SCOPE ITEM 8

IDENTIFICATION AND EVALUATION OF THREATS POSED BY *PHYTOPHTHORA* TO THE NATIVE BIOTA OF SOUTH-WEST WESTERN AUSTRALIA

Stephen A. Carstairs and Larissa E. Carstairs

1 INTRODUCTION

Phytophthora occupies an interesting position in the Oomycetes, as it forms a natural link between the saprobic watermoulds (Saprolegniales) at one extreme, and the obligate, parasitic downy mildews (Peronosporaceae) at the other. Anton de Bary (1876) combined the Greek *phyto* (plant) and *phthora* (destruction) to synthesise the generic name *Phytophthora* which literally means plant destroyer. Although all species of *Phytophthora* are saprophytic to some degree, 90% of the crown diseases of woody plants may be attributable to members of the genus (Tsao, 1990).

Phytophthora cinnamomi and its interactions with Western Australian native plant communities have been the subject of extensive research for around thirty years. A considerable body of knowledge now exists on the activity of this pathogen, particularly in causing epidemic dieback of *Eucalyptus marginata* and many other components of the native jarrah forest community. Some measure of the importance of *P. cinnamomi* is reflected by the fact that it is one of only five key threatening processes identified in the Endangered Species Protection Act of 1992 (Commonwealth). Much less is known about the threats that other species of *Phytophthora* pose to the native biota of the South West Land Division (SWLD) of Western Australia and it is that with which the current study is concerned.

1.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

During the early part of the nineteenth century, citrus trees and many other exotic plants were shipped around the world in the absence of quarantine restrictions. Not surprisingly, at least some of the *Phytophthora* spp. (including *P. cinnamomi*) known to infect citrus (Erwin & Ribeiro, 1996) were carried on the roots of imported trees to parts of the SWLD. Fraser (1956) first reported *P. cinnamomi* on Australian native vegetation in New South Wales. Podger *et al.* (1965) subsequently isolated and identified the pathogen from dieback-affected jarrah forest in Western Australia.

In order to evaluate the extent to which taxa of *Phytophthora* pose a threat to native plant communities, it is necessary to consider certain aspects of the host-pathogen interaction. Significant features of the ancient, dry sclerophyll flora of the SWLD are its richness in woody perennials and a high degree of endemicity. These characteristics are common to most species of the Myrtaceae, Proteaceae, Papilionaceae, Dilleniaceae and Epacridaceae, which represent major segments of heath, woodlands and forests of the SWLD. *P. cinnamomi* is a non-host-specific pathogen of woody perennials (Zentmyer, 1980) and some other *Phytophthora* spp. (see below) are similar in that regard. Secondly, the highly endemic nature of the native flora and the probable absence of most if not all species of *Phytophthora* until recent times, means that the putative hosts and pathogens would not have evolved together. Thus, insufficient time may have elapsed for many native plant species to adapt and develop resistance to *Phytophthora* spp.

Of 62 taxa that are presently recognised in *Phytophthora* (Erwin & Ribeiro, 1996), pathogens fall into two broadly defined categories:

- specifically (vertically) pathogenic: fungi that parasitise only one host species, or are limited to a narrow range of hosts usually included within a single genus; and
- generally (horizontally) pathogenic: fungi that attack a diverse range of hosts, usually including members of more than one family.

Our intention was to identify those taxa of *Phytophthora* that (like *P. cinnamomi*) are generally pathogenic to woody plants and as such, would be expected to pose a threat to the flora of the SWLD. We also consider the possibility that currently undescribed taxa in that category may be discovered overseas or in natural Western Australian ecosystems.

1.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

A number of factors may impinge upon the pathologist's ability to recover *Phytophthora* from natural ecosystems. These include:

- the sampling intensity employed to achieve some predetermined confidence level of detection;
- the effectiveness of substrate(s) selected for use as baits;
- seasonal and site effects on recovery; and
- incubation of host plant tissue on agar, as opposed to the use of substrate baits for detection of the fungus in samples containing soil and plant material.

In dieback-affected sites, inoculum levels of *P. cinnamomi* do not remain constant but vary from season to season (Shea *et al.*, 1980; Shearer & Shea, 1987). Differences between floristic composition in different areas may also influence inoculum levels (Shea *et al.*, 1978; Murray *et al.*, 1985).

Carstairs & Stukely (1996) examined the relationship between sampling intensity and the number of samples shown to be positive for *Phytophthora* at a particular site. They found a positive though weak relationship ($r^2 = 0.36$) for 28 infested sites. It was also found that if six samples/site were assayed and if all proved negative for *Phytophthora*, a site could then be deemed free of the fungus within confidence limits of 95%.

While detection and recovery of *Phytophthora* from recently infected plant tissue is simple, isolation from material in advanced stages of decay is difficult or impossible unless special techniques are used. In the past 25 years, development of baiting techniques and selective media for isolation of *Phytophthora* spp. from old, diseased tissues and soils, have made these pathogens among the most readily detectable (Tsao, 1990).

Since the mid 1980's, the Department of Conservation and Land Management (CALM) have managed a root-rot disease thought to be caused by an undescribed species of *Phytophthora* in the Fitzgerald River National Park (FRNP). This species, which appears to have some affinity with *P. megasperma*, is subsequently referred to as *P.* aff *megasperma* Black Point. Detection of the pathogen was difficult when techniques designed for isolation of *P. cinnamomi* were used and two factors that emerged as critical were sample collection time (season) and sample type (M. Grant, J. Webster, and R. Hart pers. comm.).

1.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

It has been known for about 30 years that species of *Phytophthora* other than *P. cinnamomi* are present in natural ecosystems in the SWLD. In the mid 1980's, Shearer *et al.* (1987; 1988) compared the pathogenicity of a range of *Phytophthora* spp. and found that some grew in jarrah as aggressively as *P. cinnamomi*. However, in *Banksia grandis*, *P. cinnamomi* was by far the most aggressive pathogen and it girdled plant stems before any host resistance could be expressed. In contrast, other species of *Phytophthora* were confined to lesions by active host resistance (Shearer *et al.*, 1988).

Since then, results obtained with analytic molecular techniques including RFLP and isoenzyme analysis, have demonstrated that certain *Phytophthora* spp., eg. *P. citricola*, *P. cryptogea*, *P. drechsleri*, and *P. megasperma*, are artificial species comprising a number of discrete but morphologically indistinguishable biological taxa (Oudemans & Coffey, 1991; Oudemans *et al.*, 1994). This suggests that some of the findings of early pathogenicity studies should be viewed with caution. Recently, pathogenicity tests have been conducted with allozyme taxa (taxa distinguished by isoenzyme analysis) on *B. grandis* in the southern karri forest (Podger & Carstairs, unpubl.). Preliminary results of that work are included in Section 4.3.

Plant diseases may be described in terms of epidemic progress curves and epidemics can be divided into those that fit simple interest (SI) type curves, or those that fit compound continuous interest (CCI) curves (van der Plank, 1963). The progress curve of avocado root-rot disease caused by *P. cinnamomi* has been described as a CCI type of epidemic (MacKenzie *et al.*, 1983).

2 OBJECTIVES

The primary objective of this work is to address Scope Item No. 8 for the *Phytophthora* and *Diplodina* canker project (1997/98). This entails evaluation of the significance of *P. megasperma* as a threat to the native biota, and investigation of the identity and importance of other *Phytophthora* spp. found in native vegetation.

Attainment of the stated objective involved work in the areas noted below:

1. Identification of *Phytophthora* taxa that are generally pathogenic to woody plants and thus represent a threat to native flora of the SWLD.

2. Evaluation of the possibilities; (a) that any new, generally pathogenic species of *Phytophthora* (on woody plants) will be discovered in natural ecosystems in the SWLD or elsewhere, and (b) that taxa of *Phytophthora* reported from Australia are representative of the genus, worldwide.

3. Assessment of methods for the detection and isolation of *Phytophthora* taxa noted in 1 (above).

4. Evaluation of the relative abilities of different taxa of *Phytophthora* to cause disease and/or mortality in native vegetation of the SWLD.

5. Preparation of a host list for *P.* aff. *megasperma* Black Point and description of an epidemic progress curve for this pathogen in natural ecosystems.

3 METHODS

3.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

Sixty-two taxa of *Phytophthora* (Erwin & Ribeiro, 1996) were assessed to determine their pathogenic characteristics and each was assigned to one of the following classes:

- host not determined;
- specifically pathogenic on herbaceous plants;
- generally pathogenic on herbaceous plants;
- specifically pathogenic on woody plants; or
- generally pathogenic on woody plants.

Taxa were deemed to be generally pathogenic to woody plants if they had been reported as pathogenic on three or more families of vascular plants and if the majority of their hosts were woody species.

3.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

Information on baiting techniques for the isolation of 15 *Phytophthora* taxa (general pathogens of woody plants) was selected from the literature (Erwin & Ribeiro, 1996) and updated with data reported for Western Australia

In the FRNP, taxa of *Phytophthora* were detected in March and August, 1997, by baiting roadside pond water with 2-6 nylon covered pears. In March, 11 ponded sites and the Gairdner River were baited for 2-4 days with 24 pears (2 pears/site). In August, 8 ponded sites were baited with 2-6 pears.

After retrieval, the nylon covers were removed and the pears were maintained in the laboratory in plastic dishes for 2-5 days to allow development of lesions. Portions of lesions were excised and incubated for a period on selective agar in Petri dishes. Mycelium of *Phytophthora* that grew from the lesions was subcultured to corn meal agar (CMA) and checked for purity after two days. The isoenzyme characteristics of pure cultures were determined using methods described by Carstairs & Stukely (1996). Up to three isolates of each allozyme class were examined microscopically to establish the morphology of their sporangia and oospores. A Contingency Chi Square test was used to compare the relative abundance of different *Phytophthora* taxa isolated from pond water in autumn and spring.

Ninety-three samples of soil and root tissue, from sites infested with *P. cinnamomi*, were assessed for the presence of the pathogen by direct plating and baiting methods. The direct method involved incubation of root material on selective agar, and subsequent isolation and identification of fungi growing from the roots. The second technique entailed incubation of *Eucalyptus sieberi* cotyledon baits in soil (850g) from each sample, followed by transfer of lesioned baits to selective agar and identification of fungi growing from the baits. These procedures were also used to assay 85 samples from sites infested with *P.* aff. *megasperma* Black Point. Contingency Chi Square tests were applied to compare *Phytophthora* recovery rates obtained with different methods.

3.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

Some heath vegetation at the side of Point Anne Road in the FRNP is affected by aerial cankers and by root-rot caused by *Phytophthora*. In order to minimise the requirement for artificial inoculation experiments, and to provide circumstantial evidence as to the identity of the causal organism(s), we decided to investigate whether any one species of *Phytophthora* was consistently present in the tissues of diseased plants. To further assist this objective, we determined whether isolates recovered from diseased plants at the roadside were conspecific with those isolated from soil or water in which the hosts were growing.

For 2-4 days, ponded water at the edge of Point Anne Road was baited for *Phytophthora* with eight nylon-covered pears. The pears were returned to the

laboratory, processed in the manner indicated already and isolations of *Phytophthora* were identified (Section 3.2).

Eleven Proteaceous plants with symptoms characteristic of root rot were excavated together with 2kg of soil adjacent to the roots. In the laboratory, plants and soil were separated for each sample. Under aseptic conditions, 12 pieces of root and shoot from each plant were placed on selective, antibiotic agar in Petri dishes and incubated for five days. Fungi growing from the plant material were subcultured to fresh CMA. The allozyme class identity of each culture of *Phytophthora* was determined using the isoenzyme technique described by Carstairs & Stukely (1996).

The soil collected with each plant was divided into two portions and placed in plastic dishes. Distilled water was added to cover the soil to a depth of 20-30mm. A pear fruit was then placed in each dish of soil and these were left on the laboratory bench for 5-7 days. Taxa of *Phytophthora* were isolated from the pears and identified as before. The Fisher-Irwin Exact Test was used to determine whether numbers of *Phytophthora* taxa recovered from ponded water, soil and plant tissues were the same.

4 RESULTS AND DISCUSSION

4.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

Of the 62 *Phytophthora* taxa reported worldwide, 31 are pathogens of herbaceous plants and 28 parasitise woody hosts. The latter include roughly equal numbers of specific and general (non-specific) pathogens (Table 1). It is notable that none of the specific pathogens of woody plants have been reported from Australia. In contrast, all of the general pathogens of woody species are present in this country (Tables 1and 2).

Table 1. Numbers of *Phytophthora* taxa reported worldwide and in Australia, classified in terms of host specialisation

Class	Number of Taxa	
	Worldwide	Australia
Host not determined	3	0
Specifically pathogenic on herbaceous hosts	22	5
Generally pathogenic on herbaceous hosts	9	4
Specifically pathogenic on woody hosts	13	0
Generally pathogenic on woody hosts	15	15
Totals	62	24

Table 2. Occurrence of species of *Phytophthora* in Western Australia and elsewhere in Australia

Species	Western Australian Ecosystems		Eastern States and Territories
	Natural	Other[1]	
General pathogens on woody hosts			
Non-caducous			
P. cambivora	NR[2]	+	+
P. cinnamomi	+	+	+
P. citricola	+	+	+
P. citrophthora	NR	+	+
P. cryptogea	+	+	+
P. drechsleri	+	+	+
P. gonapodyides[3]	+	NR	NR
P. megasperma	+	+	+
P. nicotianae	+	+	+
P. syringae[4]	NR	NR	+
Caducous			
P. boehmeriae	+	NR	+
P. cactorum	NR	+	+
P. heveae	NR	NR	+
P. hibernalis	NR	+	NR
P. palmivora	NR	NR	+
Caducity unknown			
Phytophthora spp.	+	+	+
Specialist pathogens on herb. hosts			
P. clandestina	NR	+	+
P. macrochlamydospora	NR	NR	+
P. medicaginis	NR	NR	+
P. sojae	NR	NR	+
P. vignae	NR	NR	+
General pathogens on herb. hosts			
P. erythroseptica var. *erythroseptica*	NR	+	+
P. fragariae var. *fragariae*	NR	NR	+
P. infestans	NR	+	+
P. porri	NR	NR	+

[1] Other ecosystems: horticultural and pasture.

[2] NR = Not reported.

[3] *P. gonapodyides* has been recovered from karri forest soil from the temperate SWLD of WA.

[4] Although not reported for Western Australia, *P. syringae* has been recorded from apple in South Australia and it has been reported from New Zealand.

When Contingency Chi Square analysis was used to compare the number of taxa worldwide (for each host class) with that in Australia, the two samples were found to differ significantly from one another ($\chi^2_{obs.}=14.68>\chi^2_{0.05}=7.81$; d.f.=3). Although there has been considerable speculation about the geographic origin of particular species of *Phytophthora*, the literature contains little in regard to the possible origin of the genus. Our conclusion is that *Phytophthora* originated in the Indo-China region where it evolved with the Fabaceae before migrating with members of that family into the Americas. Other members of the genus migrated westward into the Mediterranean basin and some of these specialised to become the Peronosporaceae and Albuginaceae. It follows that Australia would have few endemic species of *Phytophthora*. This accords with the observation that the assemblage of *Phytophthora* taxa in Australia is not typical of the genus worldwide. The disproportionate occurrence and success in Australia of *Phytophthora* taxa that are general pathogens of woody hosts is not unexpected in view of the preponderance of woody species in the native flora.

The 15 recognised general pathogens of woody hosts all occur in Australia (Table 1) and eight of these have been recovered from natural ecosystems in Western Australia. The latter comprise seven of ten non-caducous species and one of five caducous species (Table 2). Application of the Fisher-Irwin Exact test to the data indicated that observed recovery rates in Western Australia were unlikely ($p = 0.01$). The three non-caducous species yet to be reported from natural ecosystems in this State are *P. cambivora*, *P. citrophthora* and *P. syringae*.

An explanation for the paucity of caducous, general pathogens of woody hosts in natural Western Australian ecosystems may reside in the mode of dispersal of the asexual phase of these fungi which requires environmental conditions unlike those prevalent in the SWLD of the State. We conclude that among the 62 recognised taxa of *Phytophthora,* only those ten that are both generally pathogenic on woody plants and non-caducous pose a threat to native biota in the SWLD.

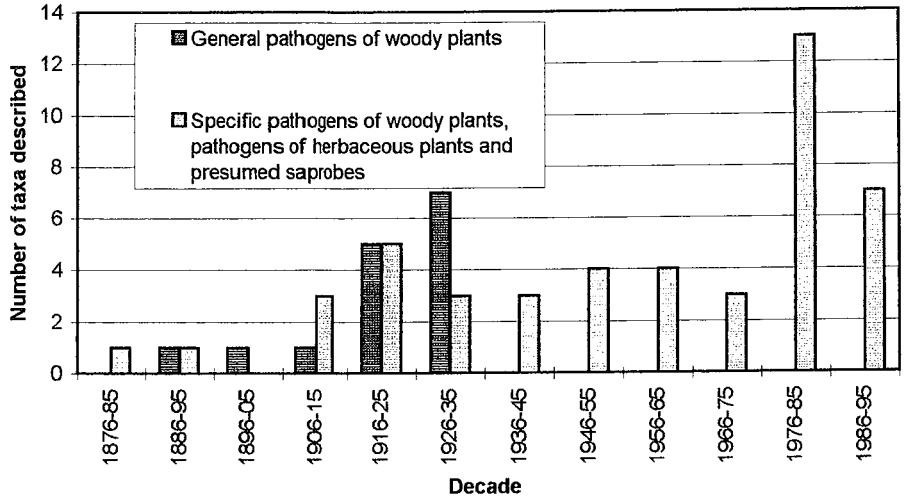
Until recently, Western Australian laboratories have relied on traditional alpha taxonomic methods for the identification of *Phytophthora* spp. based on microscopic examination of morphological characters. However, a molecular technique (CAGE of isoenzymes) has now been developed by CALM (in collaboration with the Minerals and Energy Research Institute of Western Australia) for identifying species of *Phytophthora* (Carstairs & Stukely, 1996). This technique has facilitated the assignment of some isolates to species previously unrecorded in Western Australia. These are *Phytophthora boehmeriae* (D'Souza *et al.*, 1997) and *Phytophthora gonapodyides* (Carstairs & Podger, in prep.).

The isozyme technique has also been used successfully to identify the presence of discrete taxa within the *P. megasperma* species complex, the *P. cryptogea/drechsleri* complex and the *P. citricola* complex (Carstairs & Stukely, 1996). With the continued use of molecular techniques for characterisation of isolates of *Phytophthora*, it seems likely that other currently unrecognised taxa will be identified from natural ecosystems in the SWLD.

Most species of *Phytophthora* that are generally pathogenic on woody host plants were described between 1916 and 1935 (Figure 1). No new taxa in this category have

been described since then and it appears unlikely that many non-host-specific pathogens like *P. cinnamomi*, *P. nicotianae* or *P. palmivora* remain undiscovered. As to the other categories of *Phytophthora*, an average of 3-4 new taxa are described per decade, but it is unlikely that these will pose a threat to the biota of the SWLD.

Figure 1. Numbers of descriptions of new *Phytophthora* taxa published since 1876



4.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

Apple has been reported as a satisfactory bait for detecting 11 of the 15 generally pathogenic *Phytophthora* spp. of woody plants. Other satisfactory baits include lupine radicles (10 species), *Eucalyptus* cotyledons and pear fruit (8 species), and cedar or pine needles (7 species) (Appendix 1). Between 1965 and the late 1970's, the lupine baiting technique (Chee & Newhook, 1965), or modifications of that, were most commonly used for isolation of *P. cinnamomi*. The results of quantitative tests in which the relative effectiveness of lupine radicle and eucalypt cotyledon baits were compared, showed that the latter were most sensitive for detection of *P. cinnamomi* (Marks & Kassaby, 1974). Although these are now used frequently in Western Australia, consideration of the methods summarised in Appendix 1 suggests that the best approach is to employ a combination of baits including pear fruit with eucalypt cotyledons or conifer needles (cedar or pine).

In the current study, pear baits were used successfully (in March, 1997) to recover 91 isolates of *Phytophthora* from 9 of 12 sample sites including the Gairdner River and 11 roadside ponds in the FRNP. In August, a further 160 isolates were recovered from 7 out of 8 roadside ponds in the same area. The 251 isolates were identified by

isoenzyme analysis as belonging to one or other of 10 allozyme taxa. Four of the taxa were isolated from samples collected on both dates (Table 3) and 3 of these were particularly abundant and widespread. Taxon 2(b).3 was recovered from 6 of the 13 sites that yielded *Phytophthora* and it comprised 48% of all isolations. Corresponding values for taxa 2(a).2 and 2(b).2 were 13% (5 sites) and 10% (4 sites), respectively.

When a Contingency Chi Square test was used to compare the proportional representation of the three abundant taxa in March with the equivalent values for August, the seasonal differences were found to be significant ($\chi^2_{obs.}=44.7>\chi^2_{0.05}=5.99$; 2 d.f.). Taxon 2(b).2 showed least response to season with its representation decreasing from 16% in autumn to 12% in early spring. Taxon 2(b).3 increased from 39% to 85% in the same period while 2(a).2 showed the greatest response. It was the most abundant taxon in autumn with 45% representation, but this declined to only 3% in spring.

Table 3. Allozyme taxon affinity and sporangium morphology of stated numbers of isolates of *Phytophthora* recovered from roadside pond sites in the Fitzgerald River National Park on two dates in 1997 (numbers of sites positive for each taxon are shown in parentheses)

Allozyme Taxon[1]	Morphology	Numbers of Isolates (+'ve sites) March		August	
1(a).1	Semi-papillate sporangia	3	(1)	0	(0)
2(a).1	Non-papillate sporangia	0	(0)	18	(2)
2(a).2	"	30	(4)	3	(2)
2(a).3	"	12	(1)	0	(0)
2(b).1	"	0	(0)	26	(3)
2(b).2	"	11	(3)	13	(1)
2(b).3	"	26	(2)	94	(5)
2(b).4	"	4	(1)	0	(0)
2(b).5	"	5	(2)	2	(2)
Phytophthora spp.	Not determined	0	(0)	4	(1)

[1] Taxa including (a) or (b) in their appellations are homothallic or heterothallic, respectively.

Although chance may play an important role in recovery of taxa that are not widespread or abundant, season appears to influence the likelihood of detecting some taxa of *Phytophthora* in roadside ponds. Of the ten allozyme taxa identified here, taxon 2(a).1 (*P.* aff. *megasperma* Black Point) is most often associated with root-rot diseases of Proteaceae in the FRNP or on the sand plains north of Perth. It was only recovered in spring from two of the 13 (15.4%). roadside ponds positive for *Phytophthora* in the

FRNP. Based on a proportional representation of 15.4% of all isolates in sites positive for *Phytophthora*, the calculated number of sites requiring assessment for 80% confidence in recovering *P.* aff. *megasperma* Black Point would be 9.63. In autumn, nine sites that were positive for *Phytophthora* were assessed and yet we did not recover *P.* aff. *megasperma* Black Point. Taxon 2(b).1 is another *Phytophthora* that was not recovered in autumn but was relatively common in spring (Table 3).

Of 71 samples from which *P. cinnamomi* was isolated, either directly from root tissue or indirectly from baits that had been incubated in the soil or from both, only three samples were deemed to be negative using baits, while root tissue in 18 samples failed to yield the fungus (Table 4.). The results of Chi Square analysis indicated that the observed difference in isolation rates between baits and roots was significant ($\chi^2_{obs.}=10.71>\chi^2_{0.05}=3.84$; 1 d.f.).

Table 4. Isolation outcomes (+ / -) for *P. cinnamomi* and *P.* aff. *megasperma* Black Point (BP) from substrate baits incubated in soil samples, and/or directly from root tissues that were present in the same soil samples

Baits and Roots	*P. cinnamomi*	*P.* aff. *megasperma* BP
Both negative (-)	22	25
Bait - : Root +	3	48
Bait + : Root -	18	3
Both positive (+)	50	10
Total No. of soil samples	93	86

A contrasting result was obtained for *P.* aff. *megasperma* Black Point. Out of 61 soil samples in which the pathogen was found to be present, the baiting method produced a negative result on 48 occasions, while only three failures to isolate the fungus from root tissue were recorded (Table 4). Chi Square analysis revealed that the failure rate for baits was significantly greater than that for root tissue ($\chi^2_{obs.}=43.32>\chi^2_{0.05}=3.84$; 1 d.f.).

The results of this work suggest that *P. cinnamomi* is the superior saprophyte and that it may persist well in old, diseased tissues or soils from which it is readily isolated by baiting. On the other hand, *P.* aff. *megasperma* Black Point appears to be less able in that regard. It is clear that detection methods that are suitable for one species of *Phytophthora* might be not be ideal for another.

4.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

Six allozyme taxa of *Phytophthora* were recovered from the Point Anne Road site. Five taxa were isolated from ponded water and two of these were unique to the water column. Taxon 2(a).1 (*P.* aff. *megasperma* Black Point) was recovered from diseased plants and from the soil profile but not from the water column. Both the water column and soil profile were relatively rich in *Phytophthora* species by comparison with plant tissues (Table 5).

Application of the Fisher-Irwin Exact Test showed that (out of 6 possibilities), recovery of 5 taxa in the water column and 1 not detected, with 4 taxa in the soil profile and 2 undetected, was very likely on the basis of chance ($p = 0.41$). However, the same test indicated that chance recovery of 5 taxa from the water column (1 not detected) and only 1 in plant tissue was unlikely ($p = 0.04$). Similarly, chance detection of 4 taxa in soil and one in plant tissues was improbable ($p = 0.07$).

Thus, the finding that *P.* aff. *megasperma* Black Point (taxon 2(a).1) was the only taxon of *Phytophthora* to be recovered from diseased plants (Table 5) is not explicable on the basis of chance alone. This is consistent with the suggestion that, of the six taxa assessed, only *P.* aff. *megasperma* Black Point has a pathogenic role on the hosts examined here.

Table 5. Numbers of isolates of six allozyme taxa of *Phytophthora* recovered from ponded water, soil or plant samples collected in Point Anne Road

Taxon	Water Column	Soil Profile	Plant Tissue	Totals
2(a).1	0	18	7	25
2(a).2	2	14	0	16
2(b).1	12	12	0	24
2(b).2	13	0	0	13
2(b).3	72	60	0	132
Phytophthora spp.1	4	0	0	4
Totals	103	104	7	214

The host list for *P.* aff. *megasperma* Black Point is short by comparison with the host range associated with some general pathogens of woody plants, eg. *P.cinnamomi* or *P. nicotianae*. However, the latter species have been recognised for more than half a century. So far, 22 Western Australian species belonging in eight genera of the Proteaceae have been recorded as hosts of *P.* aff. *megasperma* Black Point. Pinaceae is the only other family that includes known hosts of the pathogen (Table 6).

Table 6. Plant species from which *P.* aff. *megasperma* Black Point has been isolated in five CALM Districts

District	Plant Family	Species	Isolations (No.)
Albany	Proteaceae	*Adenanthos cuneatus*	2
		Banksia attenuata	6
		Banksia baxteri	8
		Banksia gardneri	1
		Banksia lemanniana	4
		Banksia media	3
		Conospermum distichum	1
		Daviesia sp.	1
		Dryandra circioides	1
		Dryandra cuneata	4
		Dryandra falcata	3
		Dryandra plumosa	3
		Dryandra quercifolia	2
		Dryandra tenuifolia	2
		Hakea varia	1
		Isopogon formosus	1
		Lambertia inermis	1
Esperance	Proteaceae	*Banksia speciosa*	1
		Dryandra sessilis	1
Moora	Proteaceae	*Banksia attenuata*	9
		Banksia ilicifolia	1
		Hakea prostrata	1
		Hakea sp.	2
Pemberton	Proteaceae	*Banksia occidentalis*	1
SW Capes	Pinaceae	*Pinus radiata*	3

In Section 3.1, taxa of *Phytophthora* which parasitise species in three or more families of woody plants were classified as generally pathogenic on that category of host. Since *P.* aff. *megasperma* Black Point has been recorded on just two host families, its present status is currently that of a specific pathogen. This makes it unique among other species of *Phytophthora* in Australia as it is the only one included in that class. We believe that as *P.* aff. *megasperma* Black Point receives increasing attention it will be found on a broader spectrum of hosts and eventually it will be recognised as a general pathogen of woody plants.

P. aff. *megasperma* Black Point has a regional distribution in the south-west of the State where it is mostly confined to heath and woodlands of the coastal plain. Sites infested with the fungus were assessed in the FRNP and on the sand plain north of Perth to determine whether observed disease was in an early or advanced stage of progression (Table 7). Approximately equal numbers of sites were recorded for each of the two stages. It appeared that plant populations on the different sites had either been recently infected, or the epidemic had run its course. This supports the conclusion that progress of disease associated with infestations of *P.* aff. *megasperma* Black Point fits a CCI epidemic progress curve

Table 7. Progress of disease associated with infestations of *P.* aff. *megasperma* Black Point at locations in the Fitzgerald River National Park (FRNP) and on the sand plains north of Perth (NSP)

Region	Disease Progress	Location	Dominant Plant Species
FRNP	Advanced[1]	Collett Road	*Banksia attenuata*
		North of Quaalup Road	*B. baxteri*
		North of West Mt. Barren	*B. attenuata*
	Early[2]	West of Point Anne Road	*B. attenuata, B. baxteri, B. coccinea*
		East of West Mt. Barren	*B. baxteri*
		West of West Mt. Barren	*B. attenuata*
NSP	Advanced	Cervantes Road	*B. attenuata*
		Jurien Rd 1	*B. attenuata*
			B. prionotes
		Moora-Badgingarra Road	*B. attenuata, B.menziesii, B. prionotes*
		Namegarra Road	*B. attenuata*
		Yerrumullah Road	*B. attenuata, B. menziesii*
	Early	Bibby Road	*B. attenuata*
		Cataby South	*B. attenuata*
		Jurien Road 2	*B. attenuata*
		Mimegarra Road	*B. attenuata*
		Munbinea Road	*B. attenuata*
		Yerrumullah Road	*B. attenuata, B. menziesii*

[1] Advanced: the site has been denuded of dominant plant species or most are dead.
[2] Early: the frequencies of dead or diseased, dominant plant species are low.

Podger & Carstairs (unpubl.) have conducted pathogenicity tests with allozyme taxa of *Phytophthora* and with *P. cinnamomi* on *B. grandis* in the southern karri forest. Preliminary results of this work indicate that development of lesions in *B. grandis* proceeds at a much greater rate in plants inoculated with *P. cinnamomi* than in treatments with other taxa of *Phytophthora.* In one trial, the stems of 18 *Banksia* plants that had been inoculated with *P. cinnamomi* were girdled by lesions and all the trees soon died. Although some plants inoculated with other taxa of *Phytophthora* also died, the mortality rates for those treatments did not differ significantly from the control in which *Banksia* stems were wounded but not inoculated with test fungi.

Trials of this type test the capacity of fungal mycelium to grow in live stem tissues, but not the ability of infective zoospores to invade healthy plants and cause disease. Experimentation comparing the infective abilities of several taxa of *Phytophthora* with that of *P. cinnamomi* (soil inoculation with zoospores) is the subject of another trial by Podger & Carstairs (in progress). Definitive experimentation of this type is extremely labour intensive and likely to require long term maintenance and monitoring of trial plots.

5 OUTCOMES

5.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

- Of the 62 taxa included in *Phytophthora*, those that are generally pathogenic to woody plants and are non-caducous, pose the greatest threat to the sclerophyllous flora of the SWLD. These are the first ten species listed in Table 2 (Section 4.1). All have been recorded both in Australia and overseas. Apart from *P. cambivora*, *P. citrophthora*, and *P. syringae*, the other seven species have been recovered from natural ecosystems in the SWLD. While it is unlikely that many new taxa in this class will be discovered elsewhere, we expect that the other described members of the group will be found in Western Australia.

- Molecular techniques for differentiating between taxa of *Phytophthora* are establishing an excellent track record for accuracy and efficiency in Western Australia and in other parts of Australia. However, many mycologists are reluctant to embrace the new techniques on the grounds that adequate diagnostic tools are already available for the characterisation of fungi based on microscopic examination of morphological features.

5.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

- The results of this study should provide researchers in Western Australia with a better appreciation of the degree to which species of *Phytophthora* other than *P. cinnamomi* pose a threat to native plants in the SWLD. Historically, there is little

doubt that taxa isolated from infested plant communities have not always been correctly identified and that difficulties in detection of important pathogens are experienced from time to time. A flexible methodology for the detection of potentially important, pathogenic species such as *P.* aff. *megasperma* Black Point is outlined in this report.

5.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

- Of six allozyme taxa recovered from Point Anne in the FRNP, only one (*P.* aff. *megasperma* Black Point) was considered likely to be pathogenic. *P.* aff. *megasperma* Black Point has a regional distribution and seems to be confined to heath or woodland in coastal plain ecosystems where it has eroded conservation values in the Shannon-D'Entrecasteaux National Park, the FRNP, Cape Arid National Park, and conservation reserves on the sand plain north of Perth.

- The host list of *P.* aff. *megasperma* Black Point suggests that it is specifically pathogenic on woody plant species. However, its current status in that regard should be viewed with caution until more comprehensive studies of the fungus are undertaken.

6 REFERENCES

Alavi, A. & Strange, R.N. (1979). A baiting technique for isolating *Phytophthora drechsleri*, causal agent of crown rot of *Cucumis* species in Iran. *Plant Disease Reporter* **63**, 1084-1086.

Anderson, E.J. (1951). A simple method for detecting the presence of *Phytophthora cinnamomi* Rands in soil. *Phytopathology* **41**, 187-189.

Banihashemi, Z. & Mitchell, J.E. (1975). Use of safflower seedlings for the detection and isolation of *Phytophthora cactorum* from soil and its application to population studies *Phytopathology* **65**, 1424-1430.

Campbell, W.A. (1949). A method for isolating *Phytophthora cinnamomi* directly from soil. *Plant Disease Reporter* **33**, 134-135.

Carstairs, S.A. & Stukely, M.J.S. (1996). Rapid identification of species of *Phytophthora*. Minerals and Energy Research of Western Australia Report No. 175. Department of Conservation and Land Management. Perth, WA.

Chee, K.H. & Foong, K.M. (1968).Use of cacao pod for recovering *Phytophthora* species pathogenic to *Hevea brasiliensis*. *Plant Disease Reporter* **52**, 5.

Chee, K.H. & Newhook, F.J. (1965). Improved methods for use in studies on *Phytophthora cinnamomi* Rands and other *Phytophthora* species. *New Zealand Journal of Agricultural Research* **8**, 88-95.

Dance, M.H., Newhook, F.J. & Cole, J.S. (1975). Bioassay of *Phytophthora* spp. in soil. *Plant Disease Reporter* **59**, 523-527.

de Bary, A. (1876). Researches into the nature of the potato fungus, *Phytophthora infestans. Journal of the Royal Agricultural Society of England* **12**, 139-269.

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

Erwin, D.C. & Ribeiro, O.K. (1996). *Phytophthora* diseases worldwide. The American Phytopathological Society Press, St Paul, Minnesota.

Fraser, L. (1956). Miscellaneous plants: *Phytophthora cinnamomi* attacking native plants. *Australian Plant Disease Records* **8**, 12.

Gerretson-Cornell, L. (1974). A comparative test of isolation of *Phytophthora cinnamomi* Rands between the lupin baiting and a newly devised apple trap. *Phyton* **32**, 35-36.

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

Greenhalgh, F.C. (1978). Evaluation of techniques for quantitative detection of *Phytophthora cinnamomi*. *Soil Biology and Biochemistry* **10**, 257-259.

Grimm, G.R. & Alexander, A.F. (1973). Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. *Phytopathology* **63**, 540-541.

Hargreaves, A.J. & Duncan, J.M. (1978). Detection of *Phytophthora* species in field soils by simple baiting procedures. *Soil Biology and Biochemistry* **10**, 343-345.

Harris, D.C. & Bielenin, A. (1986). Evaluation of selective media and bait methods for estimating *Phytophthora cactorum* in apple orchard soils. *Plant Pathology* **35**, 565-574.

Holliday, P. & Mowat, W.P. (1963). Foot rot of *Piper nigrum* L. (*Phytophthora palmivora*). Phytopathology Papers No. 5, Commonwealth Mycological Institute Kew, England. 62pp.

Ioannou, N. & Grogan, R.G. (1977). The influence of soil matric potential on the production of sporangia by *Phytophthora parasitica* in relation to its isolation from soil by baiting techniques (Abstr.). *Proceeds of the American Phytopathological Society* **4**, 173.

Jeffers, S.N. & Aldwinckle, H.S. (1987). Enhancing detection of *Phytophthora cactorum* in naturally infested soil. *Phytopathology* **77**, 1475-1482.

Jenkins, S.F., Jr. (1962). Preliminary studies estimating the disease potential of *Phytophthora parasitica* var. *nicotianae* in infested tobacco soils. *Plant Disease Reporter* **46**, 825-826.

Klemmer, H.W. & Nakano, R.Y. (1962). Techniques in isolation of pythiaceous fungi from soil and diseased pineapple tissue. *Phytopathology* **52**, 955-956.

Klotz, L.J. & DeWolfe, T.A. (1958). Techniques for isolating isolates of *Phytophthora* spp. which attack citrus. *Plant Disease Reporter* **42**, 675-676.

Lee, B.S. & Varghese, G. (1974). Studies on the genus *Phytophthora* in Malaysia. I. Isolation techniques, comparative morphology and physiology and reaction to antibiotics. *Malaysian. Agricultural Research* **3**, 13-21. (*Revue of Plant Pathology* **54**, 790).

Linderman, R.G. & Zeitoun, F. (1977). *Phytophthora cinnamomi* causing root rot and wilt of nursery-grown native western azalea and salal. *Plant Disease Reporter* **61**, 1045-1048.

MacKenzie, D.R., Elliot, V.J., Kidney, B.A., King, E.D., Royer, M.H. & Theberge, R.L. (1983). Application of modern approaches to the study of epidemiology of diseases caused by *Phytophthora*. In: Phytophthora. Its Biology, Taxonomy, Ecology, and Pathology. Eds.:Erwin, D.C., Bartnicki-Garcia, S. & Tsao, P.H. The American Phytopathological Society, Minnesota, USA. 392 pp.

Marks, G.C. & Kassaby, F.Y. (1974). Detection of *Phytophthora cinnamomi* in soils. *Australian. Forestry* **36**, 198-203.

Marks, G.C., Kassaby, F.Y. & Reynolds, S.T. (1972). Die-back in mixed hardwood forests of eastern Victoria. A preliminary report. *Australian Journal of Botany* **20**, 141-154.

McIntosh, D.L. (1964). *Phytophthora* spp. in soils of the Okanagan and Similkameen valleys of British Columbia. *Canadian Journal of Bot*any **42**, 1411-1415.

Mircetich, S.M. & Matheron, M.E. (1976). Phytophthora root and crown rot of cherry trees. *Phytopathology* **66**, 549-558.

Molot, P.M. & Beyries, A. (1976). Contamination in vitro de la fraise et de la tomate immature par diverses especes de *Phytophthora* (Artificial inoculation of unripe strawberry and tomato fruits with several species of *Phytophthora*). *Annals of Phytopathology* **8**, 91-94. (In French).

Murray, D.I.L., Darling, D.D. & McGann, L.R. (1985). Indirect effect of floristic composition on production of sporangia by *Phytophthora cinnamomi* in jarrah forest soils. *Australian Journal of Botany* **33**, 109-113.

Narasimham, V. & Ramakrishnan, K. (1969). Studies on wilt disease of betelvine. *Madras Agricultural Journal* **56**, 68-73.

Newhook, F.J. & Jackson, G.V.H. (1977). *Phytophthora palmivora* in cocoa plantation soils in the Solomon Islands. *Transactions of the British Mycological Soc*iety **69**, 31-68.

Okaisabor, E.K. (1971a). The mechanism of initiation of *Phytophthora* pod rot epiphytotics. In: Proceeds of the 3rd International Cocoa Research Conference (1969) Accra, Ghana. pp. 398-404.

Okaisabor, E.K. (1971b). Studies on some aspects of the soil phase of *Phytophthora palmivora* (Butl.) Butl. In: Proceeds of the 3rd International Cocoa Research Conference (1969) Accra, Ghana. pp. 484-489.

Orellana, R.G. (1954). Contribution to the study of survival, dissemination and control of *Phytophthora* of cacao. Proceeds of the. Inter-Am. Cacao Conference 5th. Turrialba, Costa Rica. Sec. *Fitopatologia* **1**, 1-10.

Oudemans, P. & Coffey, M.D. (1991). A revised systematics of twelve papillate *Phytophthora* species based on isozyme analysis. *Mycological Research* **95**, 1025-1046.

Oudemans, P., Forster, P.H. & Coffey, M.D. (1994). Evidence for distinct isozyme subgroups within *Phytophthora citricola* and close relationships with *P. capsici* and *P. citrophthora*. *Mycological Research* **98**, 189-199.

Pegg, K.G. (1977). Soil application of elemental sulphur as a control of *Phytophthora cinnamomi* root and heart rot of pineapple. *Australian Journal of Experimental Agriculture and Animal Husbandry* **17**, 859-865.

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

Ponchet, J., Ricci, P., Andreoli, C. & Augé, G. (1972). Méthodes sélectives d'isolment du *Phytophthora nicotianae* f. sp. *parasitica* (Dastur) Waterh. a partir du sol (Selective methods of isolation for *Phytophthora nicotianae* f. sp. *parasitica* (Dastur) Waterh. from the soil). *Annals of Phytopathology* **4**, 97-108.

Pratt, B.H. & Heather, W.A. (1972). Method for rapid differentiation of *Phytophthora cinnamomi* from other *Phytophthora* species isolated from soil by lupin baiting. *Transactions of the British Mycological Soc*iety **59**, 87-96.

Ricci, P. (1972). Moyens d'etude de l'inoculum du *Phytophthora nicotianae* f. sp. *parasitica* (Dastur) Waterh., parasite de l'oeillet dans le sol (*Phytophthora nicotianae* f. sp. *parasitica* (Dastur) Waterh. of carnation. Quantitative estimation of inoculum in the soil). *Annals of Phytopathology* **4**, 257-276. (In French).

Robertson, G.I. (1975). A paper disc technique for the recovery of *Pythium* spp. from soil or water. *New Zealand Journal of Agricultural Research* **18**, 409-410.

Satyprasad, K. & Romaro, P. (1980). A simple technique for isolating *Phytophthora palmivora* from soil. *Current Science* **49**, 360-361.

Schwinn, F.J. (1961). Der nachweis von *Phytophthora cactorum* (Leb. et Cohn) Schroet. im Boden (The detection of *Phytophthora cactorum* [Leb. et Cohn] Schroet. in soil). *Mitt. Bio. Bundesanst. Land-Forstwirtsch.* (Berl.-Dahlem) **104**, 42-44. (In German).

Sewell, G.W.F., Wilson, J.F. & Dakwa, J.T. (1974). Seasonal variations in the activity in soil of *Phytophthora cactorum*, *P. syringae*, and *P. citricola* in relation to collar rot disease of apple. *Annals of Applied Biology* **76**, 179-186.

Shea, S.R., Gillen, K.J. & Kitt, R.J. (1978). Variation in sporangial production of *Phytophthora cinnamomi* Rands on jarrah (*E. marginata* Sm) forest sites with different understorey compositions. *Australian Forest Research* **8**, 219-226.

Shea, S.R., Gillen, K.J. & Leppard, W.I. (1980). Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased, freely drained *Eucalyptus marginata* Sm sites in the northern jarrah forest of south western Australia. *Protection Ecology* 2, 135-156.

Shearer, B.L., Michaelsen, B.J. & Somerford, H.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., Michaelsen, B.J. & 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. & Shea, S.R. (1987). Variation in seasonal population fluctuations of *Phytophthora cinnamomi* within and between infected *Eucalyptus marginata* sites of south western Australia. *Forest Ecology and Management* **21**, 209-230.

Shew, H.D., Benson, D.M. & Grand, L.F. (1979). A comparison of baits for isolating *Phytophthora cinnamomi* from soil (Abstr.). *Phytopathology* **69**, 1045.

Stukely, M.J.C., Shearer, B.L., Tay, F.C.S., Hart, R.M. & Hart R.P. (1997). *Phytophthora* species in natural vegetation in Western Australia. Australasian Plant Pathology Society. Eleventh biennial conference, 1997. Perth, Western Australia.

Tsao, P.H. (1960). A serial dilution end-point method for estimating disease potentials of citrus Phytophthoras in soil. *Phytopathology* **50**, 717-724.

Tsao, P.H. (1990). Why many *Phytophthora* root rots and crown rots of tree and horticultural crops remain undetected. *EPPO* (Eur. Mediterr. Plant Prot. Organ.) *Bulletin* **20**, 11-17.

Turner, P.D. (1965). Behaviour of *Phytophthora palmivora* in soil. *Plant Disease Rep*orter **49**, 135-137.

van der Plank, J.E. (1963). Plant diseases: Epidemics and Control. Academic Press. New York. 349pp.

van der Scheer, H.A.T. (1971). Isolation of *Phytophthora cactorum* from soil in orchards and strawberry fields and differences in pathogenicity to apple. *Netherlands Journal of Plant Pathology* 77, 65-72.

Zentmyer, G.A. (1980). *Phytophthora cinnamomi* and the diseases it causes. The American Phytopathological Society. Minnesota.

Zentmyer, G.A., Gilpatrick, J.D. & Thorn, W.A. (1980). Methods of isolating *Phytophthora cinnamomi* from soil and from host tissue (Abstr.) *Phytopathology* **50**, 87.

Zentmyer, G.A. & Ohr, H.D. (1980). Avocado root rot. University of California Division of Agricultural Science, Berkeley. Leaflet. 2440. 15pp.

Appendix 1. Baiting techniques for the detection and isolation of generally pathogenic *Phytophthora* species of woody plants

Species	Bait Material	Conditions of Baiting and Remarks	References
P. boehmeriae	Apple slices, lupine radicles.	Immersed in 200 ml of water added to 25 g of soil; 4-10 days.	Gerretson-Cornell (1976).
	E. sieberi cotyledons.	Detached cotyledons floated on water 5-10 cm over about 850 g soil.	D'Souza *et al.* (1997).
P. cactorum	Apple fruit.	Buried partly in wet soil in large flat; 24°C, 90-100% RH, 5-6 days.	Schwinn (1961).
	Pear fruit.	Immersed partly in 50 cc of soil plus 11 cc of water; 6-7 days; pear better than apple, peach, and apricot fruit as baits.	McIntosh (1964).
	Apple fruit, pear fruit.	Buried partly in wet soil at 20°C until rot occurs; isolation from apple & strawberry soils; pear better than apple as bait.	van der Scheer (1971).
	Apple fruit.	Immersed partly in 20 mm soil layer with 5 mm of water above soil; optimum 20°C, 14 days; *P. citricola* and *P. syringae* also recovered from apple soils at lower incubation temperatures.	Sewell *et al.* (1974).
	Safflower seedlings.	Planted in steamed soil before adding 15 mm layer of test soil in contact with hypocotyl or directly to test soil; optimum 28°C, 3-4 wks; better than apple fruit; used also in quantitative assays of apple soils.	Banihashemi & Mitchell (1975).
	Strawberry fruit.	Immature fruit inoculated with soil in small hole; 22°C, 3-6 days; used for isolation from strawberry soils; also susceptible to 5 other *Phytophthora* species.	Molot & Beyries (1976).
	Apple seedlings, cotyledons.	Soil suspensions were diluted in up to 72-fold dilutions; seedlings and cotyledons incubated in 15-20 ml of suspension in glass vials; 14 days at 20°C in 16-hour photoperiod.	Harris & Bielenin (1986).
	Apple seedlings, cotyledons	Extended baiting (soil air dried, moistened for 3 days, and then flooded).	Jeffers & Aldwinckle (1987).
P. cambivora		See Dance *et al.* (1975) under *P. cinnamomi*. See Mircetich & Matheron (1976) under *P. megasperma*.	
P. cinnamomi	Apple fruit.	Holes made in fruit are filled with soil; incubated at 15-27°C, 5-10 days; suitable also for many other but not all *Phytophthora* species; however, *Pythium* and other soil fungi also colonise fruit.	W.A. Campbell (1949).
	Pineapple leaves & rooted crowns.	Immersed in 400-800 ml of water added to about 10-20 cc of soil; 21-24°C, 4 days.	Anderson (1951).
	Avocado fruit, *Persea indica* seedlings.	Fruit partly embedded in flooded soil; 27°C, 2-4 days; seedlings planted in wet soil, 2-3 days.	Zentmyer *et al.* (1960); Zentmyer (1980).

Appendix 1. (Continued)

Species	Bait Material	Conditions of Baiting and Remarks	References
P. cinnamomi	Pineapple leaves & rooted crowns.	Leaf base or root tips immersed in water-soil mixture at least 5 cm above the container bottom; 18-27°C.	Klemmer & Nakano (1962).
	Lupine radicles, excised.	Root tips immersed in large volume of water over a 20 mm soil layer; 2 days; isolation from pine soils; better than apple fruit; also susceptible to four other *Phytophthora* species.	Chee & Newhook (1965).
	Lupine radicles, excised.	Pieces 1-2 cm long floated on water 2-3 mm over 30 cc of soil in petri plate; 2-3 days; used for quantitative assays of eucalyptus soils.	Marks *et al.* (1972).
	Lupine radicles.	Immersed in 150 ml of water added to 26 g of soil; 17-24°C; 5 days; lesions distinguishable from those caused by eight other *Phytophthora* species except *P. drechsleri*.	Pratt & Heather (1972).
	Apple slices, lupine radicles.	Immersed in 200 cc of water added to 25 g of soil; 4-10 days; both baits were satisfactory.	Gerrettson-Cornell (1974).
	Eucalyptus cotyledons.	Detached cotyledons floated on water 5-10 mm over 50-60 cc of soil; 22-24°C, within 60 h; two to four times more sensitive than lupine radicles; used for quantitative assays of eucalyptus soils.	Marks & Kassaby (1974).
	Cedar needles, pine needles, lupine radicles.	Floated on or immersed partly in water added to soil; both 16 and 22°C, 3 days; conifer needles also good for *P. cambivora*, *P. citricola*, *P. cryptogea*, *P. drechsleri*, *P. hibernalis*, and a few other *Phytophthora* species.	Dance *et al.* (1975).
	Eucalyptus leaf disks.	Floated on water added to soil or roots; 20°C, 24 h; *Pythium* species interfere with results.	Linderman & Zeitoun (1977).
	Avocado leaf pieces.	Floated on water added to soil; 4 days; recovery up to 89%.	Pegg (1977).
	Eucalyptus cotyledons, lupine seedlings, pear fruit.	Floated on water added to soil at various depths; 25°C under continuous light, 3-7 days; all baits successful but more frequently with pear; used in quantitative assays.	Greenhalgh (1978).
	Persea indica seedlings.	Immersed partly in large volume of water added to 1.2-2.5 cm soil layer; 5-7 days; isolation from avocado soils.	Zentmyer & Ohr (1978).
	Azalea leaf disks, cedar needles, fir seedlings, lupine radicles.	Floated on or immersed in 100 ml of water added to 5-50 g of soil; 20°C, 1-2 days; recovery was best with azalea leaf disks and least with lupine radicles.	Shew *et al.* (1979).
	Pear fruit.	Pear baits were suspended in streams for 4 days; or were immersed in water 2.5 cm over about 1 kg soil for 5 days then lesions were plated onto selective antibiotic agar plates.	Carstairs & Carstairs (Section 2.2 Methods).
P. citricola		See Dance *et al.* (1975) under *P. cinnamomi*.	
		See Sewell *et al.* (1974) under *P. cactorum*.	
	E. sieberi cotyledons	Detached cotyledons floated on water 5-10 cm over about 850 g soil.	Stukely *et al.* (1997).
		See Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*.	

Appendix 1. (Continued)

Species	Bait Material	Conditions of Baiting and Remarks	References
P. citrophthora		See Klotz & DeWolfe (1958) and Tsao (1960)under *P. nicotianae*.	
P. cryptogea		See Dance *et al.* (1975); Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*. See Stukely *et al.* (1997) under *P. citricola.*	
P. drechsleri	Cantaloupe seed & seedlings.	Planted in wet soil mixed with diseased tissues of *Cucumis* species; 20-35°C, 7 days. See Stukely *et al.* (1997) under *P. citricola.* See Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*.	Alavi and Strange (1979).
P. gonapodyides	Apple fruit, pear fruit, citrus leaf pieces, *Begonia* petioles, lupine seedlings.	Apples and pear baits were suspended in reservoirs for 9 days; *Begonia* petioles, citrus leaf pieces, lupine seedlings were suspended in water from reservoirs for 1-4 days and plated on P_{10}ARP.	Robertson (1975).
	Lupine radicles, *E. sieberi* cotyledons.	Detached radicles and cotyledons were floated on water 2-5 cm over *circa* 1 kg of soil for 5 days then plated onto a selective antibiotic agar plate.	Carstairs & Podger (in prep.)
P. heveae		See Lee & Varghese (1974) under *P. nicotianae.* See Gerrettson-Cornell (1976) under *P. boehmeriae.*	
P. hibernalis		See Dance *et al.* (1975) under *P. cinnamomi*.	
P. megasperma	Pear fruit.	Partly immersed in water 1 cm deep over 500 cc of soil; 20°C for 3 days; for isolation from cherry soils; also good for *P. cambivora.*	Mircetich & Matheron (1976).
	Pine needles, lupine seedlings, apple fruit, pear fruit.	Needles or seedlings placed in water 5 mm deep over 100 g of soil; 16 and 22°C, 3 days; fruit buried in larger amounts of soil; 22°C, 10 days; for isolation from strawberry and raspberry soils. See Stukely *et al.* (1997) under *P. citricola.* See Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*.	Hargreaves & Duncan (1978).
P. nicotianae *(P. parasitica)*	Apple fruit, lemon fruit, orange fruit.	Soil or citrus tissues inserted into apple the same as W.A. Campbell's (1949) method. Lemon or orange placed on surface of saturated soil; 20°C, 4 or more days; also good for *P. citrophthora* and *P. syringae* from citrus soils.	Klotz & DeWolfe (1958).

Appendix 1. (Continued)

Species	Bait Material	Conditions of Baiting and Remarks	References
P. nicotianae	Lemon fruit.	Immersed partly in 150 ml of water added to 25 cc of soil; 25°C, 6 days; also good for *P. citrophthora*; used for quantitative assays of citrus soils.	Tsao (1960).
	Tobacco leaves.	Petiole end immersed in water-soil mixture as in Tsao (1960); used for quantitative assays of black shank fungus in tobacco soils.	Jenkins (1962).
	Castor bean seed.	Buried in soil; 63 h; for isolation from betel vine soils or irrigation water.	Narasimham & Ramakrishnan (1969).
	Carnation petals.	Immature petals from buds floated on soil suspension in petri plate, 24-35°C, 2-4 days; used for quantitative assays of carnation soils; also detects *P. capsici*.	Ponchet *et al.* (1972), Ricci (1972).
	Citrus leaf pieces.	Small leaf pieces from various *Citrus* species, floated on water 1-2 cm above 100 cc of soil; 22-28°C, 3-4 days; calamondin fruit equally effective as bait.	Grimm & Alexander (1973).
	Apple fruit, eggplant fruit.	Methods not given. Both fruits also good for *P. capsici*. Cocoa pod also allows isolation of *P. palmivora* and *P. heveae*.	Lee & Varghese (1974).
	Tomato fruit.	Green fruit placed in flooded soil from tomato fields after premoistening for 1 week. See Stukely *et al.* (1997) under *P. citricola*.	Ioannou & Grogan (1977).
P. palmivora	Cocoa pods.	Placed on or in soil inoculated with a loopful of soil suspension on the pod surface and incubated in a moist box; 1-4 days; for isolation from cocoa soils.	Orellana (1954).
	Apple fruit, black pepper leaves.	Soil inserted into apple the same as in W.A. Campbell's (1949) method. Black pepper leaves immersed partly in water-soil mixture; for isolation (of MF4) from black pepper soils. Black pepper leaf disks used successfully by P.H. Tsao (unpublished).	Holliday & Mowat (1963).
	Cocoa pods.	Soil inserted beneath flaps of endocarp tissue of unripe pods; for isolation from cocoa soils.	P.D. Turner (1965).
	Cocoa pods.	Soil or diseased *Hevea* tissues inserted into unripe green pods as in W.A. Campbell's (1949) apple method; 26-30°C, 4-5 days; also good for *P. meadii* and *P. parasitica*).	Chee & Foong (1968).
	Cocoa pod tissues.	Small blocks of tissue placed in wet soil; 25°C, 4 days; premoistened for 5 days if soil samples were dry.	Okaisabor (1971a,b).
	Cocoa pods and tissues	Four methods: pod on soil, soil in pod, pod tissue in flooded soil (the best), and pod tissue on soil; used in quantitative assays of cocoa soils.	Newhook & Jackson (1977).
	Colocasia esculenta roots about 2.5 cm long.	Roots autoclaved and incubated in moistened soil (50 g/ Petri plate) at 15°C for 1 week. Roots washed and plated on oatmeal agar.	Satyaprasad & Romarao (1980).
P. syringae		See Klotz & DeWolfe (1958) under *P. nicotianae*: Sewell *et al.* (1974) under *P. cactorum*.	

CONTROL OF *PHYTOPHTHORA* AND *DIPLODINA* CANKER IN WESTERN AUSTRALIA

FINAL REPORT

TO THE THREATENED SPECIES AND COMMUNITIES UNIT

BIODIVERSITY GROUP

ENVIRONMENT AUSTRALIA

DECEMBER 1998

Department of Conservation and Land Management
Locked Bag 104, Bentley Delivery Centre, WA 6983