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# 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 database. 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 widespread 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 vegetative compatibility groups (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 characterized 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

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determine whether there are shared VCGs among the different countries and hosts in southern Africa and establish a VCG tester strain database.

## 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 performed to ensure that only *Chr. austroafricana* isolates were included in this study, as *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 VCG occurs per tree (Van Heerden et al. 1997, Van Heerden and Wingfield 2001). VCGs were, therefore, determined for one isolate per tree from Malawi, Mozambique, Namibia and Zambia. To determine VCGs, mycelial plugs were transferred from the edges of actively growing cultures onto oatmeal agar (30 g rolled oats, 20 g agar and 1 L d$H_2O$). 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 2 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 1977). Reactions were assessed after 2 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 1989) 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 PCR-RFLP banding patterns described by Van der Merwe et al. (2010) for *Chr. austroafricana* (data not shown).

*Table 1.* Origin, hosts and vegetative compatibility groups (VCG) of isolates of *Chrysoporthe austroafricana* used in this study.

|  |  |  | VCG CODE |  |
|---|---|---|---|---|
| Country | Host | Isolate nr CMW[1] | 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 |
| 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 |
| 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 |

*Table 1* Continued

| | | | VCG CODE | |
|---|---|---|---|---|
| Country | Host | Isolate nr CMW[1] | Country | Testers Southern Africa |
| 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* | 11318[2] | SA19 | ZA60 |
| South Africa | *E. grandis* | 11319[2] | SA18 | ZA61 |
| South Africa | *E. grandis* | 11320[2] | SA17 | ZA62 |
| South Africa | *E. grandis* | 11321[2] | SA20 | ZA63 |
| South Africa | *E. grandis* | 11324[2] | SA9 | ZA64 |
| South Africa | *E. grandis* | 11326[2] | SA10 | ZA65 |
| South Africa | *E. grandis* | 11327[2] | SA12 | ZA66 |
| South Africa | *E. grandis* | 11330[2] | SA23 | ZA67 |
| South Africa | *E. grandis* | 11331[2] | SA22 | ZA68 |
| South Africa | *E. grandis* | 11334[2] | SA3 | ZA69 |
| South Africa | *E. grandis* | 11335[2] | SA8 | ZA70 |
| South Africa | *E. grandis* | 11337[2] | SA5 | ZA71 |
| South Africa | *E. grandis* | 11339[2] | SA1 | ZA72 |
| South Africa | *E. grandis* | 11340[2] | SA2 | ZA73 |
| South Africa | *E. grandis* | 11341[2] | SA6 | ZA74 |
| South Africa | *E. grandis* | 11342[2] | SA7 | ZA75 |
| South Africa | *E. grandis* | 11344[2] | SA14 | ZA76 |
| South Africa | *E. grandis* | 11345[2] | SA13 | ZA77 |
| South Africa | *E. grandis* | 11346[2] | SA15 | ZA78 |
| South Africa | *E. grandis* | 11347[2] | SA16 | ZA79 |
| South Africa | *Syzygium* sp. | 10036[3] | SAS1 | ZA80 |
| South Africa | *Syzygium* sp. | 10038[3] | SAS2 | ZA81 |
| South Africa | *Syzygium* sp. | 10039[3] | SAS3 | ZA82 |
| South Africa | *Syzygium* sp. | 10040[3] | SAS4 | ZA83 |
| South Africa | *Syzygium* sp. | 10047[3] | SAS5 | ZA84 |
| South Africa | *Syzygium* sp. | 10050[3] | SAS6 | ZA85 |
| South Africa | *Syzygium* sp. | 10051[3] | SAS7 | ZA86 |
| South Africa | *Syzygium* sp. | 10052[3] | SAS8 | ZA87 |
| South Africa | *Syzygium* sp. | 10053[3] | SAS9 | ZA88 |
| South Africa | *Syzygium* sp. | 10059[3] | SAS10 | ZA89 |
| South Africa | *Syzygium* sp. | 10060[3] | SAS11 | ZA90 |
| South Africa | *Syzygium* sp. | 10061[3] | SAS12 | ZA91 |
| South Africa | *Syzygium* sp. | 10062[3] | SAS13 | ZA92 |
| South Africa | *Syzygium* sp. | 10063[3] | SAS14 | ZA33 |
| South Africa | *Syzygium* sp. | 10064[3] | SAS15 | ZA93 |
| South Africa | *Syzygium* sp. | 10066[3] | SAS16 | ZA94 |
| South Africa | *Syzygium* sp. | 10067[3] | SAS17 | ZA74 |
| South Africa | *Syzygium* sp. | 10071[3] | SAS18 | ZA95 |
| South Africa | *Syzygium* sp. | 10072[3] | SAS19 | ZA96 |
| South Africa | *Syzygium* sp. | 10075[3] | SAS20 | ZA97 |
| South Africa | *Syzygium* sp. | 10080[3] | SAS21 | ZA98 |
| South Africa | *Syzygium* sp. | 10081[3] | SAS22 | ZA99 |
| South Africa | *Syzygium* sp. | 10082[3] | SAS23 | ZA100 |
| South Africa | *Syzygium* sp. | 10086[3] | SAS24 | ZA101 |
| South Africa | *Syzygium* sp. | 10087[3] | SAS25 | ZA102 |
| South Africa | *Syzygium* sp. | 10193[3] | SAS26 | ZA62 |
| South Africa | *T. granulosa* | 9327[3] | SAT1 | ZA72/ZA81 |
| South Africa | *T. granulosa* | 9339[3] | SAT2 | ZA63 |
| South Africa | *T. granulosa* | 9341[3] | SAT3 | ZA37/ZA39 |
| South Africa | *T. granulosa* | 9345[3] | SAT4 | ZA103 |
| South Africa | *T. granulosa* | 9348[3] | SAT5 | ZA104 |
| South Africa | *T. granulosa* | 9349[3] | SAT6 | ZA105 |
| South Africa | *T. granulosa* | 9350[3] | SAT7 | ZA60 |
| South Africa | *T. granulosa* | 9359[3] | SAT8 | ZA91 |
| South Africa | *T. granulosa* | 9364[3] | SAT9 | ZA106 |
| South Africa | *T. granulosa* | 9370[3] | SAT10 | ZA107 |

*Table 1* Continued

| Country | Host | Isolate nr CMW[1] | VCG CODE Country | Testers Southern Africa |
|---|---|---|---|---|
| Zambia | *Eucalyptus* sp. | 13966 | ZE1 | ZA89 |
| Zambia | *Eucalyptus* sp. | 13970 | ZE2 | ZA108 |
| Zambia | *Eucalyptus* sp. | 13975 | ZE3 | ZA108 |

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.

[1] Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) University of Pretoria, South Africa.

[2] 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.

[3] VCG identified by Heath (2005).

### 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 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).

## 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

*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.[1] | 100 | 23 |
| | *S. guineense*[2] | 62 | 32 |
| | *T. granulosa*[2] | 37 | 10 |

[1] Van Heerden and Wingfield (2001).

[2] 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 5.* Diversity based on VCGs for populations from southern Africa.

| | | | Diversity | |
|---|---|---|---|---|
| Country | Host | No. of isolates | Ĝ[3] | Hs[4] |
| Mozambique | *Eucalyptus* spp. | 14 | 50 | 9 |
| | *S. cordatum* | 23 | 79 | 19 |
| Namibia | *S. guineense* | 27 | 53 | 20 |
| South Africa | *E. grandis*[1,2] | 100 | 0.095 | 55 |
| | *Syzygium* spp.[2] | 62 | 26 | 36 |
| | *T. granulosa*[2] | 37 | 22 | 24 |

[1] Van Heerden and Wingfield (2001).
[2] Heath (2005).
[3] Maximum% of genotypic diversity (Stoddart and Taylor 1988).
[4] Normalized Shannon diversity index (Sheldon 1969).

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 programme where resistance was established to a single, highly virulent isolate of *Chr. austroafricana* (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 the 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 microsatellite 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 programmes 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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