Population Structure of *Phytophthora cinnamomi* in South Africa

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ABSTRACT

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Phytophthora cinnamomi isolates collected from 1977 to 1986 and 1991 to 1993 in two regions in South Africa were analyzed using isozymes. A total of 135 isolates was analyzed for 14 enzymes representing 20 putative loci, of which four were polymorphic. This led to the identification of nine different multilocus isozyme genotypes. Both mating types of *P. cinnamomi* occurred commonly in the Cape region, whereas, predominantly, the A2 mating type occurred in the Mpumalanga region of South Africa. A2 mating type isolates could be resolved into seven multilocus isozyme genotypes, compared with only two multilocus isozyme genotypes for the A1 mating type isolates. Low levels of gene (0.115) and genotypic (2.4%) diversity and a low number of alleles per locus (1.43) were observed for the South African *P. cinnamomi* population. The genetic distance between the Cape and Mpumalanga *P. cinnamomi* populations was relatively low (D_m = 0.165), and no specific pattern in regional distribution of multilocus isozyme genotypes could be observed. The genetic distance between the "old" (isolated between 1977 and 1986) and "new" (isolated between 1991 and 1993) *P. cinnamomi* populations from the Cape was low (D_m = 0.164), indicating a stable population over time. Three of the nine multilocus isozyme genotypes were specific to the "old" population, and only one multilocus isozyme genotype was specific to the "new" population. Significant differences in allele frequencies, a high genetic distance (D_m = 0.581) between the Cape A1 and A2 mating type isolates, significant deviations from Hardy-Weinberg equilibrium, a low overall level of heterozygosity, and a high fixation index (0.71) all indicate that sexual reproduction occurs rarely, if at all, in the South African *P. cinnamomi* population.

Additional keywords: asexual reproduction, isozyme analysis, Oomycetes, origin, population genetics.

The Oomycete *Phytophthora cinnamomi* Rands is a notorious soilborne plant pathogen with a wide host range (37). It was first recorded in South Africa in 1931 (7,34) and led to diseases of major economic importance, e.g., root disease of *Eucalyptus* and *Pinus* species (14,36) and root rot of avocado (35). It is also a pathogen of grapevines (29), commercially cultivated *Protea* species (32), and native undisturbed fynbos vegetation (33), which is a unique floral kingdom of the southwestern Cape, South Africa.

P. cinnamomi is diploid and heterothallic with two mating types, A1 and A2 (9). Sexual reproduction in heterothallic *Phytophthora* species occurs when gametangia of opposite mating type meet in host tissue, leading to the formation of oospores. Oospores can survive for long periods of time, in or outside the host. *P. cinnamomi* has a global geographical distribution, in which the A2 mating type is more frequently isolated than the A1 mating type (37,38). In South Africa, the A2 mating type has usually been associated with agricultural crops and cultivated forests (30). It was also prevalent in native forests, where both mating types sometimes occurred together at the same site. In native undisturbed fynbos vegetation and rivers draining mountain catchments, only the A1 mating type has been found (31). This was also the case in Papua New Guinea, where the A1 mating type has been recovered exclusively from native areas (1).

The center of origin of *P. cinnamomi* is unknown. Based on the observation that many plants in Asia are resistant to *P. cinnamomi*, Crandall and Gravatt (6) hypothesized that *P. cinnamomi* originated there. Zentmyer (37,38) suggested the New Guinea-Celebes-Malaysia region, possibly extending into northeastern Australia. However, recent evidence indicates that *P. cinnamomi* has been introduced into east Asia (39). Furthermore, isozyme studies on *P. cinnamomi* in Australia indicated low levels of genetic diversity and the absence of sexual reproduction, indicative of an introduced pathogen (19,20). High levels of genetic diversity were found among A1 mating type isolates from Papua New Guinea, indicating a possible center of origin for *P. cinnamomi* (19). Taiwan (13) and South Africa (33) have also been hypothesized as having indigenous *P. cinnamomi* populations.

The level of genetic diversity in fungi is usually the highest in isolates obtained from the center of origin. This has, for example, been revealed by an isozyme study with *P. infestans*, in which many isozyme alleles, the occurrence of sexual reproduction, and a high level of genetic diversity were identified for isolates from central Mexico, the presumed center of origin of this fungus, compared with elsewhere in the world (24,27). In addition, DNA fingerprinting revealed high levels of genotypic diversity in *P. infestans* isolates from central Mexico to such an extent that almost every isolate had a unique genotype (8,11). However, the presence of both mating types does not necessarily imply the occurrence of sexual reproduction, as low levels of genetic diversity were found among *P. infestans* isolates in northwestern Mexico, where both mating types were present (11). *Phytophthora* species can rapidly reproduce asexually through the formation of large numbers of sporangiospores, which can germinate either directly or differentiate into motile zoospores. Asexual, or clonal reproduction, may lead to lower levels of genotypic diversity compared with sexual reproduction.

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The aims of the current investigation were to (i) assess the level of gene and genotypic diversity in the South African *P. cinnamomi* population using isozymes, (ii) study the occurrence of particular multilocus isozyme genotypes, (iii) compare the A1 and A2 mating type populations, (iv) test for the presence of sexual reproduction in the South African *P. cinnamomi* population, and (v) study changes in population structure over time. The assessment of levels of gene and genotypic diversity will give insight into the center of origin, the frequency of sexual reproduction, and the occurrence and spread of particular genotypes in the pathogen population. This information may be important for breeding and selection for resistance against *P. cinnamomi* and for the implementation of effective disease control strategies.

MATERIALS AND METHODS

Sources of isolates. One hundred and thirty-five isolates of *P. cinnamomi* collected between 1977 and 1993 from the south and southwestern Cape region (collectively referred to as Cape) and from the northeastern parts of South Africa (collectively referred to as Mpumalanga) (Fig. 1) were selected for this study. These isolates were obtained from a broad variety of host plants. Isolates collected from 1977 to 1986 are referred to as "old" isolates and those collected from 1991 to 1993 as "new" isolates.

Isolations from soil were made by baiting with lupin seedlings (4) and *Citrus* leaf discs (12). Direct isolations from diseased host tissue were performed onto a selective medium (28). Possibly infested water was filtered through 0.3-µm Nucleopore polycarbon membrane filters (Nucleopore Corporation, Pleasanton, CA). The filters were then plated onto a selective medium (28). All isolates have been maintained in sterilized water at room temperature and are part of the culture collection of the Tree Pathology Cooperative Programme, Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein, South Africa.

Determination of mating type. The mating type of all isolates was determined by pairing with isolates of known mating type on carrot agar plates (22). Plates were incubated at 20°C in the dark and examined for the presence of oospores after 4 weeks.

Mycelium production and enzyme extraction. Isolates for isozyme analyses were cultured in clarified V8 broth at 25°C in the dark. After 5 days, excess liquid was drained from the resultant mycelial mats before further drying between sterile paper towels. Approximately 0.5 g of mycelial tissue was transferred to 1.5-ml microcentrifuge tubes, and 0.5 ml of extraction buffer (0.1 M Tris-HCl, pH 7, and 20 mg/ml of polyvinylpyrrolidone) was added before freezing in liquid nitrogen. The frozen mycelium was then ground with a rod in a microcentrifuge tube and the crude enzyme suspension absorbed onto paper wicks (21). Paper wicks were stored at –70°C until use.

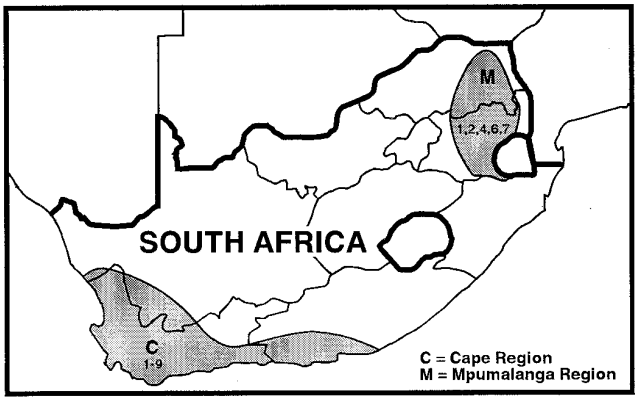

Fig. 1. Map of South Africa showing the different multilocus isozyme genotypes of *Phytophthora cinnamomi* collected from the Cape (C) and Mpumalanga (M) regions.

Isozyme analyses. Fourteen different enzymes and corresponding buffer systems were used in this study (Table 1). Procedures for starch gel electrophoresis followed those of Oudemans and Coffey (21). Buffers were prepared according to the following: morpholine citrate buffers at pH 6 (MC6), pH 7 (MC7), and pH 8 (MC8) (5); Tris-EDTA-borate buffer (TEB) (15), and a discontinuous buffer system (LiOH) (23). Enzyme-staining recipes were similar to those used by Oudemans and Coffey (21), except for final volume, as agar overlays were used to reduce costs. The staining procedure for superoxide dismutase (SOD) was identical to that of Oudemans and Coffey (21). Mycelium production, enzyme extraction, and isozyme analyses were repeated three times to ensure reliability of results. For comparison between gels, isolates representing the different alleles were used as standards.

Data analysis. Each band identified was treated as an allele of a specific locus. Because *Phytophthora* species are diploid (2), genetic interpretation of banding patterns was based on that of a diploid organism. Isozyme alleles of each isolate were scored by their presence or absence to generate a multilocus isozyme genotype. Isolates with the same overall multilocus isozyme genotype were considered clonal.

Genotypic diversity (25) was calculated on the basis of the number of multilocus isozyme genotypes in the two regions studied in South Africa (Cape and Mpumalanga), as well as in the "old" and "new" *P. cinnamomi* populations within the two regions. To compare levels of genotypic diversity ($\hat{G}$) between regions, the individual diversity values were divided by the sample size of each region (N) (16) to calculate the percentage maximum possible diversity obtained ($\hat{G}/N\%$).

Gene diversity for the four different South African *P. cinnamomi* populations was calculated using Nei's gene diversity (17). The probable mating system was determined by comparing the observed proportion of heterozygotes with the expected level of heterozygosity. Wright's fixation index, $F = 1 - (H_{obs}/H_{exp})$, was used for this purpose, in which H_{obs} is the observed mean heterozygosity per locus and H_{exp} is the expected mean heterozygosity in the population, which is the same as Nei's gene diversity (3).

Regional and temporal differentiation between *P. cinnamomi* populations were determined using differences in allele frequencies. Based on allele frequencies of the four polymorphic isozyme loci, population differentiation between the subpopulations from the Cape and Mpumalanga regions, between the "old" and "new" populations of the Cape, and between A1 and A2 mating type pop-

TABLE 1. Enzyme stains and corresponding buffer systems used for isozyme analysis of South African *Phytophthora cinnamomi* isolates

Enzyme system	EC number	Abbreviation	Buffer[z]
Oxireductases			
Diaphorase	1.6.4.3	DIA	LiOH
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH	LiOH
Isocitric dehydrogenase	1.1.1.42	IDH	MC8
Lactate dehydrogenase	1.1.1.27	LDH	LiOH
Malate dehydrogenase	1.1.1.37	MDH	LiOH
Malic enzyme	1.1.1.40	ME	LiOH
Phosphogluconate dehydrogenase	1.1.1.44	6PGD	TEB
Superoxide dismutase	1.15.1.1	SOD	LiOH
Transferases			
Adenylate kinase	2.7.4.3	AK	TEB
Hexokinase	2.7.1.1	HEX	TEB
Isomerases			
Glucose-6-phosphate isomerase	5.3.1.9	GPI	MC7
Mannose-6-phosphate isomerase	5.3.1.8	M6PI	MC6
Others			
Aconitate hydratase	4.2.1.3	ACO	MC6
Fructose-1,6-diphosphatase	3.1.3.11	F16DP	TEB

[z] Morpholine citrate buffers at pH 6 (MC6), pH 7 (MC7), and pH 8 (MC8); Tris-EDTA-borate buffer (TEB); and a discontinuous buffer system (LiOH).

ulations of the Cape were determined using an unbiased minimum genetic distance (D_m) (18).

Frequencies of genotypes at the four polymorphic isozyme loci in the "old" and "new" populations of the Cape region were tested for deviations from expected Hardy-Weinberg equilibrium using the BIOSYS-1 Statistical Package (26). No correction for clonal genotypes was conducted, as this would lead to an unacceptably small population size (n = 9).

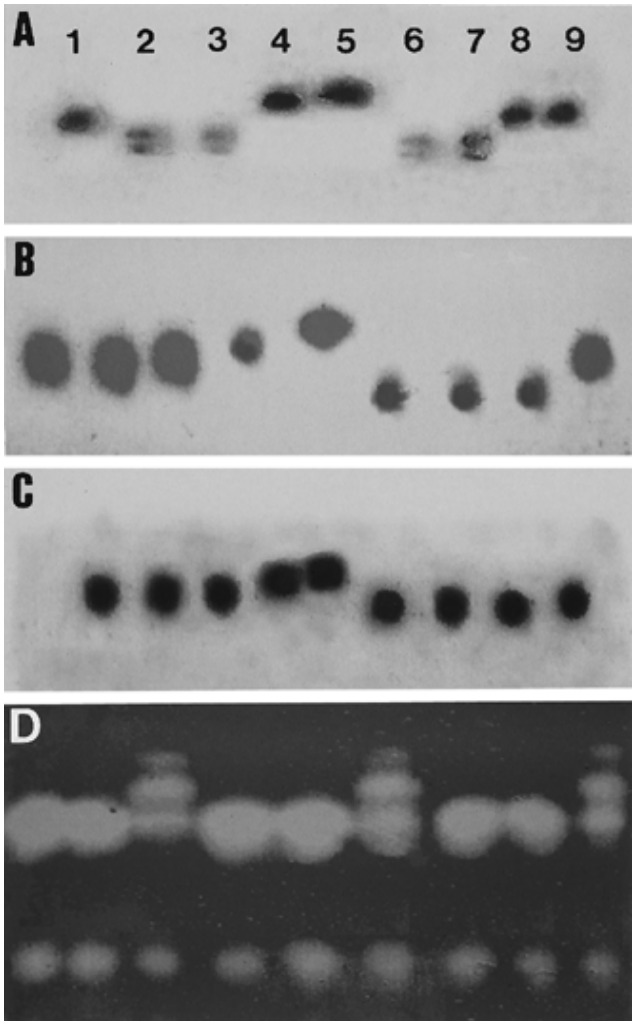

Fig. 2. Isozyme patterns for four polymorphic enzymes in South African *Phytophthora cinnamomi* isolates. Lanes 1 to 9 represent multilocus isozyme genotypes 1 to 9. **A,** Isocitrate dehydrogenase; **B,** aconitate hydratase; **C,** mannose-6-phosphate isomerase; and **D,** superoxide dismutase showing monomorphic locus at the bottom.

RESULTS

Overall diversity in South Africa. Both *P. cinnamomi* mating types were isolated from the Cape (63 A1 and 46 A2) and Mpumalanga (one A1 and 25 A2) regions. A total of 64 A1 and 71 A2 mating type isolates were obtained. The A2 mating type isolates were predominantly from commercial crops, but also from native fynbos vegetation, in contrast to A1 mating type isolates, which were predominantly from native fynbos vegetation in the Cape region. Regional distribution of mating type isolates showed that the A1 mating type was primarily in the Cape region, with only one A1 mating type isolate recovered from the Mpumalanga region. The A2 mating type occurred commonly in both regions.

Twenty putative loci were observed for the 14 enzymes examined. Polymorphisms were observed for only four enzymes, isocitric dehydrogenase (IDH), aconitate hydratase (ACO), mannose-6-phosphate isomerase (M6PI), and SOD, with the first three enzymes each having four alleles and SOD having only two alleles (Fig. 2). The other 10 enzymes examined were all monomorphic, with two putative loci observed at adenylate kinase (AK), diaphorase (DIA), hexokinase (HEX), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH). Two putative loci were also observed at SOD, of which one locus was monomorphic (Fig. 2D). Based on the 14 different alleles of the four polymorphic loci, nine different multilocus isozyme genotypes could be distinguished among the 135 isolates analyzed (Table 2). The 71 A2 mating type isolates could be resolved into seven different multilocus isozyme genotypes, with genotype 6 being the most common (Table 2). Among the 64 A1 mating type isolates analyzed, 63 were of multilocus isozyme genotype 4 and one was of multilocus isozyme genotype 5 (Table 2). The overall levels of gene and genotypic diversity for the South African *P. cinnamomi* population were H_{exp} = 0.115 and $\hat{G}/N$ = 2.4%, respectively (Table 3).

Regional and temporal diversity. In the Cape region, genotype 9 could only be identified among the "new" isolates, whereas multilocus isozyme genotypes 2, 3, 5, and 8 could only be identified in the "old" population. In the Mpumalanga region, multilocus isozyme genotype 4 was only identified in the "old" population (Table 2).

There was no association between multilocus isozyme genotype and host. The highest number of different multilocus isozyme genotypes was recovered from *Pinus* species (seven genotypes out of 11 isolates), *Ocotea bullata* (six genotypes out of 39 isolates), and *Eucalyptus* species (five genotypes out of 11 isolates). Two

TABLE 2. Alleles present in South African isolates of *Phytophthora cinnamomi* in each of the nine different multilocus isozyme genotypes

						No. of isolates						
	Locus[x]					Cape			Mpumalanga			South Africa
Genotype	IDH	ACO	M6PI	SOD	Mating type	"Old"[y]	"New"[z]	Total	"Old"	"New"	Total	total
1	BB	BB	BB	AA	A2	8	2	10	4	2	6	16
2	AB	BB	BB	AA	A2	4	0	4	1	2	3	7
3	AB	BB	BB	AB	A2	1	0	1	0	0	0	1
4	CC	CC	CC	AA	A1	52	10	62	1	0	1	63
5	DD	DD	DD	AA	A1	1	0	1	0	0	0	1
6	AB	AA	AA	AB	A2	2	19	21	7	7	14	35
7	AB	AA	AA	AA	A2	2	3	5	1	1	2	7
8	BB	AA	AA	AA	A2	3	0	3	0	0	0	3
9	BB	BB	BB	AB	A2	0	2	2	0	0	0	2
No. of alleles	4	4	4	2								

[x] IDH = isocitric dehydrogenase; ACO = aconitate hydratase; M6PI = mannose-6-phosphate isomerase; and SOD = superoxide dismutase.
[y] "Old" = collected from 1977 to 1986.
[z] "New" = collected from 1991 to 1993.

isolates recovered from the same tree represented different multilocus isozyme genotypes (1 and 2, respectively). Overall levels of genotypic diversity were low (Table 3).

Similar numbers of different alleles per locus were observed for "old" and "new" Cape and Mpumalanga *P. cinnamomi* populations. Levels of gene diversity observed for the "old" (H_{exp} = 0.072) and the "new" (H_{exp} = 0.105) *P. cinnamomi* populations from the Cape region were also similar. The slightly higher levels of gene diversity observed for the "new" *P. cinnamomi* population than for the Cape region resulted in a lower fixation index for the "new" compared with the "old" Cape *P. cinnamomi* population. The same was observed for the Mpumalanga *P. cinnamomi* population (Table 3). Allele frequencies between regional and temporal *P. cinnamomi* populations were dissimilar (Table 4). The genetic distance between the two regions (D_m = 0.165) and between the "old" and "new" (D_m = 0.164) *P. cinnamomi* populations from the Cape region was low (Table 5).

Sexual reproduction in South Africa. A low level of heterozygosity (H_{obs} = 0.033) and a high fixation index (F = 0.71) were observed for the South African *P. cinnamomi* population (Table 3). The "old" and "new" *P. cinnamomi* populations from the Cape region were analyzed for conformance to expected Hardy-Weinberg equilibrium at the four polymorphic isozyme loci. Seven of the eight Hardy-Weinberg analyses deviated significantly from the assumption of random mating. Only allele frequencies at the SOD locus in the "old" Cape population were in equilibrium. Alleles were shared among the A1 and A2 mating type populations at the SOD locus only (Table 4), without any polymorphism among the A1 mating type population. The genetic distance (D_m = 0.581) between the mating type populations was high (Table 5).

DISCUSSION

Our isozyme study on 135 isolates of *P. cinnamomi* from South Africa revealed lower levels of genotypic diversity than would be expected from a heterothallic, outbreeding organism. The low number of isozyme alleles per locus indicates that *P. cinnamomi* was probably introduced to South Africa. The small number of clones, a significant deviation from Hardy-Weinberg equilibrium for genotype frequencies, and a high level of differentiation between the A1 and A2 mating type populations indicate that sexual reproduction probably does not play an important role in the South African *P. cinnamomi* population.

The level of isozyme gene diversity for the South African *P. cinnamomi* population (H_{exp} = 0.115) is similar to that of the Australian *P. cinnamomi* population (19), as reviewed by Goodwin (10). Thus, the South African *P. cinnamomi* population is very similar to the Australian one with respect to the presence of both mating types, the apparent lack of sexual reproduction, and an introduced pathogen population.

The low level of isozyme gene diversity is also reflected in low levels of genotypic diversity for the South African *P. cinnamomi* population. Comparison of genotypic diversities revealed similarity between the two regions. The higher level of genotypic diversity observed for the Mpumalanga population might be due to the small sample size from this region. A low level of maximum genotypic

TABLE 3. Summary statistics for "old" (1977–1986) and "new" (1991–1993) *Phytophthora cinnamomi* populations from the Cape and Mpumalanga regions in South Africa based on 30 isozyme alleles from 20 putative loci

	Cape			Mpumalanga			South Africa total
	"Old"	"New"	Total	"Old"	"New"	Total	
Genotypes	1–8	1, 4, 6, 7, 9	1–9	1, 2, 4, 6, 7	1, 2, 6, 7	1, 2, 4, 6, 7	1–9
$\hat{G}$ [u]	1.9	2.7	2.6	2.9	2.5	2.8	3.3
$\hat{G}/N$ (%)[v]	2.6	7.5	2.4	20.6	20.7	10.6	2.4
A[w]	1.71	1.50	1.71	1.50	1.29	1.50	1.43
H_{obs}[x]	0.008	0.060	0.025	0.050	0.071	0.063	0.033
H_{exp}[y]	0.072	0.105	0.104	0.104	0.083	0.095	0.115
F[z]	0.89	0.43	0.76	0.52	0.14	0.34	0.71

[u] $\hat{G}$ = genotypic diversity (25).
[v] $\hat{G}/N$ (%) = the percentage of maximum possible diversity obtained.
[w] A = mean number of different alleles per locus.
[x] H_{obs} = observed heterozygosity.
[y] H_{exp} = expected heterozygosity (= Nei's gene diversity index [17]).
[z] F = Wright's fixation index.

TABLE 4. Allele frequencies of "old" (1977–1986) and "new" (1991–1993) and A1 and A2 mating type populations of *Phytophthora cinnamomi* from the Cape and Mpumalanga regions in South Africa

		Cape		Mpumalanga		Mating type	
Locus[z]	Allele	"Old"	"New"	"Old"	"New"	A1	A2
IDH	A	0.062	0.306	0.321	0.417	0.000	0.352
	B	0.212	0.417	0.607	0.583	0.000	0.648
	C	0.712	0.278	0.071	0.000	0.984	0.000
	D	0.014	0.000	0.000	0.000	0.016	0.000
ACO	A	0.096	0.611	0.571	0.667	0.000	0.634
	B	0.178	0.111	0.357	0.333	0.000	0.366
	C	0.712	0.278	0.071	0.000	0.984	0.000
	D	0.014	0.000	0.000	0.000	0.016	0.000
M6PI	A	0.096	0.611	0.571	0.667	0.000	0.634
	B	0.178	0.111	0.357	0.333	0.000	0.366
	C	0.712	0.278	0.071	0.000	0.984	0.000
	D	0.014	0.000	0.000	0.000	0.016	0.000
SOD	A	0.979	0.708	0.750	0.708	1.000	0.732
	B	0.021	0.292	0.250	0.292	0.000	0.268

[z] IDH = isocitric dehydrogenase; ACO = aconitate hydratase; M6PI = mannose-6-phosphate isomerase; and SOD = superoxide dismutase.

diversity (2.4%) in the Cape *P. cinnamomi* population is partially due to a high number of A1 mating type isolates of multilocus isozyme genotype 4.

There are three lines of evidence that indicate the absence or infrequent occurrence of sexual reproduction in the South African *P. cinnamomi* population. First, allele frequencies in only one of the eight tests were in Hardy-Weinberg equilibrium. Second, some alleles present in the A1 population were not found in the A2 mating type population (Table 4), and genetic distance (D_m = 0.581) was high between A1 and A2 mating type populations from the Cape. This was significantly higher than the genetic distance values between regions and among the "old" and the "new" *P. cinnamomi* population from the Cape. Infrequent genetic recombination is the most likely explanation for the observed high level of differentiation between mating type populations, which apparently lack a common gene pool. Third, the low level of observed heterozygosity and the high fixation index (F = 0.71) indicate that the population is not randomly mating (3).

In a review by Goodwin (10), fixation indices of various *Phytophthora* species were compared. From these, it is evident that the *P. cinnamomi* population from Papua New Guinea had a high level of heterozygosity and a fixation index of 0.34, which is consistent with a random mating population. The Australian *P. cinnamomi* population had a fixation index of 0.56, which significantly deviates from that expected of a randomly mating *Phytophthora* population (10). The South African *P. cinnamomi* population, with a fixation index of 0.71, compares well with the Australian population in this regard. Based on isozyme data, a lack of genetic recombination in the Australian *P. cinnamomi* population was demonstrated by Old et al. (20). Populations of *P. cinnamomi* in Australia and South Africa seem to approach homozygosity, as experienced for homothallic *Phytophthora* species. However, more genetic markers should be employed to determine the actual levels of heterozygosity so as to accurately identify the importance of sexual reproduction in populations of *P. cinnamomi*. Population size and regional distribution of isolates also should be considered.

Gene diversity in the South African *P. cinnamomi* population is fairly stable over time, as indicated by the low genetic distance (D_m = 0.164) between the "old" and "new" *P. cinnamomi* populations from the Cape region. Also, the two regional populations appear to be similar, as a low genetic distance (D_m = 0.165) was obtained between populations. Overall, three multilocus isozyme genotypes identified in 1977 to 1986 were not identified again in 1991 to 1993. This is also reflected in the presence of more alleles in the "old" compared with the "new" *P. cinnamomi* population in both regions. The inability to detect these specific alleles especially occurred in the Cape population, which may be due to a smaller number of isolates sampled from the "new" population. In addition, multilocus isozyme genotype 9 was only identified in the "new" Cape population. Multilocus isozyme genotype 4 could not be found in the "new" Mpumalanga population, but could have escaped isolation as it was rarely encountered in the "old" Mpumalanga population.

TABLE 5. Unbiased minimum genetic distance (D_m) among *Phytophthora cinnamomi* mating type and temporal populations from the Cape region, and among regional populations in South Africa

	D_m		
Locus[y]	A1:A2	"Old":"New"[z]	Cape:Mpumalanga
IDH	0.758	0.139	0.209
ACO	0.749	0.224	0.214
M6PI	0.749	0.224	0.214
SOD	0.066	0.070	0.021
Average	0.581	0.164	0.165

[y] IDH = isocitric dehydrogenase; ACO = aconitate hydratase; M6PI = mannose-6-phosphate isomerase; and SOD = superoxide dismutase.

[z] "Old" = collected from 1977 to 1986 and "New" = collected from 1991 to 1993.

This study does not answer the question of origin of *P. cinnamomi*. A higher level of genetic diversity would be expected in the *P. cinnamomi* population if it was indigenous to South Africa, as hypothesized by von Broembsen and Kruger (33). The low levels of gene and genotypic diversity in the South African *P. cinnamomi* population are not consistent with a South African origin. Commercial forestry in South Africa has been established in the Cape region and probably acted as a means of distribution for *P. cinnamomi* A2 mating type isolates in forestry areas. This is evident as a high number of different multilocus isozyme genotypes was observed in isolates from *Pinus* species (seven genotypes out of 11 isolates) and *Eucalyptus* species (five genotypes out of 11 isolates) in forestry plantations. A high number of different multilocus isozyme genotypes was also observed from *Ocotea bullata* (native forest tree species) (six genotypes out of 39 isolates). However, this may be partly due to a higher number of isolates analyzed from this host compared with those from *Pinus* and *Eucalyptus* species. *Ocotea* trees occur in the same areas as the commercial forest plantations, and it is possible that the A2 mating type spread from the plantations to the *Ocotea*. To determine the origin of *P. cinnamomi*, a comprehensive study on a global scale is needed in which populations from Papua New Guinea, South Africa, Australia, and Asia, in particular, are compared. Among the four geographical areas mentioned, Papua New Guinea seems the most likely option, as isozyme studies have already indicated the presence of high levels of gene diversity (19,20).

The locus for the aconitase enzyme was previously reported as heterozygous for *P. cinnamomi* populations from Papua New Guinea, China, and Taiwan (20,21). In this study, the ACO locus was always homozygous in the South African *P. cinnamomi* population. This may either be due to different enzyme buffer systems employed, which could have resulted in the detection of different alleles, or simply due to the lack of ACO heterozygotes in the South African population. The absence of heterozygosity at the ACO locus in the South African population was also observed by Oudemans and Coffey (21). This may indicate the introduction of only *P. cinnamomi* isolates homozygous at the ACO locus and the lack of genetic recombination between isolates homozygous for different alleles.

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