CHAPTER 6

Genetic structure in a global population of *Fusarium oxysporum* f. sp. *cubense*

ABSTRACT

Fusarium oxysporum f. sp. *cubense* (*Foc*) is a haploid, asexually reproducing ascomycete that causes Fusarium Wilt or Panama Disease of bananas. The fungus has been found in most banana producing areas of the world. For effective disease management knowledge of the population structure is becoming essential. In the present study, vegetative compatibility groups (VCGs) of *Foc* isolates from South Africa were determined and compared with those from other areas where Panama disease occurs. We also used eight polymorphic PCR-based microsatellite markers to characterize population structure and genetic diversity of *Foc* from diverse sources. This meta-population was divided into geographically defined sub-populations, namely those from Asia, Australia and South Africa. Genetic differentiation between the Asian and South African populations was high and gene flow minimal. Index of association (I_A) and linkage disequilibrium tests showed no evidence of recombination within the global population. This supports the view that *Foc* reproduces clonally wherever it occurs. Isolates from the three regions studied appeared to be closely related although there were groups consistent with origin.

INTRODUCTION

Fusarium oxysporum is an important causal agent of vascular wilt on many agricultural crops (Booth 1971). Pathogens belonging to this species are generally grouped according to *formae speciales* (Hawksworth 1995). Amongst these, *Fusarium oxysporum* f. sp. *cubense* (*Foc*) cause of Panama disease devastates Cavendish bananas in the two regions of South Africa (Ploetz 1990, Viljoen 2002). The pathogen is soilborne, infecting bananas through the roots and moving up into the vascular system (Stover 1962). *Foc* thus blocks the xylem vessels preventing the movement of nutrients and water and ultimately, resulting in plant death. Races are poorly defined in *Foc* and are simply based on host pathogen interactions (Stover & Buddenhagen 1986). Isolates representing different populations of the pathogen also show variation in virulence due to differences in environmental conditions (Ploetz 1990). For example, Cavendish is susceptible to *Foc* 'subtropical' race 4, which occurs in South Africa but is resistant to race 1 of the pathogen occurring in the tropics (Ploetz *et al.* 1990).

Foc is believed to have been spread worldwide through infected planting material (Pegg & Langdon 1987, Stover 1962, Ploetz 1990). The route of entry of the pathogen into South Africa is unknown due to the incomplete records of banana production in the country. It is thought that Indian labourers, who worked on sugar cane plantations in KwaZulu-Natal during colonial times, could have introduced infected rhizomes into South Africa (Ploetz *et al.* 1990, Robinson 1996).

Previous studies considering diversity amongst *Foc* isolates have used both phenotypic and genotypic markers to characterize the pathogen. The phenotypic character most commonly used to subdivide *Foc* isolates is vegetative compatibility (Ploetz & Correll 1988, Brake *et al.* 1990, Moore *et al.* 1993). This character differentiates isolates that have identical alleles at

each of the loci that govern heterokaryon formation (Correll, Kliitich & Leslie 1987, Leslie 1993). *Foc* has 21 known vegetative compatibility groups (VCGs), of which only a few are common and widespread (Ploetz & Pegg 2000). Fifteen VCGs have been found in Asia, which is believed to be the centre of origin of bananas (Ploetz & Pegg 2000). Although vegetative compatibility is a useful means of subdividing *Foc* into genetically isolated groups, it does not measure genetic relatedness among isolates. In addition, VCGs are phenotypic markers that may be subject to selection (Glass & Kuldau 1992, Leslie 1993). Therefore, neutral DNA-based genetic markers would be more suitable for analysing genetic variation within and between *Foc* populations.

DNA-based genetic markers have had a significant impact on the study of population genetics of many organisms (McDonald 1997). PCR-based genetic markers, such as microsatellites that exhibit co-dominance, produce highly reproducible results that are ideal for population genetics studies (Bruford & Wayne 1993, Queller, Strassman & Hughes 1993, Taylor *et al.* 1999). Microsatellites are tandemly repeated units of 1-5 bp that are distributed throughout the eukaryotic genome (Queller *et al.* 1993). Microsatellites can vary substantially in repeat numbers between individuals (Bruford & Wayne 1993, Dayanandan, Rajora & Bawa 1998). Microsatellites are therefore popular markers in fungal population genetics.

The aim of this study was to consider the identity of *Foc* VCGs among isolates from the two primary banana-growing regions in South Africa. Secondly, we wanted to examine the genetic structure of *Foc* in South Africa and compared this with two populations of the fungus from other parts of the world. Finally we aimed to evaluate the potential of gene flow between these populations. The genetic variation within different populations was also determined, using microsatellite markers.

MATERIALS AND METHODS

Fungal isolates

All VCG tester isolates examined in this study were from the world-wide collection of *Foc* provided by Dr. Natalie Moore, ACDI Institute, Australia, Dr S. Bentley, University of Queensland and Dr R. Ploetz, University of Florida. These included a total of 75 isolates of which 16 were from Australia, 19 from Southeast Asia and 40 from South Africa. South African isolates were obtained from the main banana-growing regions in the country and included 21 from Mmpumalanga and 19 from Kwa-Zulu Natal. These isolates were stored on filter paper at 4°C and are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (Table 1).

Generation of nit mutants and VCG determination

Isolates were characterised based on vegetative compatibility using the technique of Puhalla (1985). This technique assigns isolates to VCGs based on heterokaryon formation between complementary nitrate-nonutilizing (*nit*) mutants. *Nit* mutants are produced on media supplemented with chlorate. Nit mutants were produced for all South African isolates as well as the known VCG tester strains from elsewhere in the world and these are stored in the culture collection of FABI. All *nit* 1 and *nit* 3 mutants were paired at least twice on minimal medium (MM) (Correll *et al.* 1987) with each of the Nit M mutants representing eleven tester strains for the known VCGs. Nit M, *nit* 3 and *nit* 1 mutants of the same isolate were also paired to test for self-compatibility. Complementary *nit* mutants formed dense, wild-type growth on MM as a result of heterokaryosis and were assigned to the same VCG. Vegetatively incompatible isolates were detected by their inability to form a heterokaryon when paired and grown on MM.

Microsatellite marker analysis

DNA extractions

All 75 isolates were grown in potato dextrose broth (PDB) without shaking at room temperature for 7-10 days. The mycelium was then harvested, freeze-dried and stored at –20°C. For total DNA extraction, mycelial masses were ground to a fine powder in liquid nitrogen using a mortar and pestle. Genomic DNA was extracted from each isolate using the phenol-chloroform based extraction method described by Raeder & Broda (1985).

PCR amplifications

Eight microsatellite primers designed for *Foc* (Chapter 5) were used in PCR reactions. Each 25-µl PCR reaction contained 0.4mM of each deoxynucleoside triphosphate (dNTP), 1x PCR buffer, 1.0 pmol/µl of each primer (one primer from a primer pair fluorescently labelled), 0.25 units Expand High Fidelity *Taq* polymerase (Roche Molecular Biochemicals), and 2 ng of DNA. PCR reactions were performed on a Hybaid TouchDown PCR machine (Hybaid Limited, UK). PCR reaction conditions included an initial denaturation cycle at 96°C for 2 min followed by 10 cycles of denaturation at 94°C for 20s, primer annealing at 58°C for 45s and extension at 72°C for 45s. A further 25 cycles of denaturation at 94°C for 20s, annealing at 58°C for 40s and extension at 72°C for 50s was carried out. A final extension step at 72°C for 10 min was included. Amplified products were resolved on 1.5% or 2% (w/v) agarose gels in Tris Acetate EDTA (TAE) (pH 8.0) stained with ethidium bromide and visualised under UV illumination (Sambrook, Fritsch & Maniatis 1989).

Genescan analysis

Fluorescently labelled PCR products were combined with a 1:14 mixture of formamide and LIZ ™ standard. Samples were then separated on an ABI Prism 3100 DNA sequencer. The

sizes of DNA fragments were determined using the Genescan® 2.1 and Genotyper® 3.0 analysis software (Perkin Elmer Corp.).

Data sets were generated for the analysis of the three subpopulations based on different geographic origins (South Africa, Australia, Asia). Statistical analysis of amplified loci was performed using all data sets to determine allele frequency, genetic diversity, genotypic diversity, population differentiation and gene flow.

Population analysis

Gene diversity (H) at each locus was estimated by allele frequency for each sub-population using the formula provided by Nei (1973). A graph showing the genotypic diversity (G_{ST}) (Stoddart & Taylor 1988) against the number of loci (1000 resampling repetitions) was also plotted for all populations. This was done to determine whether the eight loci were sufficiently variable for population structure analysis for *Foc*.

Population differentiation and gene flow was calculated among subpopulations using Weir's theta (θ) (Wright's F_{ST}) for randomized datasets (Weir 1996) using the program Multilocus 1.2 (Agapow & Burt 2000). To test for the presence of partitions within the global data set, G_{st} was calculated using the equation provided by Nei (1973). Indirect estimation of gene flow (N_m) was also calculated from the estimate of G_{st}.

The mode of reproduction, i. e. the hypothesis of random association among loci, was tested using the index of association (I_A) statistic, generated with the program Multilocus. The I_A test was simulated by comparing the observed value of I_A to that expected under the null hypothesis of random mating using 1000 randomizations of the original data matrix (Agapow & Burt 2000).

The genetic distance for the three combined populations was calculated using absolute distances (D_{AD}). A distance matrix was constructed using the measure $\delta\mu^2$ (Goldstein, Pitt & Taylor *et al.* 1995). The program MICROSAT (http://human.stanford.edu/microsat) was used for this purpose. A UPGMA (Unweighted Pair-Group Method with Arithmetic mean) dendrogram based on the distance matrix was constructed in MEGA version 2.1 (Kumar *et al.* 2001). Each individual population was analysed using all the available isolates. A dendrogram for the three combined populations was also constructed, in order to evaluate genetic distance between them.

RESULTS

Generation of nit mutants and VCG determination

Nit mutants were generated for all the South African isolates and all the known testers used in this study. The proportion of *nit* 1 mutants was 90%, Nit M mutants 80% and *nit* 3 mutants 70%. Almost all isolates produced at least one Nit M mutant. Crossings of Nit M with *nit* 3 or *nit* 1 isolates produced a zone of wild-type growth where the two *nit* mutants anastomosed and produced a heterokaryon. Complementary *nit* mutants formed a dense, wild-type growth on MM and were subsequently placed in the same VCG as the tester strain. Isolates from the South African population only formed heterokaryons when they were paired with the Australian VCG 0120 tester strains. No positive reactions resulted from pairings with VCGs 0121, 0122, 0129, 01211, the other VCGs associated with race 4, or isolates representing other VCG complexes.

Population analysis

Isolates from Asia had the greatest number of alleles (35) followed by Australia (21) and South Africa (18), respectively. Asian isolates shared more alleles with Australian isolates than with isolates from South Africa (Table 2). All loci were polymorphic in the global and

than with isolates from South Africa (Table 2). All loci were polymorphic in the global and subpopulations of *Foc*, except for one (G20/G20-21), which was monomorphic for the South African population. Some alleles were unique to certain groups of isolates. South African isolates had 18 alleles of which one was unique, Australian isolates had 21 alleles and only one was unique, and those from Asia had 35 alleles of which 14 were unique (Table 2).

A total of 33 different genotypes from the world-wide population of *Foc* were identified. Only one genotype was shared across the three populations. The common genotype shared in all geographic subpopulations was genotype 1 and this was also the dominant genotype in South Africa. South African and Asian populations shared three genotypes (1, 7, and 10), while Australian and Asian populations shared three genotypes (5, 10, and 25). The highest level of genotypic diversity ($\hat{G}$ = 79%) was found in the Asian population while the Australian and South African populations had $\hat{G}$ = 50% and $\hat{G}$ = 6% respectively (Table 3). The eight markers were shown to be sufficiently variable for population structure analysis by calculating genotypic diversity (G_{ST}) and plotting this value against the number of loci using randomisation in the Multilocus program (Fig 1). Only the sample representing the South African population did not reach a plateau.

Gene diversity (*H*) values were 0.108, 0.351, and 0.501 for the South African, Australian and Asian populations, respectively (Table 3). G_{ST} between subpopulations (2.5, 8 and 15.04, respectively) could also be determined as a measure of genetic variation. The data set could be divided into partitions that correlated with the geographic origin of isolates between the subpopulations (Table 3).

Population differentiation (Weir 1996) in all possible combinations produced θ values of 0.416, 0.224, and 0.175 ($P < 0.001$) for the populations from South Africa, Australia and Asia

respectively (Table 4). The level of gene flow (N_m) was the highest between the Australian and Asian population ($\frac{1}{\theta}$= 5.69, N_m = 3.315). The amount of gene flow between the South African population and the Australian population was also high ($\frac{1}{\theta}$ = 4.46, N_m = 3.601) (Table 4). The lowest amount of gene flow was found between the South African and Asian population ($\frac{1}{\theta}$= 2.39, N_m = 1.642).

The observed values for the I_A in Australian and Asian populations fell beyond the distribution range for a recombining population (P<0.001). The analysis of the Australian and Asian populations indicated clonal patterns of reproduction. Only the South African population had non-significant P-values and the null hypothesis of random mating could not be rejected (Fig. 2).

The UPGMA dendrogram based on genetic distance between the three subpopulations grouped South African isolates together and separately from the other two geographical regions (Fig. 3). One South African isolate clustered with isolates from Southeast Asia, and two isolates from Indonesia were grouped with the South African population. The Australian isolates share several genotypes with those from Asia. The Australian and Asian isolates were interspersed in the dendrogram.

DISCUSSION

In this study, both VCGs and microsatellite markers were used to evaluate the gene diversity, genotypic diversity, mode of reproduction, gene flow and partitioning between subpopulations of *Foc* from different geographical origins. The South African population was more extensively sampled than the Australian and Asian populations.

All South African isolates were in VCG 0120, which also occurs in Australia, Southeast Asia and many other banana-producing areas worldwide. This indicates that the diversity within the South African population is low and reconfirms the idea that the fungus was introduced into this area. It also confirms the wide distribution of VCG 0120 and the success of this well-adapted clone in many parts of the world. VCG analysis also supports previous reports that strains of *Foc* have been introduced into Australia from Asia, probably with banana planting material (Brake *et al.* 1990, Moore *et al.* 1993). Thus, some genotypes from the Asian gene pool have been incorporated into the gene pool of the Australian and South African populations.

The highest gene diversity found in this study was for isolates from Asia. Gene diversity is defined as the probability of obtaining two different alleles at a locus within the population (Nei 1973). The mean of H ($\overline{H}$) was calculated to facilitate comparisons among the populations. This value is supported by a higher mean allelic diversity at the loci (Hs = 0.707) and a higher genotypic diversity $\hat{G}$ = 79%. More unique alleles and genotypes were found in the Asian population than in South Africa and Australia, providing further support for an Asian origin of the pathogen. The higher gene diversity could be the result of mutations accumulating over time (Burdon & Silk 1997). According to Fisher *et al.* (2000), unique alleles are evidence that the pathogen is native to a given region (McDonald & McDermott 1993), in this case, Asia. All living organisms including fungal populations tend to be more diverse at the centre of origin and more homogeneous in areas of introduction (Andrivon 1996). Southeast Asia is believed to be the centre of origin of both the Panama disease pathogen and its host (Ploetz & Pegg 1997). Our results support this hypothesis.

The low genotypic diversity amongst *Foc* isolates from South Africa shows that *Foc* represents a clonal lineage in this country. A limited number of genotypes are shared between

the ancestral Asian population, Australia and South Africa, where the pathogen is known to be introduced. The few identical genotypes among the three populations illustrate how *Foc* is dominated by clonal lineages, presumably reflecting the effects of clonal selection (Kumar *et al.* 1999). Asexual reproduction allows for the amplification of fit genotypes, which can occur at a high frequency within a population (Pringle & Taylor 2001, McDonald & Linde 2002). In addition, geographic isolation from populations in Southeast Asia could have resulted in genetic drift, which could contribute to differences between isolated populations over time (Brasier 1987, 1995). Selection, of genotypes favoured by the environmental conditions in areas of introduction could also have resulted in new genotypes (Lobuglio, Pitt & Taylor 1993). The I_A statistics for two of the three populations also indicate a clonal pattern of reproduction. However, the South African population showed a population structure indicative of recombination. This could be due to resampling of specific genotypes, or migration (Chen & McDonald 1996, Douhan, Peever & Murray 2002) between the different banana growing regions in South Africa.

Graphs plotting G_{ST} versus number of loci showed that the Asian and Australian populations reached a plateau. G_{ST} is defined as the probability that two individuals taken at random have different genotypes (Nei 1973). The South African isolates do not appear to represent all the variation that is present in this population. However, this population has been extensively sampled and represents the highest number of isolates in this study. The low level of genotypic diversity found in the South African population was to be expected given the fact that only a single VCG occurs in this area.

Populations of *Foc* resemble clones and differ in the distribution of genotypes. Only one genotype was most common in each of the geographical subpopulations and only one was dominant in the South African population. Greater ecological fitness or reproductive

dominant in the South African population. Greater ecological fitness or reproductive advantage over other genotypes (Pringle & Taylor 1999, Schilder *et al.* 1999) could explain the large geographical range of a specific genotype. The dominance of genotype 1 in the South African population of *Foc* could be due to genetic bottlenecks and founder effects (Gillespie 1998).

There was a good correlation between population differentiation (θ) and geographical distance between subpopulations of *Foc*. The higher gene flow and low population differentiation between the Asian and Australian populations is probably due to the close geographic proximity of the two regions. Low levels of gene flow between the South African and Asian populations could be due to a physical barrier, such as distance, that would have limited migration between the populations. Fisher *et al.* (2000) showed in their study that reduced gene flow due to geographic or reproductive isolation would result in populations with low genetic variation due to random drift. Our results showed that only three genotypes are shared between South African and Australian isolates and all other genotypes are unique to these regions. This provides evidence that bananas were introduced into new areas at different times. High levels of differentiation between populations might imply that sufficient time has elapsed for the onset of genetic drift, which can eliminate alleles from a population (Leung, Nelson & Leach 1993). High levels of population differentiation observed in this study are probably due to geographic and genetic isolation. Populations may become differentiated by isolation and the accumulation of different mutations in a population (Fry *et al.* 1992).

A UPGMA dendrogram based on genetic distances between the three *Foc* populations showed that specific groups within the *Foc* metapopulation. (A metapopulation is loosely defined as the set of individuals that inhabit a landscape). The South African isolates formed

a single group but this also included two isolates from Asia. The remainder of the Australian and Asian isolates formed a subgroup. This indicates that there is a certain degree of host specialisation or environmental adaptation influencing the evolution of *Foc* in the three geographically separated regions. It could also be that the South African population has resulted from a limited number of introductions.

Results of this study indicate that in most areas *Foc* propagates clonally. It appears that Asian populations have been extensively dispersed world-wide and only some Asian genotypes have become fixed in certain geographical areas. The extensive geographical range of certain genotypes confirms that clonal populations are well adapted to different environmental conditions. Thus, the widespread distribution of the VCG 0120, and the stability of certain genotypes within populations of *Foc,* emphasises the ability of this clonal pathogen to thrive.

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Table 1. Isolates of *Foc* from a worldwide population of the pathogen used in this study.

Isolate number[1]	Other name[2]	Origin	Cultivar	VCG[3]	Donor/ Collector
Foc 1		South Africa	Cavendish	0120	A. Viljoen
Foc 2		..		..	..
Foc 4		..	Williams	..	..
Foc 5		..		..	E. Grimbeek
Foc 6		..	Cavendish	..	..
Foc 7		..		..	..
Foc 8		..	Chinese Cavendish	..	E. Grimbeek
Foc 9		..	Williams	..	..
Foc 10		..		..	A. Viljoen
Foc 11		..	Israeli Grand Naine	..	..
Foc 12		..	DC24R22	..	..
Foc 14		..		..	..
Foc 17		..	Cavendish	..	..
Foc 18		..	Israeli Grand Naine	..	..
Foc 19		..	Williams	..	E. Grimbeek
Foc 20		..	Williams	..	..
Foc 21		..	Williams	..	..
Foc 22		..	Williams	..	..
Foc 23		..	Williams	..	..
Foc 24		..		..	A. Viljoen
Foc 27		..	Cavendish	..	..
Foc 28	Taiwan 14	Taiwan		0121	R. Ploetz
Foc 31		South Africa	Cavendish	0120	A. Viljoen
Foc 42		..	..	..	..
Foc 43		..	..	..	..
Foc 45		..	..	..	..
Foc 46	23486	Australia	Cavendish	..	N. Moore
Foc 48	Thai1-2	Thailand	Kluai Namwa	0123	..
Foc 49		Australia		0124	..
Foc 50		..		0125	..
Foc 51	Phil 6	Philippines	Latundan	0126	..
Foc 52		Australia		0129	..
Foc 53	Indo-14	Indonesia	Pisang Ambon Putih	0120	I. Djatnika
Foc 54		Philippines		0122	R. Ploetz
Foc 57	RPMW40	Malawi	Bluggoe	01214	..
Foc 58		..		01216	..
Foc 59		..		01217	..
Foc 60		Indonesia		01218	..
Foc 61		..		01219	..
Foc 109		South Africa	Cavendish	0120	E. Grimbeek
Foc 130		..	..		..
Foc 135		..	..		..
Foc 136		..	..		..
Foc 137		..	..		..
Foc 140		..	..		..
Foc 141		..	Grand Naine	..	..
Foc 144		..	Williams	..	..
Foc 145		..	..	..	..
Foc 147		..	Israeli Grand Naine	..	A. Viljoen
Foc 148		..	Israeli Grand Naine	..	..
Foc 149		..	Cavendish	..	..
Foc 150	RP 4	..	..	..	R. Ploetz
Foc 151	RP 6	..	..	..	..

Isolate number[1]	Other name[2]	Origin	Cultivar	VCG[3]	Donor/ Collector
Foc 152	RP 16	South Africa	Cavendish	0120	E. Grimbeek
Foc 153		Australia	..	..	N. Moore
Foc 229	II 5	Indonesia	Pisang Manurung	01213	R. Ploetz
Foc 231	CV-2	..	Valery	..	..
Foc 232	Indo 93	..	Cavendish	..	..
Foc 233	Indo 129	..	Kepok (BBB)	..	..
Foc 234	DMI 8	..	Pisang Capatu	..	R. Ploetz
Foc 235	22424	Australia	Ladyfinger	0120	K. Pegg, N. Moore
Foc 236	23486	..	Cavendish	..	..
Foc 237	23539	..		..	..
Foc 238	23987	..	Cavendish	..	..
Foc 239	N5631	..	Cavendish	..	..
Foc 240	W91307	..		..	..
Foc 241	W91 345	..	Lady Finger	..	..
Foc 242	22410	..	Cavendish	..	..
Foc 244	22615	..	Lady Finger	..	..
Foc 243	A2	..	Mons mari	..	R. Ploetz
Foc 245	O-1220	..	Mons	..	..
Foc 246	Jak 1	Indonesia	Pisang berangan	01213	..
Foc 247	DMI 4	..	Pisang Ambon	..	..
Foc 248	RPML1	Malaysia	Pisang mas	01213/01216	..
Foc 249	RPML24	..	Pisang awak	..	..

[1]Isolates are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.
[2]Names as used in other culture collection from donor.
[3]Vegetative compatibility groups (VCGs) a phenotypic marker used to characterise fungal isolates based on heterokaryon formation (Puhalla 1985).

Table 2. Alleles, genotype configuration for each allele, allele frequencies and gene diversity for each isolate present at eight microsatellite loci.

Locus[1]	Allele size[2]	Genotype configuration[3]	Allele frequencies		
			South Africa	Australia	Asian
G40/G41	210	A	0.975	0.812	0.315
	211	B	0	0.062	0.052
	215	C	0	0	0.21
	219	D	0.025	0.125	0.105
	222	E	0	0	0.315
			H = 0.048	H = 0.321	H = 0.743
G4/G4-5	386	A	0	0	0.21
	387	B	0	0.062	0.421
	389	C	0.925	0.5	0.263
	390	D	0.075	0.437	0.105
			H = 0.138	H = 0.555	H = 0.698
M41/M42	149	A	0.1	0.375	0.105
	150	B	0.875	0.5	0.684
	153	C	0.025	0.125	0.21
			H = 0.223	H = 0.593	H = 0.477
G16/G6-8	276	A	0	0	0.052
	277	B	0	0.125	0.052
	280	C	0.05	0.125	0.052
	281	D	0.850	0.75	0.736
	282	E	0.1	0	0.052
	285	F	0	0	0.052
			H = 0.265	H = 0.406	H = 0.444
G42/G4	203	A	0.025	0.125	0.631
	205	B	0.975	0.875	0.368
			H = 0.0487	H = 0.218	H = 0.466

Locus[1]	Allele size[2]	Genotype configuration[3]	Allele frequencies		
			South Africa	Australia	Asian
G1/MV1-15	284	A	0.075	0.5	0.421
	285	B	0.925	0.5	0.578
			H = 0.138	H = 0.5	H = 0.488
G20/G20-21	444	A	0	0	0.105
	454	B	1	0.875	0.315
	455	C	0	0	0.421
	457	D	0	0	0.0526
	460	E	0	0.125	0
	467	G	0	0	0.105
			H = 0	H = 0.218	H = 0.698
G2/G2-3	342	A	0.025	0.125	0.052
	343	B	0	0	0.105
	344	C	0.1	0	0
	345	D	0.875	0.875	0.315
	346	E	0	0	0.052
	347	F	0	0	0.052
	348	G	0	0	0.368
	353	H	0	0	0.052
			H = 0.223	H = 0.218	H = 0.743
Population size			40	16	19
No. of alleles			18	21	35
No. of unique alleles			1	1	14
No. of polymorphic loci			7	8	8
Gene diversity			0.108	0.351	0.501

[1]The eight polymorphic loci as defined in Chapter 5.
[2]Allele size ranges.
[3]Each allele of the eight loci was assigned a different letter.

Table 3. Population parameters calculated for *Foc* populations from South Africa, Australia and Asia.

Parameter	South Africa	Australia	Asia
Population size (N)	40	16	19
Number of alleles	18	21	35
Number of unique alleles	1	1	14
Gene diversity ($\overline{H}$)	0.108	0.351	0.501
Genotypic diversity (G_{ST})	2.061	0.697	1.13
$\hat{G}$	6%	50%	79%
G_{st}	0.2838	0.3418	0.0665
Reproductive mode	recombining	clonal	clonal

$\hat{G}$ -Maximum percentage (%)of genotypic diversity (G_{ST}) using the formula $\hat{G} = G/N*100$, where N is the population size (McDonald *et al.* 1994).
G_{st} -To test for the presence of partitions within a population using the equation provided by Nei (1973)

Table 4. Population parameters calculated for *Foc* populations from South Africa, Australia and Asia.

	Asia	Australia	South Africa
Asia θ		$\theta = 0.175$ $P < 0.001$ $\left(\frac{1}{\theta}\right) = 5.69$	$\theta = 0.416$ $P < 0.001$ $\left(\frac{1}{\theta}\right) = 2.39$
Australia	$Gst = 0.1311$ $Nm = 7.018$ M = 3.315		$\theta = 0.224$ $P < 0.001$ $\left(\frac{1}{\theta}\right) = 4.46$
South Africa	$Gst = 0.2334$ $Nm = 1.9363$ M = 1.642	$Gst = 3.6011$ $Nm = 1.2618$ M = 3.601	

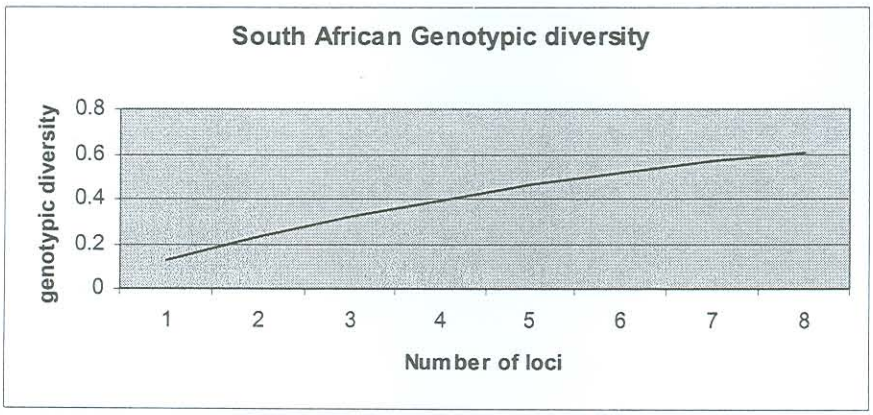
θ- theta value, population differentiation estimate Weir (1996)

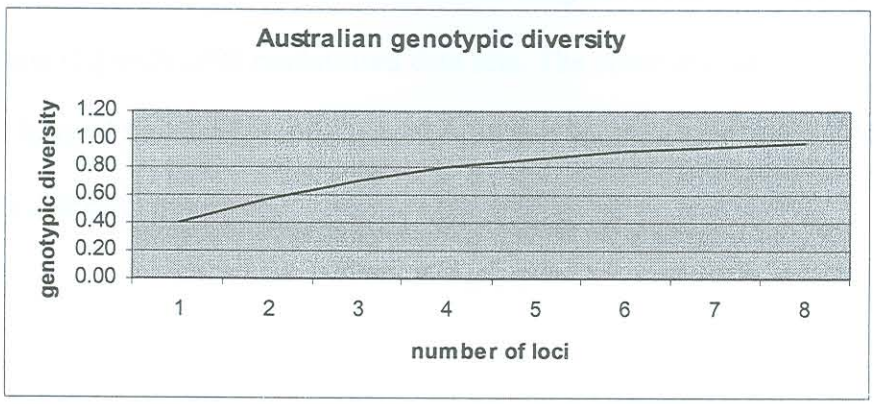
$\left(\frac{1}{\theta}\right)$- indirect estimation of gene flow from θ

Nm- indirect estimation of gene flow from *Gst* Nei (1973)

M- number of migrants from each population, an estimate from *Nm*

Fig. 1 Plot of genotypic diversity against the number of loci ($P<0.001$) for the global, VCG 0120, and three subpopulations based on geographical origin using 1000 sampling events.


South African Genotypic diversity
genotypic diversity
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
Number of loci


Australian genotypic diversity
genotypic diversity
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1
2
3
4
5
6
7
8
number of loci

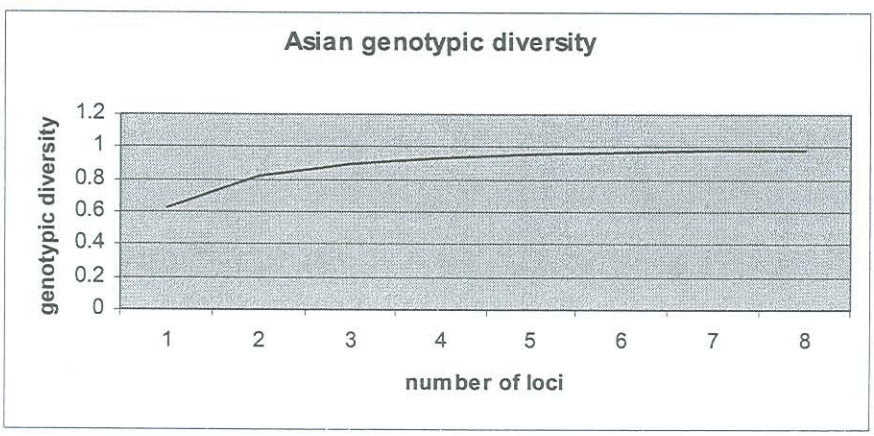
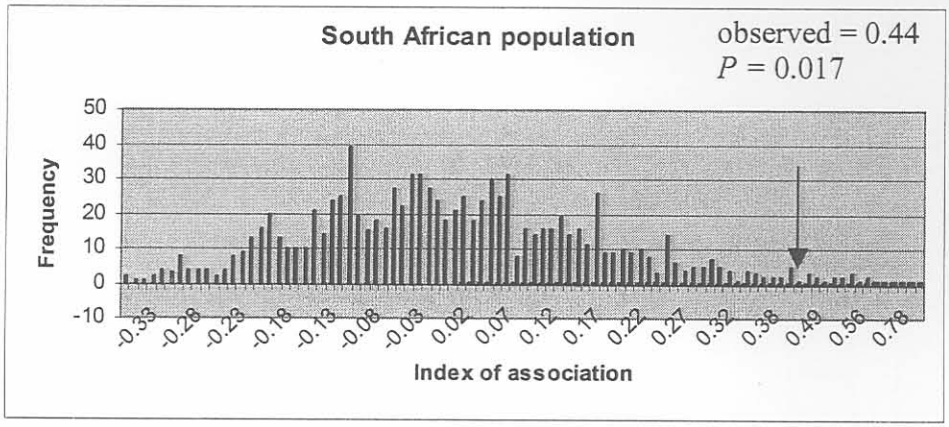
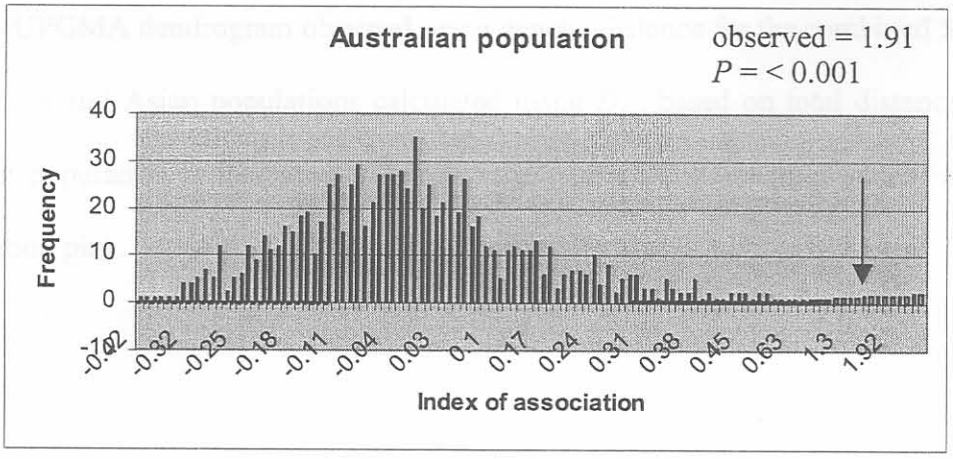

Asian genotypic diversity
genotypic diversity
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
8
number of loci

Fig. 2 Histograms representing the distribution range of clonal populations using the Index of Association (I_A) with 1000 randomised data sets. The observed values for I_A in all populations fall well beyond the distribution range for the recombining population (P<0.001) but not for the South African population.


South African population
observed = 0.44
P = 0.017
Frequency
50
40
30
20
10
0
-10
-0.33
-0.28
-0.23
-0.18
-0.13
-0.08
-0.03
0.02
0.07
0.12
0.17
0.22
0.27
0.32
0.38
0.49
0.56
0.78
Index of association


Australian population
observed = 1.91
P = < 0.001
Frequency
40
30
20
10
0
-0.32
-0.25
-0.18
-0.11
-0.04
0.03
0.1
0.17
0.24
0.31
0.38
0.45
0.63
1.3
1.92
Index of association

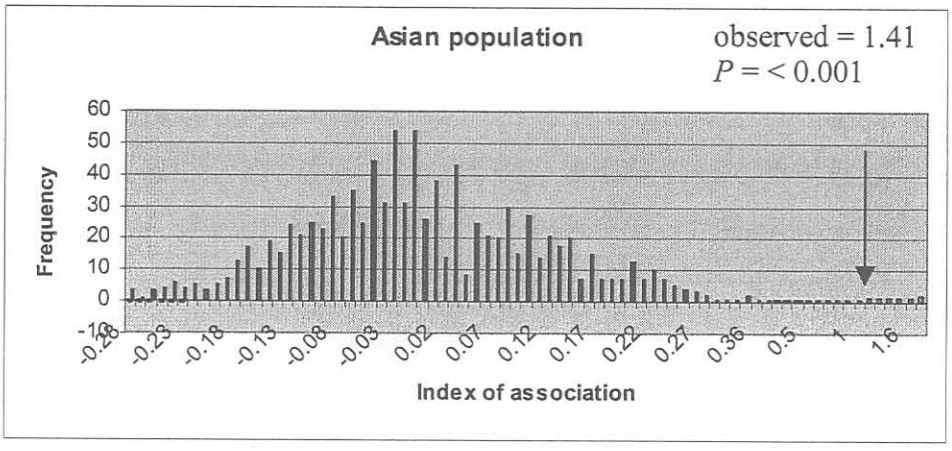
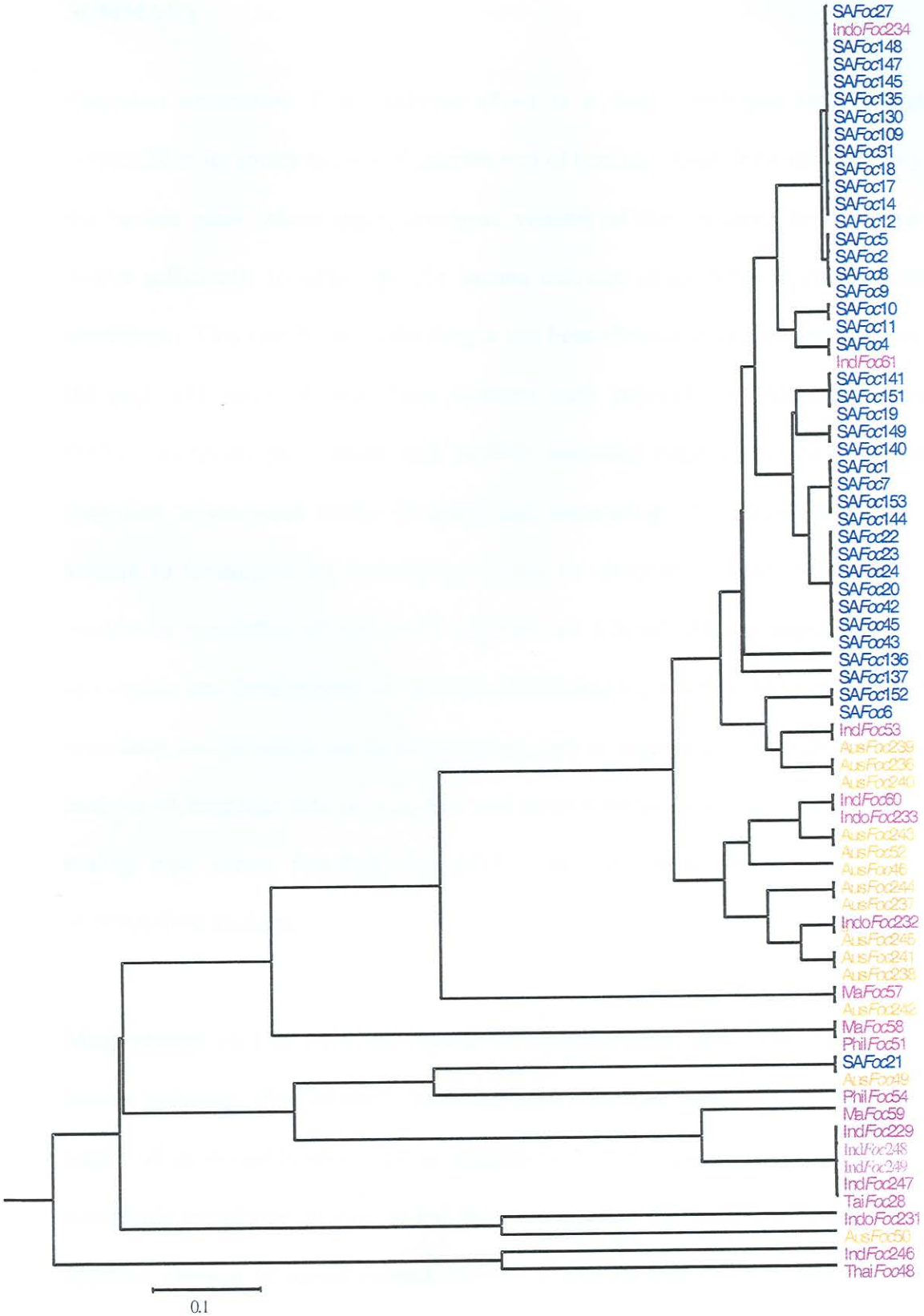

Asian population
observed = 1.41
P = < 0.001
Frequency
60
50
40
30
20
10
0
-0.28
-0.23
-0.18
-0.13
-0.08
-0.03
0.02
0.07
0.12
0.17
0.22
0.27
0.36
0.5
1
1.6
Index of association

Fig. 3 UPGMA dendrogram obtained using genetic distance for the combined South African, Australian and Asian populations calculated using D_{AD} based on total distance. The South African population is indicated with blue, the Australian population yellow and the Asian population pink.


SAFoc27
IndoFoc234
SAFoc148
SAFoc147
SAFoc145
SAFoc135
SAFoc130
SAFoc109
SAFoc31
SAFoc18
SAFoc17
SAFoc14
SAFoc12
SAFoc5
SAFoc2
SAFoc8
SAFoc9
SAFoc10
SAFoc11
SAFoc4
IndFoc61
SAFoc141
SAFoc151
SAFoc19
SAFoc149
SAFoc140
SAFoc1
SAFoc7
SAFoc153
SAFoc144
SAFoc22
SAFoc23
SAFoc24
SAFoc20
SAFoc42
SAFoc45
SAFoc43
SAFoc136
SAFoc137
SAFoc152
SAFoc6
IndFoc53
AusFoc239
AusFoc236
AusFoc240
IndFoc60
IndoFoc233
AusFoc243
AusFoc52
AusFoc46
AusFoc244
AusFoc237
IndoFoc232
AusFoc245
AusFoc241
AusFoc238
MaFoc57
AusFoc242
MaFoc58
PhilFoc51
SAFoc21
AusFoc49
PhilFoc54
MaFoc59
IndFoc229
IndFoc248
IndFoc249
IndFoc247
TaiFoc28
IndoFoc231
AusFoc50
IndFoc246
ThaiFoc48
0.1

SUMMARY

Fusarium oxysporum f. sp. *cubense* (*Foc*) is a fungal pathogen that is highly successful in its ability to cause Fusarium wilt of banana. Apart from its specificity to the banana plant (*Musa* spp.), genotypic variants of the pathogen have proven to evolve sufficiently to target specific banana cultivars under different environmental conditions. This variability in the fungus has been illustrated in numerous studies in the past, and involved phenotypic markers such vegetative compatibility groups (VCGs) and genotypic markers such as DNA amplified fingerprints (DAFs), random amplified polymorphic DNAs (RAPDs) and sequencing. This thesis has made an attempt to investigate the underlying reasons for diversity and diversification of a worldwide population of *Foc*, with emphasis on a South African population. The application and development of molecular biological tools for phylogenetic and host-specificity studies which included techniques such as vegetative compatibility studies, analysis of sequence data of a nuclear and mitochondrial gene region, searching for mating type genes, transformation of *Foc* and the development and testing of microsatellite markers.

Many studies on *Foc* have been useful in demonstrating the diverse nature of the banana pathogen. Few of these, however, have made any attempts to investigate the nature of its diversification. In an examination of the reproductive potential of a worldwide population of *Foc*, it has been proven that the fungus is clonal with an apparent absence of sexual recombination. It may be that *Foc* has become such a genetically isolated population and such an evolutionary advanced pathogen that no sexual stage will ever be found. Mutations therefore, are expected to be the primary mechanism responsible for genetic variation in *Foc*.

Sequence data of nuclear and mitochondrial gene regions with different evolutionary rates proved that *Foc* consists of several clonal lineages that maybe genetically distinct. These clonal lineages group into separate groups. The first of these groupings included the South African population while the second included isolates from the Australasian region. It is suggested that clonal lineages could be linked with distinct pathotypes of the fungus. All isolates of *Foc*, however, maintain one common link, their ability to cause disease on the banana host.

The relationship between *Foc* and the banana plant has been studied extensively many years ago on the Gros Michel cultivar. The successful transformation of *Foc* with the green fluorescent protein, however, now makes it possible to better understand the interactions between different fungal pathotypes and host genotypes. It will also substantially assist in understanding the process of fungal infection and host defence responses. Details of the interactions, especially between Cavendish varieties and the pathogen, needs to be investigated further in future.

A study of the population structure of *Foc*, using VCGs and microsatellite markers, provided valuable insights into the diversity of the South African population in relation to a worldwide collection of the fungus. Both markers showed that the South African population of *Foc* was remarkably homogeneous, strongly reminiscent of an introduced population. This was in clear contrast to the large genetic diversity apparent in populations from Southeast Asia, the reported centre of origin of the pathogen. VCGs are believed to represent clonal lineages within a species. They are useful but not ideal markers for defining diversity in a pathogen like *Foc* that has no defined genetic basis for race assignment. While VCGs are believed to represent

clonal lineages within a species, the assumption that isolates in the same VCG are genetically very similar can be misleading.

The development of microsatellite markers for *Foc* has provided an opportunity to study evolutionary relationships among populations and subpopulations of the Fusarium wilt pathogen. Differentiation between subpopulations was found to be high due to restricted gene flow and random drift. The high gene diversity revealed within the native *Foc* population is a clear indication that management of Fusarium wilt should involve the development of resistance cultivars.