Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia

Daniel HÜBERLI[1], Inez C. TOMMERUP[2], Mark P. DOBROWOLSKI[1], Michael C. CALVER[1] and Giles E. St J. HARDY[1]*

[1] *School of Biological Sciences and Biotechnology, Murdoch University, Perth WA 6150, Australia.*
[2] *CSIRO, Forestry and Forest Products, PO Box 5, Wembley WA 6913, Australia.*
E-mail: g-hardy@central.murdoch.edu.au

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Seventy-three isolates of *Phytophthora cinnamomi* were collected from diseased *Eucalyptus marginata* (jarrah) and *Corymbia calophylla* (marri) trees in two forest communities in the southwest of Western Australia. Both populations of *P. cinnamomi* were examined for phenotypic and genotypic variation. Microsatellite DNA analysis showed that all isolates were of the same clonal lineage. We show, for the first time for *P. cinnamomi*, that morphological and pathogenic variation between populations of the clonal lineage are very broad and continuous. The phenotypes examined included growth rates and colony morphology on potato dextrose agar at different temperatures, sporangial and gametangial morphology, ability to form lesions in detached jarrah and marri stems, and ability to cause deaths of clonal jarrah plants in a glasshouse trial. Phenotype variation was derived asexually. All phenotypes investigated varied independently from one another. Cluster analysis of 24 morphological and pathogenicity phenotypes identified two main clusters of isolates corresponding to each population. The ability to cause deaths in both populations ranged from killing all plants within 59 d to plants being symptomless 182 d after inoculation.

INTRODUCTION

No other soilborne pathogen has had such a large impact worldwide and over such a large range of plant hosts as *Phytophthora cinnamomi*. In Australia, this heterothallic pathogen has caused extensive destruction in many natural ecosystems to which it has been introduced, particularly in the southwest of Western Australia (WA) (Wills 1993, Shearer & Dillon 1995). It has been estimated that about 2000 of the 9000 locally indigenous plant species are susceptible to the pathogen in WA (Wills 1993). The high susceptibility of the dominant hardwood tree species, *Eucalyptus marginata* (jarrah) is a major concern. Interestingly, the codominant tree species in the jarrah forest, *Corymbia calophylla* formerly *E. calophylla* (marri), generally survives on infected sites (Podger 1972).

Despite the worldwide impact of *P. cinnamomi*, our understanding of phenotypic and genotypic variation among isolates in populations is poor. To manage and control this disease successfully, it is important to know the amount of phenotypic variation within a pathogen population. When *P. cinnamomi* was first described, Rands (1922) showed that isolates varied in pathogenicity in stem-inoculated *Cinnamomum burmanni* trees. Since then there have been numerous reports on pathogenicity, macro-morphological (colony type and growth rate) and micro-morphological (sporangial, gametangial and chlamydospores) variation among worldwide (Galindo & Zentmyer 1964, Zentmyer *et al*. 1976, Zentmyer 1980), national (Haasis, Nelson & Marx 1964, Chee & Newhook 1965, Shepherd & Pratt 1974, Dudzinski, Old & Gibbs 1993) and regional (Shepherd, Pratt & Taylor 1974) collections of isolates. Some of the studies with *P. cinnamomi* indicated the existence of host specialisation in pathogenicity (Zentmyer 1980, Zentmyer & Guillemet 1981).

Prior to 1984, the phenotypic studies of *P. cinnamomi* did not use isozyme techniques and hence were genetically structured only on the basis of mating types. Subsequently, it was shown that the Australian *P. cinnamomi* population consists of two isozyme genotypes for the A2 mating type and one for the A1 mating type, with the most common being the A2 isozyme type 1 (Old, Moran & Bell 1984, Old, Dudzinski & Bell 1988). These isozyme genotypes represent a small component of worldwide variation (Old *et al*. 1984, Oudemans & Coffey 1991) and correspond to three separate clonal lineages as shown by analysis with microsatellite loci (Dobrowolski 1999). Low levels of isozyme variation and the lack of recombination in microsatellite loci indicated that they were asexually reproducing (Old *et al*. 1984, 1988, Dobrowolski 1999). This was also the conclusion for South African isolates (Linde *et al*. 1997), although their isozyme system was different to the Australian one.

* Corresponding author.

Table 1. Details of the 73 *Phytophthora cinnamomi* isolates used in the study collected from two sites in the *Eucalyptus marginata* (jarrah) forest of Western Australia. Microsatellite variants are shown for four loci tested. Isolate rankings from the largest (rank 1) to the smallest (rank 73) mean values of growth rate at different temperatures, lesion size after 6 d in detached jarrah and *Corymbia calophylla* (marri) stems, and survival of a resistant jarrah clonal line underbark inoculated are shown.

Isolate details				Rank			
					Lesions in detached stems[c]		
	Pit No.	Year isolated	Microsat. genotype[a]	Growth on PDA[b]	Jarrah	Marri	Plant survival[d]
Jarrah site 1 isolates (J1)							
MP94	H3718	1993	cg	31	33–35[e]	16–45	4
MP62	Y	1992	4	41	2	1	68
MP97-07	4	1997	cg	46	—[f]	—	28
MP98	H3717	1993	6	50	22–23	16–45	37
MP127	H3722	1993	1	52	14–15	15	34
MP97-15	6	1997	cg	53	—	—	51
MP97-09	4	1997	cg	58	—	—	21
MP97-16	13	1997	cg	60	—	—	29
MP97-11	6	1997	cg	62	—	—	52
MP97	H3718	1993	cg	65	36	6–7	59
MP96	H3718	1993	cg	67	40	16–45	71–73
MP97-12	6	1997	cg	68	—	—	1
MP27	X	1981	cg	69	7	14	64
MP61	Y	1992	4	70	4	16–45	61
MP95	H3718	1993	cg	71	33–35	16–45	70
MP97-08	4	1997	4	72	—	—	71–73
Marri site 1 isolates (M1)							
MP81	H3718	1993	cg	3	—	—	7
MP87	H3717	1993	cg	4	19	4	71–73
MP99	H3718	1993	cg	9	16	16–45	3
MP83	H3718	1993	cg	12	—	—	35
MP82	H3718	1993	cg	14	11	11	20
MP101	Z	1993	cg	15	41	9	65
MP84	H3717	1993	cg	20	39	16–45	23
MP100	Z	1993	cg	24	43–45	16–45	40
MP80	H3718	1993	cg	27	42	3	13
MP92	H3718	1993	cg	29	31	16–45	43–44
MP89	H3718	1993	cg	32	37–38	16–45	54
MP112	H3718	1993	cg	35	43–45	16–45	45
MP90	H3718	1993	cg	40	—	—	66
MP110	H3718	1993	cg	43	29	16–45	24
MP88	H3718	1993	cg	44	26	16–45	8
MP85	H3713	1993	cg	45	—	—	36
MP91	H3718	1993	cg	47	25	16–45	15
MP102	Z	1993	cg	54	33–35	16–45	22
MP111	H3718	1993	cg	55	37–38	16–45	63
MP86	H3717	1993	cg	61	14–15	16–45	6
Jarrah site 2 isolates (J2)							
MP94-03	238	1994	cg	2	20	16–45	5
MP94-32	232	1994	cg	5	—	—	17–18
MP94-01	238	1994	cg	6	10	16–45	33
MP94-48	232	1994	cg	7	—	—	58
MP94-27	238	1994	cg	8	—	—	53
MP94-11	228	1994	cg	11	8	13	10
MP94-04	238	1994	cg	13	5	10	55
MP94-47	232	1994	cg	19	—	—	30
MP94-25	238	1994	cg	21	—	—	49
MP94-39	232	1994	cg	23	—	—	26
MP94-10	228	1994	cg	25	9	2	9
MP94-19	239	1994	cg	26	—	—	43–44
MP94-30	238	1994	cg	28	—	—	2
MP94-40	232	1994	cg	30	—	—	19
MP94-26B	238	1994	cg	33	—	—	31
MP94-49	232	1994	cg	34	—	—	56
MP94-15	228	1994	2	36	3	6–7	41
MP94-26A	238	1994	cg	37	—	—	38
MP94-22	239	1994	3	38	—	—	12

Table 1 (*cont.*)

Isolate details				Rank			
					Lesions in detached stems[c]		
Isolate No.	Pit No.	Year isolated	Microsat. genotype[a]	Growth on PDA[b]	Jarrah	Marri	Plant survival[d]
MP94-09	228	1994	cg	39	12–13	16–45	39
MP94-42	232	1994	cg	49	17	16–45	17–18
MP94-13	228	1994	2	57	21	16–45	42
MP94-37	232	1994	cg	59	—	—	69
MP94-17	228	1994	2	63	—	—	25
MP94-05	238	1994	2	64	28	16–45	57
MP94-12	228	1994	2	66	6	16–45	11
MP94-20	239	1994	2	73	—	—	67
Marri site 2 isolates (M2)							
MP94-33	232	1994	cg	1	22–23	16–45	16
MP119	W	1993	cg	10	24	8	27
MP94-18	D5416	1994	cg	16	18	5	60
MP116	W	1993	cg	17	32	16–45	48
MP71	D5416	1993	cg	18	1	12	50
MP115	W	1993	cg	22	43–45	16–45	32
MP94-28	232	1994	cg	42	—	—	46
MP118	W	1993	5	48	30	16–45	47
MP114	W	1993	cg	51	12–13	16–45	62
MP72	E5406	1993	cg	56	27	16–45	14

[a] Microsatellite genotypes: cg, common genotype; genotype 1-6 (see text for details).

[b] Growth rates (mm d^{-1}) ranked from fastest to slowest at 28 ° then at 24 ° and then 20 °C.

[c] Lesion lengths (mm) in detached stems of jarrah and marri were ranked from longest to shortest. Only 45 isolates were tested. Those isolates that did not form lesions were ranked 43–45 in jarrah stems and 16–45 in marri stems.

[d] Deaths of jarrah ranked from lowest to highest percent survival at 60 d, then 120 d, and then 182 d. Those isolates that did not cause deaths or lesions were ranked 71–73.

[e] Ranks within a range are not different from one another.

[f] Not included in experiment.

Only two phenotypic studies have used *P. cinnamomi* populations of defined isozyme genotypes (Dudzinski *et al*. 1993, Linde, Kemp & Wingfield 1999). The Australian study showed that isolates of *P. cinnamomi* vary widely in pathogenicity and this variation was not related to mating type or isozyme type (Dudzinski *et al*. 1993). Phenotypic variation in a large range of morphological and pathogenicity characters has not been examined simultaneously in a genetically structured study of *P. cinnamomi* populations.

Our initial work (Hüberli, Hardy & Tommerup 1997a) on two southwest WA populations suggested there were some differences in phenotypic variability between the two populations. The introduction of *P. cinnamomi* into these natural plant communities probably occurred with the first European settlers from between 70 to 150 years ago and strict hygiene measures were not implemented until 1980. Hence, there was a period of at least 50 years for the inadvertent movement and for phenotypic divergence of the pathogen. This study examines genotypic and phenotypic variation, including macro-morphological, micro-morphological and pathogenicity characters, among isolates of *P. cinnamomi* from two WA populations.

MATERIALS AND METHODS

Isolates

All 73 isolates of *Phytophthora cinnamomi* were collected from diseased marri and jarrah trees and plated directly onto NARPH, an agar selective for *Phytophthora* (Hüberli, Tommerup & Hardy 2000). The trees were growing in a mosaic of rehabilitated open-cut bauxite minepits at the Jarrahdale (site 1; 32.20 south, 116.04 east) and Willowdale (site 2; 32.55 south, 116.02 east) mines of Alcoa World Alumina Australia, located approx. 70 km apart. Thirty-six isolates were collected from site 1, within an 18 km radius, and 37 isolates were from site 2, within a 5 km radius. Jarrah and marri isolates from site 1 will be referred to as J1 and M1 isolates, and those from site 2 will be referred to as J2 and M2. Each of the four groups of isolates will be referred to as a sub-population. Most isolates were collected during 1992–97 and one in 1981 (Table 1). All 73 isolates were used in all experiments, except one, in which 45 isolates were used (Table 1). Isolates were maintained at 24 ± 1 °C in the dark on vegetable-8 juice (Campbell's Soups Australia) agar (V8A) (Hüberli, Tommerup & Hardy 1997b). Three weeks before pathogenicity experiments commenced, all isolates were repassaged through jarrah seedlings as a precautionary measure against loss of pathogenicity through continuous subculturing (Erwin & Ribeiro 1996). Repassaging involved inoculating seedlings separately with each of the isolates. The isolates were recovered from the lesion front after about 1 wk by plating onto NARPH, cultured on V8A and used within 2 wk.

Isolates used in the study are stored in the culture collection of G. Hardy.

Microsatellite loci analysis

All isolates were the A2 mating type as described by Hüberli *et al*. (1997a, b). For microsatellite loci analysis, isolates were grown in V8 broth, and DNA extracted and analysed with four microsatellite loci (d39, e16, g10 and g13) as described by Dobrowolski (1999).

Macro-morphological phenotypes

Inoculum discs of 5 mm diam were cut with a sterile cork borer from the colony margins of 5-d-old cultures grown on 10 ml of potato dextrose agar (PDA) (Gibco BRL) and transferred, mycelial side down, to the centre of individual Petri-plates (9 cm diam) containing 10 ml of PDA. Plates were sealed with Parafilm (American National Can, Chicago) and incubated at 20, 24, 28 or 32 ± 1 ° in the dark. There were four replicate plates per isolate-temperature treatment. A preliminary study using ten isolates showed that at 12–16 ° growth was slow with no significant differences among isolates. Therefore, radial growth was measured after 4 d at 20, 24 and 28 ° along two lines intersecting at right angles at the centre of the inoculum disc. The radial growth rate (mm d^{-1}) was calc. by taking the average of all radial measurements, subtracting the inoculum disc radius and dividing by four. Colony types of each isolate at each temperature were recorded as rosaceous, petaloid or no-pattern (Erwin & Ribeiro 1996: 110). After 16 d at 32 °, hyphal growth was very sparse and non-uniform, precluding radial growth measurements. These plates were transferred to a 24 ° incubator for up to 14 d to determine whether the 32 ° had caused stasis (defined by growth at 24 °) or death (defined by no growth at 24 °) of the cultures.

Micro-morphological phenotypes

Sporangia

Sporangia were produced aseptically using the method described by O'Gara *et al*. (1997) prior to the stage of cold-shocking the hyphal-mats. Mycelial strands were separated in water and examined at 400 × magnification for sporangia, with two replicate flasks per isolate. The length and breadth of 15 intact primary sporangia per flask were randomly selected and measured, and the length : breadth (L : B) ratio calc. Sporangial shape was recorded using the descriptors of Erwin & Ribeiro (1996).

Oogonia, oospores and antheridia

Isolates were paired individually with an A1 mating type isolate (3266, provided by M. Stukely) on V8A and incubated for 14 d at 24 ± 1 ° in the dark. There were two replicate plates per isolate pairing. For each isolate pairing, the diam of 15 oogonia and their oospores were measured per plate, and the number of paragynal associations for 100 randomly selected oogonia per plate were counted, as described by Hüberli *et al*. (1997b).

Disease and pathogenicity phenotypes

Capacity to form lesions in detached jarrah and marri stems

A total of 45 isolates was screened in three separate trials, ensuring that all detached jarrah and marri stems were inoculated within 24 h of being cut from the tree (Table 1). Isolates MP119 and MP127 were selected randomly as a representative from each mine and were included in each trial as standard isolates. Each host-isolate combination was replicated three times in the first trial and six times in the remaining two trials.

During spring, 1-yr-old green stems were cut from non-clonal jarrah and marri trees from the Jarrahdale minesite at dawn to minimise water-deficits. All side branches and leaves were immediately removed in the field, and the stems were transported to the laboratory in moist hessian bags in insulated containers. Stems with a mean diam $\pm$ SEM of 6.5 ± 0.4 mm for jarrah and 6.2 ± 0.3 mm for marri were cut into 20 cm lengths, and surface-decontaminated with 70% ethanol. In order to reduce desiccation of stems, the freshly cut ends were covered immediately with moist cotton wool and plastic film squares (6 cm^2) which were held in place with rubber bands.

A sterile scalpel was used to cut a bark-flap, about 15 mm long and 10 mm wide, at the centre of each stem, through the epidermis to the phloem. A 5 mm diam agar disc, cut from the margin of a 3-d-old culture growing on V8A, was inserted mycelium-side-down under the flap, the flap closed and the wound sealed with Parafilm. Controls were inoculated with sterile V8A discs. The tissue in contact with the inoculum disc will be referred to as the site of inoculation for all underbark wound-inoculations. Stems were incubated at 24 ° in the dark in disinfected plastic trays ($39 \times 27 \times 6$ cm) lined with moist paper towels, and sealed in plastic bags.

A preliminary experiment showed that 8 d after inoculation the rate of lesion extension began to increase more rapidly, probably as a result of stem senescence. Therefore, the total length of each externally visible lesion that extended above and below the site of inoculation was measured daily for 6 d. After the final measurement, two randomly selected stems per isolate were plated onto NARPH to confirm the presence of *Phytophthora cinnamomi*. Lesion length increments varied daily, thus the data are presented as lesion lengths at 6 d.

Capacity to cause deaths of clonal jarrah plants

This experiment was a completely randomised block design of clonal jarrah plants, underbark wound-inoculated with all 73 isolates of *Phytophthora cinnamomi*. The trial was conducted in an evaporatively-cooled glasshouse and consisted of six blocks, with one replicate plant from all isolates and four control plants in each block.

One-year-old jarrah (line 77C40; Marrinup Nursery, Alcoa World Alumina Australia, WA) plants resistant to *P. cinnamomi* (I. Colquhoun, pers. comm.) were planted into 130 mm free-draining plastic pots (Hüberli *et al*. 2000). All plants were acclimatised to the glasshouse for 3 wk prior to inoculation and hand watered daily. The average min. and max. glasshouse temperatures for the duration of the experiment were 20 ° and 29 °, respectively.

Sterile Miracloth (Calbiochem Corporation, CA) discs, 10 mm diam, were placed onto V8A (30 per plate). Each plate was inoculated with a *P. cinnamomi* isolate. Plates were incubated in the dark at 24 ° for 10 d. Plants were underbark wound-inoculated as described previously, but Miracloth discs were used as the inoculum. The bark-flap was cut upwards at approx. 5 cm above the potting medium surface. Controls were inoculated with sterile Miracloth discs.

Survival of plants was monitored daily. Plants were scored as dead when all leaves were crisp and dry as resprouting can occur from plants judged visually as moribund. Dead plants were harvested and seven 1 cm sections from above the site of inoculation were plated onto NARPH. At 26 wk, all surviving plants were harvested after measuring the distal lesion lengths from the middle of the site of inoculation upwards. Where lesions were present, stems were cut into 1 cm sections, from 1 cm into the lesion front to 6 cm up the stem and then cut longitudinally to expose the bark and wood to the NARPH medium. Visually symptomless stems were plated as above, from the site of inoculation for 6 cm up the stem. All stem sections were plated sequentially onto NARPH, incubated at 24 ± 1 ° for 14 d and the recovery of *P. cinnamomi* examined every 2 d. Stem sections were removed from those plates from which *P. cinnamomi* was not recovered, and a leaching technique was used to obtain further recoveries (Hüberli *et al*. 2000). The recovery of *P. cinnamomi* in advance of lesions is referred to as extension beyond the lesion (EBL). Colonisation refers to the total amount of stem invaded by the pathogen, which in symptomatic stems includes the lesion length and the EBL, while in symptomless stems includes only the EBL.

Data analysis

Data for parametric tests were screened for assumptions of homoscedasticity, presence of outliers, normality and non-correlations of means and variances, while all proportions were angular transformed prior to analysis. All significant main effects and interactions were compared using the Least Significant Difference test ($P = 0.05$).

Colony data were tabulated into a three-way contingency table using the variables of colony type (no-pattern, petaloid and rosaceous), temperature (20, 24 and 28 °) and isolate sub-population (J1, M1, J2 and M2). Frequencies of sporangia shapes (nine shapes excluding pyriform, see Table 2) were cross-tabulated by isolate sub-population (J1, M1, J2 and M2). Pyriform sporangia were excluded from the analysis because only one observation was made for this shape.

Lesion lengths for the standard isolates in the detached stem experiment did not vary significantly ($P > 0.30$) among the three trials and so the three trials were analysed together. The data deviated from normality and could not be corrected by transformation, so the analysis used Kruskal–Wallis Analysis of variance (ANOVA). Where there were differences, pairwise comparisons between isolate sub-populations were made using Kruskal–Wallis ANOVA. Survival times and the 95% confidence intervals were estimated for the glasshouse pathogenicity experiment.

Rank correlations were investigated among all morphological and pathogenicity characters separately for each isolate sub-population using the Spearman rank *r*. Significance of each correlation was tested at $P = 0.005$ using a Bonferroni correction to ensure that the significance level across all tests was $P = 0.05$. The interactions among isolate sub-populations *r* were investigated using the test for comparing more than two *r* (Zar 1999). Interactions that were significant were further analysed using the Tukey-type multiple comparison test (Zar 1999).

Numerical taxonomic analyses were carried out using cluster analysis with Ward's method as the agglomerative strategy and the squared Euclidean distance as the distance measure. The data set comprised of 24 variables including radial growth rates and colony types at 20, 24 and 28 ° (6 variables), sporangial dimensions (3 variables) and shapes (9 variables), oospore and oogonial dimensions (2 variables), paragyny proportions (1 variable), lesion lengths in detached marri and jarrah stems (2 variables), and mean survival time (1 variable).

RESULTS

Microsatellite loci analysis

Microsatellite analysis showed that all isolates were members of the A2 type 1 clonal lineage as described by Dobrowolski (1999). All the phenotypic variation described in this paper occurred in one clonal lineage of *Phytophthora cinnamomi*. There was no evidence for sexual reproduction by either selfing or outcrossing among the isolates. All 73 isolates were identical at two (e16 and g10) of the four loci. A total of 60 isolates were identical at all 4 loci examined and these are referred to as the common genotype as described by Dobrowolski (1999). Their genotype was d39 (122/136), e16 (169/171), g10 (118/154) and g13 (158/178/184), with microsatellite allele sizes given in base pairs. Microsatellite mutation at two loci (d39 and g13) and mitotic recombination at locus g13, produced six additional genotypes represented by 13 isolates (Table 1). These differed from the common genotype as follows: genotype 1, d39 (122/**132**); genotype 2, d39 (122/**140**); genotype 3, d39 (122/**140**) g13 (—/178/184); genotype 4, g13 (158/**176**/184); genotype 5, g13 (158/**180**/184); and genotype 6, g13 (158/178/**186**). Genotype 2 was prevalent at site 2, which also had genotypes 3 and 5 (Table 1). Genotype 3 probably arose by mitotic recombination from genotype 2. The other three variant genotypes were found only at site 1 (Table 1). Genotypes 2 and 4 were found in more than one minepit and they were represented by six and three isolates, respectively.

Macro-morphological phenotypes

Radial growth rates

Growth rates for all isolates were lower ($P < 0.001$) at 20 ° than at 24 and 28 °. Only J2 isolates had significantly ($P = 0.004$) increased growth rates between 24 and 28 °, while for the remaining isolates there were no differences between the two temperatures. The J1 isolates had lower ($P < 0.008$) growth rates at 24 and 28 ° compared to the other isolates,

and were the only group of isolates that had a growth optimum at 24 ° (Table 2). Most of the isolates from the other three sub-populations had a growth optimum at about 28 °. At 20 °, J1 isolates had reduced ($P < 0.005$) growth rates in comparison to M1 and M2 isolates (Table 2).

Growth rates at 20 ° were correlated with those at 24 ° for M1 and J2 isolates ($r > 0.74$, $P < 0.001$). Strong correlations ($r > 0.83$, $P < 0.001$) were found for growth rates at 24 and 28 ° for all sub-populations except J1 isolates which had a significantly ($P < 0.01$) lower r compared to the other sub-populations. The correlation coefficient for growth rates at 20 and 28 ° for J1 isolates was lower ($P < 0.01$) in comparison to J2 isolates. Only for J2 isolates were growth rates at 20 ° and 28 ° correlated ($r = 0.75$, $P < 0.001$).

Growth of all isolates at 32 ° was very limited (< 0.5 mm), with no hyphae growing onto the agar. This temperature caused the death of 55 isolates in 16 d. The remaining 18 isolates regrew between 5 and 10 d after transfer to 24 °.

Colony types

The log-linear model which best fitted the data involved significant two-way interactions between colony type and temperature ($\chi^2_6 = 19.92$, $P < 0.005$) and between colony type and isolate sub-population ($\chi^2_9 = 45.31$, $P < 0.001$). J2 isolates had different colony proportions at 20 and 28 ° in comparison to all isolate sub-populations, while at 24 ° they were very similar to M2 isolates (Table 2). The three colony types were more evenly represented in each isolate sub-population at 24 ° in comparison to those at 20 and 28 ° (Table 2). At 24 and 28 °, rosaceous colonies for site 1 isolates were more predominant than for site 2 isolates and the site 2 isolates had a larger proportion of no-pattern colonies at 24 ° compared to the site 1 isolates. Site 2 isolates had more consistent colonies over all three temperatures than site 1 isolates (Table 2).

Micro-morphological phenotypes

Sporangia

Sporangial lengths of J2 isolates were smaller ($P < 0.009$) than the J1, M1 and M2 isolates, while for breadths only, there were no differences ($P = 0.09$) between the sub-populations (Table 2). The site 1 isolates had larger ($P < 0.02$) L:B ratios compared to site 2 isolates (Table 2). The J2 and M2 isolates were significantly different ($P = 0.003$) from one another. Sporangia lengths and breadths were significantly correlated ($r > 0.59$; $P < 0.005$) for all sub-populations except M2 isolates.

All isolates formed non-papillate sporangia. Of the eleven sporangial shapes recorded, the dominant one was ovoid (57–74%) followed by limoniform (13–20%), ellipsoid (5–8%) and obpyriform (2–8%) (Table 2). All isolate sub-populations had less than 2.5% of conical, globose, cylindrical, distorted and obovoid sporangia. Contingency table analysis revealed an association ($P = 0.001$) between sporangia shapes and isolate sub-populations. Site 1 isolates had the lowest frequency of ovoid (57–58%) sporangia, and had the highest of limoniform (19–20%), ellipsoid (7–8%), obpyriform (7–8%) and obovoid (1–2%) sporangia compared to site 2 isolates (Table 2).

Oogonia, oospores and antheridia

There were no differences ($P > 0.39$) among the isolate sub-populations in oogonia and oospore diam and paragyny percentage (Table 2). Oospore and oosphere diam for all sub-populations were strongly correlated ($r > 0.97$; $P < 0.001$). All isolates had some paragynous antheridial associations and these were always in conjunction with amphigynous antheridia. There were ten isolates that had paragynous association averages of more than 30%, with MP97-07 (J1) and MP62 (J1) having as many as 42% and 48.5%, respectively.

Disease and pathogenicity phenotypes

Capacity to form lesions in detached jarrah and marri stems

Most isolates formed lesions within 3 or 4 d after inoculation in jarrah and marri stems, respectively, and *Phytophthora cinnamomi* was always isolated from the lesions. Isolates MP100 (M1), MP112 (M1) and MP115 (M2) did not form lesions in either host, however, the pathogen was always recovered from the site of inoculation. An additional 27 isolates failed to produce lesions in marri stems and *P. cinnamomi* was recovered from the site of inoculation (Table 1). Correlations of lesions in jarrah stems with marri stems were not significant for all sub-populations ($r < 0.68$, $P > 0.02$). Lesions in jarrah stems were always longer ($P < 0.001$) than those in marri stems for the four isolate sub-populations (Table 2). For isolates MP80 (M1), MP97 (J1), and MP101 (M1) lesion lengths in jarrah and marri stems were not different ($P > 0.05$). The M1 isolates formed smaller ($P < 0.04$) lesions in jarrah stems compared to the other three isolate sub-populations. In marri stems, there were no differences ($P = 0.66$) in lesion lengths among isolate sub-populations. Controls never had lesions and *P. cinnamomi* was never recovered from these stems.

Capacity to cause deaths of clonal jarrah plants

The first plant death occurred 6 d after inoculation for isolate MP94-11 (J2). The J1 isolate sub-population had a lower ($P < 0.05$) capacity to cause deaths compared to the other three sub-populations over the 26 wk duration of the experiment (Fig. 1). This distinction between the J1 and the other sub-populations was significant ($P < 0.05$) at 70 d after inoculation (Fig. 1).

Isolates with the highest capacity to cause deaths were MP97-12 (J1) and MP94-30 (J2) (Table 1). They killed all plants within 59 d. Isolates MP96 (J1), MP97-07 (J1) and MP87 (M1) did not cause any deaths and were considered nonpathogenic in the conditions tested (Table 1). Of these, MP96 (J1) and MP97-07 (J1) did not form lesions and *Phytophthora cinnamomi* was not recovered from these stems. Isolate MP87 (M1) did not produce lesions, however, one replicate had an EBL of 10 mm. The remaining 68 isolates, including four which killed all plants within 106–177 d, were

Table 2. Macro- and micro-morphological, and pathogenicity phenotypes of 73 isolates of *Phytophthora cinnamomi* from four sub-populations in the *Eucalyptus marginata* (jarrah) forest of Western Australia. Pathogenicity phenotypes are for underbark inoculations of detached jarrah and *Corymbia calophylla* (marri) stems with 45 isolates, and clonal plants of jarrah in the glasshouse with 73 isolates. Sub-populations were J1, M1, J2 and M2 isolates as explained in Table 1. Row values followed by the same letter are not significantly different ($P = 0.05$).

	P. cinnamomi isolate sub-populations			
Phenotype	J1 ($n = 16$)	M1 ($n = 20$)	J2 ($n = 27$)	M2 ($n = 10$)
Macro-morphology				
Growth on PDA				
Mean ± SEM growth rate (mm d^{-1})				
20 °	3.7 ± 0.1 a	4.0 ± 0.1 b	3.9 ± 0.1 ab	4.1 ± 0.1 b
24 °	5.1 ± 0.1 a	5.7 ± 0.1 c	5.4 ± 0.1 b	5.6 ± 0.1 c
28 °	4.9 ± 0.1 a	5.8 ± 0.1 b	5.7 ± 0.1 b	5.9 ± 0.1 b
Colony type (%) at:				
20 ° No-pattern	6	15	37	10
Petaloid	75	65	63	60
Rosaceous	19	20	0	30
24 ° No-pattern	12	22	44	40
Petaloid	38	28	33	30
Rosaceous	50	50	22	30
28 ° No-pattern	0	11	33	10
Petaloid	69	67	52	80
Rosaceous	31	22	15	10
Colony type consistency (%)				
Same at all temp.	25	28	44	40
Different at two temp.	75	67	52	60
Different at all temp.	0	5	4	0
Micro-morphology				
Sporangia (mean ± SEM)				
Length (μm)	57.4 ± 0.6 b	58.0 ± 0.5 b	55.2 ± 0.3 a	57.3 ± 0.7 b
Breath (μm)	35.0 ± 0.3 a	35.7 ± 0.3 a	35.9 ± 0.2 a	36.0 ± 0.4 a
Length:breadth ratio	1.65 ± 0.01 c	1.64 ± 0.01 c	1.56 ± 0.01 a	1.61 ± 0.02 b
Shapes (%)				
Ovoid	57.1 ± 3.8	58.0 ± 2.8	74.2 ± 3.1	64.7 ± 4.8
Limoniform	19.2 ± 3.7	20.3 ± 2.9	13.0 ± 1.7	18.3 ± 3.1
Ellipsoid	7.5 ± 1.3	7.2 ± 1.2	5.7 ± 1.1	5.0 ± 1.5
Obpyriform	7.9 ± 2.1	7.5 ± 1.4	2.3 ± 0.7	4.7 ± 2.2
Cylindrical	1.9 ± 0.6	1.2 ± 0.4	1.5 ± 0.5	2.3 ± 1.3
Distorted	2.5 ± 0.6	2.0 ± 0.8	1.4 ± 0.7	2.3 ± 1.6
Globose	1.5 ± 0.5	0.7 ± 0.4	0.9 ± 0.3	1.3 ± 0.5
Obovoid	1.3 ± 0.7	2.3 ± 0.9	0.7 ± 0.3	0.3 ± 0.3
Conical	1.3 ± 0.4	0.8 ± 0.5	0.2 ± 0.2	1.0 ± 1.0
Pyriform	0	0	0.1 ± 0.1	0
Gametangia (mean ± SEM)				
Oogonia diam (μm)	37.9 ± 0.2 a	38.2 ± 0.2 a	38.2 ± 0.1 a	38.3 ± 0.2 a
Oospore diam (μm)	35.4 ± 0.2 a	35.7 ± 0.2 a	35.6 ± 0.1 a	35.8 ± 0.2 a
Paragynous associations (%)[a]	18.3 ± 2.7 a	12.8 ± 1.8 a	15.2 ± 1.8 a	17.0 ± 3.7 a
Pathogenicity				
Lesion lengths (mean ± SEM) in detached stems				
Jarrah	32.3 ± 3.1	21.7 ± 2.7	36.7 ± 2.6	31.4 ± 3.0
Marri	3.1 ± 1.9	1.8 ± 0.9	2.4 ± 1.3	3.2 ± 1.4
Clonal jarrah in glasshouse				
Mean estimated plant survival (d)	297 a	201 b	197 b	188 b
Upper 95% confidence interval	421	270	262	271
Lower 95% confidence interval	209	149	148	131
Surviving plants 182 d after inoculation				
Proportion plants (%)	53	40	37	37
Recovery of *P. cinnamomi* (%)[b]	33	44	45	41
Group mean ± SEM				
Lesion length (mm)	13 ± 4.5	14 ± 3.3	21 ± 4.5	18 ± 5.7
Extension beyond lesion (mm)	3 ± 1.1	7 ± 2.1	7 ± 1.9	6 ± 3.2
Colonisation (mm)	17 ± 4.6	20 ± 4.1	28 ± 5.2	24 ± 6.2

[a] Paragynous associations always had amphigynous antheridia.

[b] Includes lesioned and symptomless plant material which were washed and leached to obtain a recovery of *P. cinnamomi* (Hüberli *et al.* 2000).

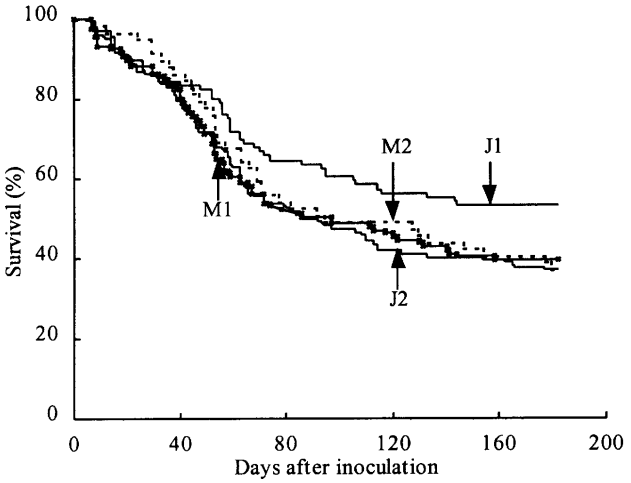

Fig. 1. Percentage survival for a clonal line of *Eucalyptus marginata* (jarrah) that was underbark wound-inoculated in the glasshouse with 73 isolates of *Phytophthora cinnamomi* from four sub-populations in the jarrah forest of Western Australia. Sub-populations were J1, M1, J2 and M2 isolates as explained in Table 1.

considered intermediate in their pathogenicity, as they were highly variable in their capacity to cause deaths over the duration of the experiment. *P. cinnamomi* was recovered from only 33% (16) of the surviving plants inoculated with J1 isolates, which was the lowest isolation percentage, compared to the other three isolate sub-populations (Table 2). Overall, the site 1 isolates generally formed smaller lesions and colonisation was lower compared to the site 2 isolates (Table 2). Eight additional recoveries of *P. cinnamomi* were obtained when the leaching technique was applied to stems from the 51 surviving plants. All control plants were symptomless except for one that died after 18 wk, but it had no lesion and *P. cinnamomi* was not isolated from this plant.

The capacity to cause deaths was not significantly correlated with the capacity to form lesions in jarrah ($-0.42 < r < 0.57$, $P > 0.11$) and marri ($-0.02 < r < 0.57$, $P > 0.11$) stems for all sub-populations. Of three isolates that produced large lesions in jarrah and marri stems (MP62 (J1), MP94-10 (J2) and MP94-15 (J2)), only MP94-10 (J2) had a relative high capacity to cause deaths in clonal jarrah (Table 1).

Correlation of morphological and pathogenicity phenotypes

All correlations were tested at $P = 0.005$ after Bonferroni correction. The correlation of growth rates on PDA at all temperatures with jarrah deaths ($-0.49 < r < 0.09$, $P > 0.05$), detached jarrah stem lesions ($-0.31 < r < 0.35$, $P > 0.26$) and detached marri stem lesions ($r < 0.57$, $P > 0.02$) was low. Isolates ranked high in pathogenicity in any of the experiments had either slow or rapid growth on PDA (Table 1). Cluster analysis of 24 morphological and pathogenicity phenotypes grouped the isolates into two distinct groups that corresponded to site of origin with 76–77% accuracy (Fig. 2). This site discrimination became less obvious as fewer phenotypic variables were used in the analysis. No one variable could be used to distinguish sites or hosts from which an isolate originated.

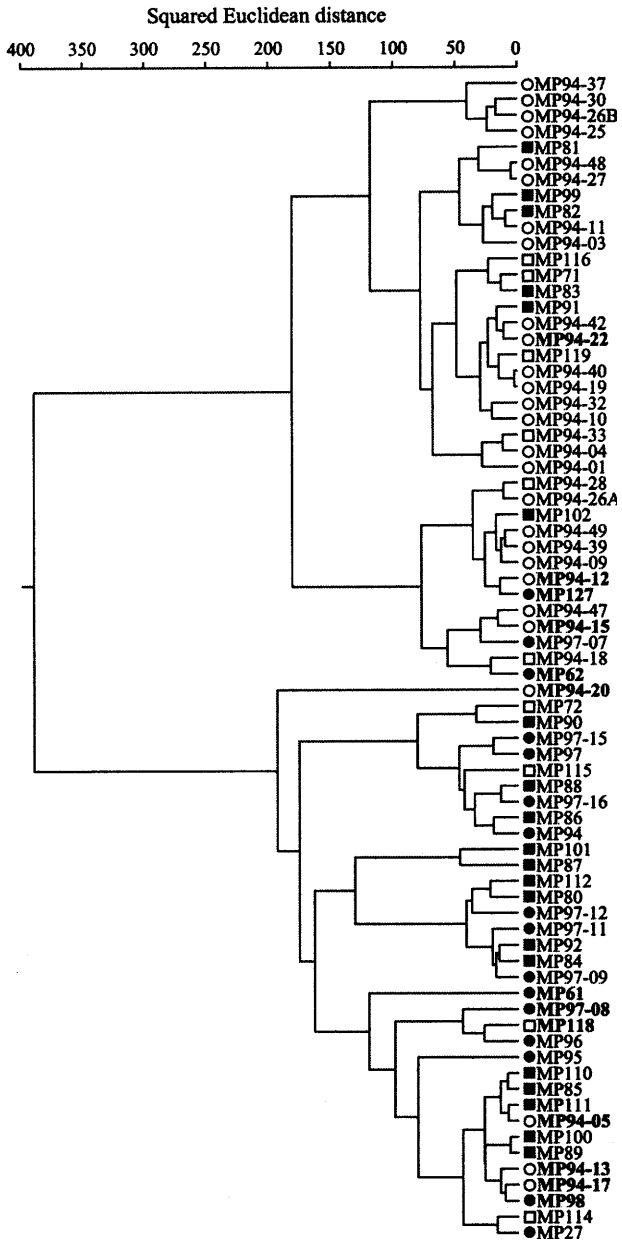

Fig. 2. Relationships among 73 isolates of *Phytophthora cinnamomi* from four sub-populations in the *Eucalyptus marginata* (jarrah) forest from Western Australia revealed by Ward's Method cluster analysis of the matrix of Squared Euclidean distance based on 24 morphological and pathogenicity characters. Sub-populations were J1 (●), M1 (■), J2 (○) and M2 (□) isolates as explained in Table 1. Variant microsatellite genotype isolates are in bold.

DISCUSSION

This study demonstrates the substantial variation in some phenotypes within a clonal lineage of *Phytophthora cinnamomi*. Remarkably, this variation occurred within two WA populations located 70 km apart. It is the first such study for *P. cinnamomi* and possibly for *Phytophthora* using many isolates, which focuses on phenotypic and genotypic variation from localised populations.

Genotypic variation

Out of the three clonal lineages of *Phytophthora cinnamomi* (Dobrowolski 1999) found in Australia, only the A2 type 1 clonal lineage was found at both our sites. The microsatellite analysis provided no evidence for sexual reproduction, as described previously (Dobrowolski 1999). Our finding is consistent with us never having isolated the A1 mating type from either site despite many attempts over 9 years. However, other studies of Australian populations of *P. cinnamomi* also found no evidence of sexual reproduction, despite the presence of both A1 and A2 mating type isolates, sometimes in close proximity (Old *et al*. 1984, 1988, Dobrowolski 1999). There was no evidence of recombination by selfing in A2 isolates.

In addition to the common genotype, we found six microsatellite genotypes that were represented in J1, J2 and M2 isolates. Two of these genotypes were found in more than one minepit. If the microsatellite mutation events causing these genotypes occurred only once, then these two groups of isolates were spread prior to or as a result of mining activity. One possibility for their dissemination is the previous and current practice of moving topsoil from one infested minepit to another during rehabilitation (Colquhoun & Hardy 2000).

The phenotypic variation we observed has arisen asexually. The large numbers of propagules produced in affected sites (Zentmyer 1980) together with differences in environmental factors between sites theoretically provide conditions for genetic variation to occur or phenotypic variation to be expressed (epigenetic differences) in the two sites. Therefore, stringent hygiene measures should be maintained between populations to prevent the introduction of any new isolates that differ in phenotype, which may result in greater disease expression.

Macro-morphological phenotypes

The growth-temperature relationship of isolates is affected by the composition of the agar medium (Shepherd & Pratt 1974, Zentmyer *et al*. 1976) and isolation method (Shepherd & Forrester 1977). Consequently, comparisons among data sets are virtually impossible unless consistent agar media are used and populations of similar genetic background are compared. The fastest growing isolates at 24 ° of our two localised populations were at the lower range of radial growth rates reported by Zentmyer *et al*. (1976), who used fresh potatoes in their agar medium. But, they were similar to South African isolates growing at 25 ° on synthetic PDA (Linde *et al*. 1999). The J1 sub-population had slower growth rates on PDA. Growth rate distinctions have previously been found in national populations (Shepherd & Pratt 1974, Linde *et al*. 1999). None of our isolates grew at 32 ° and 75% were killed after 16 d exposure to 32 °. This is in contrast to other studies where some isolates of *Phytophthora cinnamomi* have been shown to grow at temperatures greater than 34 ° on fresh PDA (Zentmyer *et al*. 1976) and on other agar media (Haasis *et al*. 1964, Shepherd *et al*. 1974).

To our knowledge, the instability of colony morphology has not been reported before for *P. cinnamomi* or any other *Phytophthora* species. Considering that all our isolates were of the same clonal lineage, our work demonstrates that colony morphology for *P. cinnamomi* is not a stable character, and should be used with considerable caution when identifying *Phytophthora* species. Overall, the rosaceous and petaloid colonies were the predominant types, which agrees with previous studies on PDA using worldwide collections (Haasis *et al*. 1964, Zentmyer 1980). However, we found that the site 2 isolates were distinct in that 40–44% formed no-pattern colonies. We also found that temperature altered colony type in up to 75% of all isolates tested and two isolates (MP81 and MP94-32) produced different colony types at each temperature.

Micro-morphological phenotypes

Relatively stable phenotypic characters were oospore, oosphere and sporangia dimensions, and sporangia shapes as described for other isolates (Stamps *et al*. 1990). Variation in these characters was low compared to paragyny proportions or growth on PDA. However, as commented earlier, comparisons among studies are difficult as nutrition affects oospore and oogonia size (Chang, Shepherd & Pratt 1974, Zentmyer, Klure & Pond 1979) and paragyny proportion (Gao *et al*. 1998). These examples emphasize that comparison of organ measurements needs to be approached with caution unless the methods used are well defined.

Currently, the genus *Phytophthora* is divided into six groups providing a practical classification (Waterhouse 1963). Sporangia were all non-papillate and their dimensions were a stable character in our study, which agrees with Waterhouse's grouping. Antheridial associations in our study were found to be highly variable, with ten isolates having more than 30% paragynous and amphigynous antheridia in a species considered to form predominately amphigynous antheridia (Stamps *et al*. 1990). These and previous observations for isolates from WA, Australia, Papua New Guinea, Europe, South Africa and America (Hüberli *et al*. 1997b, Tommerup unpubl.) indicates that paragyny is widespread in *Phytophthora cinnamomi*. These findings warrant a redescription of the species to include paragynous antheridia as a character. This makes *P. cinnamomi* the second species in group VI of the *Phytophthora* key that is able to form paragynous antheridia. But *P. cinnamomi* is distinct from the other, *P. richardiae*, in that it has both paragynous and amphigynous antheridia associated with an oogonium.

Recently, molecular techniques have shown a clear association of species according to Waterhouse's grouping by sporangial papillation form (Crawford *et al*. 1996, Cooke & Duncan 1997). However, antheridial association was not correlated with molecular grouping and was not considered a sound criterion for classifying *Phytophthora* species (Cooke & Duncan 1997). Indisputable molecular techniques for classifying *Phytophthora* species would have a distinct advantage over morphological characters since they are not subject to environmental influences.

Disease and pathogenicity phenotypes

Capacity to form lesions in detached jarrah and marri stems

We found no evidence of host specificity among marri and jarrah isolates in stem inoculations. Marri isolates were not

more aggressive in marri stems, and likewise for jarrah isolates in jarrah inoculated stems. This indicates that the capacity of isolates to form lesions in jarrah and marri stems are two independent phenotypes. Some earlier studies have raised the possibility that host specificity exists in *Phytophthora cinnamomi* pathogenicity (Zentmyer 1980, Zentmyer & Guillemet 1981). However, conclusive interpretations probably cannot be made from these studies as they used five or fewer isolates. Our conclusion of a lack of host specificity in *P. cinnamomi* is supported by recent intensive pathogenicity studies with Australian (Dudzinski *et al*. 1993), French (Robin & Desprez-Loustau 1998) and South African (Linde *et al*. 1999) isolates.

Linde *et al*. (1999) found that age of cultures had an effect on the capacity to form lesions, as indicated by Erwin & Ribeiro (1996). Unlike our methods, Linde *et al*. (1999) did not repassage the isolates through plant material prior to the tests. Hence, the differences they found amongst isolates may be due to a mixture of culture age and innate isolate phenotype.

Most of our 73 isolates were more aggressive in detached jarrah stems than marri stems. This supports the findings in studies with attached stem inoculations (Tippett, Hill & Shearer 1985, Shearer, Michaelsen & Somerford 1988) and field observations (Podger 1972) that marri is more resistant to *P. cinnamomi* than jarrah. Detached stems have been used to assess the susceptibility of numerous woody plant species to *P. cinnamomi* and other *Phytophthora* species. (Sewell & Wilson 1959, Dixon, Thinlay & Sivasithamparam 1984, Matheron & Mircetich 1985, Dolan & Coffey 1986, Hansen, Hamm & Roth 1989, Scott, Wicks & Lee 1992). They have not been used with any *Eucalyptus* species although detached roots have been, with varying success (Tippett *et al*. 1985, Shearer, Michaelsen & Warren 1987). Shearer *et al*. (1987) found that the growth of *Phytophthora* species, including *P. cinnamomi*, in detached jarrah roots correlated with the growth in intact stems in the field. In a similar study with marri and jarrah, Tippett *et al*. (1985) did not find a correlation between lesion length in attached stems of trees in the forest and in the detached roots.

Out of 73 isolates, only MP94-10 (J2) had a high capacity to form lesions in both hosts and had a high capacity to cause deaths. Rapid early colonisation of the pathogen was not correlated with deaths and there are a number of possibilities for this. Firstly, plants were physiologically different in the two experiments, and secondly, lesion lengths and death of plants may not correlate. In another environmentally-controlled inoculation experiment, we found that some plants with large lesions survived longer than others with small lesions (Hüberli *et al*. 1998). This is similar to our observations for inoculated clonal jarrah in a field trial (Hüberli, unpubl.). Thirdly, lack of a correlation may be due to differences in inoculum. An agar-based inoculum used in the detached stem experiment may give the pathogen a competitive edge over a non-food-based inoculum, such as the colonised Miracloth discs used in the glasshouse pathogenicity experiment.

In both experiments, differences among isolates' capacity to cause disease was five-fold or more, indicating that this capacity is a variable phenotype and may be influenced by many factors. Also, it may be a multigenic character given the breadth of behaviour of the pathogen clonal lineage in a jarrah clonal line. Consequently, we recommend the detached jarrah stem test not be used in discrimination of pathogenicity among isolates, but it may be useful in rapidly determining the susceptibility of plant hosts. This test would be beneficial in breeding programmes and rehabilitation of infected minepits with resistant plant lines. However, more *Eucalyptus* species would need to be tested with many *P. cinnamomi* phenotypes to develop it as a routine procedure.

Capacity to cause deaths of clonal jarrah plants

Considerable variation in pathogenicity existed among isolates from the two sites and among minepits. The capacity to cause deaths ranged from killing all trees in 54 d to non-pathogenic, with most isolates being intermediate. This large variation in pathogenicity has been reported for Australian (Dudzinski *et al*. 1993), French (Robin & Desprez-Loustau 1998) and South African (Linde *et al*. 1999) national collections of isolates, but not from local collections. Dudzinski *et al*. (1993) found a 2.3-fold difference in the capacity to cause deaths among their 18 A2 type 1 isolates from Australia-wide collections compared to our five-fold difference among isolates from local populations. This disparity between the two studies may be due to differences in the clonal lines of jarrah and the physiological age of plants.

These large amounts of variation in two regional forest communities of the pathogen clonal lineage have important implications for the screening of plants resistant to *Phytophthora cinnamomi*. For example, MP97-12 and MP94-30, which killed all plants within 59 d would infer the host was susceptible, while those isolates that were non-pathogenic or intermediate in our study would falsely imply the host was resistant to *P. cinnamomi*. Our results suggest that the jarrah clonal line 77C40 indeed has lower resistance against our isolates than those used previously (McComb *et al*. 1990) for assessing resistance of jarrah. The large variation in pathogenicity phenotypes also indicates that a set of different isolates having a wide spectrum of pathogenicity is required to test for resistance of different hosts.

The capacity to cause deaths by the majority of J1 isolates was low. Individual J1 isolate pathogenicities contributed to a sub-population phenotype that had a lower capacity to cause deaths. This is also reflected by the lower recovery of J1 isolates from the surviving plants compared to the other three sub-populations. However, low recovery was not necessarily due to the pathogen being dead, as a small proportion were recovered by a leaching technique (Hüberli *et al*. 2000).

A complication in the prevention of *P. cinnamomi* spread is the lack of fine-scale mapping of the pathogen distribution in forests and of recognition that symptomless infected trees may be present. Standard isolation practises of plating tissue directly onto selective media may not detect *P. cinnamomi* in plants (Hüberli *et al*. 2000). In the present study, we had similar evidence of *P. cinnamomi* not being detected in plant tissue until it was extensively leached before plating as in Hüberli *et al*. (2000). Failure to detect the pathogen by direct plating could result in misclassification of sites and inadvertent contamination of unaffected sites. Also, it could provide opportunities for introductions of new phenotypes to affected

sites. Diagnostic tools that detect *P. cinnamomi* in its low-active states with high reliability are urgently required.

Correlations of morphological and pathogenicity phenotypes

Phenotypic variation was independent and continuous such that isolate sub-populations overlapped with one another. No single or small group of characters could be identified to separate the population into sub-species units as 24 variables were required to obtain 76% differentiation of sites. No distinction was due to the host from which isolates were derived. The range of variation indicates these phenotypic traits are quantitative. The segregation of isolates by site in the dendrogram implies that phenotypic divergence has occurred in the two populations. While it was not within the scope of this study to determine the factors that contributed to phenotypic divergence, a number of possibilities could have contributed including selection, high mutation rates, inherited expression differences (epigenetic differences) and/or stochastic events.

No significant relationship was found between radial growth rates and pathogenicity phenotypes for the 73 isolates tested. This finding conflicts with the report of Linde *et al*. (1999) who found a significant correlation between lesion length and growth on agar. They postulated a causal relationship and fitted a non-linear equation to the data. In our study, we found no causal relationship. Two methodological differences that could contribute to Linde's correlations are the use of agar as an inoculum source or the lack of isolate repassaging prior to the experiments as discussed earlier. Consequently, as growth rates on agar were independent of the capacity to cause deaths or lesions in our study, we do not recommend using PDA as a quick screen for highly pathogenic isolates in jarrah breeding and selection programs as suggested for the *Eucalyptus smithii* selection program in South Africa (Linde *et al*. 1999).

Our findings of large phenotypic variation among isolates derived asexually from one clonal lineage have important taxonomic, management and resistance screening implications. We have shown that there are stable and non-stable taxonomic characters for *Phytophthora cinnamomi*. The non-stable characters such as paragyny and colony morphology on PDA warrant an adjustment of the species description to include these phenotypes. Within a site or minepit, we found a large variation among isolates in the capacity to cause disease in jarrah and marri. Microsatellite evidence indicated that dissemination of *P. cinnamomi* isolates between minepits had occurred prior to or during mining. We would suggest that in affected ecosystems a minimal soil movement policy is adopted to reduce the chance of introductions of new phenotypes. For resistance screening programs, it is extremely important to use isolates most pathogenic to the host in question.

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REFERENCES

Chang, S. T., Shepherd, C. J. & Pratt, B. H. (1974) Control of sexuality in *Phytophthora cinnamomi*. *Australian Journal of Botany* **22**: 669–679.

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

Colquhoun, I. J. & Hardy, G. E. St J. (2000) Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the *Eucalyptus marginata* (jarrah) forest of Western Australia. *Plant Disease* **84**: 116–127.

Cooke, D. E. L. & Duncan, J. M. (1997) Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of the ribosomal RNA gene repeat. *Mycological Research* **101**: 667–677.

Crawford, A. R., Bassam, B. J., Drenth, A., Maclean, D. J. & Irwin, J. A. G. (1996) Evolutionary relationships among *Phytophthora* species deduced from rDNA sequence analysis. *Mycological Research* **100**: 437–443.

Dixon, K. W., Thinlay & Sivasithamparam, K. (1984) Technique for rapid assessment of tolerance of *Banksia* spp. to root rot caused by *Phytophthora cinnamomi*. *Plant Disease* **68**: 1077–1080.

Dobrowolski, M. P. (1999) *Population and sexual genetics of Phytophthora cinnamomi in Australia using microsatellite markers*. PhD thesis, Murdoch University, Perth, WA.

Dolan, T. E. & Coffey, M. D. (1986) Laboratory screening technique for assessing resistance of four avocado rootstocks to *Phytophthora cinnamomi*. *Plant Disease* **70**: 115–118.

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

Erwin, D. C. & Ribeiro, O. K. (1996) *Phytophthora Diseases Worldwide*. American Phytopathological Society Press, St Paul, MN.

Galindo, J. & Zentmyer, G. A. (1964) Mating types in *Phytophthora cinnamomi*. *Phytopathology* **54**: 238–239.

Gao, Z. M., Zheng, X. B., Lu, J. Y. & Ko, W. H. (1998) Effect of culture media on antheridial configuration in *Phytophthora boehmeriae*. *Canadian Journal of Botany* **76**: 2177–2179.

Haasis, F. A., Nelson, R. R. & Marx, D. H. (1964) Morphological and physiological characteristics of mating types of *Phytophthora cinnamomi*. *Phytopathology* **54**: 1146–1151.

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

Hüberli, D., Hardy, G. E. St J. & Tommerup, I. C. (1997a) Morphological and physiological characteristics of two *Phytophthora cinnamomi* populations. In *Eleventh Biennial Conference of the Australasian Plant Pathology Society*: 307. Perth, WA.

Hüberli, D., Hardy, G. E. St J., Tommerup, I. C. & Colquhoun, I. J. (1998) Temperature changes resistance of clonal *Eucalyptus marginata* to *Phytophthora cinnamomi*. In *Seventh International Congress of Plant Pathology*: 2.5.4, Edinburgh.

Hüberli, D., Tommerup, I. C. & Hardy, G. E. St J. (1997b) The role of paragynous and amphigynous antheridia in sexual reproduction of *Phytophthora cinnamomi*. *Mycological Research* **101**: 1383–1388.

Hüberli, D., Tommerup, I. C. & Hardy, G. E. St J. (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* **29**: 164–169.

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

Linde, C., Kemp, G. H. J. & Wingfield, M. J. (1999) Variation in pathogenicity among South African isolates of *Phytophthora cinnamomi*. *European Journal of Plant Pathology* **105**: 231–239.

McComb, J. A., Bennett, I. J., Stukely, M. J. C. & Crane, C. (1990) Selection and propagation of jarrah for dieback resistance: a progress report. *Combined Proceedings of the International Plant Propagators' Society* **40**: 86–90.

Matheron, M. E. & Mircetich, S. M. (1985) Seasonal variation in susceptibility of *Juglans hindsii* and Paradox rootstocks of English walnut trees to *Phytophthora citricola*. *Phytopathology* **75**: 970–972.

O'Gara, E., McComb, J. A., Colquhoun, I. J. & Hardy, G. E. St J. (1997) The infection of non-wounded and wounded periderm tissue at the lower stem

of *Eucalyptus marginata* by zoospores of *Phytophthora cinnamomi*, in a rehabilitated bauxite mine. *Australasian Plant Pathology* **26**: 135–141.

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

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

Oudemans, P. & Coffey, M. D. (1991) Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. *Mycological Research* **95**: 19–30.

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

Rands, R. D. (1922) Stripe canker on cinnamon, caused by *Phytophthora cinnamomi* n. sp. *Announcements of the Institute of Plant Diseases No. 54*. Department of Agriculture Industry and Business, Batavia

Robin, C. & Desprez-Loustau, M. L. (1998) Testing variability in pathogenicity of *Phytophthora cinnamomi*. *European Journal of Plant Pathology* **104**: 465–475.

Scott, E. S., Wicks, T. J. & Lee, T. C. (1992) The development of an assay for resistance to *Phytophthora cambivora* in almond rootstocks using shoots excised from tissue cultures. *Plant Pathology* **41**: 639–645.

Sewell, G. W. F. & Wilson, J. F. (1959) Resistance trials of some apple rootstock varieties to *Phytophthora cactorum*. *Journal of Horticultural Science* **34**: 51–58.

Shearer, B. L. & Dillon, M. (1995) Susceptibility of plant species in *Eucalyptus marginata* forest to infection by *Phytophthora cinnamomi*. *Australian Journal of Botany* **43**: 113–134.

Shearer, B. L., Michaelsen, B. J. & Somerford, B. 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.

Shepherd, C. J. & Forrester, R. I. (1977) Influence of isolation method on growth rate characteristics of populations of *Phytophthora cinnamomi*. *Australian Journal of Botany* **25**: 477–482.

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

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

Stamps, D. J., Waterhouse, G. M., Newhook, F. J. & Hall, G. S. (1990) Revised tabular key to the species of *Phytophthora*. *Mycological Papers* **162**: 1–28.

Tippett, J. T., Hill, T. C. & Shearer, B. L. (1985) Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. *Australian Journal of Botany* **33**: 409–418.

Waterhouse, G. M. (1963) Key to the species of *Phytophthora* De Bary. *Mycological Papers* **92**: 1–22.

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

Zar, J. H. (1999) *Biostatistical Analysis*. 4th edn. Prentice Hall, NJ.

Zentmyer, G. A. (1980) *Phytophthora cinnamomi and the Diseases it Causes*. [Monograph No. 10.] American Phytopathological Society, St Paul, MN.

Zentmyer, G. A. & Guillemet, F. B. (1981) Evidence of strains of *Phytophthora cinnamomi*. *Plant Disease* **65**: 475–477.

Zentmyer, G. A., Klure, L. J. & Pond, E. C. (1979) The influence of temperature and nutrition on formation of sexual structures by *Phytophthora cinnamomi*. *Mycologia* **71**: 55–67.

Zentmyer, G. A., Leary, J. V., Klure, L. J. & Grantham, G. L. (1976) Variability in growth of *Phytophthora cinnamomi* in relation to temperature. *Phytopathology* **66**: 982–986.

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