% survival after 10 years in the field. The major portion of the mortality in the resistant clones (and family) occurred within the first two years, no new mortality occurred over the last five years (Figure 1).
- Lesion length was similar for the survivors within the resistant clones (Table 1).

TABLE 1. Ten year field survival and resistance testing mean lesion lengths

PARENT TREE	NUMBER PLANTED	% ALIVE 1999	STEM DIP SCORE (mm)
CF 1 cuttings	25	48%	18.1 (11, 12-22)*
CF 1 seedlings	20	24%	21.3 (6, 11-32)
CF 2	20	80%	25.2 (15, 14-48)
CB 1	20	45%	19.4 (8, 11-38)
CB 2	20	0%	-
CB 4	18	6%	45 (1)
CB 5	20	0%	-

* (number of trees tested and range of scores)

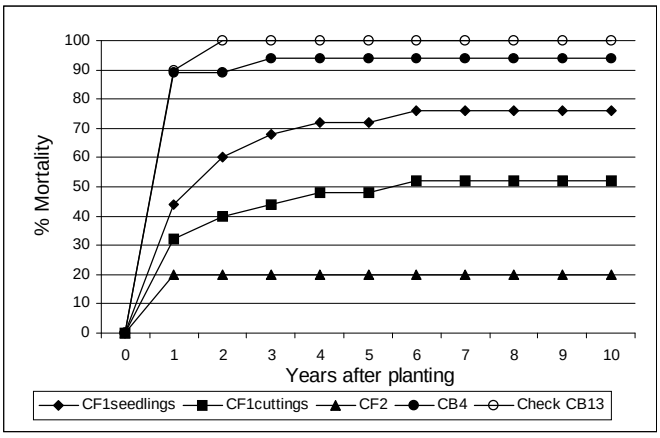


FIGURE 1. Clonal mortality percent at OSU Botany Farm planting.

2) 1993 Plantings (two sites):

- >50% mortality occurred in the first year, very little mortality has occurred between 1995 and 1997 (Figure 2)
- Mean mortality in April 1997 was 82% at Quosatana (Q), and 71% at Flannigan (F), but some families performed inconsistent over the two sites (Figure 3).
- In 1997, family 510015 had the lowest mortality at both sites (44% at Q, 56% at F), and no family had 100% mortality over both sites (Figure 3).
- The correlation of family mean mortality over these two sites with the 1989/1990 branch inoculation tests at OSU was low to moderate and varied somewhat by site and age of field assessment. The family mean correlation between the two sites was also low to moderate, depending on age (Table 2).

TABLE 2. Correlation of percent mortality between two sites

Months After Planting	Correlation
3	0.215
4	-0.213
7	0.194
14	0.104
19	0.408**
31	0.485**
39	0.526**
43	0.430**
49	0.321*

* sig. at $p \leq 0.1$ **sig. at $p \leq 0.05$

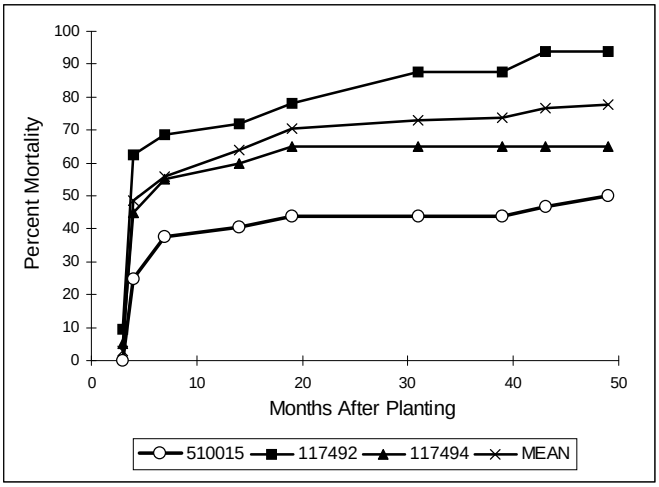


FIGURE 2. Cumulative percent mortality over two sites established in 1993 for three families and the mean of all 28 families.

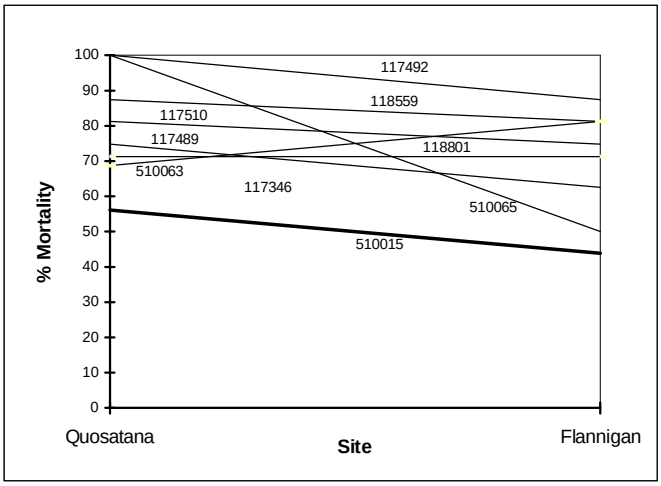


FIGURE 3. Comparison of percent mortality on two sites in April 1997 (49 months after planting).

3) 1998 Plantings (3 sites):

- Seedlings from the four control crosses, involving clones CF1, CF2, or progeny of 510015 as one or more parents were among the very best for resistance at all three sites (using lesion score or percent of trees infected). Open-pollinated progeny of 510015 also showed relatively small lesion scores at all sites
- Several of the "randomly" selected families had low lesion scores, particularly Quosatana (Figure 4).
- Nearly all families in the OSU raised beds had a very high percentage of trees infected (Figure 4), and relatively high lesion score.
- Family mean correlations across sites were low to moderate, and varied depending on the sites and whether the checklots were included (Table 3).

TABLE 3. Correlations between family mean lesion codes between three field test sites.

	Camas Valley	Quosatana	OSU Raised Beds
Camas Valley		0.292**	0.524**
Quosatana	0.118		0.297**
OSU Raised Beds	0.389*	0.041	

Above diagonal n = 107
Below diagonal n = 97 (excludes checklots)

DISCUSSION

The relatively high survival after 10 years in the field of some clones at the OSU Botany Farm is encouraging. However, the reason for the early mortality from *P. lateralis* of some ramets of these resistant clones is unexplained at this point.

Results to-date indicate that there is a very low frequency (perhaps <1%) of phenotypic selections from natural populations that exhibit strong resistance similar to the best clones at the OSU Botany farm.

Information on the types and numbers of resistance mechanisms is not available at this point.

The best selections to date appear to rate highly for resistance regardless of screening method. Recent efforts in the resistance program have concentrated on branch lesion testing from thousands of phenotypic selections. Many of the top 10% of selections from this technique will be evaluated with a root test to confirm resistance.

The low correlations among the three sites planted in 1998 are disappointing, and may be due to an array of factors, including magnitude and nature of the resistance in most of the "random" families, the type of assessment made in 1998, infection level, and the timing of the assessment at the three sites.

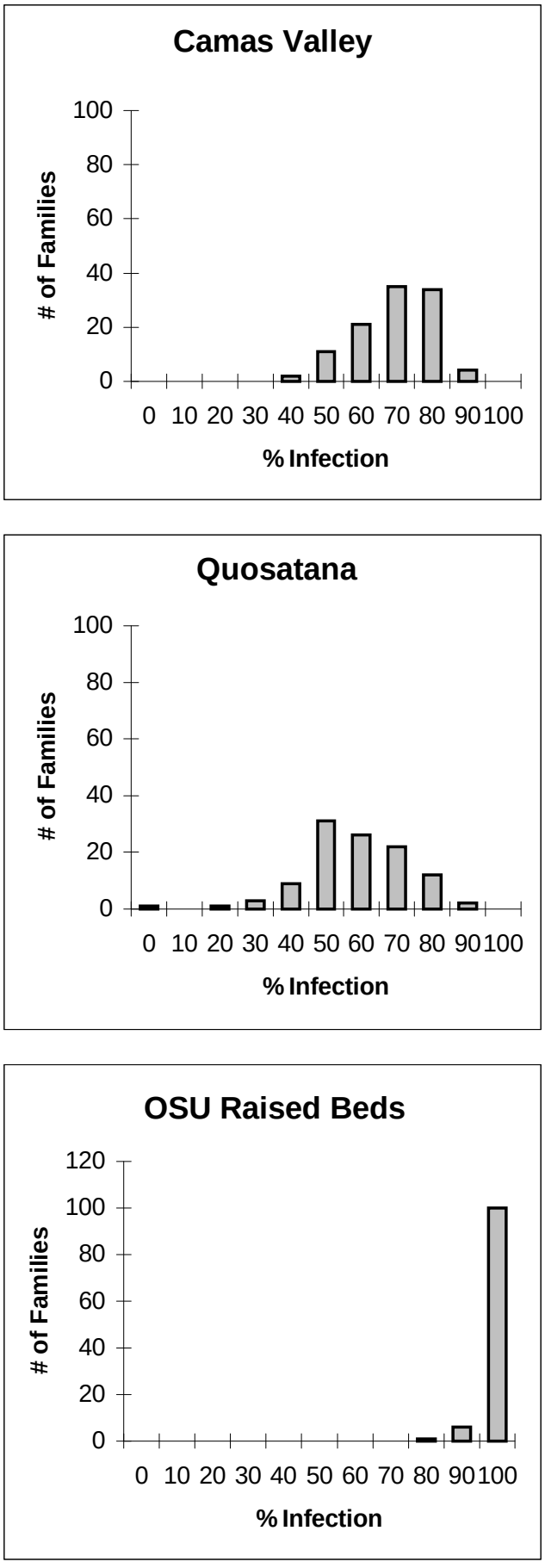


FIGURE 4. 1998 Field site distribution of family mean percent infection.

ACKNOWLEDGEMENTS

The establishment and maintenance of these plantings involved an array of OSU, Forest Service, and BLM personnel, with funding from a US Forest Service Forest Health and Protection Special Technology Development Project. A Special thanks to Mary Brennan for artistic design and construction of the poster.

BAITING OF PHYTOPHTHORA SPP. WITH THE RHODODENDRON LEAF TEST

Karin Themann and Sabine Werres *

Federal Biological Research Station, Institute for Plant Protection in Horticulture, Messeweg 11/12, D - 38104 Braunschweig
*corresponding author

INTRODUCTION

Rhododendron leaves with a well developed cuticle can be used as a very sensitive and easy to handle baiting method to screen large amounts of root, soil and water samples for the presence of living *Phytophthora* propagules. These baits trapped a wide range of *Phytophthora* species which are important pathogens of trees and other woody plants. Furthermore, the method can successfully detect low concentrations of *Phytophthora* propagules. The method can also be used to verify the success of the chemical control of *Phytophthora* species, for example in nurseries. Only living and vital propagules of the *Phytophthora* species can enter the tissue of the Rhododendron leaves during the test procedure.

In the following a detailed guideline is presented. This guideline gives a summary of our experiences so far with the Rhododendron leaf test and should answer all the questions asked by the colleagues who have started to use this bait test.

MATERIAL

For the baiting procedure

material	*comment:*
plastic container (size: 11,5 x 18,5 x 5 cm)	*• of course you can take containers of another size, but it is important that the combination of container size, root and soil sample and the thickness of the water layer above the root and soil sample is identical to our standard (due to the oxygen influx, etc.)*
sterilized demineralized water	*• deionized water can perhaps also be used, we have not tried it* *• do not use tap water, it may be too chlorinated*
well developed leaves of *Rhododendron catawbiense* 'Cunningham's White'	*• it may be that leaves of other Rhododendron species and cultivars trap Phytophthora species as well as R. catawbiense ‚C.W.', we have never proved this* *• it is very important that the leaves have a well developed cuticula, otherwise Pythium species can invade the tissue as fast as Phytophthora spp.* *• do not use leaf discs as a bait; preparing leaf discs means setting wounds and the wounds enable Pythium spp. and bacteria to enter the leaf tissue very quickly*
incubation chambers with circa +20°C and +15°/10°C	*• 20°C: we usually incubate at room temperature because it is between 18° and 22°C* *• low temperatures are helpful to trap Phytophthora species which prefer low temperatures for vegetative growth, e.g. P. syringae*

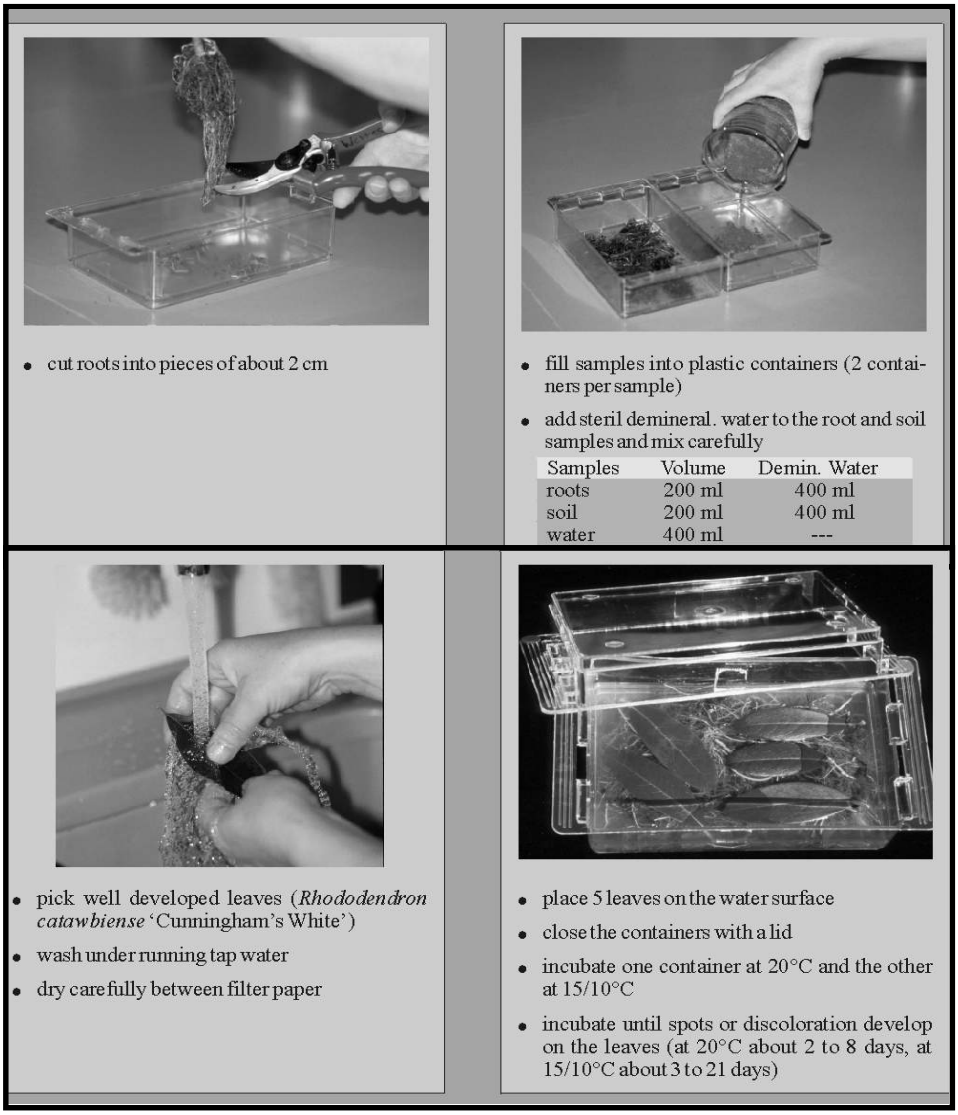
For the isolation procedure

material	*comment:*
solution with e.g. 0.037 % active chlorine for surface desinfection	*• you can also take other solutions and chemicals for surface desinfection, but then you should test concentration and desinfection period for the leaves*
sterile demineralized water	
sterile filter paper	
carrot piece agar (15 g carrot pieces and 15 g agar per 1000 ml deion. water without supplements like fungicides and antibiotics!)	*• try to use media without antibiotics and fungicides etc. because many supplements can suppress vegetative growth and/or development of other structures of many Phytophthora species* *that means: the leaves could have trapped Phytophthora but due to the supplements in the agar medium the fungi perhaps do not grow out of the leaf tissue into the selective medium* *• there are some Phytophthora species which grow better on oat meal agar than on carrot piece agar, e.g. P. katsurae, P. capsici. Take oatmeal agar for these species if you transfer hyphae from the outgrowing culture on carrot agar*
oatmeal agar	
incubation chamber with +20°C	

METHOD

The baiting procedure

method	*comment:*
wash the freshly picked Rhododendron leaves under running tap water and dry them carefully between filter paper	• *pick the leaves not earlier than you really want to place them on the water surface, otherwise it may be that they start to wilt and then they are no longer an attractive bait for Phytophthora* • *it is very important that you dry the surface of the leaves carefully, otherwise they do not swim on the water surface. And that makes the recognition/discovery of leaf spots very difficult*
place 5 leaves per container on the water surface	• *the leaves should swim and should not touch the soil or root layer on the bottom of the container (that prevents the trapping of pathogens like Cylindrocladium scoparium, which cannot swim actively to the leaves)*
close the containers with a lid	• *do not close hermetically, air exchange must be possible*
incubate one container at room temperature (about 20°C) and the other one at 15°C/10°C	• *incubation with light is better than in the dark,when we incubate at room temperature we always use the light intensity and the natural day length in our laboratory* • *if you use an incubation chamber give light for about 10-12 hours at 20 °C and 12 hours at 15°C following by 12 h darkness at 10°C*
incubate until spots or discolour-ation develop on the leaves (at room temperature: about 2 to 8 days, at 15/10°C about 3 to 21 days)	• *the development of spots depends on a lot of different factors as there are the Phytophthora species, the physiology of the fungi in the samples, contamination of the samples with fungicides etc.; so it may be that spots appear later than within 8 days* • *the very first spots which can appear are very small watersoaked looking areas in the leaf tissue which can only be recognized if the leaf is held against the light* • *take the first leaves for isolation as soon as the spots develop, do not take all leaves at the same date if you are not sure that the characteristic 'Phytophtora spots' have been developed*

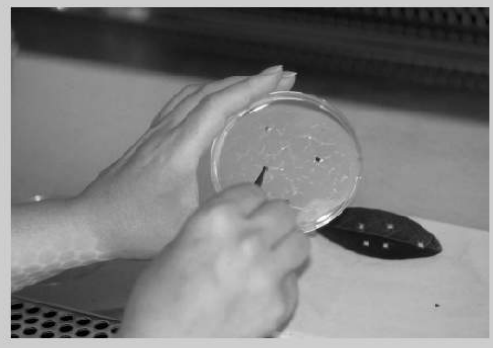


The isolation procedure

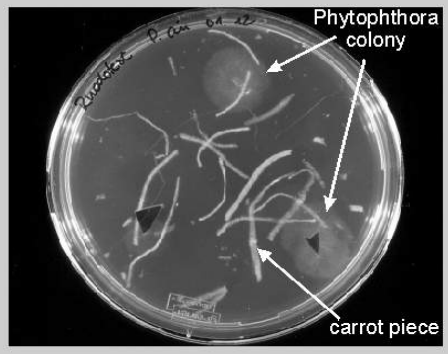
method	*comment:*
wash leaves with characteristic discolouration carefully under running tap water	• *it is very important to wash the leaves very carefully because that is the easiest way to eliminate most of the bacteria and other microorganisms living in the dirt on the cuticula, you can take a brush for the washing procedure*
surface desinfect the leaves for 1 - 2 minutes	
wash with sterilized demineral. water (2-3 times)	
dry carefully with sterile filter paper	• *careful drying of the cuticula is very important to prevent bacteria growth on the agar medium (most of the water is near the vessels and veins!)*
cut out tissue pieces around the spots or discoloured tissue	• *cut tissue pieces only out of the middle part of the leaf, most of the Pythium species enters the tissue through the leaf stalk or through the leaf tip (the cuticula starts to macerate preferabily at the leaf tip and the leaf stalk which are usually under water during the incubation period)* • *tissue pieces should not be larger than about 3 x 3 mm*
place the tissue pieces on carrot piece agar	• *stick them into the agar so that half of the tissue piece is in the agar*
incubate the Petri dishes at 20°C in the dark	
look for hyphae and other structures under the microscope	• *most of the homothallic Phytophthora species prefer the surroundings of the carrot pieces to produce oospores* • *take into consideration that more than one Phytophthora species can be present in a single root, soil or water sample*
prepare hyphal tip cultures for taxonomic investigations	

- wash leaves with characteristic spots carefully under running tap water

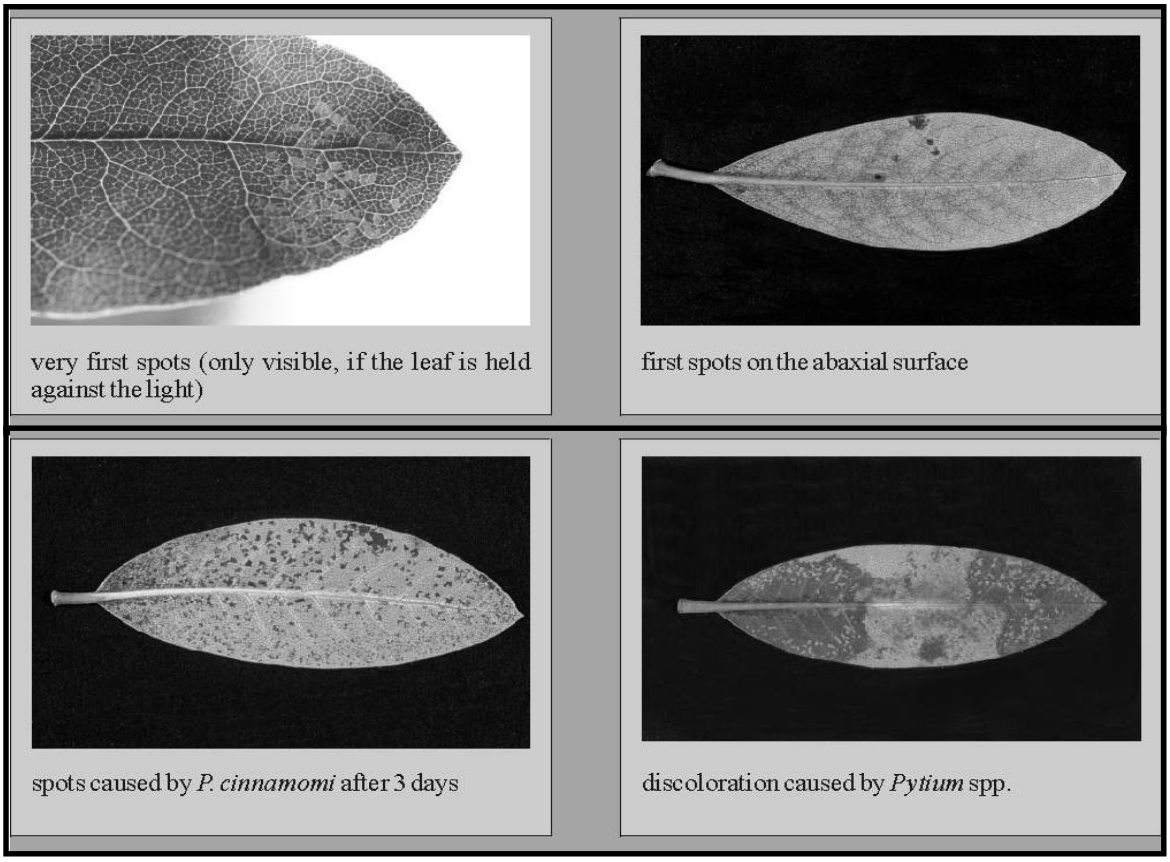
- surface desinfect the leaves for about 1 - 2 minutes (solution with 0.037 % active chlorine)
- wash 2 - 3 times in sterilized demineral. water
- dry with sterile filter paper



- cut out tissue pieces around the spots
- place the tissue pieces on carrot piece agar
- incubate the Petri dishes at 20 °C in the dark



- *Phytophthora* spp. growing out of the leaf tissue pieces on carrot piece agar
- prepare hyphal tip cultures for taxonomic investigations



very first spots (only visible, if the leaf is held against the light)

first spots on the abaxial surface

spots caused by *P. cinnamomi* after 3 days

discoloration caused by *Pytium* spp.

GENERAL COMMENTS

Bait tests work with living organisms. That means, there may be variations in the incubation periods, in the kind of leaf spots, in the trapping rates, etc. What is of importance is to prepare a positive and a negative control to control the bait test itself and to get a feeling for the development of the leaf spots. For positive control in all studies a growing culture of a *P. cinnamomi* and of a *P. syringae* isolate were taken. For negative control sterile carrot piece agar was taken. The culture of one Petri dish (or the agar of one Petri dish, negative control) was mixed with 100 ml of a sterile sand-soil mixture (1 + 3 v/v) and 500 ml sterile *aqua demin*. was added. The *P. cinnamomi* isolate causes leaf spots at room temperature (18-22°C), always within 3 days and the *P. syringae* isolate within 3-4 days when incubated at 15°/10°C.

REFERENCES

Themann, K. und Werres, S. (1997): *In vitro* comparison of different diagnostic methods for the detection of *Phytophthora* species in water. *Proceedings of the 9th International Congress on Soilless Culture, St. Helier, Jersey 12.-19. April 1996, ISOSC, Wageningen*, 535-550.

Werres: S., Hahn, R. und Themann (1997): Application of different techniques to detect *Phytophthora* spp. in roots of commercial produced *Chamaecyparis lawsoniana. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **104**(5), 474-482.

Themann K. und Werres, S. (1998): Verwendung von Rhododendronblättern zum Nachweis von *Phytophthora*-Arten in Wurzel- und Bodenproben. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **50**(2), 37-45.

http://www.bba.de/phytoph/diagn_r.htm or
http://www.bba.de/phytoph/diagnose.htm

SURVEY OF PHYTOPHTHORA SPECIES FROM SOILS SURROUNDING DISEASED CHESTNUT SPECIES IN NORTH AMERICA

A.M.Vettraino[1, 2], A. Vannini [1], N. Anselmi[1], and D.W. Fulbright[2]

[1]Department of Plant Protection, University of Tuscia, Viterbo (Italy
[2]Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824 (USA)

SUMMARY

New records of *Phytophthora* spp. associated with declining chestnut trees in plantation in USA are reported. Declining trees showed typical symptoms of Ink disease of European chestnut. Among the species isolated, *P. cinnamomi* and *P. gonapodyides* have been identified.

INTRODUCTION

Since the loss of the American chestnut (*Castanea dentata*) to chestnut blight at the turn of the 20th century, nut-tree breeders, foresters, and chestnut farmers in North America have turned to other species of chestnut, including Chinese chestnut (*C. mollissima*), Japanese chestnut (*C. crenata*) and interspecific hybrids between Japanese and European chestnut (*C. sativa*) for nut production. In Europe, "ink disease", associated with several species of *Phytophthora* of which *P. cambivora* and *P. cinnamomi* are the most common species isolated, is responsible for a serious dieback of chestnut. This disease was reported in North America in 1932 and was thought to be responsible for a widespread dieback of chestnut reported to occur throughout the 19th century (Crandall *et al.*1945). Because chestnut blight was so devastating in the 20th century, little work was done on ink disease in North America and it is not entirely clear what species were responsible for the "ink disease" symptoms.

In the past few years, scattered reports of chestnut root rot of newly planted trees in orchards in the North America have surfaced. Because the symptoms suggest ink disease of European chestnut, the problem has been simply referred to as *Phytophthora* root rot.

Objectives of the Study

The objectives of the this study were to 1) identify and differentiate the species of *Phytophthora* found on chestnut species in North America; and, 2) determine the effect these species have on the various chestnut species and varieties planted in North America.

MATERIALS AND METHODS

ELISA

Tissue samples from disease trees in Michigan were assayed in 1998 using the Agri-Screen detection kit for *Phytophthora* (Neogen Corp.,Lansing, MI). Small pieces of tissue from root, crown and/or main stems were assayed following the recommended procedures of the manufacture.

Phytophthora isolation

Each soil sample contained chestnut fine or coarse roots collected from the base of the tree, about 50 cm from the collar. After collection, soil samples were moistened and incubated at 20°C for 3 days. In plastic containers, about 200 ml of soil was 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 (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) (Robin, 1991). *Phytophthora* isolates were maintained on carrot agar (CA) (Brasier, 1969) at 20° in darkness and sub-cultured at 4-wk intervals.

Figure 1. Symptoms of ink disease on Chinese chestnut in Michigan .

Isolate identification

Colony morphology assessment was carried out on 10-day-old cultures grown in 9 mm Petri dishes at 20°C in darkness. Sporangia were produced by placing a disk of mycelium from a 7-day-culture grown on CA, where the soil extract was prepared according to Chee and Newhook (1965)

RFLP patterns of ITS1- 5,8S - ITS2 region of rDNA were generated according to the methodology reported by Cooke and Duncan (1997) with some modifications, among which the use of *Rsa* I instead of *Taq* I restriction enzyme.

RESULTS AND DISCUSSION

Flame shaped necroses at the bases of the trunk, similar to those caused by *P. cambivora* and *P. cinnamomi* on European chestnut, were observed at the base of the symptomatic trees analyzed (Figure 1). ELISA assays for *Phytophthora* were conducted in 1998 on

tissues from trees thought to have symptoms of ink disease. Strong positive recordings were found for at least 3 sites in Michigan (Prust, Kewadin and Muskegon). Both root and stems were assayed, and in all cases the root assays gave the strongest readings (Table I).

In June and July, 1999, the locations resulting in positive ELISA assays in 1998 and other sites not included in the ELISA study were sampled and *Phytophthora* isolates recovered (Table I). Cultures are currently being characterized by microscopy and ITS analysis. A site at the National Colonial Farm, Accokeek, Maryland, not included in the ELISA assays of 1998, was also included in the sampling.

So far, only two isolates recovered from the soil around symptomatic trees have been analyzed based on their ITS1 and ITS2 sequences. One of the isolates is from the Prust site in Michigan and the other is from the NCF site in Maryland . Other isolates are currently being studied.

The NCF isolate from Maryland is clearly *P. cinnamomi* based on morphology and ITS analysis when compared with standard strains (Figure 2). The isolate recovered from the Prust site in Michigan, based on ITS sequence, appears to be *P. gonapodyides*, however, it shows hyphal swellings that are not typical of the species. This morphological feature has been reported for some North American isolates of this species (Brasier *et al.* 1993).

TABLE I. Response of ELISA tests and isolation of Phytophthora spp. From soil of the 5 chestnut orchards investigated.

Place of sampling	ELISA	Origin of sampling	N° trees samples	N° trees positive	N° isolates	Species	Date of sampling
Prust, Michigan	Positive	Symptomatic trees	4	2	3	*P. gonapodyides*	June, 1999
Kewadin, Michigan	Positive	Dead tree	1	1	5	*Phytophthora* spp.	July, 1999
Central Lake, Michigan	Positive	Symptomatic trees	4	2	7	*Phytophthora* spp	July, 1999
Muskegon, Michigan	Positive	mud	-	-	4	*Phytophthora* spp	July, 1999
NCF, Maryland	n.d.	Symptomatic trees	3	1	4	*P. cinnamomi*	July, 1999

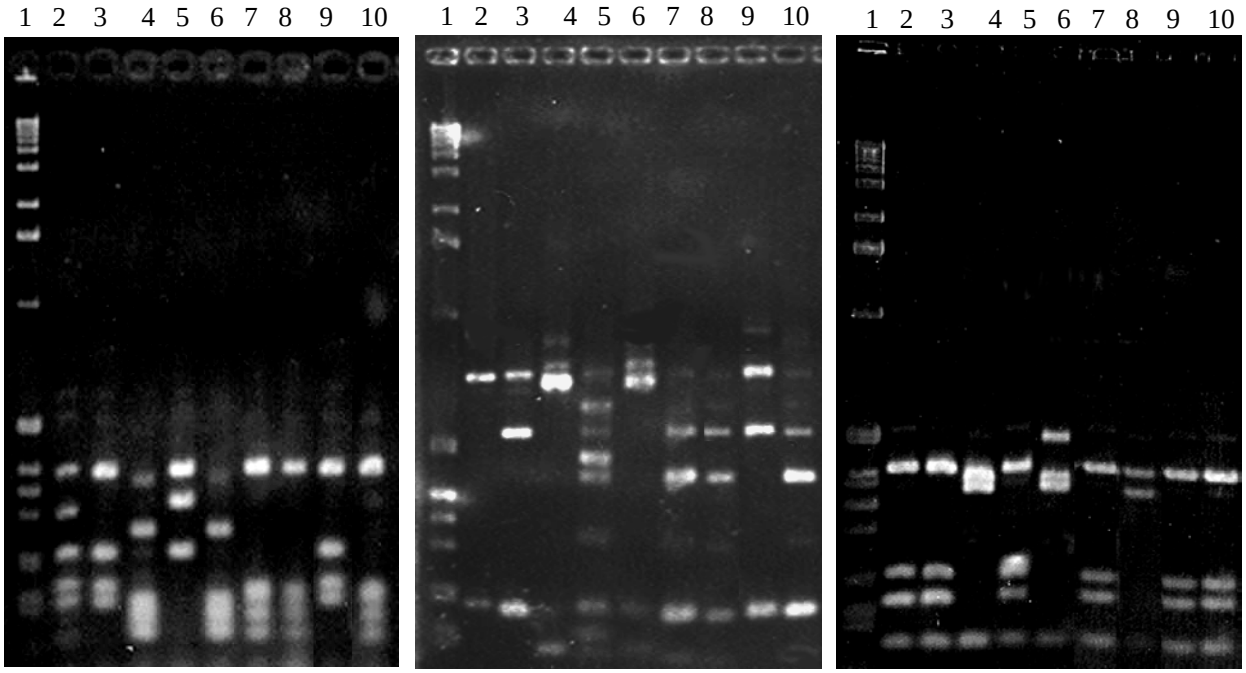


Figure 2. ITS1 and 2 RFLP analysis of the ribosomal RNA gene repeat of several standard strains and two unknowns.

Lane 1: 1 Kb Ladder
Lane 2: P. cambivora
Lane 3: P. cinnamomi
Lane 4: P cryptogea
Lane 5: P. drechsleri
Lane 6: P. erythroseptica
Lane 7: P. gonapodyides
Lane 8: P. megasperma
Lane 9 unknown-NCF
Lane 10: unknown-Prusr

ACKNOWLEDGEMENTS

This research was supported by the Michigan Agricultural Experiment Station, Midwest Nut Producers Council, Fowler Nurseries Newcastle, CA, Northern Nut Growers Association and The American Chestnut Foundation.

We gratefully acknowledge and thank Mario Mandujano for his technical assistance.

REFERENCES

Brasier, C.M., 1969. The effect of light and temperature on reproduction in vitro in two tropical species of *Phytophthora*. Transactions of the British Mycological Society 52, 105-113.

Brasier, C.M., Hamm, P.B. and Hansen, E.M. 1993. Cultural characters, proteins patterns and unusual mating behaviour of *Phytophthora gonapodyides* isolates from Britain and North America. Mycol. Res. 97: 1287-1298.

Chee, K.H. and Newhook. F.J., 1965. Improved methods for use in studies on *Phytophthora cinnamomi* Rands and other *Phytophthora* species. N.Z.J. Agric. Res. : 88-95

Cooke, D.E.L. and Duncan J.M., 1997. Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of the ribosomal RNA gene repeat. Mycol. Res. 101: 667-677

Crandall, B.S., Gravatt, G.F. and Ryan, M.M., 1945. Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. Phytopathology 35: 162-180.

Robin, C., 1991. La maladie de l'encre du chene rouge (*Quercus rubra*, L.) causee par *Phytophthora cinnamomi* Rands. Perspectives pour l'amelioration genetique de la resistance. These de l'Universite de Bordeaux II. 140pp.

Waterhouse, G.M., 1966. *Phytophthora cambivora*. CMI Description of Pathogenic Fungi and Bacteria. No. 112. Commowealth Mycological Institute, Kew, Surrey , England.

PCR DIAGNOSIS OF *PHYTOPHTHORA LATERALIS*

Oregon State University, Corvallis, OR
Loretta M. Winton and Everett M. Hansen

Introduction

A sequencing project of forest *Phytophthora* species revealed an opportunity for the development of a molecular tool for the diagnosis of *P. lateralis*. Deletions in both of the ITS regions of the nuclear ribosomal genes in *P. lateralis* were used to design complementary PCR primer sequences that amplify a 719 base-pair fragment only if *P. lateralis* DNA is present in the sample. This approach has previously been demonstrated for *P. citricola* in European forest trees (Shubert *et al* 1999). Because the lack of a 719 bp PCR product is taken as evidence that *P. lateralis* DNA is not present within the limits of detectability, an internal control is included as a check on the success or failure of the PCR reaction. The control primers are universal primers based upon conserved sequences of the nuclear ribosomal small subunit and amplify a 400 base-pair fragment of this region in plants, protists, and true fungi.

This poster demonstrates initial tests of the *P. lateralis* specific primer set on diverse substrates, including Port Orford cedar (POC) wood, roots, and surrounding soil and sets the stage for ongoing experiments on stream water.

Materials & Methods

DNA Extraction. As we have so far been unsuccessful at DNA extraction directly from soils, we have employed a baiting technique using POC foliage to attract zoospores in soil samples flooded with deionized water. To extract DNA, baits were frozen in liquid nitrogen, ground in a beadmill, and incubated at 65 ºC for 1 hour in a CTAB buffer. After chloroform extraction, DNA was precipitated with isopropanol. DNA extractions directly from POC root and wood samples were performed similarly except they were further purified to reduce PCR inhibitors by passing the extract over QiaAmp Spin Columns (Qiagen). Water samples were filtered through .3 uM membranes. Membranes were then frozen in liquid nitrogen, ground in a beadmill, and boiled in 200 ul of Instagene Matrix (BioRad).

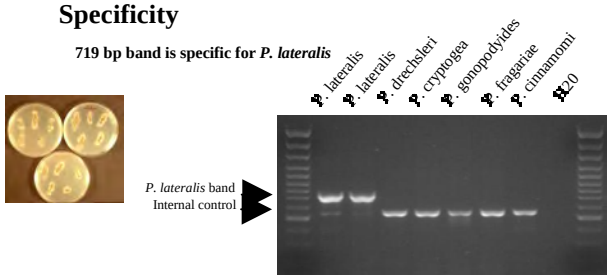
PCR. PCR was performed in a 15 ul multiplex reaction (1X enzyme buffer, 200 uM dNTP, .4 uM *P. lateralis* primers, .1 uM universal internal control primers, .8 U RedTaq DNA polymerase (Sigma), and 1 ul template DNA). The thermal cycler was programmed for 30 sec. annealling at 52 ºC and 1 min. extension at 72 ºC.

P. lateralis primers:
Platf 5'-TTA GTT GGG GGC TTC TGT TC
Platr 5'-AGC TGC CAA CAC AAA TTT C
Universal internal control primers (White *et al* 1990):
NS1 5'-GTA GTC ATA TGC TTG TCT C
NS2 5'-GGC TGC TGG CAC CAG ACT TGC

Truck Wash Site. Washing equipment to prevent spread of *P. lateralis* is recommended, but efficacy has not previously been tested. We compared the standard seedling bioassay (6 week test), with soil baiting and isolation (10 day test), and soil baiting and PCR of the baits (4 day baiting, 1 day PCR). Soil from the road surface, from the roadside ditch, and washed from the road grader was tested.

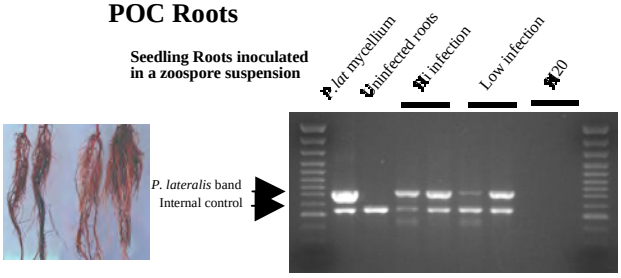
Results

Specificity



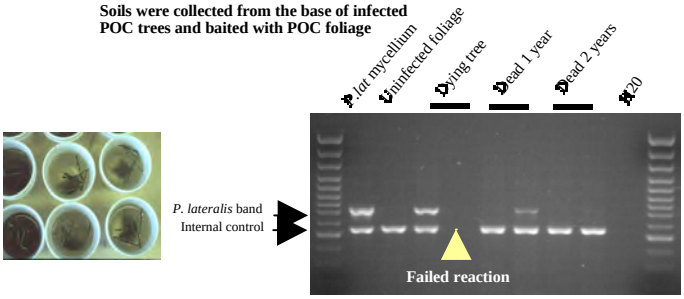
Conclusion: The diagnostic primers amplify neither the closely related *Phytophthora* species nor the other common Oregon forest *Phytophthora* species tested.

POC Roots



Conclusion: PCR primers can detect *P. lateralis* in artificially inoculated seedling roots within 5 weeks of inoculation.

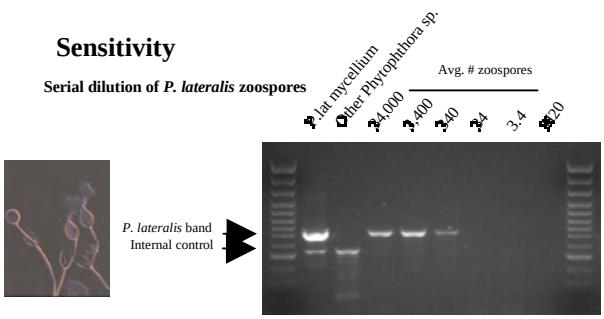
Soils



Conclusion: Baits extracted for PCR can be used to diagnose *P. lateralis* in the soils under infected living trees and trees which have been dead for up to 1 year.

Sensitivity

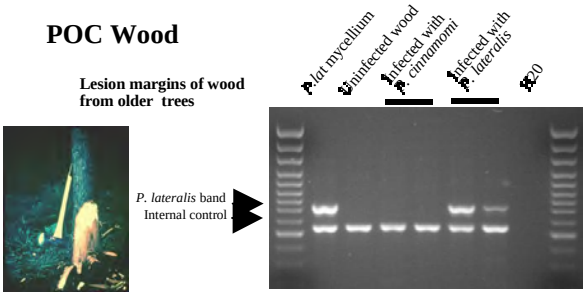
Serial dilution of *P. lateralis* zoospores



Conclusion: At present, our current detection limit is in the hundreds of zoospores.

POC Wood

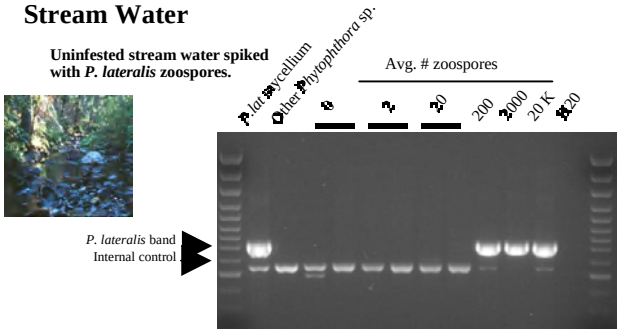
Lesion margins of wood from older trees



Conclusion: PCR primers can discern between the visually similar lesions caused by *P. cinnamomi* & *P. lateralis*.

Stream Water

Uninfested stream water spiked with *P. lateralis* zoospores.



Conclusion: PCR may potentially detect *P. lateralis* in infested streams when zoospores are concentrated on a filter membrane.

Truck Wash

Comparison of PCR results and traditional culturing and seedling bioassay methods for truck wash. Soils from various locations at the truck wash site were baited with POC foliage. Baits were processed for both PCR and culturing on selective media.

(+ *P. lateralis* detected, - *P. lateralis* not detected, ? Equivocal, nd not done)

Location	PCR[1]	Culture[2]	Bioassay
Disease-free road surface	-	-	nd
Disease-free road surface	-	-	nd
Roadside ditch, dead POC's	?	+	+
Roadside ditch, dead POC's	+	-	+
Roadside ditch, dead POC's	+	-	+
Roadside ditch, live POC	+	+	nd
Soil from blocked culvert	+	-	nd
Road grader blade	-	-	+
Road grader wheels	-	-	+
Soil from infested stream	?	-	nd
Roadside ditch, live POC	+	-	nd
Roadside ditch, dead POC	+	-	nd
Dying POC roots	+	-	nd

1. DNA was extracted from POC baits and used in PCR.
2. Most baits were overgrown with Pythium in culture and P. lateralis may have been present but undetected.

Conclusions

°The *P. lateralis* primer set amplifies only *P. lateralis* DNA.

°PCR diagnosis can be performed on both root and stem tissues.

°Soils can be tested for *P. lateralis* by PCR processing of foliage baits.

°PCR diagnosis can be performed upon soils beneath both living & dead trees by processing of foliage baits.

°PCR may potentially detect *P. lateralis* in infested stream water.

°PCR diagnosis is more sensitive than traditional culturing techniques and is not hampered by other, fast-growing fungi.

°More work must be done to decrease the limit of detectability to less than 100 zoospores in water.

°Soil & stream sampling problems may pose a barrier to decreasing the limit of detectability.

°Advances in real-time quantitative PCR may prove useful for comparative studies.

Literature Cited

Shubert, R, G Bahnweg, J Nechwatal, T Jung, DEL Cooke, JM Duncan, G Muller-Starck, C Langebartels, H Sandermann Jr, & W Oßwald. 1999. Detection and quantification of Phytophthora species which are associated with root-rot diseases in European deciduous forests by species-specific polymerase chain reaction. Eur. J. For. Path. 29: 169-188.

White, TJ, T Bruns, S Lee, . Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. Ed. By MA Innis, DH Gelfand, JJ Sninsky, TJ White. New York: Academic Press Inc. pp. 315-321.

PARTICIPANT LIST

Peter Angwin
USDA Forest Service Shasta Trinity National Forest
2400 Washington Ave, Redding CA 96001 USA
Phone:(530) 242-2336 Fax:(530) 242-2233
pangwin/r5_shastatrinity@fs.fed.us

Nabil Atalla
Bureau of Land Management
3040 Biddle Road, Medford OR 97504 USA
Phone:(541) 770-2396 Fax:(541) 770-2400
nabil_atalla@blm.gov

Stan Barras
USDA Forest Service
PO Box 96090, Washington DC 20090
Phone:(202) 205-1528 Fax:(202) 205-6207
sbarras/wo@fs.fed.us

Maria Cristina Bianco
Dipartimento di Patologia vegetale, via G. Amendola
165/A, Bari 70126 Italy
Phone:0039 080 5442920 Fax:0039 080 5442916
cristina.bianco@agr.uniba.it

Andrew Bower
USFS, Dorena Genetic Resource
34963 Shoreview Rd, Cottage Grove OR 97424
Phone:(541) 942-5526 Fax:(541) 942-4331
abower@fs.fed.us

Clive Brasier
Forestry Commission Research
Alice Holt Lodge, Farnham Surrey,
United Kingdom GU10 4LH
Phone:44 420 22255 Fax:44 420 23653
c.brasier@forestry.gov.uk

Ana Cristina Coelho
E.S.E. Universidade do Algarve, Campus de Penha
8000 Faro, Algarve Portugal
Phone:351-89-800100 Fax:351-89-864675
Acoelho@ualg.pt

Ian Colquhoun
Kwinana Alumina Refinery
PO Box 252, Applecross WA 6167 Australia
Phone:618 9316 5246 Fax:618 9316 5167
Ian.Colquhoun@alcoa.com.au

David Cooke
Scottish Crop Research Inst,
Fungal & Bacterial Plant Pathology
Invergowrie, Dundee DD2 5DA Scotland
dcooke@scri.sari.ac.uk

Teresa de Gioia
Dipartimento di Biologia e Patologia vegetale,
via G. Amendola, 165/A, 70126 Bari, Italy
Phone:39 80 544292 Fax:39 80 5442906
teresa.degioia@agr.uniba.it

Claude Delatour
INRA, Lab Pathology
E. 5H280, Champenoux France
Phone:33 3 83 39 40 55 Fax:33 3 83 39 40 69
delatour@nancy.inra.fr

Marie Desprez-Loustau
INRA,Unite de Recherches en Saute de Vegetal - BP 81
33883 Villenave d'Ornon Cedex, France
Phone:05 56 84 32 10 Fax:05 56 84 32 22
loustau@bordeaux.inra.fr

Donald Goheen
USDA Forest Service
2606 Old Stage Rd, Central Point OR 97502USA
Phone: (541) 858-6126 Fax: (541) 858-6110
dgoheen@fs.fed.us

Ellen Goheen
USDA Forest Service
2606 Old Stage Rd, Central Point OR 97502 USA
Phone: (541) 858-6125 Fax: (541) 858-6110
egoheen@fs.fed .us

Donald Grant
521 E Anderson Road, Sequim WA 98382 USA
Phone:(360) 683-4498

Annmarie Hamilton
Irish Forestry Board, Forest Protection, Research
Laboratory
Newtownmount Kennedy, Wicklow Ireland
Phone:(01) 2819511 Fax:(01) 2810465
Amhamilton.coillte@indigo.ie

Everett Hansen
Oregon State University, Botany and Plant Pathology
2085 Cordley Hall, Corvallis, OR 97331 USA
Phone:(541) 737-5243 Fax:(541) 737-3573
hansene@ava.bcc.orst.edu

Giles Hardy
Murdock University, School of Biological Sciences
Murdock 6150 Australia
Phone:61 8 9360 6272 Fax:61 8 9 360 6303
g-hardy@central.murdoch.edu.au

Marilia Horta
Universidade De Algarve, Campus De Gambelas
8000 Faro, Algarve Portugal
m_horta@hotmail.com

Thomas Jung
Institute of Forest Botany/Pathology
Am Hachanger 13, Freising D-85354 Germany
Phone:0049-8161-714874 Fax:0049-8161-714576
Jung@bot.forst.uni-muenchen.de

Alan Kanaskie
Oregon Dept of Forestry
2600 State St, Salem OR 97310 USA
Phone:(503) 945-7397 Fax:(503) 945-7376
alan.kanaskie@odf.state.or.us

Andre Levy
French Forest Health Dept
50 Chemin d'Artigues, 33150 Cenon, France
Phone:33-5-56-40-46-46 Fax:33-5-56-32-44-84
Levy.dsf@wanadoo.fr

Nicola Luisi
Dipartimento di Biologia, via G. Amendola
165/A, Bari 70126 Italy
Phone:0039 080 5442920 Fax:0039 080 5442916
luisin@agr.uniba.it

Beniot Marcais
INRA, Lab Pathology
E. 5H280, Champenoux France
Phone:33 3 83 39 40 55 Fax:33 3 83 39 40 69
marcais@nancy.inra.fr

Katy Marshall
USDA Forest Service
2606 Old Stage Road, Central Point OR 97502
Phone:(541) 858-6124 Fax:(541) 858-6110
Kmarshall01@fs.fed.us

Eric Martz
Siskiyou National Forest, Powers Ranger District
Powers OR 97466
Phone:(541) 439-3011
Emartz@fs.fed.us

Marion Maurel
INRA, Station de Pathologie Vegetale
BP 81, 33883 Villenave d'Ornon Cedex France
Phone:05 56 84 32 26 Fax:05 56 84 32 22
maurel@ferrade.bordeaux.inra.fr

Michael McWilliams
Oregon Dept of Forestry
2600 State St, Salem OR 97333 USA
Phine:(503) 945-7395 Fax:(503) 945-7376
mmcwilliams@odf.state.or.us

Ana Moreira
Estacao Agronomica Nacional
Quinta Do Harques 2780, Oerias Portugal
Phone:351-89-800935 Fax:351-89-818419
ean@mail.telepac.pt

Kenneth Old
CSIRO Forestry & Forest Products
PO Box 4008, ACT 2604, Kingston Australia
Phone:61 6 2818329 Fax:61 6 2818312
ken.old@ffp.csiro.au

Wolfgang Osswald
University of Munich, Forestbotany/Phytopathology
Am Hochanger 13, 85354 Freising Germany
Phone 08161-714577 Fax:08161-714576
Osswald@bot.forst.uni-muenchen.de

Paul Reeser
Oregon State University, Botany & Plant Pathology
2082 Cordley Hall, Corvallis OR 97331 USA
Phone:(541) 737-5242 Fax:(541) 737-3573
Reeserp@ava.bcc.orst.edu

Cecile Robin
INRA, Station de Pathologie Vegetale
BP 81, 33883 Villenave d'Ornon Cedex France
Phone:05 56 84 32 10 Fax:05 56 84 32 22
robin@bordeaux.inra.fr

Don Rose
USDA Forest Service, Siskiyou National Forest
PO Box 440, Grants Pass OR 97528 USA
Phone:(541) 471-6500
Drose/r6pnw_siskiyou@fs.fed.us

Ian Smith
Centre for Forest Tree Tech, Dept of Natural Resources
PO Box 137, Heidelberg, Victoria 3084 Australia
Phone:61 3 94508647 Fax:61 3 94508644
ian.w.smith@nre.vic.gov.au

Richard Sniezko
USDA Forest Service, Dorena Genetic Resource Center
34963 Shoreview Road, Cottage Grove OR 97424
Phone:(541) 942-5526 Fax:(541) 942-4331
Rsniezko@fs.fed.us

Jean-Claude Streito
LNEV
38 rue Sainte Catherine, 54043 Nancy cedex France
Phone:03 83 30 41 51 Fax:03 83 32 00 45
lnpv.nancy@wanadoo.fr

Wendy Sutton
Oregon State University, Botany and Plant Pathology
2085 Cordley Hall, Corvallis OR 97331
Phone:(541) 737-5242 Fax:(541) 737-3573
suttonw@ava.bcc.orst.edu

Frank Tainter
Clemson University, Dept of Forest Resources
Clemson SC 29634 USA
Phone:(864) 656-4856 Fax:(864) 565-3304
ftntr@clemson.edu

Inez Tommerup
Forestry & Forest Prod, CSIRO
CSIRO Perth, PO Box 440, Private Bag PO,
Wembley 6014 Australia
tomi@ccmar.csiro.au

Nick Vagle
USDA Forest Service, Siskiyou National Forest
Grants Pass OR 97528
Phone:(541) 471-6810 Fax:(541) 471-6514
nvagle/r6pnw_siskiyou@fs.fe

Andrea Vannini
Universita Dello Tuscia, Dipartimento Protezione Plante
VIA S. Camillo De Lellis, Viterbo 01100 Italy
Phone:390761 357449 Fax:390761 357473
Vannini@unitus.it

Annamaria Vettranio
Universita Della Tuscia, Dipartiento Protezione Plante
VIA S. Camillo De Lellis, Viterbo 01100 Italy
Phone:390761 357449 Fax:390761 357473
vettrain@unitus.it

Tonia Walchhuetter
Melbourne University
387 Hawthorn Rd, Caufield South,
Victoria 3162 Australia
Phone:03 9344 5039 Fax:03 9347 5460
tonianw@email.com