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Genetic diversity among isolates of *Phaeomoniella chlamydospora* on grapevines

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Abstract. *Phaeomoniella chlamydospora* is one of the main causal agents of Petri disease and esca of grapevines. Although it is known to have a coelomycete synanamorph, no teleomorph has thus far been reported for *P. chlamydospora*, and its disease cycle remains largely unknown. The present study compared the genetic diversity of *P. chlamydospora* isolates from different grapevine-growing countries using amplified fragment length polymorphisms. Sixty-three isolates from South Africa and 25 from grapevine regions in Australia, France, Iran, Italy, New Zealand, Slovenia and the USA were studied. Two primer combinations were used producing 138 scorable markers, of which 33% were polymorphic. An unweighted paired group method of arithmetic averages analysis showed a high similarity (≥94.5%) among the different isolates. The overall low level of genetic variation confirmed asexual reproduction to be dominant in the field. Different genotypes were found among isolates of *P. chlamydospora* within the same grapevine, suggesting multiple infections from different inoculum sources. Isolates from different production areas and countries had a high percentage of similarity and clustered together, indicating the absence of genotype–geographic structure. The presence of the same genotype in different vineyards and production areas suggests that long-range dispersal through aerial inoculum or infected plant material play an important role in genotype distribution.

Additional keyword: *Vitis vinifera*.

Introduction

Petri disease and esca are vascular diseases that cause dieback and stunted growth of grapevines (*Vitis* spp.) worldwide. Petri disease is caused by a combination of *Phaeomoniella* (*P.*) *chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams and several species of *Phaeoacremonium* (*Pm.*) W. Gams, Crous & M.J. Wingf. (Scheck *et al*. 1998; Mugnai *et al*. 1999; Groenewald *et al*. 2001; Mostert *et al*. 2005). More often, *P. chlamydospora* has been associated with typical Petri disease symptoms than species of *Phaeoacremonium* (Mugnai *et al*. 1999; Chicau *et al*. 2000; Edwards and Pascoe 2004). The same fungi, in conjunction with the wood rotting basidiomycetes *Fomitiporia mediterranea* M. Fischer and to a lesser extent *Stereum hirsutum* (Willd.:Fr) Pers, are associated with esca symptoms (Larignon and Dubos 1997; Mugnai *et al*. 1999; Ari 2000; Cortesi *et al*. 2002; Fischer 2002). Petri disease often occurs on young grapevines (1–5 years old) and has caused significant losses in newly planted vineyards (Bertelli *et al*. 1998; Mugnai *et al*. 1999; Morton 2000; Pascoe and Cottral 2000). Although esca affects mostly vines older than 15 years, younger vines have also been observed with esca symptoms (Edwards *et al*. 2001).

Several studies have examined genetic diversity of *P. chlamydospora*, although these studies focused mainly on variation within a single country. Analysis of genetic variation conducted using a combination of RAMS (Random Amplified Micro or Mini Satellites), RAPD (Random Amplified Polymorphic DNA), AFLP and UP-PCR (Universally Primed Polymerase Chain Reaction) markers generally showed low levels of diversity in populations of *P. chlamydospora* from France, Italy and New Zealand (Borie *et al*. 2002; Pottinger *et al*. 2002; Tegli *et al*. 2000). Of the different techniques used by Pottinger *et al*. (2002), AFLPs gave the greatest amount of variation and good insight into genotype distribution. The overall low

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level of genetic diversity supported the observation that only asexual reproduction occurs in vineyards. However, Tegli *et al*. (2000) reported low levels of gametic disequilibrium, suggesting that cryptic sex could be occurring. Borie *et al*. (2002) found genetic variation among isolates of *P. chlamydospora* in France indicating recombination, and they concluded that this probably occurred via the parasexual cycle.

The aim of this study was to investigate the genetic diversity among isolates of *P. chlamydospora* on grapevines at an intra-vine, intra-vineyard and inter-vineyard level using AFLPs. Knowledge pertaining to the distribution of different genotypes would shed light on the sources of inoculum and pathogen dispersal, and thus aid in refining control strategies. Isolates of *P. chlamydospora* were collected from different positions on the same vine, different vines within a vineyard and different vineyards within South Africa. Selected isolates from different grape producing countries, Australia, France, Italy, Iran, New Zealand, Slovenia and the USA were included to test for genotype-geographic distribution.

Methods

Fungal isolates

A total of 88 isolates of *P. chlamydospora* was investigated in this study (Table 1). Sixty-three isolates were obtained from grapevines in South Africa. The rootstock, trunk (scion) and cordons (scion) of diseased grapevines were cut into disks and surface sterilised using the following protocol: 30 s in 70% ethanol, 2 min in 1% sodium hypochloride and 15 s in 70% ethanol. Small pieces of tissue were cut from just below the surface, around and in the darkened vascular tissues, placed onto potato-dextrose agar (PDA, Biolab, South Africa) amended with streptomycin (1 mL/L) and incubated at 25°C in the dark. Single-conidium isolations were made from colonies identified as *P. chlamydospora*. Three vines were sampled more intensively as illustrated in Fig. 1. A total of 12, seven and five isolates were obtained from vines Nietvoorbij 1/1 (sampled from both cordons), Zandrift 1/2 and Zandrift 1/5, respectively. Intra-vineyard variation was studied in four vineyards, Honeydew, Zandrift 1, Zandrift 2, Zandrift 3, with respectively eight, four, two and three vines sampled. Inter-vineyard variation in South Africa was studied by collecting isolates from six vineyards representing four grapevine growing districts (Paarl, Robertson, Stellenbosch and Swartland). Isolates representative of different grapevine regions in other countries were obtained from Australia (five), France (three), Italy (nine) and New Zealand (five).

Table 1. Isolates of *Phaeomoniella chlamydospora* obtained from *Vitis vinifera* for AFLP analysis

Country	Region/District	Farm	Vineyard/ vine[A]	Vine position[B]	Accession number
South Africa	Paarl	Honeydew	1/1	C	L.M. 38
South Africa	Paarl	Honeydew	1/1	T	L.M. 39
South Africa	Paarl	Honeydew	1/2	C	L.M. 40
South Africa	Paarl	Honeydew	1/4	C	L.M. 299
South Africa	Paarl	Honeydew	1/4	B	L.M. 300
South Africa	Paarl	Honeydew	1/4	R	L.M. 301
South Africa	Paarl	Honeydew	1/5	T	L.M. 303
South Africa	Paarl	Honeydew	1/5	C	L.M. 302
South Africa	Paarl	Honeydew	1/6	C	L.M. 304
South Africa	Paarl	Honeydew	1/8	T	L.M. 310
South Africa	Paarl	Honeydew	1/9	T	L.M. 312
South Africa	Paarl	Honeydew	1/9	C	L.M. 311
South Africa	Paarl	Honeydew	1/10	R	L.M. 316
South Africa	Paarl	Zandrift	1/2	G5	L.M. 143
South Africa	Paarl	Zandrift	1/2	T8	L.M. 146
South Africa	Paarl	Zandrift	1/2	C11	L.M. 148
South Africa	Paarl	Zandrift	1/2	C13	L.M. 150
South Africa	Paarl	Zandrift	1/2	C16	L.M. 152
South Africa	Paarl	Zandrift	1/2	C17	L.M. 153
South Africa	Paarl	Zandrift	1/2	C20	L.M. 154
South Africa	Paarl	Zandrift	1/3	R3	L.M. 156
South Africa	Paarl	Zandrift	1/3	R4	L.M. 196
South Africa	Paarl	Zandrift	1/3	T6	L.M. 158
South Africa	Paarl	Zandrift	1/4	T9	L.M. 171
South Africa	Paarl	Zandrift	1/5	R2	L.M. 130
South Africa	Paarl	Zandrift	1/5	G5	L.M. 131
South Africa	Paarl	Zandrift	1/5	C13	L.M. 137
South Africa	Paarl	Zandrift	1/5	C14	L.M. 138
South Africa	Paarl	Zandrift	1/5	C19	L.M. 183
South Africa	Paarl	Zandrift	1/5	C20	L.M. 141
South Africa	Paarl	Zandrift	2/5	T	L.M. 101
South Africa	Paarl	Zandrift	2/5	C	L.M. 102

(*Continued next page*)

Table 1. Continued

Country	Region/District	Farm	Vineyard/ vine[A]	Vine position[B]	Accession number
South Africa	Paarl	Zandrift	2/18	R	L.M. 97
South Africa	Paarl	Zandrift	2/18	C	L.M. 99
South Africa	Paarl	Zandrift	3/5	R	L.M. 94
South Africa	Paarl	Zandrift	3/5	T	L.M. 95
South Africa	Paarl	Zandrift	3/8	T	L.M. 110
South Africa	Paarl	Zandrift	3/16	T	L.M. 104
South Africa	Paarl	Zandrift	3/16	C	L.M. 105
South Africa	Paarl	Zandrift	4/14	B	L.M. 107
South Africa	Robertson				L.M. 3
South Africa	Stellenbosch	Inge Bein			L.M. 10
South Africa	Stellenbosch	Nietvoorbij	1/1	R1	L.M. 700, F.H. 1
South Africa	Stellenbosch	Nietvoorbij	1/1	R2	L.M. 701, F.H. 2
South Africa	Stellenbosch	Nietvoorbij	1/1	R3	L.M. 702, F.H. 3
South Africa	Stellenbosch	Nietvoorbij	1/1	R3.5	L.M. 703, F.H. 4
South Africa	Stellenbosch	Nietvoorbij	1/1	R4	L.M. 704, F.H. 5
South Africa	Stellenbosch	Nietvoorbij	1/1	G5	L.M. 705, F.H. 6
South Africa	Stellenbosch	Nietvoorbij	1/1	T9	L.M. 708, F.H. 9
South Africa	Stellenbosch	Nietvoorbij	1/1	C14Left	L.M. 710, F.H. 11
South Africa	Stellenbosch	Nietvoorbij	1/1	C18Left	L.M. 711, F.H. 12
South Africa	Stellenbosch	Nietvoorbij	1/1	C14Right	L.M. 712, F.H. 13
South Africa	Stellenbosch	Nietvoorbij	1/1	C19Right	L.M. 715, F.H. 16
South Africa	Stellenbosch	Nietvoorbij	1/1	C20Right	L.M. 740
South Africa	Swartland	De tuine	1/2	C	L.M. 37
South Africa	Swartland	De Tuine	1/3	C	L.M. 210
South Africa	Swartland	De Tuine	1/4	T	L.M. 211
South Africa	Swartland	De Tuine	1/6	C	L.M. 215
South Africa	Swartland	De Tuine	1/7	T	L.M. 222
South Africa	Swartland	De Tuine	1/7	C	L.M. 221
South Africa	Swartland	De Tuine	1/8	C	L.M. 223
South Africa	Swartland	De Tuine	1/9	T	L.M. 217
South Africa	Swartland	De Tuine	1/9	R	L.M. 219
Italy	Bandinelli				L.M. 683, Bb16
Italy	Friuli				L.M. 674, 389.79.95
Italy	Lombardia				L.M. 671, 229.I.95
Italy	Puglia				L.M. 680, 330.U2.95
Italy	Tuscany				L.M. 682, RM1
Italy	Sicily				L.M. 686, 205.03
Italy	Umbria				L.M. 675, 191P195
Italy	Umbria				L.M. 676, 202.P1.25
Italy	Veneto				L.M. 673, 1101.95
Australia	New South Wales, Hunter Valley				L.M. 662, VPRI 30667
Australia	New South Wales, Wentworth				L.M. 664, VPRI 22914
Australia	South Australia, Clare Valley				L.M. 661, VPRI 30666
Australia	South Australia, Markaranka				L.M. 663, VPRI 30668
Australia	South Australia, Padthaway				L.M. 665, VPRI 22925
New Zealand	Auckland				L.M. 658, A9
New Zealand	Blenheim(1)				L.M. 656, B2
New Zealand	Blenheim(2)				L.M. 657, Bcb1
New Zealand	Canterbury				L.M. 659, PchLV
New Zealand	Gisborne				L.M. 655, G1
France	Alsace				L.M. 735, LCP 933884
France	Dordogne				L.M. 738, LCP 974026
France	Gironde				L.M. 736, LCP 974025
Iran	South Iran, Shool				L.M. 746
Slovenia					L.M. 755
USA	California				L.M. 789, CBS 101571

[A]The first number distinguishes the different vineyards on the same farm and the second number the different vines in the same vineyard.
[B]Positions sampled per vine: rootstock (R), graft union (G), trunk (T) and cordon (C).

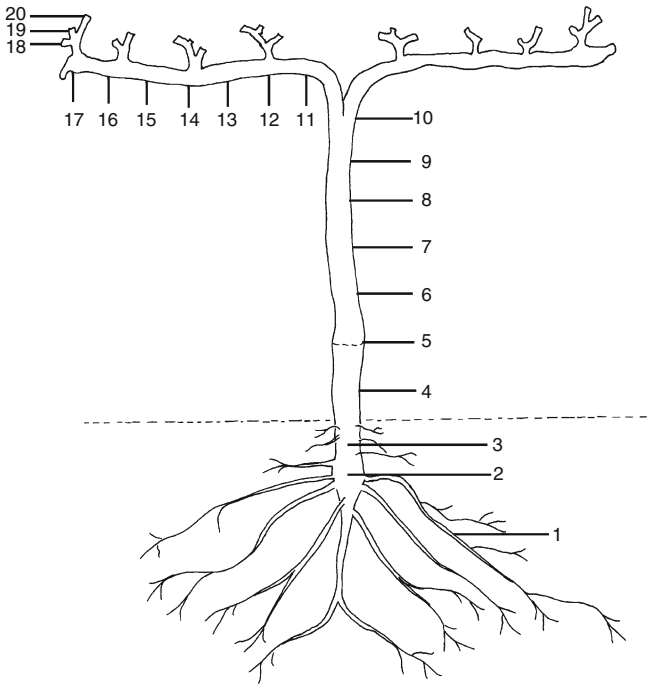

Fig. 1. Positions sampled on different grapevines: 1 = roots; 2–4 = rootstock; 5 = graft union; 6–10 = trunk, and 11–20 = cordon.

Single isolates were obtained from Iran, Slovenia and the USA. Isolates are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, with representative strains deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

DNA extraction

Isolates were plated onto 2% malt extract agar (MEA, Biolab, South Africa) and incubated for ~14 days at 24°C. Genomic DNA was extracted from 200 mg fungal mycelium using the FastDNA Kit (Bio101, Carlsbad, CA) according to the manufacturer's instructions. The concentration of DNA was estimated with SmartLadder (Eurogentec, Belgium) on a 1% (w/v) agarose gel containing ethidium bromide at 0.1 µg/mL in 0.5 × TAE buffer (0.4 M Tris, 0.05 M sodium acetate and 0.01 M EDTA, pH 7.85) and visualised under UV light. The DNA was stored at −20°C.

AFLP analysis

The procedure for AFLPs was modified from that of Vos *et al*. (1995). Restriction enzyme digestion and adaptor ligation were conducted using 10 ng of DNA, 10 U of *Eco*RI [New England BioLabs (NEB), Beverly, MA], 2 U of *Mse*I (NEB), 50 mM NaCl, 1 × T4 DNA ligase buffer (NEB), 10 mM $MgCl_2$, 1 mM ATP, 10 µg bovine serum albumin, 100 pmol *Mse*I-adapters, 10 pmol *Eco*RI-adapters and 40 U T4 DNA ligase (NEB) made up to a final volume of 11 µL with dH_2O. This reaction was carried out at 37°C for 12 h. After ligation, 25 µL dH_2O were added to the reaction mixture. The preselective PCR was performed with 4 µL diluted restriction/ligation mixture, 1.2 pmol of primer *Eco*RI-0 (Vos *et al*. 1995), 6.5 pmol of primer *Mse*I-0 (Vos *et al*. 1995), 1.5 mM $MgCl_2$, 0.5 U *Taq* DNA polymerase (Bioline, Luckenwalde, Germany), 1 × Bioline *Taq* polymerase buffer and 0.1 mM of each dNTP in a total volume of 20 µL. Amplification was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) starting with an initial 2 min step at 72°C followed by 25 cycles of 94°C for 20 s, 56°C for 30 s and 72°C for 2 min. The preselective amplification was monitored by electrophoresis on a 0.8% (w/v) agarose gel as described above. The preamplified DNA was diluted 1 : 1 with dH_2O and used as template for selective amplification. The selective amplification was prepared with 1.5 µL diluted preselective amplification mix, 5 ng *Eco*RI, 30 ng *Mse*I, 1.5 mM $MgCl_2$, 0.5 U Bioline *Taq* DNA polymerase, 1 × Bioline *Taq* polymerase buffer and 0.1 mM of each dNTP in a total volume of 10 µL. Twenty-two different selective primer combinations were screened against three isolates of *P. chlamydospora*. Of these, primer combinations *Eco*RI-T/*Mse*I-CC and *Eco*RI-A/*Mse*I-CG (Applied Biosystems) gave good resolution and an appropriate number of polymorphic bands, and were subsequently used for all isolates. Amplification was performed for 20 cycles using the following cycle profile: 94°C for 20 s, annealing step (see below) for 30 s and 72°C for 2 min. The annealing temperature in the first cycle was 66°C, which was subsequently reduced in each cycle by 1°C for the next nine cycles, and continued at 56°C for the last nine cycles. Selective PCR products (2 µL), amplified with the different primer combinations for each isolate were mixed with 0.5 µL of 6-carboxy-X-rhodamine labelled GeneScan 500 size standard (Applied Biosystems) and made up to a final volume of 25 µL with deionised formamide. The products were denatured at 96°C for 5 min and snap-cooled on ice. The samples were analysed using an ABI Prism 310 Genetic Analyser (Applied Biosystems, Foster City, USA). After electrophoresis, the patterns were extracted with Genescan 3.1 (Applied Biosystems).

Data analysis

Fingerprints were analysed with Bionumerics software (v2.5, Applied Maths, Kortrijk, Belgium). Polymorphic loci were identified as bands of equal molecular weight that were well resolved and were present in some isolates and absent in others. Fragments smaller than 40 and larger than 450 nucleotides were excluded from the analysis. The similarity among the AFLP patterns was calculated with Jaccard's correlation coefficient for each primer combination. A cluster analysis was performed by the Unweighted Pair-Group Method of Arithmetic Averages (UPGMA) on the average of the combined dataset. The reproducibility of the AFLP procedure was tested with five separate digestion-ligation reactions, preselective PCR and selective PCR using one isolate of *P. chlamydospora*.

Results

A total of 138 scorable bands were produced, of which 33% were polymorphic. The reproducibility test gave the same banding pattern in each instance showing that the results were consistent. The primer combination, *Eco*RI-T/*Mse*I-CC produced 50 bands, of which 13 were polymorphic. The *Eco*RI-A/*Mse*I-CG combination produced 88 bands, of which 32 were polymorphic. The level of overall similarity among the isolates was high (≥94.5%). However, several different genotypes were present (Fig. 2). Isolates with a 100% similarity were scored as the same genotype.

The UPGMA analysis identified four major clades (Fig. 2). The genetic similarity within these clades was 97.8% for clade I, 97.2% for clade II, 95.8% for clade III and 94.5% for clade IV. Each major clade contained a combination of isolates from different countries, indicating that genotypes tend not to group according to geographic origin. Clade I consisted of 41 South African, one Australian and two Italian isolates. Clade II contained 13 isolates from South Africa, and four from Australia. Clade III had four South African,

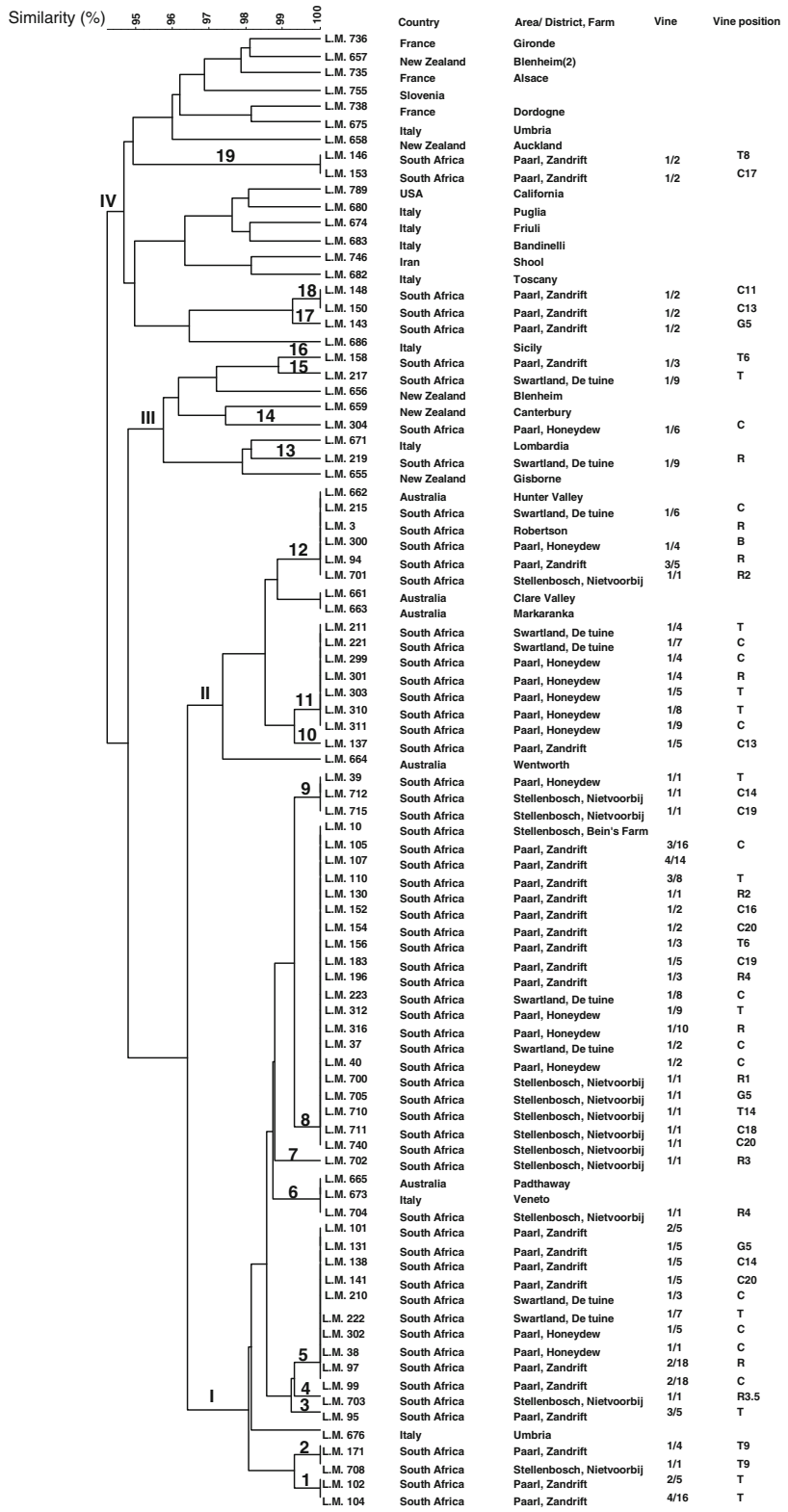

Fig. 2. Dendogram depicting patterns of genetic similarity among 88 isolates of *Phaeomoniella chlamydospora*, based on AFLP analysis using two primer combinations, calculated using Jaccard's correlation coefficient and UPGMA cluster analysis. R = rootstock, G = graft union, T = trunk and C = cordon. Roman numerals indicate major clades. Numbers indicate different genotypes found among South African isolates.

three New Zealand and one Italian isolate. Clade IV, the geographically most diverse clade, had three isolates from France, one from Iran, six from Italy, two from New Zealand, one from Slovenia, five from South Africa and one from the USA.

More than one isolate was obtained from 14 South African vines in the Paarl, Stellenbosch and Swartland regions. With the exception of vine Zandrift 2/18, each vine had more than one genotype. The maximum number of genotypes in a vine was seven (Nietvoorbij 1/1). This specific vine also had five isolates with the same genotype isolated from the roots, graft union and each cordon (L.M. 700, L.M. 705, L.M. 710, L.M. 711 and L.M. 740). Six vines (Zandrift 1/2, Zandrift 1/3, Zandrift 1/5, Zandrift 2/18, Honeydew 1/4, Nietvoorbij 1/1) had two or more isolates with the same genotype.

Intra- and inter-vineyard variation is illustrated in Fig. 3 and summarised in Table 2. Each vineyard in South Africa contained more than one genotype and the maximum number of genotypes found in a vineyard was eight. The number of isolates from each vineyard differed due to variation in isolation success.

Several genotypes were observed in each country for which multiple strains were available (Table 2). The South African isolates represented several different, as well as the same genotype. Mostly different genotypes were observed for the isolates from the other countries. However, two Australian isolates from Clare Valley and Markaranka had the same genotype. An Italian isolate (L.M. 673) had the same genotype as an Australian (L.M. 665), and South African (L.M. 704) isolate. A further Australian (L.M. 662) isolate was similar to five South African isolates (L.M. 3, L.M. 94, L.M. 215, L.M. 300, L.M. 701).

Discussion

Genetic variation among isolates of *P. chlamydospora* collected from several countries with grapevines exhibiting Petri disease or esca was investigated using AFLPs. Even though there was a high similarity among the *P. chlamydospora* isolates (≥94.5%), several genotypes were distinguished. The low levels of genetic variation confirmed asexual reproduction to be dominant in vineyards. Currently, the genus *Phaeomoniella* is known from a single species, *P. chlamydospora*, which falls within the *Chaetothyriales* (Crous and Gams 2000), and lacks any known teleomorph connection. A second species of *Phaeomoniella* has recently been isolated as an endophyte on *Pinus*, but it also lacks any

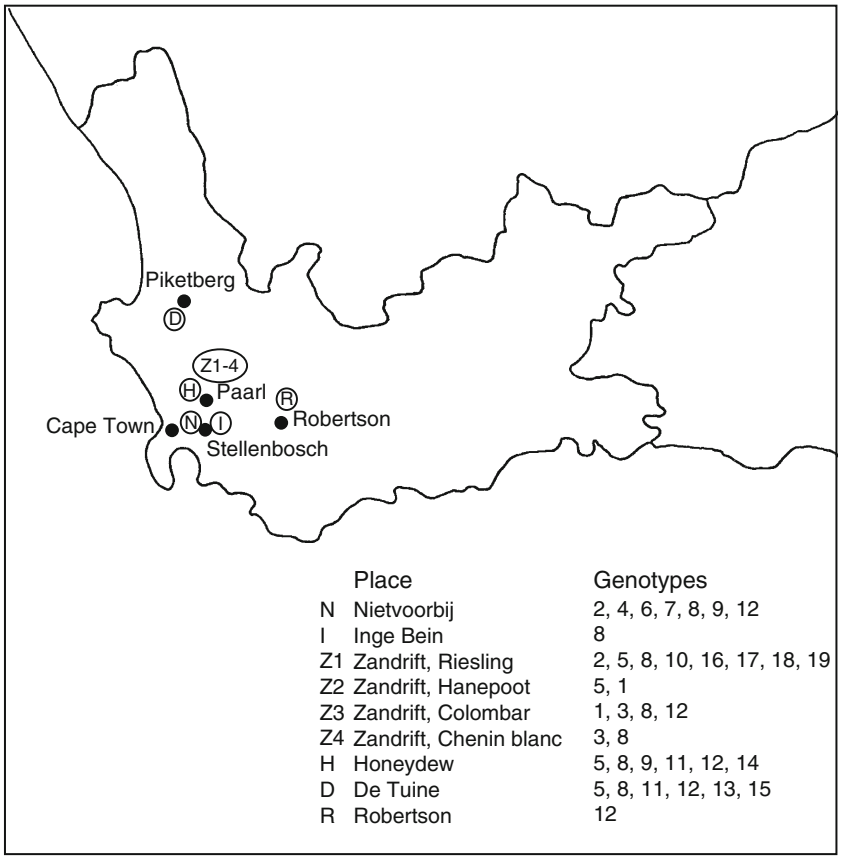

Fig. 3. Map of the Western Cape of South Africa showing the places where isolates of *Phaeomoniella chlamydospora* was sampled. The legend indicates the different genotypes found in each vineyard.

Table 2. Number of *Phaeomoniella chlamydospora* isolates obtained and genotypes observed in countries and vineyards investigated

Location	Number of isolates	Number of genotypes
Australia	5	5
France	3	3
Italy	9	9
New Zealand	5	5
South Africa[A]	63	19
Paarl, Honeydew, Alfons	13	6
Paarl, Zandrift, Colombar	5	4
Paarl, Zandrift, Riesling	17	8
Paarl, Zandrift, Hanepoot	4	2
Swartland, De Tuine, La Rochelle	9	6

[A]Multiple vineyards listed where more than one vine was sampled.

teleomorph affiliation (R. C. Summerbell, pers. comm.). The mechanisms for genetic variation in apparently asexual fungi are mostly unknown, but have been ascribed to mutation, somatic hybridisation and heterokaryosis (Burdon and Silk 1997; Schoustra 2004). Isolates of *P. chlamydospora* from New Zealand representing different genotypes all fell into the same mycelial compatibility group (MCG) (Pottinger *et al*. 2002). The presence of a single MCG among isolates of *P. chlamydospora* could favour anastomosis and the subsequent parasexual cycle.

Some genotypes were found in more than one country. Similar clonal lineages of *P. chlamydospora* in different countries could be due to single introduction events from the same inoculum source (grapevine cuttings). Other studies have also reported finding similar genotypes in different countries. Tegli *et al*. (2000) found with RAMS markers that one isolate from South Africa and the USA were similar to two different genotypes found among Italian isolates. Pottinger *et al*. (2002) also found corresponding genotypes of two Italian isolates among their New Zealand isolates. Even though the current study did not aim to resolve the geographic origin of *P. chlamydospora*, more extensive sampling from the areas where cultivars used in viticulture originated, namely Eurasia and the USA (Weaver 1976; De Blij 1983; Unwin 1991), would aid in understanding this question.

The isolates from different grape growing regions in Australia, France, Italy and New Zealand mostly had different genotypes. The nine Italian isolates all represented different genotypes, which is in agreement with the findings of Pottinger *et al*. (2002), who reported that five of the six Italian isolates they studied represented different genotypes. Pottinger *et al*. (2002) found that three of the New Zealand isolates (L.M. 658, L.M. 657 and L.M. 655) had the same genotype using AFLPs, but different genotypes with UP-PCR analysis. The reason the AFLPs resolved less genotypes than observed in the present study is because Pottinger *et al*. (2002) used two and three nucleotide extensions on the selective primers of their AFLPs (AA/CAT and GA/CAT) that selected for fewer fragments and produced only 36 scorable bands.

Inter-vineyard comparisons showed that different genotypes, as well as the same genotype, were present in different vineyards in South Africa. Inter-vineyard genetic variation was also observed by Pottinger *et al*. (2002), who concluded that multiple introductions of *P. chlamydospora* occurred into New Zealand. The widespread occurrence of the same genotype in different areas of South Africa is probably due to the extensive sampling done in this country. The occurrence of the same genotype in different production areas indicates that either the material came from the same infected nursery, or that long-range dispersal has taken place.

Up to eight different genotypes were found within a single vineyard. Multiple genotypes were also detected within single vineyards in Italy, France and New Zealand (Tegli *et al*. 2000; Borie *et al*. 2002; Pottinger *et al*. 2002). This result confirms that vineyards are exposed to several sources of inoculum (Tegli *et al*. 2000).

Multiple isolates obtained from 14 vines were used to establish intra-vine genotype variation. In some vines, only one genotype was found, suggesting that only one infection took place, from where it spread through the vine. Histopathology of grapevines artificially inoculated with *P. chlamydospora* has shown hyphae inside the xylem vessels (Pascoe and Cottral 2000; Lorena *et al*. 2001). Although conidia of *P. chlamydospora* have not been observed in vascular tissues, conidia of *Pm. aleophilum* have been observed in the vascular vessels of artificially inoculated vines (Feliciano and Gubler 2001). Hence, the possibility cannot be excluded that *P. chlamydospora*, which also exhibits a yeast-like growth phase in culture, can spread throughout the vascular system of an infected vine. It is, however, also possible that multiple infections by the same genotype could occur. In the other vines, between two and seven genotypes were found, showing that multiple infection events are common.

The control of Petri disease and esca is focused mainly on preventative measures, since there are no effective curative chemicals. The occurrence of the same genotype in different countries supports the fact that *P. chlamydospora* can occur in apparently healthy rooted grapevine cuttings (Bertelli *et al*. 1998), and seemingly disease-free grapevines (Gräfenhan *et al*. 2005) and could be distributed via exported grapevine material. Measures that can be taken to ensure clean planting material includes using disease-free grafting material, hot water treatment of rootstock cuttings, clean grafting procedures and hot water treatment of dormant nursery grapevines before planting (Mugnai *et al*. 1999; Crous *et al*. 2001; Fourie and Halleen 2004).

In summary, a high level of similarity was found among the isolates of *P. chlamydospora* in South Africa and other countries investigated. This indicates that *P. chlamydospora* primarily reproduces asexually in the field. However, different genotypes were detected intra-vine, intra- and inter-

vineyard, and among different production areas, indicating that infections occur via different sources of inoculum. Aerial inoculum of *P. chlamydospora* can infect pruning wounds (Larignon and Dubos 1997; Sparapano *et al*. 2000; Eskalen and Gubler 2001; Feliciano *et al*. 2004), making the protection of wounds during grafting and pruning of the utmost importance.

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