Population structure of *Chrysoporthe austroafricana* in southern Africa determined using Vegetative Compatibility Groups (VCGs)

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Summary

Chrysoporthe austroafricana is one of the most damaging pathogens of *Eucalyptus* trees in southern Africa. It also occurs on non-native *Tibouchina granulosa* trees and native *Syzygium* species. Additional isolates of the pathogen from previously unstudied countries in the region have become available from survey studies. The aim of this study was to use VCGs to consider the diversity in populations of isolates collected in various countries in southern Africa (Malawi, Mozambique, Namibia, South Africa and Zambia) and from different hosts. We also wanted to determine whether there are shared VCGs among these countries and hosts in southern Africa and establish a VCG tester strain data base. Results showed a high diversity amongst isolates from different countries and hosts, but suggested little movement of VCGs among countries or hosts based on the available isolates. A total of 108 VCG tester strains were identified for southern Africa.

1 Introduction

Chrysoporthe austroafricana Gryzenh. & M.J. Wingf. is a well known fungal pathogen of plantation-grown *Eucalyptus* species in southern and eastern Africa (WINGFIELD et al. 1989; CONRADIE et al. 1990; GRYZENHOUT et al. 2004; ROUX et al. 2005; NAKABONGE et al. 2006). It was first reported as *Cryphonectria cubensis* (Bruner) Gryzenh. & M.J. Wingf. in 1989 (WINGFIELD et al. 1989), causing disease and death of *Eucalyptus* trees in plantations in South Africa. *Chrysoporthe austroafricana* has subsequently been reported from Malawi, Mozambique, Zambia (NAKABONGE et al. 2006) and Namibia (VERMEULEN et al. 2011), infecting non-native *Eucalyptus* species (ROUX et al. 2005; NAKABONGE et al. 2006), native *Syzygium cordatum* Hachst., *Syzygium guineense* (CD.) (HEATH et al. 2006; NAKABONGE et al. 2006; VERMEULEN et al. 2011) and non-native *Tibouchina granulosa* Cogn.: Britton (MYBURG et al. 2002).

Infection of *Eucalyptus* species with *Chr*. *austroafricana* is associated with cankers that girdle the trees resulting in cracking, swelling and shedding of the bark. In younger trees it results in stem girdling, wilting and rapid tree death (WINGFIELD et al. 1989, CONRADIE et al. 1990). Infections of *Syzygium* species and *Tibouchina* species with *Chr*. *austroafricana* can be very difficult to detect and are in some cases only visible on a single branch or around wounds, characterized by dying branches and in some cases stem cankers, especially on *Tibouchina* species (MYBURG et al. 2002, HEATH et al. 2006, NAKABONGE et al. 2006). Both perithecia and pycnidia of *Chr*. *austroafricana* are often visible on the dead, cracked bark of cankers, sometimes resulting in a yellow discolouration of the bark (NAKABONGE et al. 2006)

There is substantial evidence to suggest that *Chr*. *austroafricana* is native to Africa. This is based on its wide-spread presence on native *S. cordatum* and *S. guineense* in southern African countries (HEATH et al. 2006, NAKABONGE et al. 2006, VERMEULEN et al. 2011), and pathogenicity trials showing that native *S. cordatum* is more tolerant to infection by this

pathogen than non-native *Eucalyptus* clones (HEATH et al. 2006). Symptoms on native *S. cordatum*, and particularly *S. guineense*, are also less severe than those observed on *Eucalyptus* species, and death of these native trees due to infection by *Chr*. *austroafricana* has not been observed (HEATH et al. 2006, NAKABONGE et al. 2006, VERMEULEN et al. 2011). Despite extensive collections from other eucalypt growing regions of the world, *Chr. austroafricana* has not been detected elsewhere. VAN HEERDEN and WINGFIELD (2001), suggested that *Chr*. *austroafricana* was introduced into South Africa based on the low diversity observed with VCG's for a population from non-native *Eucalyptus* spp. in South Africa, and the misconception, at that time, that *Chr*. *austroafricana* was synonymous to *Chr*. *cubensis* (VAN HEERDEN and WINGFIELD 2001). Using microsatellite markers, HEATH (2005), later showed that *Chr*. *austroafricana* has a high level of genetic diversity in South Africa, as would be expected of a native pathogen (TSUTSUI et al. 2000, LIU and MILGROOM 2007, STUKENBROCK and MCDONALD 2008, LINDE et al. 2009).

No information is available on the movement of *Chr*. *austroafricana* among countries in southern Africa. *Chrysoporthe austroafricana* is able to cross-infect non-native *Eucalyptus* species and *T. granulosa*, presumably from native Myrtales (HEATH et al. 2006) illustrating a host shift (SLIPPERS et al. 2005). For instance, HEATH (2005) showed that there are shared VCGs between populations from *Syzygium* and *Eucalyptus* species (5 VCGs) and populations from *Syzygium* species and *T. granulosa* (1 VCG) in South Africa. This information is not available for VCGs shared among different countries, or among hosts within other countries in southern Africa. It is also unknown whether the VCGs previously characterised in South Africa occur elsewhere. The aim of this study was to determine the diversity of populations of *Chr*. *austroafricana* from Malawi, Mozambique, Namibia and Zambia based on VCG diversity. Furthermore, we wanted to determine whether there are shared VCGs among the different countries and hosts in southern Africa and establish a VCG tester strain data base.

2 Materials and methods

2.1 Fungal isolates

Chrysoporthe austroafricana isolates were collected from *S. guineense* trees in Namibia and deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1). These isolates were collected from three localities in the Caprivi region of the country in 2007 and 2008. Samples of bark from the roots, stems and branches of trees growing along the banks of the Zambezi and Kavango rivers were collected as described in VERMEULEN et al. (2011) and isolations were made according to GRYZENHOUT et al. (2009).

Additional isolates from Malawi, Mozambique and Zambia (NAKABONGE et al. 2006) and those representing previously identified *Chr*. *austroafricana* VCGs from *Eucalyptus, Syzygium and Tibouchina* species in South Africa (VAN HEERDEN and WINGFIELD 2001; HEATH 2005) were obtained from the CMW culture collection (Table 1). The identities of the newly collected isolates from Namibia were confirmed as *Chr. austroafricana* using a PCR-RFLP (restriction fragment length polymorphisms) fingerprinting technique developed by VAN DER MERWE et al. (2010). This was done to ensure that only *Chr*. *austroafricana* isolates were included in this study, since *Chr. cubensis* and *Chr*. *deuterocubensis,* that are morphologically similar to *Chr*. *austroafricana,* are also known from Africa and co-occur with *Chr. austroafricana* in some countries (NAKABONGE et al. 2006; VERMEULEN et al. 2011).

2.2 Vegetative compatibility studies

Previous studies have shown that only one vegetative compatibility group occurs per tree (VAN HEERDEN et al. 1997, VAN HEERDEN and WINGFIELD 2001). Vegetative Compatibility Groups (VCGs) were, therefore, determined for one isolate per tree from Malawi,

Table 1. Origin, hosts and Vegetative Compatibility Groups (VCG) of isolates of *Chrysoporthe austroafricana* used in this study.

Country	**Host**	**Isolate nr [A]CMW**	**VCG**	
			Country	Testers Southern Africa
Malawi	*Eucalyptus* sp.	17105	ME1	ZA1
Malawi	*Eucalyptus* sp.	17108	ME2	ZA2
Malawi	*Eucalyptus* sp.	17109	ME3	ZA3
Malawi	*Eucalyptus* sp.	17115	ME4	ZA4
Malawi	*Eucalyptus* sp.	17118	ME5	ZA5
Malawi	*Eucalyptus* sp.	17132	ME6	ZA6
Malawi	*Eucalyptus* sp.	17133	ME7	ZA7
Malawi	*S. cordatum*	17098	MS1	ZA8
Mozambique	*Eucalyptus* sp.	13878	MOE1	ZA9
Mozambique	*Eucalyptus* sp.	13881	MOE2	ZA10
Mozambique	*Eucalyptus* sp.	13882	MOE3	ZA11
Mozambique	*Eucalyptus* sp.	13886	MOE4	ZA12
Mozambique	*Eucalyptus* sp.	13887	MOE2	ZA10/ ZA66/ ZA70
Mozambique	*Eucalyptus* sp.	13888	MOE2	ZA10
Mozambique	*Eucalyptus* sp.	13889	MOE2	ZA10
Mozambique	*Eucalyptus* sp.	13916	MOE5	ZA13
Mozambique	*Eucalyptus* sp.	13918	MOE6	ZA14
Mozambique	*Eucalyptus* sp.	13930	MOE7	ZA15
Mozambique	*Eucalyptus* sp.	13931	MOE7	ZA15
Mozambique	*Eucalyptus* sp.	17084	MOE8	ZA16
Mozambique	*Eucalyptus* sp.	17087	MOE9	ZA17
Mozambique	*Eucalyptus* sp.	17094	MOE10	ZA18
Mozambique	*S. cordatum*	13874	MOS1	ZA19
Mozambique	*S. cordatum*	13875	MOS2	ZA20

Country	Host	Isolate nr [A]CMW	VCG	
			Country	Testers Southern Africa
Mozambique	*S. cordatum*	13876	MOS3	ZA21
Mozambique	*S. cordatum*	13877	MOS4	ZA22
Mozambique	*S. cordatum*	13890	MOS5	ZA23
Mozambique	*S. cordatum*	13891	MOS6	ZA24
Mozambique	*S. cordatum*	13892	MOS7	ZA25
Mozambique	*S. cordatum*	13893	MOS8	ZA26
Mozambique	*S. cordatum*	13894	MOS9	ZA27
Mozambique	*S. cordatum*	13895	MOS10	ZA28
Mozambique	*S. cordatum*	13897	MOS11	ZA29
Mozambique	*S. cordatum*	13900	MOS12	ZA30
Mozambique	*S. cordatum*	13904	MOS13	ZA31
Mozambique	*S. cordatum*	13907	MOS14	ZA32
Mozambique	*S. cordatum*	13908	MOS15	ZA33
Mozambique	*S. cordatum*	13909	MOS16	ZA34
Mozambique	*S. cordatum*	13921	MOS17	ZA35
Mozambique	*S. cordatum*	13922	MOS3	ZA22
Mozambique	*S. cordatum*	13925	MOS18	ZA36
Mozambique	*S. cordatum*	13926	MOS6	ZA24/ ZA37
Mozambique	*S. cordatum*	13927	MOS2	ZA20
Mozambique	*S. cordatum*	13932	MOS19	ZA38
Mozambique	*S. cordatum*	13935	MOS20	ZA39
Namibia	*S. guineense*	23707	NS1	ZA40
Namibia	*S. guineense*	24268	NS2	ZA41
Namibia	*S. guineense*	24269	NS3	ZA42
Namibia	*S. guineense*	24272	NS4	ZA43
Namibia	*S. guineense*	24273	NS5	ZA44

Country	Host	Isolate nr [A]CMW	VCG	
			Country	Testers Southern Africa
Namibia	*S. guineense*	24276	NS6	ZA45
Namibia	*S. guineense*	24278	NS5	ZA44
Namibia	*S. guineense*	24281	NS7	ZA46
Namibia	*S. guineense*	24282	NS8	ZA47
Namibia	*S. guineense*	24285	NS8	ZA47
Namibia	*S. guineense*	24291	NS8	ZA47
Namibia	*S. guineense*	28240	NS9	ZA48
Namibia	*S. guineense*	28241	NS10	ZA49
Namibia	*S. guineense*	28244	NS11	ZA50
Namibia	*S. guineense*	28247	NS8	ZA47
Namibia	*S. guineense*	28249	NS8	ZA47
Namibia	*S. guineense*	28255	NS12	ZA51
Namibia	*S. guineense*	28259	NS13	ZA52
Namibia	*S. guineense*	28260	NS14	ZA53
Namibia	*S. guineense*	28263	NS15	ZA54
Namibia	*S. guineense*	28265	NS16	ZA55
Namibia	*S. guineense*	28266	NS13	ZA52
Namibia	*S. guineense*	28269	NS17	ZA56
Namibia	*S. guineense*	28270	NS18	ZA57
Namibia	*S. guineense*	28271	NS19	ZA58
Namibia	*S. guineense*	28371	NS20	ZA59
Namibia	*S. guineense*	32953	NS21	ZA12
South Africa	*E. grandis*	[B]11318	SA19	ZA60
South Africa	*E. grandis*	[B]11319	SA18	ZA61
South Africa	*E. grandis*	[B]11320	SA17	ZA62
South Africa	*E. grandis*	[B]11321	SA20	ZA63

Country	**Host**	**Isolate nr** [A]**CMW**	**VCG**	
			Country	Testers Southern Africa
South Africa	*E. grandis*	[B]11324	SA9	ZA64
South Africa	*E. grandis*	[B]11326	SA10	ZA65
South Africa	*E. grandis*	[B]11327	SA12	ZA66
South Africa	*E. grandis*	[B]11330	SA23	ZA67
South Africa	*E. grandis*	[B]11331	SA22	ZA68
South Africa	*E. grandis*	[B]11334	SA3	ZA69
South Africa	*E. grandis*	[B]11335	SA8	ZA70
South Africa	*E. grandis*	[B]11337	SA5	ZA71
South Africa	*E. grandis*	[B]11339	SA1	ZA72
South Africa	*E. grandis*	[B]11340	SA2	ZA73
South Africa	*E. grandis*	[B]11341	SA6	ZA74
South Africa	*E. grandis*	[B]11342	SA7	ZA75
South Africa	*E. grandis*	[B]11344	SA14	ZA76
South Africa	*E. grandis*	[B]11345	SA13	ZA77
South Africa	*E. grandis*	[B]11346	SA15	ZA78
South Africa	*E. grandis*	[B]11347	SA16	ZA79
South Africa	*Syzygium* spp.	[C]10036	SAS1	ZA80
South Africa	*Syzygium* spp.	[C]10038	SAS2	ZA81
South Africa	*Syzygium* spp.	[C]10039	SAS3	ZA82
South Africa	*Syzygium* spp.	[C]10040	SAS4	ZA83
South Africa	*Syzygium* spp.	[C] 10047	SAS5	ZA84
South Africa	*Syzygium* spp.	[C] 10050	SAS6	ZA85
South Africa	*Syzygium* spp.	[C] 10051	SAS7	ZA86
South Africa	*Syzygium* spp.	[C] 10052	SAS8	ZA87
South Africa	*Syzygium* spp.	[C] 10053	SAS9	ZA88
South Africa	*Syzygium* spp.	[C] 10059	SAS10	ZA89

Country	**Host**	**Isolate nr** [A]**CMW**	**VCG**	
			Country	Testers Southern Africa
South Africa	*Syzygium* spp.	[C] 10060	SAS11	ZA90
South Africa	*Syzygium* spp.	[C] 10061	SAS12	ZA91
South Africa	*Syzygium* spp.	[C] 10062	SAS13	ZA92
South Africa	*Syzygium* spp.	[C] 10063	SAS14	ZA33
South Africa	*Syzygium* spp.	[C] 10064	SAS15	ZA93
South Africa	*Syzygium* spp.	[C] 10066	SAS16	ZA94
South Africa	*Syzygium* spp.	[C] 10067	SAS17	ZA74
South Africa	*Syzygium* spp.	[C] 10071	SAS18	ZA95
South Africa	*Syzygium* spp.	[C] 10072	SAS19	ZA96
South Africa	*Syzygium* spp.	[C] 10075	SAS20	ZA97
South Africa	*Syzygium* spp.	[C] 10080	SAS21	ZA98
South Africa	*Syzygium* spp.	[C] 10081	SAS22	ZA99
South Africa	*Syzygium* spp.	[C] 10082	SAS23	ZA100
South Africa	*Syzygium* spp.	[C] 10086	SAS24	ZA101
South Africa	*Syzygium* spp.	[C] 10087	SAS25	ZA102
South Africa	*Syzygium* spp.	[C]10193	SAS26	ZA62
South Africa	*T. granulosa*	[C] 9327	SAT1	ZA72/ ZA81
South Africa	*T. granulosa*	[C] 9339	SAT2	ZA63
South Africa	*T. granulosa*	[C] 9341	SAT3	ZA37/ ZA39
South Africa	*T. granulosa*	[C] 9345	SAT4	ZA103
South Africa	*T. granulosa*	[C] 9348	SAT5	ZA104
South Africa	*T. granulosa*	[C] 9349	SAT6	ZA105
South Africa	*T. granulosa*	[C] 9350	SAT7	ZA60
South Africa	*T. granulosa*	[C] 9359	SAT8	ZA91
South Africa	*T. granulosa*	[C] 9364	SAT9	ZA106
South Africa	*T. granulosa*	[C] 9370	SAT10	ZA107

Country	**Host**	**Isolate nr** [A]**CMW**	**VCG**	
			Country	Testers Southern Africa
Zambia	*Eucalyptus* sp.	13966	ZE1	ZA89
Zambia	*Eucalyptus* sp.	13970	ZE2	ZA108
Zambia	*Eucalyptus* sp.	13975	ZE3	ZA108

A Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) University of Pretoria, South Africa

B VCG identified by VAN HEERDEN and WINGFIELD (2001). CMW used by VAN HEERDEN and WINGFIELD (2001) has been redeposited under the numbers used in this study.

C VCG identified by HEATH (2005)

ME=Malawi *Eucalyptus* VCG

MS=Malawi *Syzygium* VCG

MOE=Mozambique *Eucalyptus* VCG

MOS=Mozambique *Syzygium* VCG

NS=Namibia *Syzygium* VCG

SA=South African *Eucalyptus* VCG as designated by VAN HEERDEN and WINGFIELD (2001)

SAS=South Africa *Syzygium* VCG Heath (2005) unpublished

SAT=South Africa *Tibouchina* VCG Heath (2005) unpublished

ZE=Zambia *Eucalyptus* VCG

ZA=southern Africa VCG Testers

Mozambique, Namibia and Zambia. To determine VCGs, mycelial plugs were transferred from the edges of actively growing cultures onto oatmeal agar (30g rolled oats, 20g agar and 1L dH_2O). Two isolates were placed 2 cm apart on 6.5 cm diameter Petri dishes. A single isolate from each tree was tested against all other isolates in all possible combinations. Plates were sealed with Parafilm and incubated at 25°C in the dark for two weeks. VCGs were then identified based on the ability of different isolates to merge and form confluent mycelium, or to form a barrage reaction along the line of contact (ANAGNOSTAKIS 1997). Reactions were assessed after two weeks and reactions were scored as vegetatively compatible or vegetatively incompatible. Where a barrage formed between two isolates at the line of contact, it was scored as incompatible and where two isolates merged to form confluent mycelium it was scored as compatible. Representative VCGs from Malawi, Mozambique, Namibia and South Africa (HEATH 2005; VAN HEERDEN and WINGFIELD 2001) were then compared with each other as described above to determine whether there were shared VCGs in the different countries of southern Africa. All VCG tests were repeated once to confirm the results.

2.4 Statistical analyses of VCG data

Genotypic diversity (G) was determined for larger populations from Mozambique and Namibia as proposed by STODDART and TAYLOR 1988. To compare VCG diversity levels between populations from different areas, the genotypic diversity (G) was divided by the sample size (N) to obtain maximum percentage of genotypic diversity ($\hat{G}$) (STODDART and TAYLOR 1988; MCDONALD et al. 1994). A second parameter used was the Shannon Index (SI) (BOWMAN et al. 1971; GROTH and ROELFS 1986) that takes into account the frequency and evenness of the distribution of a particular phenotype. SI was converted into normalized

Shannon diversity index (H_s). H_s was used to compare populations of different sizes and as an indication of phenotypic diversity based on VCGs (SHELDON 1969).

3 Results

3.1 Fungal isolates

Twenty-seven isolates resembling *Chrysoporthe* species, based on morphology, were obtained from *S. guineense* in the Caprivi region of Namibia (Katima Mulilo, Island View and Popa Falls). One hundred and five additional isolates were obtained from the CMW culture collection, including eight isolates from *Eucalyptus* species and one isolate from *S. cordatum* in Malawi, fourteen isolates from *Eucalyptus* species and twenty-three isolates from *S. cordatum* in Mozambique and three isolates from *Eucalyptus* species in Zambia. The additional fifty-six isolates were from South Africa (Table 1), representing isolates of VCGs previously identified by HEATH (2005) from *S. cordatum* (26 isolates) and *T. granulosa* (10 isolates), and VAN HEERDEN and WINGFIELD (2001) from *Eucalyptus* species (20 isolates). All the isolates from Namibia were positively identified as *Chr*. *austroafricana*, matching the banding patterns described by VAN DER MERWE et al. (2010) for *Chr*. *austroafricana* (data not shown).

3.2 Vegetative compatibility studies

Chrysoporthe austroafricana isolates from Malawi (8 isolates / 8 VCGs), Mozambique (37 isolates / 30 VCGs), Namibia (27 isolates / 21 VCGs) and Zambia (3 isolates / 2 VCGs) represented 61 VCGs (Tables 1 and 2). Very few VCGs were shared among different hosts

Table 2. Number of VCGs identified for *Chrysoporthe austroafricana* population in southern Africa.

Country	Host	No. of isolates	No. of VCGs
Malawi	*Eucalyptus* spp.	7	7
	S. cordatum	1	1
Mozambique	*Eucalyptus* spp.	14	10
	S. cordatum	23	20
Namibia	*S. guineense*	27	21
Zambia	*Eucalyptus* spp.	3	2
South Africa	*Eucalyptus* spp. [a]	100	23
	S. guineense [b]	62	32
	T. granulosa [b]	37	10

[a] VAN HEERDEN and WINGFIELD (2001)

[b] HEATH (2005)

Table 3. VCGs of *Chrysoporthe austroafricana* shared between hosts in southern Africa, including data from this study and those published by HEATH (2005) and VAN HEERDEN and WINGFIELD (2001).

Host	*Tibouchina*	*Eucalyptus* spp.	*Syzygium* spp.
Tibouchina	10	3	4
Eucalyptus spp.		39	4
Syzygium spp.			68

Table 4. VCGs of *Chrysoporthe austroafricana* shared between different countries in southern Africa.

Distribution	Malawi	Mozambique	Namibia	South Africa	Zambia
Malawi	8	0	0	0	0
Mozambique		30	1	5	0
Namibia			21	1	0
South Africa				50	1
Zambia					2

(Table 3) and countries (Table 4) in southern Africa. Several pairs of isolates that were incompatible with each other (i.e. 2 different VCGs) had the ability to form a compatible reaction with a third isolate (Table 1). These isolates could either belong to VCG clusters or are closely related VCGs similar to *Cry. parasitica* (CORTESI et al. 1996).

3.4 Statistical analyses of VCG data

A high diversity was observed for the Namibian ($\hat{G}$ = 53%, $H_{s=}$ 20) and the Mozambican ($\hat{G}$ = 65%, H_s = 28) population. For the population (Table 5) from Mozambique the diversity was high for both the populations from *Eucalyptus* ($\hat{G}$ = 50%, $H_{s=}$ 9) and *S. cordatum* ($\hat{G}$ = 79%, $H_{s.}$= 19). Limited numbers of isolates were available from Malawi and Zambia and no meaningful statistical analyses could be conducted for these countries. All of the isolates from Malawi, however, represented unique VCGs, while the three isolates from Zambia represented two unique VCGs (Table 2).

Table 5. Diversity based on VCGs for populations from southern Africa.

Country	Host	No. of isolates	Diversity	
			$\hat{G}$[c]	Hs[d]
Mozambique	*Eucalyptus* spp.	14	50	9
	S. cordatum	23	79	19
Namibia	*S. guineense*	27	53	20
South Africa	[ab]*E. grandis*	100	0.095	55
	[b]*Syzygium* spp.	62	26	36
	[b]*T. granulosa*	37	22	24

[a] VAN HEERDEN and WINGFIELD (2001)

[b] HEATH (2005)

c Maximum % of genotypic diversity (STODDARD and TAYLOR 1988)

d Normalized Shannon diversity index (SHELDON 19)

4 Discussion

The high population diversity observed for *Chr. austroafricana* in southern Africa supports the view that it is native to Africa (HEATH et al. 2006). The genetic diversity of Mozambican and Namibian populations based on VCGs was higher than that observed for the South

African populations studied by HEATH (2005) and VAN HEERDEN and WINGFIELD (2001)(Table 5). The high diversity observed in Mozambican and Namibian populations suggests that the centre of diversity of *Chr*. *austroafricana* is most likely in a country other than South Africa. This is further supported by the high diversity for the Mozambique population from both native *S. cordatum* and non-native *Eucalyptus* species. Although inadequate population samples exist for Malawi and Zambia, the isolates obtained for this study all belonged to different VCGs. This is comparable with the number of VCGs seen per population for the closely related fungus *C. parasitica* in its native range (China 64 isolates / 54 VCGs and Japan 79 isolates / 71 VCGs) (LIU and MILGROOM 2007).

Although the population sizes for *Chr. austroafricana* from the various countries of southern Africa were not all optimal, the available evidence suggests little movement of *Chr*. *austroafricana* among countries in southern Africa. A very limited number of shared VCGs were observed among the different countries for which isolates were available. This suggests that these populations have been present in these countries for a long period with little introduction of new genotypes from the outside. The same is true for movement of genotypes among hosts of *Chr*. *austroafricana* in southern Africa. It is believed that *Chr*. *austroafricana* underwent a host jump from native Myrtales (*Syzygium* species) to non-native Myrtales (*Eucalyptus* species) (HEATH et al. 2006; SLIPPERS et al. 2005). The limited number of shared VCGs between native and non-native hosts could be indicative that the host jump was not recent or that the founder population has not yet been sampled. Most likely, however, a single VCG was responsible for the host jump.

Forestry in South Africa is based on a clonal program where resistance was established to a single, highly virulent isolate of *Chr*. a*ustroafricana* (VAN HEERDEN and WINGFIELD 2001). Currently, breeding programmes rely on natural infection of clones in trials to obtain information on disease susceptibility of future planting material. It has been

shown that different VCGs can differ in their pathogenicity to hosts (VAN HEERDEN and WINGFIELD 2001; TSROR LAHKIM and LEVIN 2003; ELMER et al. 1999). Although pathogenicity has not been linked to VCG types in this study, our results showed that a high diversity of VCGs exists outside South Africa. It is thus possible that the high diversity of VCG types also indicate diverse levels of pathogenicity and that introduction of such genotypes would pose a threat to the existing trees planted in South Africa.

Pathogen populations that are more diverse are able to better adapt to changes in host resistance than pathogen populations that are genetically uniform (MCDONALD et al. 1989; DELMOTTE et al. 1999; MCDONALD and MCDERMOTT 1993). This implies that more diverse populations will be able to more quickly overcome the resistance of clones selected for their tolerance to specific pathogens. *Eucalyptus* plantations in southern Africa, and other areas of the world, depend on planting disease tolerant hybrids and clones of species to reduce the impact of Chrysoporthe canker (ALFENAS et al. 1983; VAN DER WESTHUIZEN et al. 1992; VAN HEERDEN and WINGFIELD 2001; WINGFIELD and ROUX 2002). It is thus important to understand the diversity of *Chr. austroafricana* in southern Africa to insure continued control of this pathogen and to in future screen susceptibility of *Eucalyptus* clones used in forestry industry to different VCG's.

The VCG tester strains developed in this study enable investigation of some level of population diversity. It also allows a relatively cheap and easy system to obtain at least basic information on this pathogen without the use of expensive molecular tools such as microsattelite markers. A system of VCG tester strains have been developed for the related pathogen *C. parasitica* that was introduced into North America and Europe from Japan and China (CORTESI et al. 1998; ROBIN et al. 2000). In these countries, the database is useful to trace the history and origin of introductions and movements among areas. They also provide information on the reproduction of *C. parasitica* in these areas, and to evaluate the possible

success of biological control programs using hypovirulence, which is highly dependent on the clonality of the pathogen population (GURER et al. 2001; MILGROOM and CORTESI 1999; MILGROOM et al. 2008; ADAMCIKOVA et al. 2009; JANKOVSKY et al. 2010). The situation for *Chr. austroafricana* is, however, different because this is a native pathogen of which the representative population diversity has not yet been fully sampled and new VCGs are continuously produced. In this regard, developing a VCG tester database with the same functionality as that available for *C. parasitica* is challenging.

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