PROJECT 4

THE CONTROL AND MANAGEMENT OF *PHYTOPHTHORA MEGASPERMA* IN THE NATIVE PLANT COMMUNITIES OF WESTERN AUSTRALIA

Stephen A. Carstairs and Larissa E. Newcombe

1 INTRODUCTION

Of the five species of *Phytophthora* currently known to infest large areas of the south-west of Western Australia, *Phytophthora cinnamomi* is by far the most virulent pathogen and the most frequent cause of serious disease in native vegetation. In contrast, the ubiquitous *Phytophthora citricola* often appears to cause only asymptomatic infestations. In their review of dieback in Western Australia, Podger *et al.* (1996) addressed the problems for conservation and commerce posed by *P. cinnamomi*.

While extensive research on *P. cinnamomi* has been conducted in Western Australia since the mid 1960's, research on *Phytophthora megasperma* has a more recent history. Although *P. megasperma* was first recorded in Western Australian native vegetation in the 1960's (Podger *et al.* 1996), it was not until 1986 that a decision was made to conduct research aimed at controlling and managing disease caused by the pathogen in native plant communities.

In the spring and early summer of 1990 Hart, Simpson and Associates (1991) surveyed the extent and causes of dieback in the Moora District of the Department of Conservation and Land Management (CALM), and reported that *P. megasperma* and *P. cinnamomi* were the most abundant *Phytophthora* spp. in the region and had the greatest impact on the vegetation. They concluded that little was known about these species of *Phytophthora* in the district and that while few plant communities were infected, the two pathogens posed a threat to the vegetation. However, it was felt that the pathogens could be completely controlled in the Moora District. This is at variance with Podger *et al.* (1996) who argued that if the

autonomous spread of epidemics caused by *P. cinnamomi* continues unabated, the fungus might ultimately occupy all suitable niches available to it.

The Albany and Esperance Districts of CALM are the only other regions in the south-west where concern has been expressed that *P. megasperma* will cause serious dieback in native vegetation. Moore *et al.* (1991) reported that in the Fitzgerald River National Park (FRNP) there were two confirmed infestations of *P. megasperma*, at the east side of the park, and two infestations of *P. cinnamomi.* The latter fungus appeared to be the more aggressive pathogen, infesting 6 km of the Bell Track. Since the Moore *et al.* (1991) management plan for the FRNP was published, *P. megasperma* infestations at the west side of the park have been confirmed. Monitoring dieback caused by species of *Phytophthora* in the FRNP has been a management priority for CALM as the park is one of only two International Biosphere Reserves in Western Australia. It is also one of the most botanically rich areas in Western Australia, supporting about 1750 plant species representing 20% of the state's described taxa (Moore *et al.* 1991). Western Australian Proteaceae are usually very susceptible to infection by *P. cinnamomi*, and as this family is represented by about 130 species in the FRNP, it has been anticipated that losses due to dieback may seriously reduce the park's conservation and recreational values (Moore *et al.* 1991).

The climates of Moora District and FRNP are similar in that both experience cool, wet winters and hot, dry summers. Rainfall in both regions is fairly predictable, and occurs mainly between May and August. Although annual rainfall varies throughout both regions, coastal areas average 500-600 mm and this gradually decreases moving inland.

The Moora District and FRNP are also topographically and geologically similar. Both regions comprise a coastline with dunes, followed inland by broad coastal plains with numerous swamps, depressions and large areas lacking runoff. Archaean granitic rock of the Yilgarn Block is represented on the inland plains in both regions.

Low *Banksia* woodland occurs on the well drained, sandy plains in Moora District, with *Melaleuca* occupying swamps and wet areas. *Banksia* shrubland and heath is common throughout the plains of the FRNP, with woodlands occurring alongside rivers and in swamps.

The first initiative to integrate research on *P. megasperma* was that taken by Environment Australia (EA) and CALM in 1990. Among other programmes, EA Project 209 (*Phytophthora* and *Diplodina* canker control in W.A.) focussed research on the control and management of *P. megasperma* in native plant communities of Western Australia. The project required three scope items to be addressed and these constitute the objectives stated below.

2 OBJECTIVES

The major objectives of this work were:

- To determine genetic variability within *P. megasperma*, and to investigate the significance of oospores to this.
- To determine the occurrence of *P. megasperma* in the native plant communities of Western Australia.
- To investigate control measures for *P. megasperma.*

Podger *et al.* (1996) assessed research conducted in Western Australia on *P. megasperma* and reported that systematic studies on it were yet to be completed. They also noted that research had focussed principally on the biology and distribution of the pathogen, but the capacity of *P. megasperma* to damage native vegetation had not been fully evaluated. In this report we address the scope items, and those omissions identified by Podger *et al.* (1996).

3 METHODS

Attainment of the stated objectives (scope items) of this project involved research on a number of different topics. Methodology is presented below in sections numbered 3.1 through to 3.6. Background information is also included in most sections.

3.1 SYSTEMATICS OF *P. MEGASPERMA*

Our understanding of the systematics of northern hemisphere biotypes of *P. megasperma* is improving through studies such as that by Forster and Coffey (1993). Nevertheless, finding it one of the most taxonomically difficult species of *Phytophthora*, Erwin and Ribeiro (1996) concluded that until the various taxa within *P. megasperma* are characterised by more definitive studies, any "new" *megasperma*-like isolates would have to be accommodated in the descriptions of Drechsler (1931) and Hansen and Maxwell (1991).

Currently, *P. megasperma* consists of a number of discrete biotypes from various hosts (Table 1, and see Table 46.1 in Erwin and Ribeiro, 1996). Among other morphological characters, biotypes of *P. megasperma* produce non-caducous, internally proliferating, non-papillate sporangia. *P. megasperma* is homothallic and produces abundant smooth oogonia (42-52 µm diameter) in host tissues and in axenic culture. Antheridia are mainly paragynous, although some are amphigynous. These characters were used by the CALM Vegetation Health Service to distinguish field isolates of *P. megasperma* from other species of the genus. Fuller descriptions of *P. megasperma* are provided by Hansen and Maxwell (1991) and Erwin and Ribeiro (1996).

Rapid and accurate identification of *Phytophthora* spp. is essential to the plant pathologist. Carstairs and Stukely (1996) showed that identification of *P. cinnamomi* by hyphal morphology (presence of botryose hyphal swellings) was reliable, faster and less expensive than the alternative method of using cellulose acetate gel electrophoresis (CAGE) of isoenzyme patterns to identify the fungus. However, other species of *Phytophthora* lack botryose hyphal swellings and biotypes of *P. megasperma* are indistinguishable from one another on that basis.

Using the procedures of Carstairs and Stukely (1996), Bellgard and Carstairs (Carstairs *et al.* 1996) determined the isoenzyme profiles of 78 Western Australian field isolates of *Phytophthora* which had the morphological traits of *P. megasperma* noted above. To determine the taxonomic affinities of these isolates, their isoenzyme profiles were compared with those of reference isolates of *P. megasperma* biotypes and with some other species of *Phytophthora* obtained from C.M. Brazier.

3.2 ALLOZYME VARIATION, GENOTYPIC DIVERSITY AND GEOGRAPHIC DISTRIBUTION OF *P. MEGASPERMA* IN WESTERN AUSTRALIA

Swofford and Selander's (1981) computer program, for the analysis of allelic variation in genetics, was used to determine the mean number of alleles per locus (Pa) and the proportion of structural genes (loci) that were polymorphic (P1) in Western Australian biotypes of *P. megasperma.* Diversity measures (H) were determined using Shannon and Weaver's (1949) method.

3.3 MYCELIAL GROWTH AND OOGONIUM PRODUCTION IN BIOTYPES OF *P. MEGASPERMA*

Mycelial growth and oogonia were studied in *P. megasperma* using eight electromorph 1 isolates including equal numbers derived from the Albany and Mooora Districts, and four electromorph 3 isolates from the latter District.

A 9.6 mm diameter disc of agar bearing mycelium was transferred from an actively growing colony of *P. megasperma* to cornmeal agar (CMA) in a petri dish. Three replicate dishes were inoculated for each isolate. Linear radial growth of each isolate was measured horizontally and vertically 24 and 48 hours after mycelial transfer.

Seventeen to nineteen days after mycelial transfer, the number of oogonia produced in each of ten, 2 mm diameter fields of view were counted for each dish and the diameters of ten mature oogonia were measured. Numbers of oogonia produced in the presence of phosphonate were also determined for three replicate dishes of each isolate. All dishes were incubated under light at 24°C.

3.4 OUTCROSSING IN HOMOTHALLIC *PHYTOPHTHORA* SPP. AND THE SIGNIFICANCE OF OOSPORES FOR *P. MEGASPERMA*

The considerable interest that researchers have shown in modes of reproduction and the significance of oospores in *Phytophthora* spp. is due to the influence that sexual recombination has on the epidemiology and control of disease.

By comparison with asexual reproductive processes, an expected result of sexual reproduction is elevated genetic diversity as a consequence of recombination. Tooley *et. al.* (1985) used isoenzyme electrophoresis to compare populations of *P. infestans* from Central Mexico with those from USA and Canada. In the Mexican populations, where the two mating types (A1 and A2) of *P. infestans* occurred in a ratio of 1:1, four loci were polymorphic, and 15 multilocus genotypes were observed. The observed genotype frequencies at two polymorphic loci (GPI and PEP) were not significantly different from those expected for populations in Hardy-Weinberg equilibrium. Until 1985 only the A1 mating type was detected in USA and Canada. Fewer (four) multilocus genotypes occurred in these populations, and they were not in Hardy-Weinberg equilibrium (Tooley *et al.* 1985).

Oospores of the heterothallic *Phytophthora infestans* appear to be long-term perenating spores (Fry *et. al.* 1992; Drenth *et. al.* 1995), and in some species of *Phytophthora* oospores can persist in soil for many years (Duncan 1980; Duncan and Cowan 1980). Oospores may permit *Phytophthora* to survive harsh environmental conditions until the climate again becomes favourable for pathogenic activity.

By growing genetically different isolates of the homothallic *Phytophthora sojae* in dual culture, several researchers (Bhat and Schmitthenner 1993; Forster *et. al.* 1994; Whisson *et. al.* 1994; 1995) have demonstrated that hybrid oospores can be generated *in vitro*. Some crosses produced relatively few (4-10%) hybrid spores (Forster *et. al.* 1994; Whisson *et. al.* 1994), while others generated high proportions (76%) of hybrid oospores (Bhat and Schmitthenner 1993). The observation of *in vitro* outcrossing in a homothallic species raises the possibility that this may be a feature of field populations of homothallic *Phytophthora* spp.

in Western Australia. If so, an increase in genetic diversity would be expected and this may have implications for disease control.

Oudemans *et al.* (1994) identified five electrophoretic subgroups (CIT 1-5) within a worldwide collection of 125 isolates of homothallic *P. citricola*. Some field isolates from Western Australia shared a high degree of isoenzyme affinity with a CIT 3 standard isolate obtained from Michael Coffey of Riverside, California, USA (Carstairs, unpubl.). Using CAGE, Newcombe and Carstairs (Carstairs *et al.* 1996) consistently resolved two NADP-IDH specific enzymes in Western Australian isolates of CIT 3 and argued that the isenozymes were localised in different sub-cellular compartments. One enzyme was localised in the cytosol and the other in protoplastids. The isoenzyme associated with protoplastids (NADP-IDH-2) was polymorphic, and it was shown that an isolate which produced a three-banded NADP-IDH-2 phenotype was a heterozygote produced by oosporogenesis as opposed to heterokaryogenesis.

CAGE isoenzyme electrophoresis identified two biotypes of *P. megasperma* (electromorphs 1 and 3), which had alleles in common for 9 of 12 loci, but had fixed differences in alleles for three other loci (see subsection 4.1). Three putative isoenzyme loci were polymorphic, each having two alleles. Assuming that the genotypes of these biotypes originated by oosporogenesis, it should be possible to use the relative genotype frequencies to derive an estimate of outcrossing.

Fry *et al.* (1992) defined populations of *P. infestans* as units including isolates from a geographically defined location. The isolates of *P. megasperma* used in this study were recovered from Moora District. These included 14 isolates of electromorph 1 and four of electromorph 3. Usually, only a single isolate from any one infection site was assessed. The isolates of CIT 3 *P. citricola* were obtained from a small area about 40 Km NE of the d'Entrecasteaux National Park. Forty-seven infection sites were positive for CIT 3 and on average three samples (range 1-29) from each site were tested for the presence of the pathogen.

The allozyme data were analysed using BIOSYS (Swofford and Selander 1981), a computer program for the analysis of allelic variation. Outcrossing rates were estimated using the following formula:

$$t = \frac{Ho}{4pq - Ho} \quad \ldots\ldots 1.$$

where t is an estimate of the outcrossing rate, Ho is the observed heterozygote frequency, p is the frequency of one of the allozymes at a locus, and q is equal to 1-p.

3.5 OCCURRENCE OF *P. MEGASPERMA* IN NATIVE PLANT COMMUNITIES OF WESTERN AUSTRALIA

Podger (1968, 1972) noted that although disease caused by *P. cinnamomi* occurred in almost the entire range of landforms in the south-west of Western Australia, it was more frequent in wet situations along drainage lines and in broad valleys than on dry slopes and ridges. Shearer and Tippett's (1989) observations concurred with those of Podger (1968, 1972) and they described a dendritic pattern of infection which coincided with the presence of streams in shallow valleys.

Podger *et al.* (1996) identified three distinctive disease syndromes due to infection by *P. cinnamomi*. Our aim has been to identify disease syndromes associated with infection by *P. megasperma*. In order to achieve this we have visited infested sites in the Moora District and FRNP. Discussions have been held with CALM officers and private consultants working on dieback in these areas. Hart, Simpson and Associates' (1991) report on dieback in Moora District has also been consulted.

To estimate the abundance of *P. megasperma* in wet situations throughout the south-west of Western Australia, five to twelve water bodies in each of four CALM Districts, were sampled to detect the presence of *Phytophthora* spp. Pear baits were incubated in the water bodies for up to four days. Lesions forming on the baits were transferred to a selective agar medium and *Phytophthora* spp. growing from the lesions were subsequently isolated and identified.

3.6 CONTROL OF *P. MEGASPERMA*

Podger *et al.* (1996) outlined a comprehensive approach to management of disease caused by *P. cinnamomi*. The three main elements of this approach included:

- implementation of practices which ameliorate the damaging effects of *P. cinnamomi* in areas where the pathogen is already established.

- containment or reduction of autonomous spread of disease at the boundaries of existing infestations.

- reduction of the rate of vectored spread associated with establishment of new disease centres.

Although considerable research has been directed towards all three elements, phosphonate treatment appears to offer the only promising management option available for disease control in established infections and restriction of autonomous spread. Otherwise, current approaches to management must still be based almost exclusively on hygiene strategy (Podger *et al.* 1996).

Earlier attempts to evaluate the sensitivity of *P. megasperma* to phosphonate indicated that while the growth rates of some isolates were depressed by the fungicide, Western Australian isolates were usually insensitive having an ED_{50} value of 482.5 $\mu g\ ml^{-1}$ (Bellgard *et al.* 1995). In recent months we have re-examined the effect of phosphonate on field isolates of *P. megasperma*.

To determine the effect that phosphonate has on the growth rates of *P. megasperma* and *P. cinnamomi*, isolates were grown on CMA containing different concentrations of the chemical. To minimise its' oxidation to phosphate, the phosphonate was added after CMA had been autoclaved and cooled to 58°C. CMA contains about 0.38mM phosphate (Bompeix and Saindrenan, 1984). Dishes of CMA were inoculated with the test fungi in the manner

described already (section 3.3). Twelve isolates of *P. megasperma* and four of *P. cinnamomi* were used in this work. These included:

- four isoenzymically identical Albany (1) *P. megasperma* isolates retrieved from the Fitzgerald River National Park in the CALM Albany District;

- four isoenzymically identical Moora (1) *P. megasperma* isolates retrieved from the sand plain north of Perth;

- four isoenzymically identical Moora (3) *P. megasperma* isolates retrieved from the sand plain north of Perth;

- four isoenzymically identical *P. cinnamomi* isolates retrieved from the Stirling River National Park in the CALM Albany district.

P. cinnamomi was grown on CMA supplemented with phosphonate at concentrations of 0 (control),1.7, 3.3, 6.7, or 16.6 $\mu g\ ml^{-1}$, while *P. megasperma* was grown on media containing 0, 6.7, 16.6, 33.3, or 50 $\mu g\ ml^{-1}$ phosphonate. Each treatment was replicated three times. Estimates of radial mycelial growth rate, oogonium production and oogonium size were obtained using methods described already (section 3.3). After 28 days' incubation, three plugs of agar ($5mm^2$) bearing mycelium were transferred from each treatment to fresh CMA in petri dishes. The plugs were examined periodically for emergence of live hyphae and those that failed to produce growth within four days were considered to be dead.

One-way analysis of variance (ANOVA) was used to test for significant differences ($P = 0.05$) between mean values of the undernoted measurements:

- growth rates of the four isolates in each isoenzymically identical group at each of the five phosphonate concentrations.

- growth rates of the three *P. megasperma* groups at each of the five phosphonate concentrations.

- number of oogonia produced by different isolates of *P. megasperma* on CMA. This data was log transformed before analysis.

- number of oogonia produced by isolates of *P. megasperma* at different concentrations of phosphonate.

- diameter of oogonia produced by different isolates of *P. megasperma* on CMA.

- diameter of oogonia produced by isolates of *P. megasperma* at different concentrations of phosphonate.

Tukey's test (MINITAB, 1996) was used to identify any pattern of difference determined by one-way ANOVA. All statistical tests were conducted using the Minitab (1996) statistical computer package.

To determine the capacity of *P. megasperma* to infect plant tissue that had been treated with phosphonate, an isolate was tested against *Eucalyptus sieberi* seedlings that had been treated with the chemical at concentrations of 0 (control), 125, 250, or 500µg ml^{-1}. As a further control, *P. cinnamomi* was also tested against pre-treated *E. sieberi* and a species of *Pimelia*. One isolate each of *P. megasperma* (Albany 1) and *P. cinnamomi* were grown separately over the surface of *E. sieberi* seedlings. Two infested seedlings were subsequently immersed in soil extracts in 500 ml dishes. Thirty *E. sieberi* cotyledon baits were treated with phosphonate and added to the dishes of soil extract. In the case of *P. cinnamomi*, 30 leaves from *Pimelia* plants were similarly treated with phosphonate and added to the dishes. Each treatment was replicated three times. After seven days the cotyledon and leaf baits were transferred to selective antibiotic agar. If *Phytophthora* colonies grew from the baits within three days, they were recorded as being infected. This experiment was not repeated.

The incidence of *P. megasperma* on unsealed (not bituminised) road surfaces was investigated by collecting 1 kg samples from pavements from four CALM Districts in the south-west of Western Australia and baiting them with *E. sieberi* cotyledons and *Lupinus angustifolius* radicals for two, five-day periods. After each period, a minimum of 20 lesioned baits were

transferred to selective antibiotic agar and *Phytophthora* spp. growing from the baits were isolated and identified.

4 RESULTS AND DISCUSSION

Results and discussion are presented in subsection form, coinciding with the description of methods in section 3.

4.1 SYSTEMATICS OF *P. MEGASPERMA*

Using 12 putative loci, nine electromorph classes were resolved for 78 Western Australian field isolates of *P. megasperma*, three reference isolates of *P. megasperma* and related species. The multi-locus genotypes of the isolates in each electromorph class are presented in Table 1. None of the 12 loci were monomorphic for the isolates of *P. megasperma* examined here.

The entire sample of Western Australian isolates of *P. megasperma* was included in five electromorph classes with the majority of isolates belonging in class1 (Table1). This isoenzyme class was widespread, and occurred in five coastal CALM Districts, extending from Eneabba in the north to Cape Arid in the south-east. Particularly high isolation frequencies were recorded for the Albany and Moora Districts, thus suggesting that class 1 might have a disjunct distribution. The second most frequently recovered group of isolates was electromorph class 2. Isolates of this class were recovered only in Albany and Moora Districts. The three other electromorph classes found in Western Australia were infrequently isolated in the Moora District but were not detected elsewhere in the state.

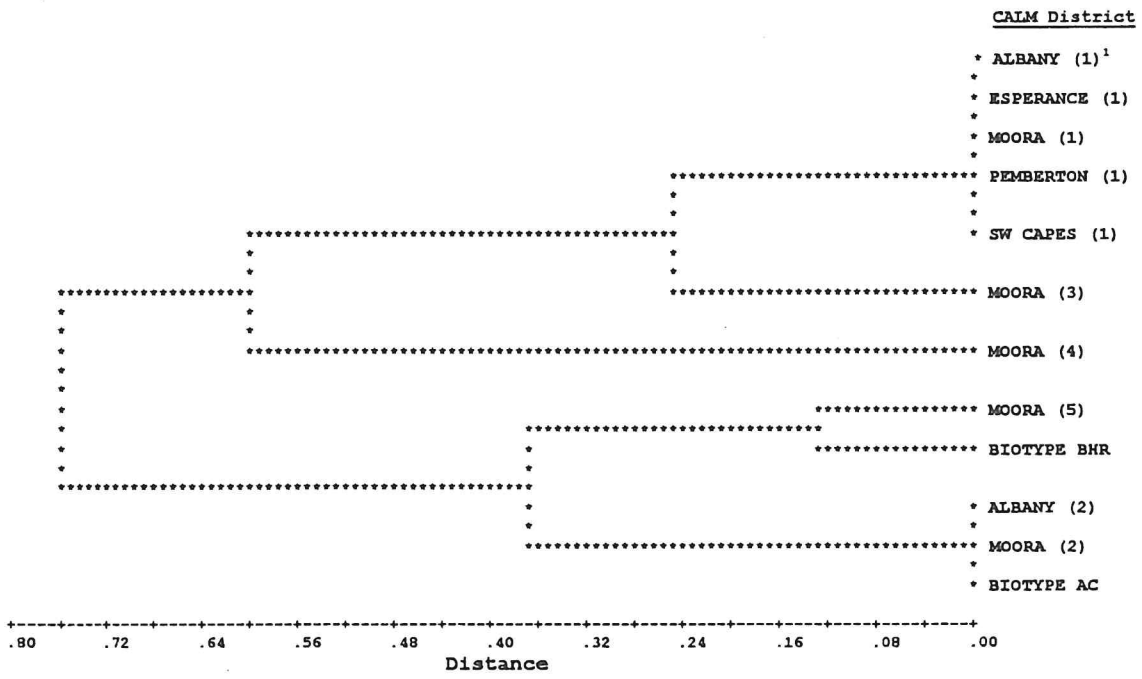
Table 1. Genotypes of putative loci (isoenzymes) of field isolates of *Phytophthora megasperma* from Western Australia, reference types of *P. megasperma*, and some related *Phytophthora* spp.

Electromorph Class	Number[1]	CALM District/ Reference[2]	Biotype[3]/ Species	Isoenzyme[4] Locus											
				FUM	HK	IDH	LDH	MDH1	MDH2	ME	MPI	6PG1	6PG2	PGI	TPI
1	42	Albany	None Matched	BB	AB	BB	DD	EE	BB	CC	CC	BB	BB	CC	AA
	4	Esperance		BB	AB	BB	DD	EE	BB	CC	CC	BB	BB	CC	AA
	14	Moora		BB	AB	BB	DD	EE	BB	CC	CC	BB	BB	CC	AA
	1	Pemberton		BB	AB	BB	DD	EE	BB	CC	CC	BB	BB	CC	AA
	3	SW Capes		BB	AB	BB	DD	EE	BB	CC	CC	BB	BB	CC	AA
2		Reference	Apple-Cherry	AA	DD	CC	CC	CC	DD	DD	FF	BB	BB	DD	EE
	3	Albany	(AC)	AA	DD	CC	CC	CC	DD	DD	FF	BB	BB	DD	EE
	5	Moora		AA	DD	CC	CC	CC	DD	DD	FF	BB	BB	DD	EE
3	4	Moora	None Matched	BB	AB	BB	DD	FF	AA	CC	CC	BB	BB	BB	AA
4	1	Moora	None Matched	CC	CC	EE	BB	EE	CC	CC	DD	BB	BB	CC	EE
5		Reference	Broad Host Range	AA	DD	AC	CC	DD	DD	CC	DD	BB	BB	BD	CC
	1	Moora	(BHR)	AA	DD	CC	CC	DD	DD	CC	DE	BB	BB	DD	CC
6		Reference	Douglas Fir	AA	BB	DD	CC	DD	CC	BB	BB	AA	AA	CC	DD
7		Reference	*P. medicaginis*	BB	EE	EE	DD	DD	EE	AA	AA	CC	CC	AA	DD
8		Reference	*P. sojae*	CC	DD	CC	DD	AA	EE	EE	CC	BB	BB	AA	EE
9		Reference	*P. trifolii*	CC	BB	CC	AA	BB	BB	AA	CC	BB	BB	BB	BB

1. Number of isolates of this class found in each CALM district.
2. Reference isolates were obtained from C. M. Brasier.
3. Biotypes refer to those identified by Hansen and Marshall (1991) for *P. megasperma*.
4. Isoenzyme abbreviations stand for: FUM = Fumerase; HK = Hexokinase; IDH = Isocitrate Dehydrogenase; LDH = Lactate Dehydrogenase; MDH1 = Malate Dehydrogenase locus 1; MDH2 = Malate Dehydrogenase locus 2; ME = Malic Enzyme (NADP) dependent; MPI = Mannose Phosphate Isomerase; 6PG1 = Glucose-6-Phosphate Dehydrogenase locus 1; 6PG2 = Glucose-6-Phosphate Dehydrogenase locus 2; PGI = Phospho-Glucose Isomerase; and TPI = Triose Phosphate Isomerase.

Rogers' distance measure (Rogers, 1972) was used to calculate a distance matrix from the isoenzyme genotype data presented in Table 1, and from this a UPGMA cluster analysis (Sneath and Sokal, 1973) was conducted to generate a dendrogram (Figure 1).

Figure 1. A cluster analysis of electromorphs of Western Australian isolates of *P. megasperma*, and some reference biotypes of *P. megasperma*. Numbers in parentheses[1] refer to electromorph classes found in stated districts.



Albany and Moora isolates of electromorph class 2 clustered with biotype AC with which they were isoenzymically identical. Biotype AC is most commonly isolated from apples and cherries in the northern hemisphere. The Moora isolate of electromorph class 5 clustered with biotype BHR, and these fungi were isoenzymically very similar. Biotype BHR of *P. megasperma* has a broad host range in the northern hemisphere. These were the only two Western Australian electromorph classes whose systematic affinities matched reference biotypes of *P. megasperma* currently held by us. None of the local isolates of *P. megasperma* could be matched with reference isolates from leguminous hosts.

The genetic distance between Moora District electromorph classes 3 and 1 was substantially less than the distances between all other field isolates of Western Australian *P. megasperma*.

Taking into account the high degree of isoenzyme fidelity between these classes (Table 1), we are of the opinion that they may represent incipient taxa, and that their close relationship is unique among the five isoenzyme classes found here.

4.2 ALLOZYME VARIATION, GENETIC DIVERSITY AND GEOGRAPHIC DISTRIBUTION OF *P. MEGASPERMA* IN WESTERN AUSTRALIA

In the sample of 78 isolates of *P. megasperma* that was examined isoenzymically, three electromorph classes (1,2 and 3) were represented by four or more isolates (Table 1). For each of these three biotypes we determined the proportion of loci that were polymorphic (Pl) and the mean number of alleles per locus (Pa), to derive estimates of intra-class genetic variation (Table 2). A locus was recorded as polymorphic if more than one putative allele was found within a biotype. Genotypic diversity was also determined (Table 2). By comparison with other species of *Phytophthora* the three biotypes of *P. megasperma* were lacking in genetic variation. *P. cactorum* was the least genetically variable species of three examined by Oudemans and Coffey (1991), but Western Australian groups of *P. megasperma* appear to have even less allozymic variation than that species.

P. megasperma electromorph classes 2 and 3 were represented by small sample sizes, thus the low estimates of variation and diversity in these biotypes may be due to chance. However, sample size is less likely to account for the small degree of genetic variation found in electromorph class 1. Australian populations of introduced *Phytophthora* spp. (eg. *P. cinnamomi*), are less genetically variable than populations of the same species overseas (Old *et al.* 1984; Carstairs and Stukely, 1996; Irwin *et al.* 1995). It may be that only a small number of *P. megasperma* electromorph class 1 individuals were introduced into Western Australia, and that these represented a subset of the genetic diversity possessed by the pathogen in its natural setting. This restriction of genetic diversity is not uncommon, as small samples of individuals from large populations inhabit new sites and become isolated from the original genepool. Examples are well documented among agricultural plants, and in *P. infestans* in Europe and North America (Fry *et al.* 1992).

Table 2. Measures of genetic variation and diversity in three electromorph classes of Western Australian isolates of *P. megasperma*, and three other species of *Phytophthora*.

Variation Within Species	No. of Isolates	Measures of Variation			Biotype Diversity (H)
		Pl[1]	Pa[2]	Genotypic Diversity (H)	
P. megasperma Electromorph class					
1	64	0.08	1.08	0	N/A
2	8	0	1.00	0	N/A
3	4	0.08	1.08	0	N/A
P. cactorum[3]	47	0.11	1.20	0.076	N/A
P. cambivora[3]	25	0.23	1.30	0.537	N/A
P. cinnamomi[3]	81	0.22	1.50	0.617	N/A
District					
Albany	45	N/A	N/A	N/A	0.11
Esperance	4	N/A	N/A	N/A	0
Moora	25	N/A	N/A	N/A	0.523
Pemberton	1	N/A	N/A	N/A	0
SW Capes	3	N/A	N/A	N/A	0

1. Pl = the proportion of loci which were polymorphic.
2. Pa = the mean number of alleles per locus.
3. Adapted from Oudemans and Coffey (1991).

Biotype diversity in CALM Districts ranged from between zero in Districts with only one electromorph class, to 0.11 in Albany District, and 0.523 in Moora District (Table 2). Biotype diversity in Moora District was comparable to that determined for *Phytophthora cambivora* and *P. cinnamomi* by Oudemans and Coffey (1991). Compared to other districts in the south-west of Western Australia Moora District appears to be rich in biotypes of *P. megasperma* with five different electromorph classes isolated to date.

Several hypotheses may account for the high biotype diversity observed for Moora District relative to other areas. Small sample sizes of isolates examined from some districts probably influenced the observed outcome. A second factor influencing the result, may be that a greater variety of habitats are present in Moora District and this might have favoured establishment of a broader spectrum of biotypes than in other areas. Thirdly, influx of infested material may have occurred over a greater period of time in the Moora District than elsewhere. More likely a combination of these factors is involved.

4.3 MYCELIAL GROWTH AND OOGONIUM PRODUCTION IN BIOTYPES OF *P. MEGASPERMA*

Isolates of biotype 3 from Moora District had the slowest growth rates on CMA apart from one biotype 1 isolate from Albany. The three other biotype 1 isolates from Albany had similar growth rates to biotype 1 isolates from Moora District (Table 3).

Diameters of oogonia of biotype 1 from Moora were much smaller than those of the same biotype from Albany and biotype 3 from Moora (Table 3).

There were considerable differences between the numbers of oogonia produced on CMA by various isolates of *P. megasperma*. Mean numbers of oogonia ranged between 0 and 118.43 in a 2 mm field of view (Table 3). Biotype 3 isolates from Moora produced relatively large numbers of oogonia compared to isolates of biotype 1 from the same District. Most variation in oogonial production was observed among biotype 1 isolates from Albany (Table 3).

Table 3. Growth rates, oogonial diameters and numbers of oogonia formed by two biotypes of *P. megasperma* on CMA with or without phosphonate. Values followed by the same letter are not significantly different (α=0.05).

Isolate Number	CALM District	Radial Growth[1]	Oogonia Diameter[2]		Oogonia Production	
					CMA[3]	Phosphonate[4]
		mm (s.e.)	µm (s.e.)	Range (µm)	Mean (s.e.)	Mean (s.e.)
2385Pm	Albany (1)[5]	5.29 (0.073)^{de}	52.35 (0.783)^{c}	39.52-62.40	11.20 (2.403)^{d}	0
2386Pm	Albany (1)	5.08 (0.172)^{bc}	50.02 (1.046)^{c}	40.56-59.28	4.07 (0.866)^{b}	3.13 (0.457)
2387Pm	Albany (1)	5.31 (0.105)^{de}	N/A	N/A	0^{a}	0
2389Pm	Albany (1)	4.29 (0.25)^{a}	49.58 (0.913)^{c}	42.64-62.40	118.43 (15.444)^{g}	122.70 (12.777)
2394Pm	Moora (1)	5.60 (0.238)^{e}	N/A	N/A	0^{a}	0
2396Pm	Moora (1)	5.18 (0.112)^{ce}	45.28 (0.892)^{b}	33.28-53.04	4.03 (0.657)^{b}	0
2397Pm	Moora (1)	5.55 (0.110)^{de}	45.31 (0.947)^{b}	34.32-57.20	5.77 (1.143)^{bc}	0
2398Pm	Moora (1)	5.20 (0.146)^{ce}	41.46 (0.741)^{a}	32.24-53.04	11.47 (2.311)^{cd}	0
2399Pm	Moora (3)	4.47 (0.129)^{ab}	N/A	N/A	0^{a}	0
2400Pm	Moora (3)	4.60 (0.074)^{abc}	51.93 (0.908)^{c}	41.60-62.40	35.07 (3.126)^{e}	0
2401Pm	Moora (3)	4.88 (0.229)^{abcd}	50.89 (0.516)^{c}	43.68-54.08	75.03 (5.862)^{fg}	0
2403Pm	Moora (3)	4.38 (0.091)^{a}	52.48 (0.910)^{c}	41.60-61.36	49.93 (4.320)^{ef}	0

1. Mean 24 hr radial growth on CMA at 24°C (n=6).
2. Diameters of 10 oogonia for each of three replicates per isolate (n=30).
3. Numbers of oogonia on unsupplemented CMA in a 2 mm diam. field.
4. Numbers of oogonia on CMA supplemented with phosphonate at 6.7 µg ml^{-1} in a 2 mm diam. field.
5. Numbers in brackets indicate electromorph affinity.

Only two biotype 1 isolates from Albany produced oogonia in the presence of phosphonate though the chemical did not affect the magnitude of oogonial production in those isolates (Table 3).

Far more variation was apparent in mycelial growth and oogonium production within biotypes 1 and 3 of *P. megasperma,* and between isolates of biotype 1 from Moora and Albany, than had been identified in the allozyme diversity study reported in earlier sections. As might be expected, Western Australian biotypes of *P. megasperma* seem to possess enough variation to enable them to exploit different environmental conditions. The significance of outcrossing and the production of outcrossed oospores by biotypes of *P. megasperma* is dealt with in the next subsection.

4.4 OUTCROSSING IN HOMOTHALLIC *PHYTOPHTHORA* SPP. AND THE SIGNIFICANCE OF OOSPORES FOR *P. MEGASPERMA*

Fifty-three CIT 3 *P. citricola* isolates were recovered from forty-seven sites. In this sample of isolates, one polymorphic locus (IDH-2) with two alleles was identified. Three multilocus genotypes (electromorphs) were observed for IDH-2 (Table 4), and this was to be expected given that for one polymorphic locus with two alleles the expected number of electromorphs (EM) may be determined from the following formula:

$$3^n = \text{the No. of EM's} \quad \ldots\ldots\ldots\ldots \quad 2.$$

where n is the number of polymorphic loci with two alleles. Thus, for CIT 3 with one polymorphic locus the expected number of electromorphs is three.

Table 4. Multilocus genotypes (electromorphs) of CIT 3 *P. citricola* and *P.megasperma* as determined by CAGE. N/A = not available.

Species/ Population	Electromorph	Locus GPI	Locus IDH-2	Locus MDH-1	Locus MDH-2	Number Observed
P. citricola (CIT 3)	CIT 3.1	aa	aa	aa	cc	28
	CIT 3.2	aa	ab	aa	cc	1
	CIT 3.3	aa	bb	aa	cc	24
P. megasperma	Moora (1)	cc	N/A	bb	bb	14
	Moora (3)	bb	N/A	cc	aa	4

In the sample of 18 isolates of *P. megasperma* biotypes 1 and 3, three loci were polymorphic, each having two alleles. So the expected number of electromorphs (genotypes) in this sample of isolates would be 27, nine more than the number of isolates examined. Only two electromorphs were observed however (Table 4). A Chi Square test to compare the expected and observed frequencies of occurrence of the multilocus genotypes at the GPI and MDH-1 loci of *P. megasperma* indicated that there was a significant difference ($X^2_{obs} > 25.85$, $X^2_{0.05} = 7.815$ with 3 df), suggesting that the loci were not randomly associated and might be linked.

Chi Square analysis of observed genotype frequencies of the three polymorphic loci of *P. megasperma* indicated that in all cases they departed significantly from expected, and had a deficiency of heterozygotes (Table 5). Further examination of Table 5 reveals that this was also the case for the IDH-2 locus of CIT 3 *P. citricola*, which like *P. megasperma* is a homothallic species. The heterothallic species, *P. infestans* (Mexico), used for comparative purposes in this study, gave quite different results. The GPI locus was at Hardy-Weinberg equilibrium, while the PEP locus appeared to have a significant excess of heterozygotes (Table 5).

In homothallic *P. megasperma* and CIT 3 *P. citricola*, estimated outcrossing rates were zero and 0.019 respectively (Table 6). In contrast, the estimated mean outcrossing rate in *P. infestans*, a heterothallic species, was 0.816. The absence of any observed outcrossing for *P. megasperma* is likely to be an underestimate. When it is assumed that the outcrossing rate in this species should be the same as that in CIT 3 *P. citricola*, it is possible to determine the

expected number of heterozygotes for any of the three polymorphic loci in *P. megasperma* given that:

$$\text{Expected No. of Hets.} = N \times \frac{t \times 4pq}{1 + t} \quad \text{.....3.}$$

where N is the number of isolates tested, t is the estimated outcrossing rate, p is the frequency of one of the two alleles at a locus, and q = 1-p. When t is estimated to be 0.019 for *P. megasperma* the expected number of heterozygotes in a sample of 19 isolates would be 0.232. Therefore it is not surprising that recombinant *P. megasperma* isolates were undetected in this study. The estimates of outcrossing that we determined are the first reported for species in the Oomycetes.

A high mean estimate of outcrossing (0.816) was determined for the heterothallic *P. infestans* (Table 6). For this species to outcross and produce oospores both A1 and A2 mating types must be present in the same host. There is evidence that some loci (GPI) are in Hardy-Weinberg equilibrium and that oospores most probably persist in the soil for many years (Drenth *et. al.* 1995). These observations suggest that in the Mexican population of *P. infestans*, oospores are an important source of novel genotypes. Field populations of *P. infestans* are ephemeral, and Fry *et. al.* (1992) have described the biology of this species as fitting the concept of a metapopulation (Olivieri *et. al.* 1990). Here, a large number of sites can each support a single distinctive population, each of which has a given probability of extinction. Vacant sites are recolonised from within the metapopulation. This concept of the biology of *P. infestans* would account for the observed rapid displacement in Europe of "old" genotypes of the fungus and replacement with fitter ones (Spielman *et. al.* 1990, Spielman *et. al.* 1991).

Estimated outcrossing rates in *P. megasperma* and CIT 3 *P. citricola* were either zero or low by comparison with *P. infestans* (Table 6). The zero value for *P. megasperma* is likely to be an underestimate since the frequencies of rare alleles at each of three polymorphic loci were low (0.22). It is unlikely that heterozygotes would be recovered when outcrossing is rare (in the order of 0.019). Presumably the estimates of outcrossing were low because the incidence of selfing in homothallic species (where s = 1-t) is high. Alternatively, the values may

Table 5. Summary of genotypic frequencies of loci found to be polymorphic in two Western Australian homothallic species of *Phytophthora* and a heterothallic species from Mexico.

Species/ Population	Locus	Genotype	Observed frequencies	Expected frequencies	Chi Square
P. citricola (CIT 3)	IDH-2	aa ab bb	28 1 24	15.2 26.6 11.2	50.045[3]
P. infestans (Mexico)[1]	GPI[2]	aa ab bb	2 12 36	1.2 13.44 35.28	0.574
	PEP	aa ab bb	32 15 3	38.52 10.73 0.75	9.554[3]
P. megasperma	GPI	bb bc cc	4 0 14	0.89 6.22 10.89	17.98[3]
	MDH-1	bb bc cc	14 0 4	10.89 6.22 0.89	17.98[3]
	MDH-2	aa ab bb	4 0 14	0.89 6.22 10.89	17.98[3]

1. Results obtained from Tooley *et al.* 1985.
2. Rare allele frequencies were pooled.
3. Observed frequencies were significantly different from expected at the 0.05 level and for 1 degree of freedom.

indicate that heterozygotes are disadvantaged in some way relative to homozygotes, and are thus being overlooked. Carstairs and Newcombe (Carstairs *et al.* 1996) investigated the latter possibility by comparing the competitive abilities of homozygous (aa) and heterozygous (ab) individuals of CIT 3 *P. citricola* (IDH-2) to infect host baits. They found that while there was no significant difference in the abilities of the zoospores of the two genotypes to infect the baits, there appeared to be some post-infection selection in favour of the homozygote, although not enough to explain the observed deficiency of heterozygotes.

Table 6. Estimates of outcrossing (t) for two homothallic species of *Phytophthora* from Western Australia and a heterothallic species from Mexico.

Species/ Population	Locus	Heterozygote Frequencies	t
P. citricola (CIT 3)	IDH-2	0.019	0.019
P. infestans (Mexico)	GPI	0.24	0.806
	PEP	0.3	0.825
P. megasperma	GPI	0	0
	MDH-1	0	0
	MDH-2	0	0

The most likely explanation for low outcrossing rates in the homothallic species is that intensity of selfing would be high relative to outcrossing. This is in contrast to dioecious or heterothallic species which must outcross to complete the sexual phase and produce oospores.

Even though outcrossing in the homothallic species was low, the significance of the process should not be under-estimated. Obviously the potential to generate new variants, as a consequence of recombination is a feature of field populations of homothallic species in Western Australia. If the metapopulation concept applies to homothallic species, it may be that field populations of *P. megasperma* in the south-west of Western Australia will at some stage be displaced by new and fitter genotypes. Given that novel *P. infestans* genotypes in Europe were fitter and more resistant to fungicides than the genotypes they replaced (Fry *et. al.* 1992), a similar situation may arise here and existing population of *P. megasperma* might be displaced by phosphonate-resistant genotypes.

4.5 OCCURRENCE OF *P. MEGASPERMA* IN NATIVE PLANT COMMUNITIES OF WESTERN AUSTRALIA

Many species of Proteaceae, Epacridaceae, Dilleniaceae, Xanthorrhoeaceae, and Fabaceae which constitute the understorey and shrub layers in forested areas of the south-west of Western Australia are commonly killed by *P. cinnamomi* at infested sites (Shearer and Tippett, 1989).

Through reference to private records and data held by CALM we have established a list of host species from which three biotypes of *P. megasperma* have been isolated in five CALM Districts (Table 7). The isolated biotypes are assumed to be responsible for disease or mortality of the host species. Biotype 1 was the most frequently recovered electromorph and except for one isolation from *Pinus radiata* in SW Capes District, all were from species of Proteaceae. Biotypes 2 and 3 were recovered from species in Proteaceae and other families known to be susceptible to *P. cinnamomi*.

Species from which unidentified biotypes of *P. megasperma* have been recovered are listed in Table 8. Combining the information in Tables 7 and 8, it is apparent that 31 proteaceous species were susceptible to *P. megasperma*, while the incidence of hosts in other plant families appears to be relatively rare. This is in contrast to *P. cinnamomi* which has a broad range of hosts spanning many families. Hart, Simpson and Associates (1991) found no evidence of high mortality rates in understorey species due to *P. megasperma* in the Moora District but it is possible that deaths in the understorey were overlooked.

A summary of Hart, Simpson and Associates' (1991) assessment of dieback associated with *Phytophthora* spp. in the Moora District is presented in Table 9. *P. megasperma* was recovered from 72% of the sites that were positive for *P. megasperma* or *P. cinnamomi*, while the corresponding value for *P. cinnamomi* was 33%. Only 5% of sites yielded isolations of both pathogens, but this result probably under-estimates dual frequency of occurrence as Hart, Simpson and Associates (1991) sampled only 1.5 (average) dead or dying plants per site.

Table 7. Plant species from which biotypes of *P. megasperma* were isolated. Figures in parentheses indicate the number of times that the fungi were isolated from a particular host.

District	Isoenzyme Electromorph 1	Isoenzyme Electromorph 2	Isoenzyme Electromorph 3
Albany		Casuarinaceae *Allocasuarina campestris* (1)	
	Proteaceae *Adenanthos cuneatus* (2) *Banksia attenuata* (6) *Banksia baxteri* (8) *Banksia gardneri* (1) *Banksia lemanniana* (4) *Banksia media* (3) *Conospermum distichum* (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)		
		Xanthorrhoeaceae *Xanthorrhoea platyphylla* (1)	
Esperance	Proteaceae *Banksia speciosa* (1) *Dryandra sessilis* (1)		
Moora			Myrtaceae *Leptospermum* sp. (1)
	Proteaceae *Banksia attenuata* (9) *Banksia ilicifolia* (1) *Hakea prostrata* (1) *Hakea* sp. (2)	Proteaceae *Banksia prionotes* (1)	Proteaceae *Banksia attenuata* (2)
Pemberton	Proteaceae *Banksia occidentalis* (1)		
SW Capes	Pinaceae *Pinus radiata* (3)		

Table 8. Plant species from which unidentified biotypes of *P. megasperma* have been recovered.

Albany District[1]	Moora District[2]
	Epacridaceae
	Leucopogon conostephioides
Proteaceae	Proteaceae
Banksia nutans	*Adenanthos cygnorum*
Banksia repens	*Banksia grandis*
Banksia speciosa	*Banksiahookeriana*
Hakea incrassata	*Banksia menziesii*
	Hakea auriculata

1. Information obtained from the Vegetation Health Service (VHS) of CALM data base.
2. Information obtained from Hart, Simpson & Assoc. 1991.

During 1996 we monitored disease at many of the sites visited by Hart, Simpson and Associates (1991), and tested 10 water bodies for the presence of *Phytophthora*. Three of the water bodies were found to be positive for *P. megasperma* (Table 10), and in each case that fungus was recovered in addition to *Phytophthora* spp. other than *P. cinnamomi*. *P. megasperma* seemed to be relatively rare in these water bodies compared with other species of *Phytophthora*.

That *P. megasperma* and *P. cinnamomi* were seldom associated with each other may be due to site history. The pathogen which becomes established first at a site may occupy all suitable niches and exclude the other pathogen. The relative frequencies with which *P. megasperma* and *P. cinnamomi* were recovered from dying plants or water bodies in Moora District probably reflects their abundance in the area. At sites infected with *P. cinnamomi* or *P. megasperma* disease impact was greater than that in areas from which other *Phytophthora* spp. were recovered.

Podger *et al.* (1996) concluded that within the genus *Phytophthora*, *P. cinnamomi* was by far the most destructive pathogen on native vegetation. Further, where annual rainfall is between 400 mm and 800 mm, damage caused by *P. cinnamomi* is likely to be localised and confined to areas prone to seasonal waterlogging. Among other sources, these conclusions were drawn from observations made by Hart, Simpson and Associates (1991) who assessed dieback in the Moora District, where annual rainfall is between 500 mm and 600 mm. A summary of their observations for *P. megasperma* and *P. cinnamomi* is presented in Table 9. In *Banksia*

woodland on draining soils, *P. cinnamomi* was associated with isolated deaths at only one site and the ratio of *Banksia* woodland sites infested with *P. cinnamomi* to wetland sites was 1:7 (Table 9). At 86% of the latter sites the frequency of dead or dying Proteaceae was high.

Table 9. Numbers of plants killed by *P. megasperma* and *P. cinnamomi* in habitats of differing hydrology in Moora District. Data reported from Hart, Simpson and Associates (1991).

	Disease Expression by *Phytophthora* species			Totals	*P. megasperma: P. cinnamomi*
	H[1]	S[2]	I[3]		
Banksia woodland					
P. megasperma	0	0	2	2	
P. cinnamomi	0	0	1	1	2:1
Road Drain					
P. megasperma	5	0	1	6	
P. cinnamomi	3	1	0	4	
P. megasperma + *P. cinnamomi*	1	0	0	1	7:5
Wetland					
P. megasperma	9	3	6	18	
P. cinnamomi	5	0	1	6	
P. megasperma + *P. cinnamomi*	1	0	0	1	19:7
Totals	24	4	11	39	

1. H: High frequency of Proteaceae dead and/or dying.
2 S: Scattered Proteaceae deaths.
3. I: Isolated Proteaceae deaths.

In the Moora District, *P. megasperma* has caused similar damage to that attributable to *P. cinnamomi*. The numbers of *Banksia* woodland and wetland sites infested with *P. megasperma* were two and nineteen, respectively (Table 9). Isolated deaths were observed in infested *Banksia* woodland sites, whereas high plant mortality rates were noted in 47% of wetland sites infested with *P. megasperma* (Table 9).

It appears that the disease syndrome associated with *P. megasperma* in *Banksia* woodland differs from that in wetlands only in numbers of host mortalities. In Moora District mortality of very susceptible plant species, Proteaceae in particular, is high in or adjacent to wetland areas, but an advancing disease front is not present upslope on well drained soils. Spot

infections in *Banksia* woodlands appear to be ephemeral by comparison with infections in wet areas.

In the FRNP, fire followed by droughting of regenerating vegetation has obscured the symptoms of disease caused by *P. megasperma* (Figures 2).

In their review of dieback in Western Australia, Podger *et. al.* (1996) predict that the grey sands and seasonally inundated flats of the coastal plain will probably be infested and irreparably damaged by *P. cinnamomi* within decades. It is likely that *P. megasperma* will similarly infest this area over the same timescale. However, its' role in damaging the coastal plain vegetation is likely to be minor relative to that of *P. cinnamomi*.

Table 10. Recovery of *P. megasperma* and other species of *Phytophthora* from water bodies in four CALM Districts.

CALM District	Season	No. of Water Bodies Tested	No. of Water Bodies negative for *Phytophthora*	No. of Water Bodies Positive for				
				P. megasperma	*P. megasperma* + *P. cinnamomi*	*P. megasperma* + *Phyt.* Spp.	*P. cinnamomi* + *Phyt.* spp.	*Phyt.* spp.[1]
Albany	Summer	12	3	1 (5)[2]	0	2 (11:13)	0	6 (52)
Dwellingup	Winter	7	2	0	0	0	1 (1:0)	4 (145)
	Spring	8	0	0	0	0	4 (16:132)	4 (296)
Manjimup	Winter	5	0	0	0	0	0	5 (45)
Moora	Spring	10	0	0	0	3 (25:181)	0	7 (670)
Total		**42**	**5**	**1 (5)**	**0**	**5 (36:194)**	**5 (17:132)**	**26 (1208)**

1. *Phyt.* spp.= species of *Phytophthora* other than *P. megasperma* and *P. cinnamomi*, i.e. principally biotypes of *P. citricola* and *P. cryptogea/drechsleri*.

2. Numbers in brackets refer to the number of isolates of these species that were recovered. Identification was based on morphological characters.

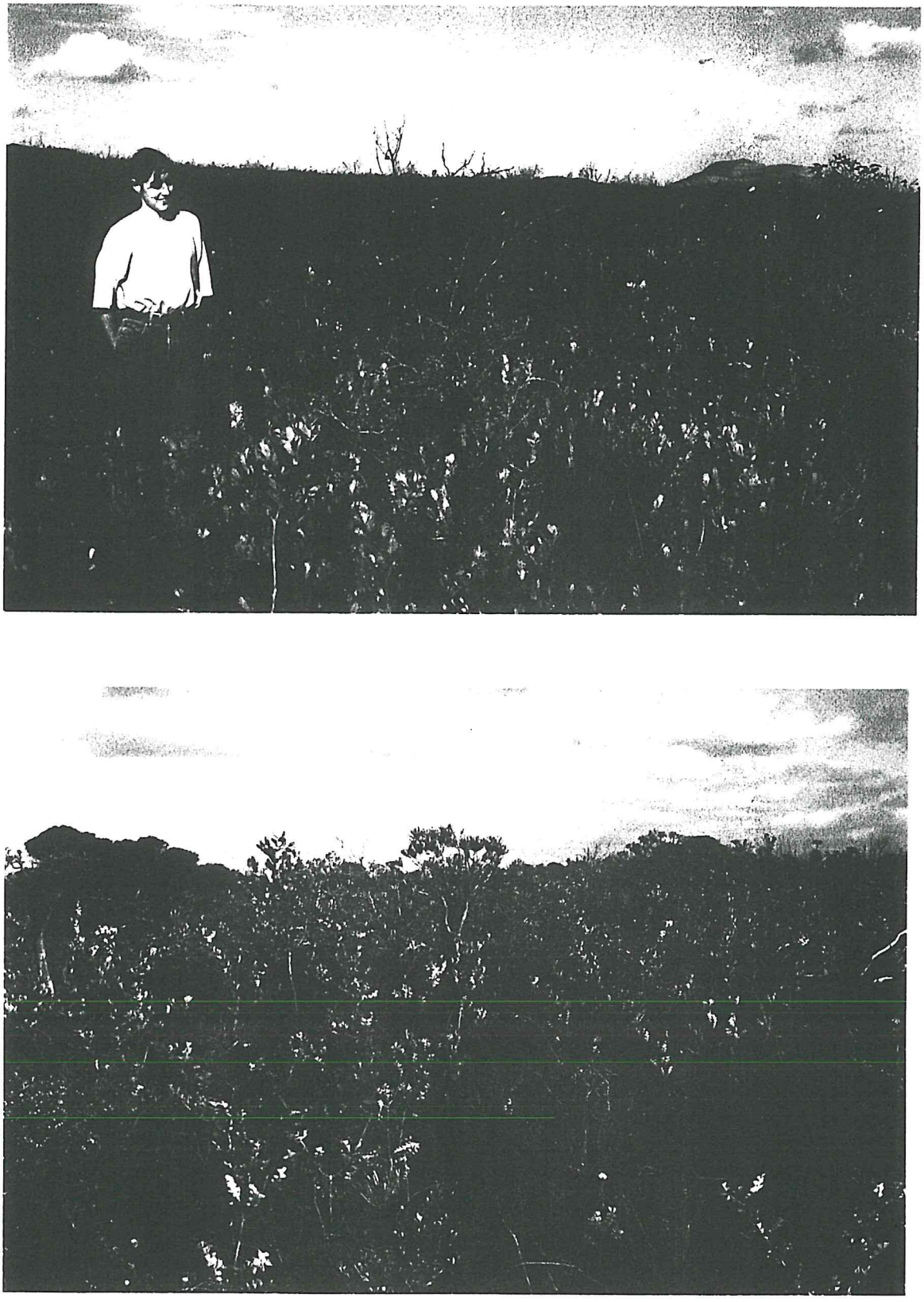


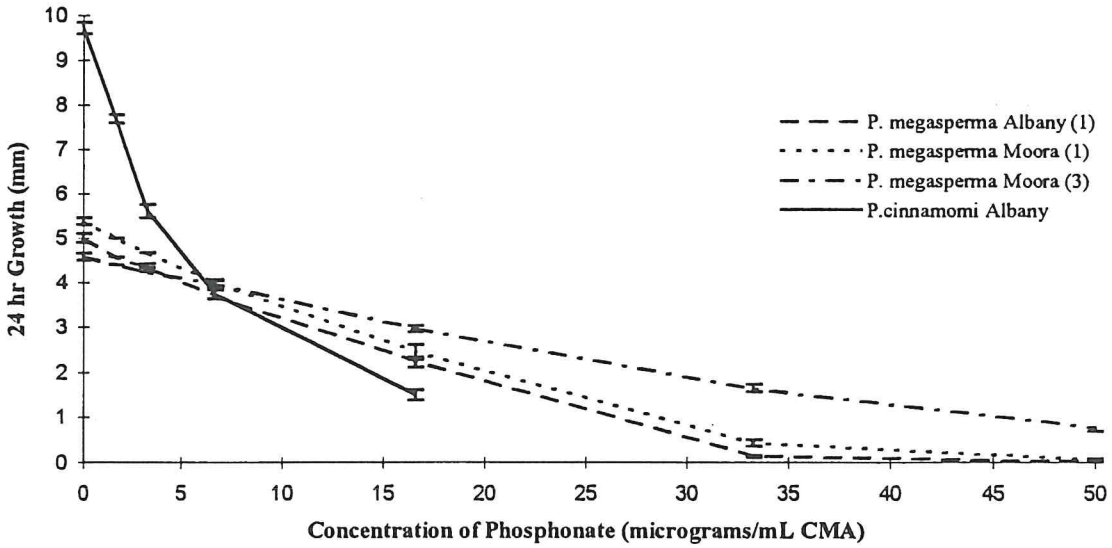
Figure 2. Drought is obscuring disease symptoms caused by *P. megasperma* in the vicinity of **a)** East Mount Barren; and **b)** West Mount Barren in the FRNP.

4.6 CONTROL OF *P. MEGASPERMA*

Research on phosphonate has been directed mainly towards minimising the degree of damage caused by *P. cinnamomi* in established infection centres. Our objective was to compare the sensitivity of *P. megasperma* and *P. cinnamomi* to phosphonate.

The responses of *P. cinnamomi* and *P. megasperma* (biotypes 1 and 3) to increasing concentrations of phosphonate are presented in Figure 3. At low concentrations, mycelial growth of *P. cinnamomi* was inhibited more severely than that of *P. megasperma.*

Figure 3. Mean radial growth of *Phytophthora* spp. on CMA containing different concentrations of phosphonate (n = 4).



Biotype 1 isolates of *P. megasperma* from Moora and Albany showed similar responses to phosphonate and were strongly inhibited at concentrations in excess of 30 $\mu g\ ml^{-1}$. Biotype 3 isolates from the former location were least sensitive of the test fungi (Figure 3).

The mean ED_{50} value that we obtained for four isolates of *P. cinnamomi* was comparable to that recorded by Komorek (1994) (Table 11). However, the combined ED_{50} for 26 *P. megasperma* isolates (ED_{50}=482.5$\mu g\ ml^{-1}$) reported by Bellgard *et al.* (1995), was substantially greater than the corresponding values that we determined for biotypes of that

species (Table 11). We repeated the phosphonate tests on *P. megasperma* and *P. cinnamomi*, and obtained similar results. Thus, Western Australian biotypes of *P. megasperma* appear to be more sensitive to phosphonate than hitherto believed.

Table 11. Growth of two biotypes of *P. megasperma* and *P. cinnamomi* on CMA containing phosphonate (P) at stated concentrations. Values followed by the the same letter are not significantly different (α=0.05).

Isoenzyme group	Growth on CMA (mm)	% inhibition of growth on P-amended CMA						ED_{50}[1]
		1.7 μg ml^{-1} P	3.3 μg ml^{-1} P	6.7 μg ml^{-1} P	16.6 μg ml^{-1} P	33.3 μg ml^{-1} P	50 μg ml^{-1} P	μg ml^{-1}
P. megasperma Albany (1)	5.0 ± 0.103^{b}	N/A[2]	N/A	25.71^{b}	55.22^{b}	96.91^{c}	99.68^{b}	14.74[3]
P. megasperma Moora (1)	5.38 ± 0.08^{c}	N/A	N/A	25.64^{b}	54.24^{b}	91.75^{b}	98.68^{b}	15.08
P. megasperma Moora (3)	4.58 ± 0.776^{a}	N/A	N/A	13.19^{a}	35.51^{a}	64.46^{a}	82.89^{a}	24.69
P. cinnamomi Albany	9.71 ± 0.128	20.82	42.25	61.43	84.66	N/A	N/A	4.99

1. ED_{50} refers to the concentration of phosphonate expected to inhibit mycelial growth by 50%. These were determined from the means of three replicates of four isolates.
2. N/A = Not applicable as isolates were not tested at these concentrations.
3. ED_{50} was determined from the means of two replicates for one isolate.

In *Banksia* sprayed twice with 40% phosphonate, phosphite levels attained a minimum concentration of 100 μg ml^{-1} before declining to 10 μg ml^{-1} after 12 months (Komorek pers. comm.; Komorek *et al.* 1995). Since *P. megasperma* biotypes 1 and 3 were sensitive to much lower concentrations (*in vitro*), it is suggested that spread of the pathogen may be limited by treatment with phosphonate.

At phosphonate concentration of 6.7 μg ml^{-1}, oospore production on CMA was suppressed in all but two biotype 1 isolates of *P. megasperma* (Table 3). At phosphonate concentrations greater than this, oospore production was suppressed in all isolates. The ability of *P. megasperma* to survive in the presence of phosphonate differed between isolates. Some isolates died at low concentrations of phosphonate, but survived at higher concentrations, while one died only at the highest concentration tested. Isolates of *P. cinnamomi* were not killed in any treatment (Table 12).

The evidence presented here indicates that phosphonate is likely to contain the spread of *P. megasperma* and that production of infective oospores will be suppressed. In some cases the pathogen will probably not survive in treated plants and sensitive biotypes may be eradicated.

Table 12. Survival of isolates of *P. megasperma* and *P. cinnamomi* after 28 days. Each value is the number (out of nine) of agar plugs bearing mycelium which had survived treatment at stated concentrations of phosphonate.

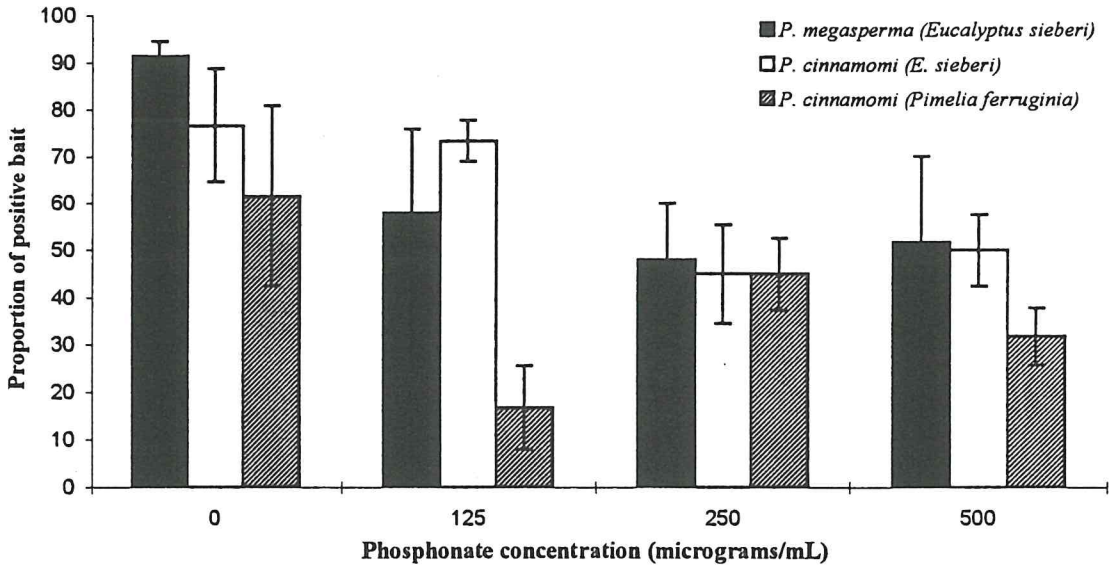
Isolate	Phosphonate Concentration (µg ml^{-1})						
	Control 0	1.7	3.3	6.7	16.6	33.3	50
P. cinnamomi							
292Pc	9[1]	9	9	9	9	N/A	N/A
293Pc	9	9	9	9	9	N/A	N/A
294Pc	9	9	9	9	9	N/A	N/A
2383Pc	9	9	9	9	9	N/A	N/A
P. megasperma							
Albany (1) 2385Pm	9	N/A	N/A	9	9	9	9
Albany (1) 2386Pm	9	N/A	N/A	9	9	9	9
Albany (1) 2387Pm	9	N/A	N/A	9	9	9	9
Albany (1) 2389Pm	9	N/A	N/A	9	**8**	9	9
Moora (1) 2394Pm	9	N/A	N/A	**0**	9	9	9
Moora (1) 2396Pm	9	N/A	N/A	9	9	9	9
Moora (1) 2397Pm	9	N/A	N/A	**1**	9	9	9
Moora (1) 2398Pm	9	N/A	N/A	9	9	9	**0**
Moora (3) 2399Pm	9	N/A	N/A	9	9	9	9
Moora (3) 2400Pm	9	N/A	N/A	9	9	9	9
Moora (3) 2401Pm	9	N/A	N/A	9	9	9	9
Moora (3) 2403Pm	9	N/A	N/A	9	9	9	9

1. Three plugs of agar containing hyphae, from each of three replicate cultures per treatment were incubated on fresh CMA and examined for growth after 1-4 days.

In an unrepeated experiment, we infected phosphonate-treated plant baits with *P. megasperma* (biotype 1) and *P. cinnamomi* and attempted to re-isolate the fungi after seven days. Both

species produced copious numbers of sporangia in all treatments. *P. megasperma* was recovered less frequently from phosphonate-treated *E. sieberi* baits than from untreated baits (Figure 4). Similar results were obtained for *P. cinnamomi* using two different types of bait. Although the results of these experiments are inconclusive, zoospores of either species appear less able to infect treated than untreated plant tissue. This suggests that treatment of infected plant communities with phosphonate could reduce the autonomous spread of the pathogen.

Figure 4. Proportion of phosphonate-treated baits from which *Phytophthora* was recovered after seven days' incubation in the presence of zoospores of *P. megasperma* or *P. cinnamomi*.



According to Komorek (pers. comm.) material costs (including aircraft hire) for double application of phosphonate by air would be about $500 per hectare. It may be that such high costs, and the continued need for follow up applications would limit use of the chemical to plant species or communities of high conservation value.

Tables 13 and 14 list the Declared Rare Flora (DRF) and Priority plant species in Moora District and FRNP that may be at risk of infection by *P. megasperma*. In deriving these lists, particular attention was paid to the type of habitat in which the species were located together with details of associated vegetation. These species are not equally at risk of infection by *P. megasperma*. Recently some DRF in the FRNP were surveyed specifically to assess their

habitats for degradation by *P. megasperma*. Not all DRF could be located as assessments were made after the spring flowering period.

Plants of *Verticordia creba* (population 5) on the Twertup Track were located. This population appeared to be at risk of infection by *P. megasperma*. A drain directing water off the Twertup Track had been cut into the DRF population. Mature *Banksia quercifolia* and *Andersonia* sp. downslope of the drain, had died or were dying possibly due to infection by *Phytophthora*, although this was not verified. Soil samples from this site were tested and found to be negative for *Phytophthora*.

As the sites with *V. creba* (population 2) and *Adenanthos glabrescens* subsp. *exasperatus* (population 2) are in close proximity, they were assessed as a single site. Here, in the presence of healthy *Banksia* spp., seedlings of *Regelia* sp. and *Dryandra* spp. were dead or dying. Seedlings of *Regelia* sp. were tested for the presence of *Phytophthora* spp., as were three soil samples, but the fungi were not detected. The site appears to be *Phytophthora*- free, and since the hydrology of the habitat was not altered during road maintenance, we conclude that the risk of infestation with *P. megasperma* is low.

A site with *Comesperma lanceolatum* in a 12-year-old *Banksia baxteri* community was assessed. Some *B. baxteri* plants were dead or dying, but *Phytophthora* could not be detected in samples from the vegetation.

Populations 1A and 1B of *Adenanthos dobagii* occur some 300 m apart in low shrubland on sandy soil. The site supporting these populations had been recently burned and as the DRF were not in flower none of the plants could be located. Two dying *Dryandra quercifolia* plants and soil samples from this site, and another nearby location on Telegraph Track were tested for *Phytophthora* spp. but the fungi were not isolated.

Sites bearing *Verticordia pityrhops* populations 1A and 1B, *Stylidium galiodes* population 1 and *Adenanthos ellipticus* on the slopes of East Mt. Barren were also assessed. At these sites associated vegetation was rich in *Phytophthora*-susceptible species of the Proteaceae, and although high frequencies of mortality due to *P. megasperma* have been observed there (M. Grant pers. comm.), the habitats appear to be well drained and not threatened by extensive

Table 13. Declared Rare Flora (DRF) and Priority plant species which occur in the sand plain north of Perth (Western Australia) and which may be at risk from *P. megasperma*.

Species	Priority 1994	Recommended Priority 1996[1]	Districts[2]	Habitat and Associated vegetation
Presumed Susceptible to *Phytophthora*				
Andersonia gracilis	2	2	D	Sand over ironstone or seasonally damp sandy clay flats near swamps. With *Calothamnus* and *Verticordia*.
Calytrix chrysantha	2	3	Ca, I, TS	Sand over gravel in low woodland with *Banksia* and *Eucalyptus* species. Sometimes in seasonal swamps.
Calytrix eneabbensis	2	3	Ca, Co, I	Sand over laterite in low woodland with *Eucalyptus, Banksia, Adenanthos, Xylomelum* and *Hakea* species.
Conospermum densiflorum subsp. *Unicephalatum*	1	1 DRF	VP	Low lying clay soil
Conospermum scaposum	1	1 DRF	D	Sand on clay in *Banksia* and *Melaleuca* heath. Seasonal wet areas or gentle slopes above drainage lines.
Darwinia acerosa	DRF	DRF	VP	Rocky soil on and near granite outcrops.
Daviesia debilior subsp. *debilior*	2	2	Ca, Co, D	Shallow sand over lateritic gravel or clay in low heath.
Dryandra mimica	DRF	DRF	VP	Sand in low, open *Banksia* woodland.
Dryandra platycarpa	2	4	Ca, Co, D	Flat sandy sites on hilltops or in swampy areas. With *Adenanthos, Xylomelum, Hakea* and *Banksia* species.
Dryandra pteridifolia subsp. *vernalis*	3	2	Co, D	Sand over lateritic gravel in low heath with *Dryandra, Eucalyptus,* and *Hibbertia* species.
Dryandra stricta	1	3	Ca, Co, D, TS	Lateritic rises in sand or clay in scrub. Low damp areas.
Grevillea althoferorum	1	1 DRF	Ca	Sand and gravelly loam on low rises or in low heath with *Grevillea, Banksia, Lambertia, Hibbertia* and *Jacksonia*.
Grevillea biformis subsp. *cymbiformis*	2	1	Ca	Sand in low heath with *Grevillea, Verticordia, Hakea* and *Jacksonia* species.
Grevillea curviloba	1	1 DRF	Ca, D	Swan region in sand or loam in winter-wet areas in heath or open woodland.
Grevillea delta	1	2 DRF	Co, D	Loamy clay or gravel over sedimentary rock along seasonal drainage lines. With *Banksia tricuspis*.
Grevillea synapheae subsp. *pachyphylla*	2	1 DRF	D	Sand above creeks, gravelly lateritic rises in low woodland with *Eucalyptus* and *Allocasuarina* species.
Grevillea uncinulata subsp. *florida*	3	1 DRF	VP	In heath , sometimes beneath *Banksia* or *Dryandra* woodland in sand and lateritic gravel.

Hensmania stoniella	2	3	Ca, Co, D	Sand or peaty sand over clay or laterite in low *Banksia* woodland.
Hypocalymma serrulatum	2	3	D	Sand over clay in *Banksia* heath or woodland, often in drainage lines or low damp areas.
Hypocalymma tetrapterum	2	3	D	Sandy loam over clay. Creeklines in marri, jarrah, wandoo woodland.
Hypocalymma xanthopetalum var. *linearifolium*	2	2	Ca, D	Sand on laterite in heath. Can be with *Banksia* scrub.
Leucopogon glaucifolius	2	3	D, I	Sand in low *Banksia* and *Eucalyptus* woodland.
Lysinema elegans	2	3	D	Edge of damp depressions on sand in low *Banksia* woodland with *Adenanthos*, and *Jacksonia* species.
Malleostemon sp. Cooljarloo	1	1	D	Sand over gravel and clay in low winter-wet areas with low scrub of *Banksia, Verticordia* and *Astartea* species.
Monotoca leucantha	2	3	Ch, VP	Low rises or ridge crests with quartzite gravel in shrub mallee with *Dryandra, Eucalyptus* and *Melaleuca* species.
Patersonia spirafolia	2	2 DRF	D	On sand over laterite in low heath.
Persoonia filiformis	2	2	Ca, D	On sand over lateritic gravel in low open heath, sometimes on the upper slopes of mesas.
Verticordia argentea	1	2	Ca, Co	Sand over loam in open shrubland with *Verticordia, Eucalyptus* and *Banksia* species
Verticordia blepharophylla	2	2	Ca, Co, D	Sand and gravel in swampy areas and drainage lines with *Anigozanthos, Banksia, Eucalyptus,* and *Melaleuca* spp.
Verticordia fragrans	1	3	Ca, D	Sand with lateritic gravel or clay loam. Low woodland with *Eucalyptus, Banksia* and *Verticordia* species.
Verticordia luteola	1	1	VCL	Sand in low heath with low woodland of *Eucalyptus, Banksia, Allocasuarina,* and *Jacksonia* species.
Unknown Susceptiblity to *Phytophthora*				
Dampiera tephrea	1	1 DRF	D, I, TS	Heath or low open woodland in sandy clay or loam.
Phlebocarya pilosissima subsp. *teretifolia*	1	2 DRF	D	Lateritic sand in low shrubland with *Banksia* and *Eucalyptus* species in valleys
Scaevola eneabba	1	1 DRF	Co	Sand heath.
Tetratheca remota	1	1 DRF	D	Sand and lateritic gravel in open heath with *Hakea, Lambertia*, and *Calothamnus* species & sedges.

1. These recommendations were made by Patrick and Brown (1996).
2. Shire abbreviations for the Moora District: Ca - Carnamah Shire, Ch - Chittering Shire, Co - Coorow Shire, D - Dandaragan Shire, I - Irwin Shire, TS - Three Springs Shire, VCL - Vacant Crown Land, VP - Victoria Plains Shire.

Table 14. Species in the Fitzgerald River National Park that may be at risk from *P. megasperma*.

Species	Priority 1994	Recommended Priority 1995[1]	No. Popl[ns] & Shire[2]	Habitat and Associated Vegetation
Presumed Susceptible to *Phytophthora*				
Adenanthos dobagii	DRF	DRF	2 Alb	Sandy soil in low shrubland.
Adenanthos ellipticus	DRF	DRF	4 Rav, Jer	Shallow siliceous soil over granite outcrops in dense scrub. Population in a gully.
Adenanthos glabrescens subsp. *Exasperatus*	3	3	3 Rav, Jer	In gravel and on rocky hill slopes in open scrub.
Andersonia echinocephala	3	3	1 Gno	Slopes. Shallow sandy, rocky soil with dense scrub.
Astroloma micophyllum	2	2	Widespread	Mallee scrub in sandy gravelly soils.
Grevillea fistulosa	2	2	4 Rav	Endemic to FRNP. On open heathland scrub in sand.
Leucopogon denticulatus	2	2	2 Gno, Jer	Open heath on clayey sand.
Montoca sp.	1	1	1 Jer	Open shrub mallee in shallow rocky quartzite soil.
Sphenotoma drummondii	3	3	1 Rav	Summits and slopes of rocky peaks. Shallow soil over granite or quartzite in woodland or montane heath.
Verticordia creba	DRF	DRF	3 Rav, Jer	Red loam over decomposed spongolite in open scrub and mallee along drainage lines.
Verticordia helichrysantha	DRF	DRF	1 Rav	Shallow sand over lateritic gravel over spongolite in low open heath close to the coast.
Verticordia pityrhops	2	2 DRF	1 Rav	Wave cut platform in shallow soil over quartzite in scrub.
Unknown Susceptibility to *Phytophthora*				
Astroloma sp.	2	2	1 known	Mallee heath in clay over lateritic clay.
Chorizema trigonum	3	3	6 Rav	Coastal mallee heath or shrubs on sandy clay.
Comesperma lanceolatum	2	2	1 Rav	*Banksia baxteri* shrubland on slope in sand.
Coopernookia georgei	DRF	DRF	4 Rav	Shallow siliceous soil over quartzite in rocky gullies with thick scrub.
Dodonea trifida	3	3	2 Jer	Red loam over laterite or spongolite.
Eucalyptus acies	3	4	2	Loam and sand over quartzite in mallee scrub.
Eucalyptus chrysantha	2	2	4 Rav	Shallow soil over quartzite outcrops.
Gastrolobium parvuliflorum	3	3	4 Rav, Jer	Course sandy soil over spongolite, or loam over granite, usually in river beds with shrubs.
Gonocarpus hispidus	1	1	1 Rav	Summit and slopes in shallow, stony soil over quartzite.
Grevillea infundibularis	DRF	DRF	2 Rav	Shallow soil amongst quartzite boulders with scrub.

Hakea hookeriana	2	2	2 Rav	Shallow rocky quartzite soil with mallee and scrub.
Leptospermum confertum	2	2	2 Rav	Well drained quartzite sand on exposed wave cut platform with closed heath.
Melaleuca coccinea subsp. *penicula*	2	2	2 Rav, Jer	North-central FRNP. Sandy loam over clay and granite with open scrub.
Melaleuca pomphostoma	3	3	3 Rav	Moderately drained clay loam in *Eucalyptus* woodland.
Stylidium galiodes	DRF	DRF	3 Rav	Mountain peaks in shallow soil amongst quartzite rocks with *Eucalyptus* scrub and mallee.
Verticordia longistylis	3	2	1 Rav	A rocky spongolite platform above the Fitzgerald River valley with *Banksia* and *Hakea* scrub.

1. These recommendations were made by Robinson and Coates (1996).
2. Shire abbreviations for the Fitzgerald River National Park in the Albany District: Alb - Albany Shire, Gno - Gnowangerup Shire, Jer - Jerramungup Shire, Rav - Ravensthorpe Shire.

infection . Further surveys to assess the possible impact of *P. megasperma* on DRF in the FRNP are planned for the immediate future (E. Hickman pers. comm.).

Podger (1968, 1972) observed that there was a marked tendency for dieback disease (caused by *P. cinnamomi*) to be most extensive in areas with a history of frequent or recent utilisation. Aerial photographs showed a strong association between the occurrence of dieback caused by *P. cinnamomi* and the presence of roadways. Where patches of dieback were located in areas remote from logging operations or roads there was evidence of the passage of vehicles or heavy equipment used in fire suppression, mining exploration, or firewood cutting. Consequently, Podger (1972) concluded that inoculum of *P. cinnamomi* was being dispersed with soil moved during road building and logging operations.

Podger *et al.* (1996) indicated that hygiene strategies which reduce the rate of vectored spread and establishment of new centres of infestation remain central to control of disease caused by *P. cinnamomi*. In their assessment, the principal element in the existing dieback management strategy (CALM Policy No.3;1991) is to prevent all spread of disease attributable to *Phytophthora* spp. Accordingly, prescriptions for hygienic practice during road repair and maintenance include demarcation of areas infested with *Phytophthora*.

We tested road surfaces in four CALM Districts for the presence of *P. megasperma* but the fungus was not detected in any of 382 samples (Table 15). An alarmingly high proportion (32%) of the road samples tested positive for *Phytophthora* spp. and isolations were obtained from all Districts. A small proportion (4.4%) of the samples from Dwellingup and Manjimup were positive for *P. cinnamomi* (Table 15).

Clearly, unsealed road surfaces can support populations of *Phytophthora* spp. and although *P. megasperma* was not recovered here, it is likely that the fungus would be present on some surfaces. Local Government and Main Roads records revealed that in Albany and Moora Districts there are about 5000 km of unsealed road surface (Table 16), and in Moora District 25% of *P.* megasperma and 38.5% of *P. cinnamomi* infection sites were associated with culverts or road drains (Table 9). It seems that hygienic practice along these roads has not been consistently effective.

Water points in the south-west of Western Australia are used for road construction and fire suppression. We tested water points and water bodies for the presence of *P. megasperma* in four CALM Districts.. A very high proportion (91%) of the water bodies were at some time positive for *Phytophthora*, and 17.1% were positive for *P. megasperma* (Table 10). Only water bodies from Albany and Moora Districts yielded *P. megasperma*.

Table 15. Recovery of *P. megasperma* and other species of *Phytophthora* from unsealed road surfaces in four CALM Districts.

CALM District	Season	No. Samples Tested	No. Samples -'ve for *Phytophthora*	No. Road Samples Positive (+'ve) for		
				P. megasperma	*P. cinnamomi*	*Phyt.* spp.[1]
Albany	Summer 1996-97	42	40	0	0	2
Dwellingup	Spring 1996	36	9	TBD[2]	5	24
Manjimup	Winter 1995	154	80	0	8	69
	Winter 1996	70	55	TBD	4	12
Moora	Winter 1996	80	66	0	0	14
Total		**382**	**250**	**0**	**17**	**121**

1. *Phyt.* spp.= species of *Phytophthora* other than *P. megasperma* and *P. cinnamomi*, i.e. principally biotypes of *P. citricola* and *P. cryptogea/drechsleri*.
2. TBD = To be determined isoenzymically. When examined microscopically, none of the *Phytophthora* isolates produced morphological characters consistent with *P. megasperma*.

Table 16. Total lengths of unsealed and sealed roads in stated areas.

CALM District	Area	Road Surface	
		Unsealed (km)[1]	Sealed (km)[2]
Albany			
	Jerramungup	1140.87	235.91
	Ravensthorpe	1335.08	232.0
Moora	Carnamah	497.52	287.63
	Coorow	675.09	256.72
	Dandaragan	1017.84	490.78
Totals		4666.40	1503.04
Albany	FRNP[3]	280.0	0.1

1. Unsealed refers to unformed, formed, and gravel roads.
2. Sealed refers to bituminised roads.
3. FRNP: Fitzgerald River National Park.

Podger *et al.* (1996) made several recommendations in regard to hygienic practices for control of dieback caused by *P. cinnamomi.* These were:

- that manuals pertaining to dieback hygiene be revised and promptly distributed.

- that terminology used in dieback management be revised, standardised and published.

- that progressive refinement of hygiene strategies be undertaken to reduce risk of infestation during forestry operations and road maintenance or construction.

- that CALM's research program give priority to applied projects related to management needs.

All of these recommendations may be applied to control of dieback associated with *P. megasperma.* However, management of dieback (*P. megasperma*) in the Albany and Moora Districts may be more difficult to coordinate and control than disease caused by *P. cinnamomi* in State Forest. Land in these Districts may be in a variety of public and private tenures. While both CALM and the Environmental Protection Authority (EPA) have regulatory powers which cover all tenures, Podger *et al.* (1996) observed that this is not always used in a coordinated manner.

To facilitate formal and coordinated control of disease caused by *P. cinnamomi* in Districts such as Albany and Moora, Podger *et al.* (1996) made the following recommendation:

- that joint planning and management arrangements be assisted by the establishment of regional coordination groups for industries and organisations that conduct extensive operations with a potential to disseminate the pathogen and spread disease. CALM should provide the necessary technical advice and support.

This recommendation may also apply to management of disease caused by *P. megasperma,* and Podger *et al.* (1996) cite The Northern Sand Plains Dieback Working Party as providing a useful model of a regional coordination group.

5 OUTCOMES

- Systematic relationships between biotypes of *P. megasperma* in the south-west of Western Australia, and reference biotypes of *P. megasperma* have been defined.

- Measures of variation in *P. megasperma* have been determined, and while Western Australian biotypes are allozymically quite invariant, it was shown that some biotypes vary both morphologically and in their response to phosphonate.

- Although the distribution of *P. megasperma* in the south-west of Western Australia appears to be disjunct, it has been recovered from five coastal CALM Districts. *P. megasperma* appears to be confined to coastal plains.

- Outcrossing events within *P.* megasperma biotypes in the field are probably rare, but still frequent enough to maintain some diversity and generate novel genotypes. Oospores probably play a significant role in the cycle of epidemics involving *P. megasperma* biotypes in Western Australia.

- *P. megasperma* is able to kill many species within the Proteaceae, but few hosts have been reported in other plant families.

- Spot infections by *P. megasperma* in *Banksia* woodland on draining soils are probably ephemeral and are characterised by isolated or very few deaths in proteaceous species. In and immediately adjacent to wetlands, *P. megasperma* is able to inflict a high degree of mortality on many susceptible species within the Proteaceae.

- On coastal plains, *P. megasperma* is likely to occupy all habitats available to it within decades. However, by comparison with the irreparable damage *P. cinnamomi* is anticipated to cause on the coastal plains, *P. megasperma* is likely to play only a lesser role.

- Phosphonate treatment is expected to contain the spread of *P. megasperma* based on the observed sensitivity of the fungus to the chemical.

- Phosphonate may control the autonomous spread of *P. megasperma* to uninfected susceptible species.

- Phosphonate may be fungicidal to some *P. megasperma* isolates.

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FINAL REPORT

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ENVIRONMENT AUSTRALIA

MAY 1997

Department of Conservation and Land Management
Locked Bag 104, Bentley Delivery Centre, W.A. 6983

Environment Australia
Biodiversity Group