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Genetic variation among *Sclerotium* isolates from Benin and South Africa, determined using mycelial compatibility and ITS rDNA sequence data

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Abstract. Damping-off and stem rot of cowpea caused by *Sclerotium rolfsii* has previously been reported in Benin, where the pathogen showed variation in growth and sclerotia production among isolates. Pathogenicity, mycelial compatibility group (MCG) tests and rDNA sequence analyses were conducted on different isolates of *S. rolfsii* and *S. delphinii* collected from different hosts and geographical areas in Benin and South Africa. All the isolates, when inoculated into soil and planted with cowpea, caused damping-off and stem rot symptoms. Aggressiveness among isolates varied depending on the host from which each was isolated. Isolates originating from cowpea produced the highest disease incidence followed by isolates from peanuts. Four MCGs were distinguished among 66 isolates. Isolates from the same hosts tended to group into the same MCG. The incidence of damping-off and stem rot of cowpea, expressed as percentage diseased plants, varied among MCGs. Plants inoculated with MCG2 displayed the highest disease incidence, whereas MCG4 resulted in the least. Parsimony analysis of ITS DNA sequence data supported a close affinity of the *Sclerotium* spp. but showed genetic variation among isolates with no grouping based on host of origin.

Additional keywords: *Athelia rolfsii*, PCR, ribosomal RNA, *Sclerotium* rot.

Introduction

Sclerotium rolfsii (teleomorph *Athelia rolfsii*) is a soilborne plant pathogenic fungus that causes disease in over 500 plant species throughout the world (Punja 1985; Harlton *et al*. 1995; Cilliers *et al*. 2000; Okabe and Matsumoto 2000, 2003; Okabe *et al*. 2000). Recently, *S. rolfsii* was also reported as the causal agent of damping-off and stem rot on cowpea, *Vigna unguiculata*, in the Ouémé Valley, Benin (Adandonon 2000; Kossou *et al*. 2001) where yield losses reached up to 40% (Anonymous 1995). In a previous study, variation in growth rate, number and time to first production of sclerotia by *S. rolfsii* isolates on potato-dextrose agar (PDA), was observed among isolates collected from different villages in the Ouémé valley (Adandonon 2000). Also, considerable variability among *S. rolfsii* isolates from Brazil was detected in terms of the number, size and location of sclerotia on the medium surface (Almeida *et al*. 2001). Two related species, *Sclerotium delphinii* and *Sclerotium coffeicola*, which are reported to occur on ornamental bulbs and coffee, respectively, produce larger sclerotia (Aycock 1966; Harlton *et al*. 1995; Okabe *et al*. 1998, 2000) that are lighter brown to orange in colour (Stevens 1931; Harlton *et al*. 1995; Punja and Damiani 1996). It is not clear whether these differences in host occurrence and morphology of sclerotia are sufficient to warrant a separate species designation for these isolates (Harlton *et al*. 1995; Punja and Damiani 1996).

Punja and Grogan (1983) found that field isolates of *S. rolfsii* from various hosts and geographical areas, even within the same area, showed variation in growth rate, numbers and size of sclerotia, and mycelial compatibility. More recently, Punja and Sun (2001) detected 68 mycelial compatibility groups (MCGs) among 128 isolates of *S. rolfsii.*

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Nalim *et al*. (1995) identified 25 MCGs among *S. rolfsii* isolates collected from Texas, and used molecular markers to show that all isolates within an MCG had identical genotypes. Some MCGs also shared the same sequence for the internal transcribed spacer (ITS) region of rDNA.

Molecular markers are increasingly being used to characterise populations of plant pathogens (Michelmore and Hubert 1987; Bruns *et al*. 1991; Okabe *et al*. 2000, 2001; Tyson *et al*. 2002). The polymerase chain reaction (PCR) and DNA sequencing, among others, have been used to study fungal populations (Taylor *et al*. 1999, 2000; Almeida *et al*. 2001). Since no studies of this nature have been reported on *S. rolfsii* isolates from Benin, the objective of this study was to use pathogenicity, MCGs and DNA sequence analysis to determine the extent of variation amongst *S. rolfsii* and *S. delphinii* isolates collected from diverse hosts and geographical areas in Benin and South Africa.

Methods

Fungal isolates and culture maintenance

The isolate designations and other relevant information of the 66 *Sclerotium* spp. isolates included in this study are listed in Table 1. Fifty-five isolates of *S. rolfsii* were from cowpea in Benin as follows: one each from 33 different sites (over 19.5 sq km) in Agonguey,

Table 1. Designations, areas, mycelium compatibility group (MCG) and disease incidence on cowpea in a greenhouse for *Sclerotium rolfsii* and *S. delphinii* isolates from Benin and South Africa

MCG	Designation number of isolates[A]	Areas of isolation	Incidence[B] ± s.e.[C] (%)	MCG	Designation number of isolates[A]	Areas of isolation	Incidence[B] ± s.e.[C] (%)
1	Agg-10	Agonguey-OB	83.33 ± 8.33	2	**Agl-01**	Agonlin-OB	91.67 ± 8.33
1	Agg-11	‘	83.33 ± 0.00	2	**Agl-02**	‘	100.0 ± 0.00
1	Agg-12	‘	83.33 ± 7.71	2	Agl-03	‘	91.67 ± 8.33
1	Agg-13	‘	75.00 ± 0.00	2	Agl-04	‘	100.0 ± 0.00
1	**Agg-14**	‘	83.33 ± 8.83	2	Agl-05	‘	91.67 ± 8.33
1	Agg-15	‘	75.00 ± 0.00	2	Agl-06	‘	100.0 ± 0.00
1	Agg-20	‘	75.00 ± 0.00	2	**Agl-07**	‘	100.0 ± 0.00
1	Agg-21	‘	83.33 ± 8.83	2	Agl-08	‘	100.0 ± 0.00
1	Agg-22	‘	91.67 ± 8.33	2	**Agl-09**	‘	91.67 ± 8.33
1	Agg-23	‘	83.33 ± 8.83	2	Agl-10	‘	91.67 ± 8.33
1	Agg-24	‘	83.33 ± 8.83	2	Agl-11	‘	100.0 ± 0.00
1	Agg-30	‘	75.00 ± 16.7	1	Dan-01	Dannou-OB	83.33 ± 8.33
1	**Agg-31**	‘	91.67 ± 8.33	1	Dan-02	‘	83.33 ± 8.33
1	Agg-32	‘	91.67 ± 8.33	1	Dan-03	‘	83.33 ± 8.33
1	Agg-33	‘	83.33 ± 8.33	1	Dan-04	‘	91.67 ± 8.33
1	Agg-34	‘	91.67 ± 8.33	1	**Dan-05**	‘	91.67 ± 8.33
1	Agg-35	‘	75.00 ± 0.00	1	**Dan-06**	‘	83.33 ± 8.33
1	Agg-36	‘	83.33 ± 8.33	1	**Gan-03**	Gangban-OB	75.0 ± 16.70
1	Agg-37	‘	83.33 ± 8.33	1	Gan-05	‘	75.0 ± 16.70
1	Agg-41	‘	83.33 ± 8.33	1	Gan-06	‘	83.33 ± 8.33
1	**Agg-42**	‘	91.67 ± 8.33	1	Gan-09	‘	91.67 ± 8.33
1	Agg-43	‘	75.00 ± 16.70	1	Gan-12	‘	75.00 ± 0.00
1	Agg-44	‘	91.67 ± 8.33	3	**Vil-01**	Viljoens-SA	50.00 ± 6.40
1	Agg-45	‘	91.67 ± 8.33	3	Vil-02	‘	41.67 ± 8.33
2	Agg-46	‘	91.67 ± 8.33	3	**Vil-03**	‘	33.33 ± 8.33
2	Agg-47	‘	100.0 ± 0.00	3	**Vil-04**	‘	41.67 ± 8.33
2	**Agg-48**	‘	100.0 ± 0.00	3	Vil-05	‘	33.33 ± 8.33
2	Agg-49	‘	91.67 ± 8.33	3	Vil-06	‘	50.00 ± 6.40
2	Agg-52	‘	91.67 ± 8.33	3	Vil-07	‘	33.33 ± 8.33
2	Agg-53	‘	91.67 ± 8.33	3	Vil-08	‘	33.33 ± 8.33
2	**Agg-54**	‘	83.33 ± 8.33	3	**Vil-09**	‘	58.33 ± 8.33
2	**Agg-55**	‘	91.67 ± 8.33	3	**Vil-10**	‘	41.67 ± 8.33
2	Agg-57	‘	83.33 ± 7.71	4	**Nel-01**	Nelspruit-SA	30.42 ± 5.71
	Control 1		00.00 ± 0.00		Control 2		00.00 ± 0.00

[A]Isolate numbers in bold were those selected for PCR amplification and DNA sequencing. Agg, Agl, Dan and Gan represent *S. rolfsii* isolates collected from cowpea from Agonguey, Agonlin, Dannou and Gangban, respectively, in the Ouémé valley, Benin (OB); Vil represents *S. rolfsii* isolates from peanut in Viljoenskroon (Viljoens) in South Africa (SA) and Nel represents *S. delphinii* for the isolate from bambara groundnut in Nelspruit, South Africa.

[B]Incidence is expressed as percentage diseased plants.

[C]SE = standard error at 5% according to the General Linear Model test.

11 sites (over 14 sq km) in Agonlin, six sites (over 11 sq km) in Dannou and five sites (over 16 sq km) from Gangban. Eleven isolates were from South Africa (Cilliers *et al*. 2002), ten from peanut (*Arachis hypogea*) and one *S. delphinii* isolate from bambara groundnut (*Vigna subterranea*). All isolates were collected during 2000 from cowpea, except those from Viljoenskroon and Nelspruit which were collected in 2002 from peanuts and bambara groundnut, respectively. Cultures were maintained on PDA slants and stored at room temperature.

Pathogenicity tests

The variation in pathogenicity of the isolates was evaluated using a *Sclerotium*-susceptible cowpea cultivar, Tchawé kpayo (Adandonon 2000). Millet (*Panicum miliaceum*) seed soil inoculum techniques were used to inoculate the soil (Weideman and Wehner 1993; Adandonon 2000). Five 5-mm-diameter discs cut from the actively growing edge of a *S. rolfsii* colony on PDA were used to inoculate 50 g of the millet seeds. Prior to inoculation, the millet seeds were steeped in water for 48 h and autoclaved for 45 min at 120°C. Inoculated millet seeds were incubated for 21 days at 27°C, and then used to inoculate the soil. Sandy loam soil was used and pasteurised by aerated steam (60°C for 60 min) and stored for 21 days before inoculation with the isolates (Pieczarka and Abawi 1978). Inoculum was air-dried in a paper bag, lightly ground and passed through nested sieves with 3-mm-diameter openings. The soil inoculation was done 2 days before planting by mixing 10 g of the *S. rolfsii* millet seed inoculum with 1 kg steam-disinfected sandy loam soil in a pot. There were 68 treatments (66 isolates and two controls) arranged in a randomised block design with three replicates. The two controls were pasteurised soil (1 kg) unmixed or mixed with 10 g non-inoculated steeped millet seed, respectively. Seeds of the cultivar Tchawé kpayo (with 98% viability) were surface sterilised in 1% NaOCl for 2 min, rinsed twice in sterile distilled water and then planted at the rate of four seeds per pot (one replicate) filled with inoculated soil. Pots were kept in the greenhouse at temperatures varying between 23 and 30°C. The percentage of damped-off seedlings was recorded after 3 days, followed at 7-day intervals until 30 days after planting.

To fulfil Koch's postulates, dying seedlings were removed at each observation, and at least one plant from each pot was submitted to agar culture assays to verify the presence of *Sclerotium* spp. (Brantner and Windels 1998). The reisolated fungus was cultured on PDA and colony characteristics recorded and compared with the original isolates. Data representing disease percentages (damping-off and stem rot) were transformed with arcsine transformation (Brantner and Windels 1998). Analyses were performed using the SAS System (SAS 1997) and mean separations were done according to the General Linear Model test.

Mycelial compatibility groups (MCG)

All isolates were paired against each other on PDA to observe mycelial interactions (Harlton *et al*. 1995; Cilliers *et al*. 2000). Three isolates were simultaneously paired on one dish and assigned to different MCGs based on the presence of a barrage zone between the isolates. Mycelial plugs (6-mm-diameter) taken from the edge of 5- to 7-day-old colonies were placed ~25 to 35 mm apart in 100 × 15 mm Petri dishes and incubated at 29 ± 1°C. Each pairing was repeated and all isolates were paired with themselves as controls. The pairings were examined macroscopically after five days for the presence of barrage zones in the region of mycelial contact (Punja and Grogan 1983). A pH-sensitive medium (50 mg/L bromocresol green) described by Powell (1995) was used to confirm the total number of identified MCGs. Petri dishes with pairing isolates on pH-sensitive medium were incubated for 5–7 days at 25°C in the dark. Incompatible reactions were distinguished by a coloured line in the reaction zone between the colonies, as viewed at a slight angle from the bottom of the plate. Compatible reactions were distinguished by merged colonies with no detectable line. All pairings were performed twice.

Population analysis

Each MCG was assigned a number (MCG1-MCG4). The most common MCG in samples was determined by identifying the number of representatives in each group. The genotypic diversity (G_{ST}) of the population was determined as proposed by Stoddart and Taylor (1988). The maximum percentage of genotypic diversity ($\hat{G}$), for the total set of isolates, was determined by dividing the genotypic diversity by the total sample size (G_{ST}/N). In the present study, $\hat{G}$ instead of the Shannon Index (*SI*) was used for comparative purposes.

Preparation of DNA

Representative isolates of each of the MCGs were selected for DNA sequence analysis. Selected isolates (Table 1) were grown at 25°C in 20 mL of nutrient broth enriched with 16 g/L glucose and shaken at 100 rpm. After 2 weeks, the mycelial mat was harvested, freeze-dried and ground to a fine powder using liquid nitrogen. Deoxyribonucleic acid preparation was done using the FTA system (Whatman-BioScience, Abington, UK). A small amount of the ground mycelium was applied to the FTA matrix, which was divided into grids for several samples per matrix and left to dry thoroughly. A section with no applied mycelium served as a negative control. A small square (2 mm × 2 mm) was cut from the matrix, placed into a sterile 0.2 mL PCR tube and washed three times with 100 µL of FTA reagent (Whatman-BioScience, Abington, UK) for 5 min. The matrix was then rinsed twice with 100 µL of sterile distilled water. The FTA discs were vacuum dried, placed on ice and PCR was conducted immediately.

PCR and gel electrophoresis

Nineteen representative isolates (Table 1) were compared by means of rDNA internal transcribed spacer sequence analysis, including the 5.8 S gene, using primers ITS1 and ITS4 (White *et al*. 1990). PCR was done using 50 µL volumes, each containing 250 µM of each dNTP (TaKaRa), 1 × PCR buffer, 20 pmol of each primer (ITS 1 and ITS 4) and 2.5 U of *Taq* polymerase (TaKaRa). Thirty PCR cycles were performed on a Perkin-Elmer 2400 thermocycler using the following conditions: one initial denaturation at 95°C for 2 min, a denaturation step at 93°C for 30 s followed by annealing at 55°C for 45 s and extension at 72°C for 90 s. PCR was concluded with a final extension at 72°C for 7 min. PCR products were visualised using ethidium bromide under ultraviolet light after electrophoresis on a 1.2% w/v agarose gel at 2 V/cm for 1 h.

Sequencing reaction mix and sequence analysis

Deoxyribonucleic acid sequencing reactions were carried out using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied BioSystems) according to the manufacturer's specifications. Analysis of sequences was done using the Phylogenetic Analysis Using Parsimony (PAUP*) 4.0b10 computer software (Swofford 1998). A heuristic parsimony analysis with random step-wise addition and tree-bisection-reconnection (TBR) was used to construct a phylogram. The confidence of branches was determined using a bootstrap analysis with 1000 replicates. Previously published sequences of seven *S. delphinii* isolates (GenBank accessions: AB075316, AB075313, AB075311, AB075317, AB075315, AB075314, AB075312) and one *Athelia rolfsii* isolate (AB042626) were included in this study (Okabe and Matsumoto 2003). Trees were rooted by the outgroup (*S. coffeicola*), represented by sequence data from GenBank (AB075319).

Results

Pathogenicity and incidence

All the *Sclerotium* isolates caused damping-off and stem rot symptoms on cowpea. No lesion development was observed with controls. However, there was variation among isolates and virulence varied depending on the host of origin. Isolates collected from cowpea caused the highest disease incidence followed by those from peanuts. The lowest disease incidence was recorded with the *S. delphinii* isolate collected from bambara groundnut.

Mycelial compatibility groups (MCGs) and population analysis

During the MCG test, antagonism zones developed between incompatible isolates and mycelium thinned out in the region of interaction. In all selfed pairings, the hyphae fused and no barrage zones developed. Barrage zones always developed between mycelia of isolates that were vegetatively incompatible, whereas compatible isolates had fused mycelia and dense growth associated with abundant sclerotia production in the contact zone. Isolates that were vegetatively compatible were grouped in the same MCG. Four MCGs were identified for the 66 *Sclerotium* isolates: MCG1, MCG2, MCG3 and MCG4 (Table 1). The method using a pH indicator confirmed the identification of four MCGs.

The MCG phenotype correlated with host of origin: cowpea isolates, from a total area of 60.5 km^2, were MCG1 or 2, peanut isolates were MCG3 and the single *S. delphinii* isolate from bambara groundnut was MCG4. Conversely, isolates from one geographical region or even one subregion belonged to several MCGs (Table 1). For example, isolates from a subregion (Agonguey-Benin, over 19.5 km^2) were members of both MCG1 and 2. Isolates from South Africa grouped in MCG3 and 4. An MCG could also contain isolates from different areas, for example MCG1 contained isolates from three villages and MCG2 from two. MCG1 was the most frequent (53%), followed by MCG2 (30%), MCG3 (15%) and MCG4 (one isolate).

The genotypic diversity (G_{ST}) for the total number of genotypes (MCGs) was 2.307 and the maximum percentage of genotypic diversity ($\hat{G}$) was 3.5%. Furthermore, the incidence of damping-off and stem rot of cowpea expressed as percentage diseased plants varied among MCGs. MCG2 showed the highest levels of disease incidence (94.2%), which is significantly ($P < 0.05$) different from that of MCG1 (83.6%), whereas MCG4 caused the least disease on cowpea (30.4%) that was significantly ($P < 0.05$) lower than that of MCG3 (41.7%).

ITS-PCR and DNA sequencing

Gel electrophoresis of PCR products yielded a band of approximately 700 bp. Different clades and sub-groups were inferred from the phylogenetic analysis, yielding diverse associations. There was no apparent clustering of isolates according to host or village of origin, although isolates from the same country grouped together. Isolates from cowpea and peanut were in the same clade, but the *S. rolfsii* isolates from cowpea formed a group separate from peanut isolates. This sub-group is supported by a bootstrap value of 62%. Other subgroups were found and some isolates from the same area (Agonguey) were in a separate subgroup. For example, AGG55, AGG14 and AGG31 were in a clade different from that of other isolates from Agonguey (Fig. 1). The one isolate from bambara was in the same clade with *S. delphinii* reference isolates. Moreover, isolates from Benin were in the same major clade with peanut isolates from South Africa and this is supported by a 100% bootstrap value. The *S. delphinii* (NEL01) isolate from bambara groundnut and *S. delphinii* reference isolates formed a separate subgroup from other *S. rolfsii* isolates. Furthermore, isolates from different MCGs had nucleotide sequence that clustered into the same subgroup. Thus, AGG14 and AGG31 of MCG1 were in the same subgroup as AGG55 of MCG2, indicating that the isolates had similar ITS DNA sequences. This clade is supported by a bootstrap value of 62%. Some isolates from the same MCG were in different subgroups. For instance, AGG14 and GAN03 from MCG1 were in two different subgroups, and this separation was supported by a bootstrap value of 62% (Fig. 1).

Discussion

In this study, all the *S. rolfsii* and *S. delphinii* isolates caused damping-off and stem rot symptoms on cowpea. However, variation in disease incidence was observed; pathogenic variation has been well demonstrated in many species of fungi (Punja 1985; Chulze *et al*. 2000; Van Heerden and Wingfield 2001) including *Sclerotium* spp. (Punja 1985).

Results from this study showed that the isolates from Benin were represented by two MCGs, as were those from South Africa. Only four MCGs were identified within the 66 isolates collected from different hosts and geographic areas. The method described by Powell (1995) resulted in the same MCG groupings that were observed on PDA medium. Harlton *et al*. (1995) screened a worldwide collection of *S. rolfsii* isolates and identified 49 MCGs from 119 isolates. Similarly, Punja and Grogan (1983) showed that *S. rolfsii* could be placed in MCGs based on mycelial interactions and they identified 25 groups from 72 isolates. Moreover, 13 MCGs were identified among 23 *S. rolfsii* isolates collected from different hosts and regions of Brazil (Almeida *et al*. 2001). In the current study, however, the four MCGs detected among the 66 isolates indicate that a low level of phenotypic differentiation exists.

The fact that isolates originating from the same area within the Ouémé valley were placed in different MCGs suggests occurrence of genetic differences within the subpopulations

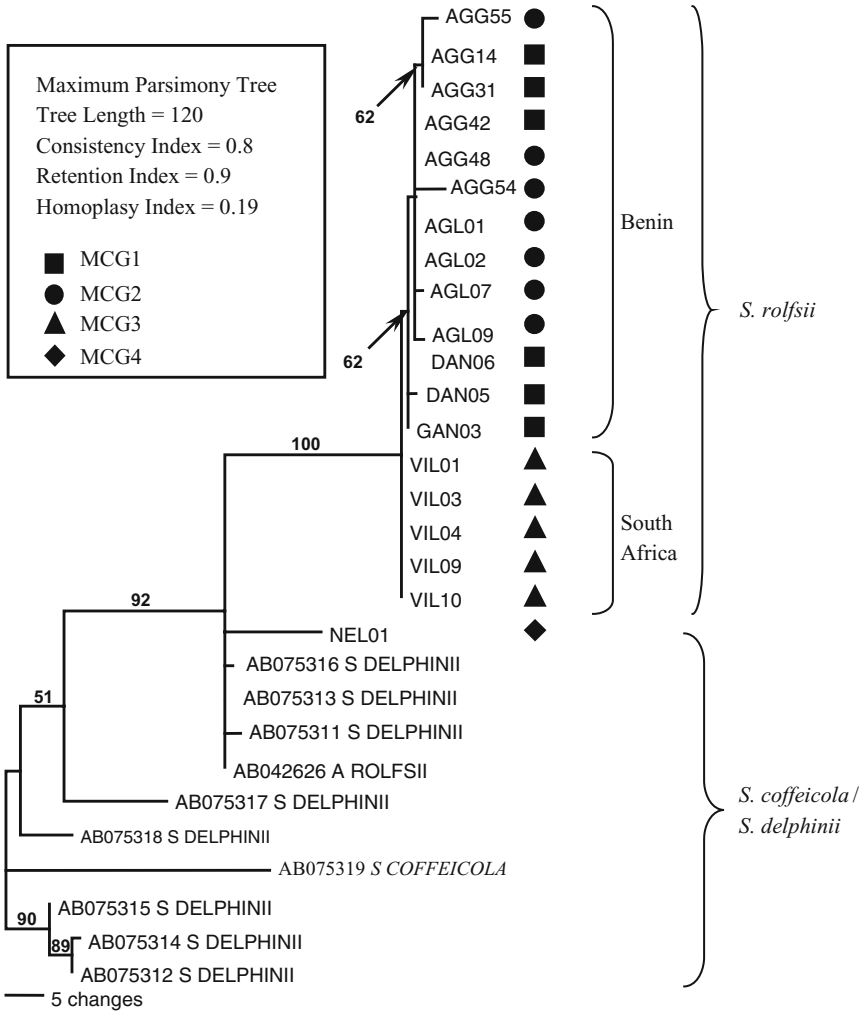

Fig. 1. Phylogenetic tree of ITS sequences from *Sclerotium rolfsii* and *S. delphinii*, using the PAUP* program. The numbers at the nodes are boostrap percentages. Previously published sequences of seven *S. delphinii* isolates (AB075316, AB075313, AB075311, AB075317, AB075315, AB075314, AB075312) and one *Athelia rolfsii* isolate (AB042626) were included in this study. *S. coffeicola* (AB075319) was used as an outgroup (Okabe and Matsumoto 2003). Agg, Agl, Dan and Gan represent isolates from cowpea in Agonguey, Agonlin, Dannou and Gangban, respectively, in the Ouémé valley, Benin; Vil represents isolates from peanut in Viljoenskroon in South Africa and Nel for the isolate from bambara groundnut in Nelspruit, South Africa.

due to migration. Basidiospores may be involved in spread since colonies arising from basidiospores would be variable enough to be in a new MCG (Punja and Grogan 1983; Harlton *et al*. 1995). Carlile (1986) suggested that a single MCG could predominate in an area if a fungal isolate colonised a new area or host and then spread vegetatively. Also, the low number of MCGs could suggest that this species was introduced relatively recently (Harlton *et al*. 1995). If genetic divergence occurs over time among individuals, through recombination and migration for instance, new MCGs could result. In contrast, the recovery of a particular MCG of *S. rolfsii* in widely different geographic areas may have resulted from spread because of agricultural practices (Harlton *et al*. 1995). Therefore, the two MCGs found in Agonguey in the present study could be attributed to migration. The very low number of MCGs found in this study could suggest that either the introduction of *S. rolfsii* is very recent in Benin, or the type of reproduction is asexual with a low level of migration. Another possible explanation could be that the sampling area is restricted, compared with that of previous studies (Punja and Grogan 1983; Harlton *et al*. 1995).

Based on the MCGs, the genotypic diversity (G_{ST}) for the studied isolates was estimated at 2.3. Genotypic diversity is a reliable measure that can be used to compare data from different pathosystems with different sample sizes (Milgroom *et al*. 1992; McDonald and McDermott 1993). Therefore, the *S. rolfsii* population of the present study was genetically uniform, compared with previous reports on the pathogen (Punja and Grogan 1983; Harlton *et al*. 1995; Cilliers *et al*. 2000; Cilliers *et al*. 2002).

Deoxyribonucleic acid sequence analyses showed diverse clades and sub-groups that did not correspond to host or origin of the isolates. These results are similar to those of Cilliers *et al*. (2000), Harlton *et al*. (1995) and Almeida *et al*. (2001). Cilliers *et al*. (2000) compared MCGs and ITS regions among isolates of *S. rolfsii* and reported that there was no apparent clustering according to host or geographic origin for the MCGs. Similarly, Harlton *et al*. (1995) found that unique individuals were not necessarily correlated to the host nor restricted in geographical range, and that clonally derived isolates within an MCG appeared to share ITS restriction sites. The latter also indicated that members within one MCG that were subjected to different evolutionary constraints, could possess the same vegetative compatibility alleles but different ITS sequences. In our study, isolates from different MCGs had nucleotide sequences that clustered into the same clade. The highly similar genetic backgrounds among isolates from different MCGs could differ at only one or few genetic loci that determine cultivar specificity, or specific virulence or pathogenicity (Dobinson *et al*. 1998; Skiba and Pang 2003).

ITS-DNA sequence analyses showed that all *S. rolfsii* isolates grouped together with *A. rolfsii* and some *S. delphinii* reference isolates. In an earlier ITS study, a close relationship was detected between *S. delphinii* and *S. rolfsii* (Harlton *et al*. 1995; Okabe *et al*. 1998). These two species are currently differentiated based on sclerotial morphology and host range with *S. delphinii* producing the largest sclerotia (Stevens 1931; Okabe *et al*. 1998). Harlton *et al*. (1995) reported that *S. rolfsii* and *S. delphinii* grouped together but separately from *S. coffeicola*. Moreover, Okabe *et al*. (2000) and Okabe and Matsumoto (2003) constructed a phylogenetic tree based on ITS-RFLP analysis and found a close relationship between *S. rolfsii* and *S. delphinii*. Based on their similarity, *S. rolfsii* was designated as *S. rolfsii* var. *rolfsii* and *S. delphinii* as *S. rolfsii* var. *delphinii* (Boerema and Hamers 1988; Harlton *et al.* 1995). Therefore, results in the current study could indicate that there is a close affinity of the *Sclerotium* spp. but showed diversity among isolates with no grouping based on host or geographic origin. In this study, we were able to gain significant insight into the variability among isolates of *S. rolfsii* and *S. delphinii* from Benin and South Africa. More isolates would be required to determine the full extent of genetic diversity among the *Sclerotium* spp. and particularly within *S. delphinii* isolates. However, the possibility exists that the *S. rolfsii* isolates from Benin and South Africa added to the phylogenetic tree in this study, may represent a new pathovar and this will be investigated in future.

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