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Isolation, Purification and Characteristics of Strains of *Sclerotium rolfsii* in Ghana

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The physiological and morphological characteristics of five strains of *Sclerotium rolfsii* identified by the phenomenon of aversion were studied. The appearance of the five strains of *Sclerotium rolfsii* on PDA plates was similar and mycelial dry weights in PDB after 6 day incubation varied statistically and the dry weight declined due to autolysis after 6 days differed according to the strains. Autolysis was moderate in strain SrXLL and extreme in strains SrEL1 and SrEL2. However, extensional growth on PDA was fastest in strains SrEL1 and SrEL2, moderate in strains SrXLL and SrXA1 and slowest in strains SrXA2. Strain SrXLL produced a mean number per Petri plate of 185 larger and heavier weight spherical, or ellipsoidal white sclerotia, which turned brown on maturing, whereas the remaining four strains formed 324 to 364 smaller and lighter weight sclerotia per Petri plate. Strain SrXLL with the smaller number of sclerotia had the heavier sclerotial weight and the greatest sclerotia diameter and volume, which was significantly different ($P < 0.05$) from those of the remaining four strains. A clear relationship was established between the number of sclerotia on one hand and the size and weight of sclerotia on the other hand. The larger sized sclerotia produce more emerging hyphae on germination than the smaller sized ones and the greater number of hyphae compensated the smaller number of sclerotia during establishment of strain SrXLL in the habitat.

Keywords: *Sclerotium rolfsii*, Hyphae, Mycelia, Sclerotia, Strain.

INTRODUCTION

Sclerotium rolfsii is a widely occurring facultative soil parasite of economic importance causing disease of several economic crop plants in the world including *Albemuschus esculentus*, *Allium cepa*, *Arachis hypogea*, *Elaeis guineensis*, *Nicotiana tabacum*, *Phaseolus* spp, *Xanthosoma mafaffa* and *Colocasia esculenta* (Synonym *Colocasia antiquorum*) (Aycock, 1966). It is prevalent in warm temperate and subtropical regions and its numerous hosts are as a result of its ability to produce oxalic acid and cell wall degrading enzymes and fast growth rate (Punja, 1985).

The fungus has two vegetative phases that are ecologically distinct. First, there is the mycelial development that forms the heavy white growth from which the fungus gains the common name “white mould”. This might also be referred to as the growth phase or pathogenic phase of the fungus. Secondly, there is the abundant production of sclerotia which enables the fungus to survive adverse environmental conditions (Aycock, 1966; Boyle, 1961; Punja, 1985). The rate of infection by mycelium entering through wounds is higher than that of mycelium attacking by direct penetration. The delay in infection during direct penetration is due to two causes. First, penetration process which is known to be principally mechanical rather than chemical would need some time to be accomplished. Also a process which takes up even more time precedes penetration in *S. rolfsii* and several other soil pathogenic fungi, such as *Rhizotonia solani*. The growing hyphae collect into aggregations called infection cushions, which adhere tightly to the host surface. From beneath the infection cushions emerge penetrating hyphae which breach the covering layer of the host organ and invade the host.

S. rolfsii is able to thrive in soil for a long time in the absence of a host as a saprophyte provided organic matter is present. The growth of *S. rolfsii* in nature is almost always prominent at or near the soil surface, and host organs in that zone are commonly attacked by the fungus. Its distribution in the superficial zones of soil has led to the suggestion that it has a high demand for oxygen. On the other hand, because *S. rolfsii* requires external sources of thiamine or at least one of the moieties which form thiamine, namely pyrimidine, it is encouraged to grow at or near the soil surface which is rich in organic matter.

It is estimated that damage caused by the fungus in Ghana ranges from 5 to 30 per cent (Addison and Chona, 1971). Annually, large quantity of *Xanthosoma mafaffa* and *Colocasia esculenta* are destroyed by the fungus in the country resulting in decline of cultivation of the crops. Probably, there are strains among *Sclerotium rolfsii* occurring in the country. However, literature is silent on physiological and morphological characteristics of *Sclerotium rolfsii* strains occurring in the country. This work was directed to identify strains among *Sclerotium rolfsii* in Ghana and study their physiological and morphological characteristics.

MATERIALS AND METHODS

Strains of *Sclerotium rolfsii*

Five isolates of *Sclerotium rolfsii* were isolated at different locations in Legon and Achimota from *Xanthosoma mafaffa* and *Elaeis guineensis.* Through the test of phenomenon of aversion the isolates were identified as strains (Table 1). All chemicals used in the investigation were from Oxoid Limited, Basingstoke, Hampshire, England, United Kingdom.

Table 1: Location, host plant and designation of *S. rolfsii* strains and a checker-board showing results of pairing of the five strains on PDA at 30°C to test for aversion phenomenon

Location	Host plant	*S. rolfsii* strains designation	SrXLL	SrXA1	SrXA2	SrEL1	SrEL2
Legon Campus	*Xanthosoma mafaffa*	SrXLL	-	+	+	+	+
West Legon	*Xanthosoma mafaffa*	SrXA1	+	-	+	+	+
Achimota	*Xanthosoma mafaffa*	SrXA2	+	+	-	+	+
Legon Campus	*Elaeis guineensis*	SrEL1	+	+	+	-	+
West Legon	*Elaeis guineensis*	SrEL2	+	+	+	+	-

(+: incidence of aversion; -: no aversion)

Cocoyam varieties

Five varieties of cocoyam in Ghana were used in this study. Four were 'Amankani fitaa' (white cocoyam), 'Amankani fufuo' (light coloured cocoyam), 'Amankani Kyirepe' (hard cocoyam), 'Amankani pa' (proper cocoyam) belonging to *Xanthosoma mafaffa* and the remaining one, *Colocasia esculenta*. All varieties of cocoyam supplied by the Agriculture Research Station of the University of Ghana at Kade, Ghana have been described (Abbiw, 1990; Karikari, 1971).

Isolation, purification and maintenance of *S. rolfsii* strain cultures

Mature brown-coloured sclerotia were carefully removed with a pair of sterile fine forceps from the host plant and placed in distilled water to wash off any adhering soil particles. They were then surface-sterilized by immersing them in 1.0% Sodium hypochlorite for three minutes, rinsed after that in three changes of sterile distilled water and inoculated onto sterile PDA in sterile Petri dishes. The growing mycelium was subcultured after 4 days. A second sub-culturing was again done to ensure a completely pure culture. Stock culture of each *S. rolfsii* strain was maintained on PDA slants in MaCartney's tubes in the refrigerators, and subcultured fortnightly. Whenever an experiment was to be carried out, the fungus was raised on PDA in sterile Petri dishes for 4 days and the mycelium then used as the inoculum. The inoculum was always taken from the advancing edge of the culture. The culture was allowed to grow for 12 days in cases where mature sclerotia were required as inoculum.

Test of phenomenon of aversion among *S. rolfsii* strains

The mycelium compatibility group (MCG) used to determine strain of *S. rolfsii* (Almeida *et al*., 2001; Punja and Grogan, 1983) was based on the phenomenon of aversion among *S. rolfsii* strains as reported first by Epps *et*

al., 1951. The test of aversion was employed to determine strains among *S. rolfsii* in the study. About 20ml of PDA was poured into each of 9.0cm diameter sterile Petri dishes and allowed to solidify. Phenomenon of aversion was determined by inoculating one plate with two 3mm diameter mycelial inocula, one at the edge of the plate and the other diametrically opposite at the other edge. The inocula belonged to two different strains or were of the same strain. The five strains were paired in all combinations. The inoculated plates were incubated at 30°C for 12 days. They were then examined to see whether the two colonies grew into each other-which would occur when there was no aversion - or form a barrage zone between two colonies when the growing edges come close to each other, indicating incidence of aversion.

Assessment of radial growth on solid agar medium

About 20ml of the medium was poured into each of 9.0cm - diameter sterile Petri dish and allowed to solidify. Two diameters at right angles to each other were drawn at the bottom of the Petri dishes. Each plate was inoculated at the intersection of the two diameters with a 3mm disc of the mycelium from the growing edge of 4 day old culture of the fungus. The diameter of each growing culture was measured along the two diameters and the mean for the replicates calculated.

Determination of the dry weight and diameter of mycelium

The mycelium of cultures in liquid medium was collected on a funnel-shaped previously dried and weighed Whatman's No. 1 filter paper. The filter paper with the harvested mycelium was dried in an electrically heated oven (Gallenkamp oven 300 plus series, A. Gallenkamp and Co. Ltd. London, England) at 60°C for 24 hours. The filter paper carrying the dried mycelium was weighed using electronic balance (Electronic Balance ER-108A, A & D Co. Ltd., Tokyo, Japan) after being allowed to cool in desiccators. The dry weight of the mycelium was then calculated. The pH of all media, culture filtrates in the study was measured using a pH meter (TOA pH meter, HM-60s OSK - 11478, Ogawa Seiko Co. Ltd., Ogawa, Japan). The diameter of the sclerotium was measured under the microscope using the eye piece graticule (Graticule Limited, Tonbridge, Kent, England, United Kingdom).

Estimation of number of sclerotia formed on agar medium plates

Some of the *S. rolfsii* strains produced a large number of sclerotia on agar medium plates. In order to ensure an accurate counting, the culture was divided into sectors with lines drawn at the bottom of the Petri dish with a filt pen into eight equal sectors. It was then easier to count accurately the sclerotia in each sector using a Tally counter and the total number calculated.

Determination of fresh weight and volume of mature sclerotia

A reliable method to determine fresh weight of the mature tiny sclerotia adopted in this investigation was to weigh together 100 randomly selected sclerotia. Four determinations were made for each *S. rolfsii* strain and the mean per 100 sclerotia calculated. The weighing was done with an electronic balance (Electronic Balance ER-108A, A & D Co. Ltd., Tokyo, Japan). The procedure used was similar to that of weight determination. One hundred mature sclerotia were randomly selected from the culture plate and put in a 10ml measuring cylinder containing 5ml of distilled water. The subsequent rise in the level of the water represented the volume of the sclerotia. Four determinations were made for each *S. rolfsii* strain and the mean per 100 sclerotia calculated.

***S. rolfsii* germination test**

S. rolfsii cultures were raised on blocks of cocoyam cormel tissue in Petri dishes at 30°C for 12 days. Forty mature and brown sclerotia were then selected randomly and seeded on fresh PDA Petri plates and incubated at 30°C. The cumulative number of germinated sclerotia after 12, 24, and 36 hours was recorded.

Data analysis

Statistical analysis of data was performed by one-way and multifactor analysis of variance (ANOVA) and the least significance differences (LSD) of the means set at Confidence Limits of 95% ($P < 0.05$) (Kershaw, 1973).

RESULTS

Phenomenon of aversion

The method employed identified the isolates of *S. rolfsii* obtained from Achimota and Legon as strains (Table 1). There was no aversion (formation of a barrage zone) when two inocula of the same strain grew on the plate which identified compatible reactions (control). On the other hand, aversion occurred in all the pairings of two different strains. The formation of a barrage zone between two colonies was indicative of an incompatible reaction between the two strains proving conclusively that SrXLL, SrXA1, SrXA2, SrEL1 and SrEL2 deserved to be recognized as true strains.

Comparative rate of growth of the different strains of *S. rolfsii* and production of sclerotia on PDA

There were differences in the rates of growth of the five strains (Table 2). The five strains could be separated into three categories of fast-growing, slow-growing and intermediate. The fast-growing ones were strains SrEL1 and SrEL2 which covered the agar medium of 9cm in diameter in 72 hours, while the slow-growing strain SrXA2

took 96 hours to cover the plate. Strains SrXLL and SrXA1 formed the intermediate group which covered the PDA plate in 84 hours.

The five *S. rolfsii* strains differed in the number of sclerotia they formed (Table 3). The diameter, volume and weight of the sclerotia also differed among the strains. Strain SrXLL formed significantly fewer sclerotia of 185 per Petri plate than the rest where the number ranged from 324 to 364 per Petri plate. Surprising, strain SrXLL with the smaller number of sclerotia had the heavier sclerotial weight and the greatest sclerotia diameter and volume. The value of all three parameters (sclerotial weight, diameter and volume) of strain SrXLL was significantly different ($P < 0.05$) from those of the remaining four strains. However, unlike the number of sclerotia formed, the three parameters mentioned above were significantly different ($P < 0.05$) among those four strains. Applying the confidence limits at 95%, the four strains could be placed in descending order of magnitude of SrEL2, SrXA2, SrXA1 and SrEL1.

Table 2: Growth of the five strains of *S. rolfsii* on PDA at 30°C

S. rolfsii strain	Mean diameter ± standard error (S.E) cm							
	Period of incubation (h)							
	12	24	36	48	60	72	84	96
SrXLL	1.1 ± 0.02a	2.0 ± 0.04c	3.3 ± 0.04b	4.5 ± 0.03c	5.7 ± 0.04c	6.8 ± 0.04b	9.0 ± 0.03a	NM
SrXA1	1.1 ± 0.02a	1.9 ± 0.02b	2.7 ± 0.03a	4.0 ± 0.03b	5.3 ± 0.03b	7.0 ± 0.04b	9.0 ± 0.03a	NM
SrXA2	1.1 ± 0.02a	1.8 ± 0.02a	2.6 ± 0.04a	3.8 ± 0.02a	5.1 ± 0.04a	6.5 ± 0.04a	8.8 ± 0.02a	9.0 ± 0.0
SrEL1	1.1 ± 0.03a	2.4 ± 0.02e	3.9 ± 0.03c	5.4 ± 0.02d	7.2 ± 0.03e	9.0 ± 0.00	NM	NM
SrEL2	1.2 ± 0.02b	2.3 ± 0.02d	3.9 ± 0.02c	5.3 ± 0.02d	6.7 ± 0.02d	9.0 ± 0.00	NM	NM

NM = No measurement after Petri plate had been covered.
Means in the vertical rows bearing different letters are significantly different ($P < 0.05$).

There were significant difference ($P < 0.05$) in sclerotial production and the number, weight and size of sclerotia formed, which could be used to distinguish the strains. Strain SrXLL stood out clearly among the strains. It formed the smallest number of sclerotia, but the sclerotia were statistically the largest and heaviest (Table 3).

Table 3: Formation of sclerotia by the five strains of *S. rolfsii* growing on PDA at 30°C

S. rolfsii strain	Mean values of parameters ± S.E. recorded for sclerotia of 12 day-old cultures			
	Total number of sclerotia/plate*	Weight of 100 sclerotia ($x10^{-2}$mg)	Volume of 100 sclerotia ($x10^{-2}cm^3$)	Sclerotium diameter (µm)
SrXLL	185 ± 20.5a	27.8 ± 0.002d	20.0 ± 0.00d	84.6 ± 0.3d
SrXA1	356 ± 42.5b	23.6 ± 0.002b	13.0 ± 0.03a	65.3 ± 0.3b
SrXA2	324 ± 12.5b	25.5 ± 0.001c	15.0 ± 0.03b	75.9 ± 0.3c
SrEL1	332 ± 44.0b	21.7 ± 0.002a	13.0 ± 0.03a	58.3 ± 0.3a
SrEL2	364 ± 28.0b	26.0 ± 0.002c	18.0 ± 0.03c	76.9 ± 0.3c

*Corrected to the nearest whole number.

Means in the vertical rows bearing the same letters are not significantly different ($P > 0.05$).

Comparative rate of growth of the different strains of *S. rolfsii* in PDB

Mycelium growth test shows that whereas *S. rolfsii* strain SrEL1 and SrEL2 again had the greatest mean mycelium dry weight of 0.31g in 6 days, the mean dry weight of 0.26g of strain SrXA1 also after 6 days was lower than the mean dry weight of the mycelium of 0.28g of strain SrXA2 which grew slowest on the PDA plate (Fig. 1). All the strains grew rapidly and attained the highest mycelial dry weight in 6 days. The dry weight declined due to autolysis after 6 days (Fig. 1). The rate of autolysis, however, differed according to the strains. The decline was gentle in strain SrXLL and sharper in strains SrEL1 and SrEL2. From Fig. 1 the calculated percentage loss in mean dry weight from the 6th to 12th day in strain SrEL2, SrEL1 SrXA1, SrXA2 and SrXLL was 16.1, 12.9, 11.5, 10.7 and 3.5 per cent, respectively. Interestingly, the growth of all the strains corresponded to drastic decrease of the culture pH, which reduced from a range of 5.3 – 6.1 at the beginning of the cultivation to 2.4 - 2.7 by the 6th day and rose slightly to 2.6 - 3.2 on the 10th day. The culture media from the 4th day to the end of the incubation period was very acidic.

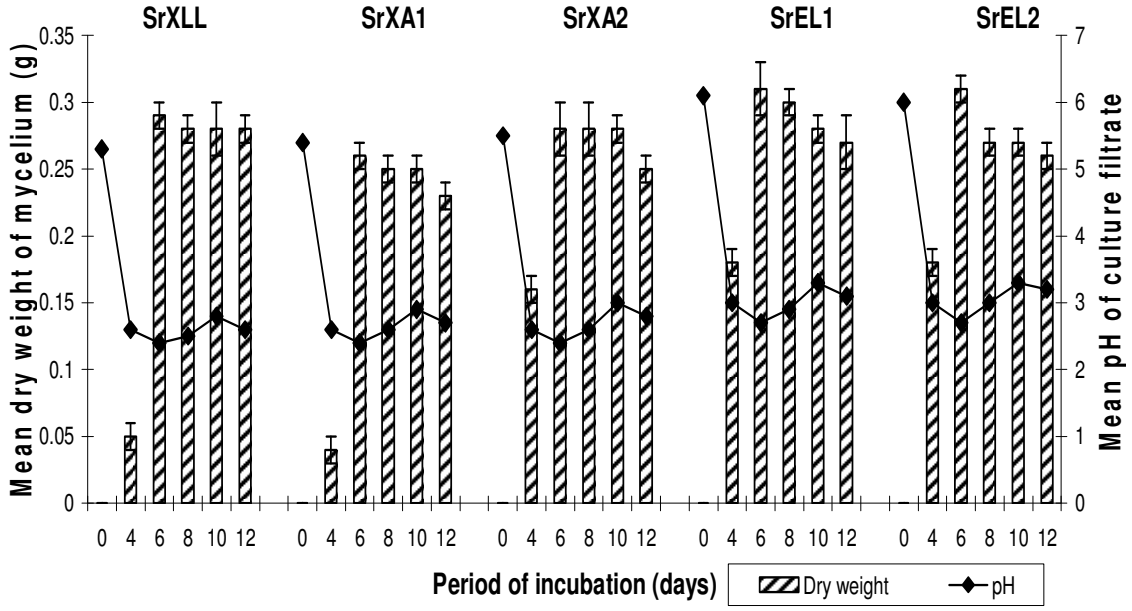

Fig. 1: Mean dry weight of mycelium of the five strains of *S. rolfsii* grown at 30°C for 12 days and mean pH of the culture filtrate during growth of the fungi in PDB.

Formation of sclerotia by *S. rolfsii* strains growing on cormel tissues of cocoyam varieties

Variation occurred in both growth and sclerotium formation in the *S. rolfsii* strains - cocoyam variety combinations (Table 4). *Sclerotium rolfsii* strains SrXLL and SrXA2 produced sclerotial initial earliest on the *X. mafaffa* var. 'Amankani pa' blocks, while strains SrXA1, SrEL1 and SrEL2 produced the sclerotial initials earliest on *C. esculenta* (Table 4). The longest time for the sclerotial initials of strains SrXLL, SrXA1, SrEL1 and SrEL2 to appear were recorded in cultures on *X. mafaffa* var. 'Amankani kyirepe' and the longest time taken by strain SrXA2 to produce sclerotial initials was recorded on *X. mafaffa* var. 'Amankani fufuo' and *C. esculenta*. The majority of the sclerotial initials matured in 24 hours. The longest time was 36 hours by strain SrXLL sclerotial initials on *X. sagittifoilium* var. 'Amankani fitaa' and strain SrEL2 on *X. mafaffa* var. 'Amankani fitaa' and *X. mafaffa* var. 'Amankani kyirepe'. There was a significantly heavier sclerotium production on the *C. esculenta* blocks. The highest mean numbers of sclerotia on the blocks of *X. mafaffa* varieties formed by strains SrXLL, SrXA1, SrXA2, SrEL1 and SrEL2 were 105, 157, 183, 311, and 251, respectively. The corresponding mean numbers on the blocks of *C. esculenta* were 280, 870, 633, 976 and 1,023, respectively. The mean volume of 100 mature sclerotia formed on *C. esculenta* was either identical to that of sclerotia on the *X. mafaffa* varieties (strains SrXLL, SrXA1 and SrEL1) or smaller in some cases (strains SrXA2 and SrEL2).

Sclerotia of strains SrXLL and SrEL2 had greater germination capacity than those of strains SrXA1, SrXA2 and SrEL1 (Fig. 2). Sclerotia formed on the different cocoyam varieties did not germinate to the same degree. Sclerotia of strains SrXLL, SrXA1 and SrEL2 formed on *X. mafaffa* var. 'Amankani kyirepe' germinated better than those formed on the other cocoyam varieties, while the best

substrate for strains SrXA2 and SrEL1 was *X. mafaffa* varieties 'Amankani fitaa' and 'Amankani fufuo', respectively. *Colocasia esculenta* consistently supported the production of the largest number of sclerotia when blocks of cormels were inoculated with the various strains.

The mean number of sclerotia per block of *X. mafaffa* varieties ranged from 80 to 311, compared to a range of 280 to 1,023 sclerotia per block of *C. esculenta* (Table 4). In addition, among the *S. rolfsii* strains, strain SrEL2 was the most prolific, confirming the results in Table 2, with a mean range of 181 to 1,023 sclerotia per block, and strain SrXLL the least productive with a range of 70 to 280 per block (Table 4).

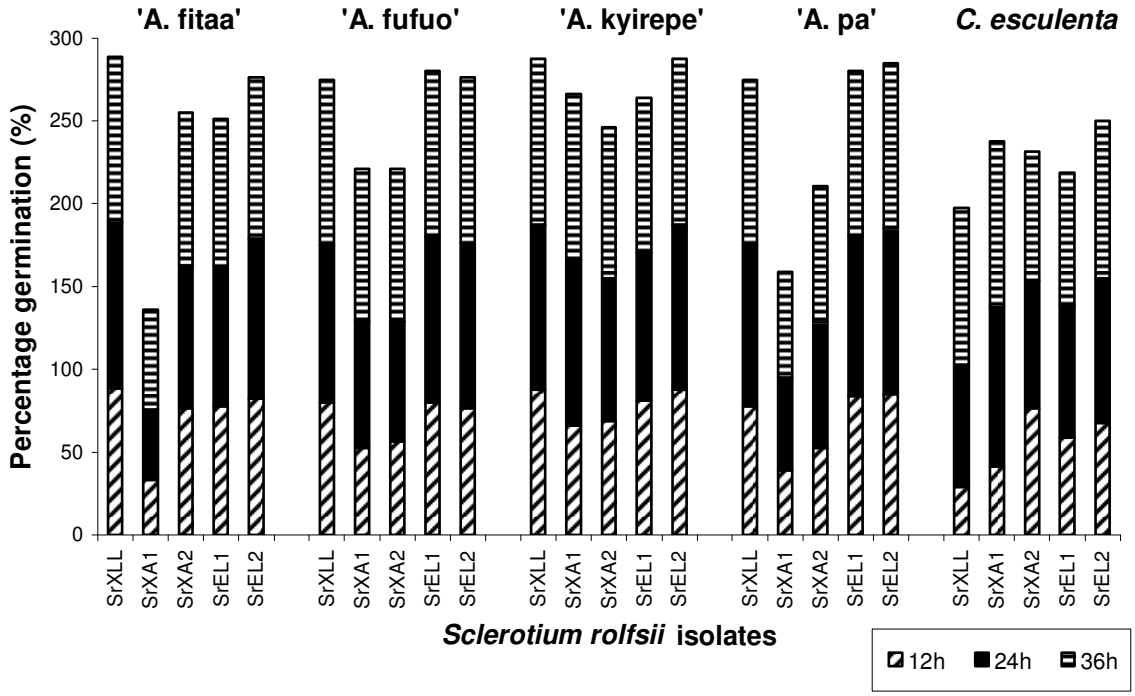

Fig. 2: Percentage germination of sclerotia formed by *S. rolfsii* strains on different varieties of *Xanthosoma mafaffa* ('Amankani fitaa', 'Amankani fufuo', 'Amankani kyirepe', 'Amankani pa') and *Colocasia esculenta* at 30°C (percentage germination on PDA plates).

DISCUSSION

The use of phenomenon of aversion (mycelial compatibility groups- MCG) successfully showed the studied strains as true strains. This reliable method of identifying strains of *S. rolfsii* was first demonstrated by Epps *et al*., 1951. Since that publication, a lot of studies employing this method have been conducted to identify strains of *S. rolfsii* (Almeida *et al*., 2001; Punja and Grogan, 1983). Thirty strains were grouped by this method and no difference were observed among the three experiments of mycelial compatibility groups (MCG), random amplified polymorphic DNA (RAPD) and intergenic transcribed spacers (ITS)-5.8S rDNA restrictive fragment analysis and sequencing (Almeida *et al*., 2001). In all the *S. rolfsii* studied, the mycelia appeared similar in density and extensional growth was linear. Generally, *S. rolfsii* is a fast-growing fungus (Coventry *et al*., 2002; Punja, 1988; Punja *et al*., 1985; Shew and Beute, 1984) and it was noteworthy that within the first 12 hours of incubation the diameter of the culture increases from the initial 3mm of the disc of inoculum to 11-12mm. An interesting relationship was established between the number of sclerotia on one hand and the size and weight on the other hand for the strains. The larger sized sclerotia produced more emerging hyphae on germination than the smaller sized ones. The smaller number of larger sized sclerotia was compensated by the greater number of hyphae they produce which helps the establishment of Strain SrXLL in the habitat. Similar distinguishing sclerotial features in two strains were noticed in earlier studies (Abeygunawardena and Wood, 1957). Strain SR4 was isolated from tomato plants in Sri Lanka and Strain SR7 from groundnut plants in Ghana. SR4 produced numerous spherical sclerotia whereas SR7 produced a much smaller number of irregularly shaped sclerotia. In addition, the morphological variation of the sclerotia observed among strains in this study was similar to strains obtained from soybean and other crops used by Punja and Damiani, 1996 as references for species identification within the genus *Sclerotium*. In similar studies

published, authors reported great variability in relation to the number and size of sclerotia as well as the disposition on PDA media surface (Almeida *et al*., 2001).

In PDB, all the strains attained the highest mycelial dry weight in 6 days followed by gradual autolysis to the 12th day of incubation. During the growth, there was a decrease in the culture pH caused mainly by the secretion of large amounts of oxalic acid (Punja and Damiani, 1996). A similar pattern of culture pH was reported (Ludwig and Haltrich, 2002), which showed a drastic decrease of the culture pH, which dropped from approximately 5.0 at the beginning of cultivation to 3.0 - 3.5 after 6 days and only started to rise slowly at the end of the cultivation after 14 days. The susceptibility of the cocoyam varieties is indicative of its role as substrate for sclerotium formation and hence the survival of the *S. rolfsii* strains in the habitat. Furthermore, the variation in sclerotium formation vindicates the observed differences among the strains. *Colocasia esculenta* cormel was a more favourable substrate for sclerotium formation by *S. rolfsii*.

CONCLUSION

There are strains of *S. rolfsii* with distinctive characteristics in Ghana, an indication that some morphological and physiological difference noticed among the five strains was reliable indicators. All five cocoyam varieties supported the production of sclerotia, with *Colocasia esculenta* cormel been a more favourable substrate for *S. rolfsii*.

Table 4: Formation of sclerotia by the different *S. rolfsii* strains on blocks of tissue of cormels of the different cocoyam varieties at 30°C

S. rolfsii strain	*Xanthosoma mafaffa* variety					
	'Amankani fitaa'			'Amankani fufuo'		
	a. Mean time (hrs) of first appearance of sclerotia **b**. Mean time (hrs) after which melanin first developed	Mean number of sclerotia per block after 12 days	Mean volume (cm^3) of 100 mature sclerotia	**a.** Mean time (hrs) of first appearance of sclerotia **b.** Mean time (hrs) after which melanin first developed	Mean number of sclerotia per block after 12 days	Mean volume (cm^3) of 100 mature sclerotia
SrXLL	a. 144 ± 9.8 b. 36 ± 6.9	80 ± 8.2	0.20 ± 0.0	a. 150 ± 6.0 b. 24 ± 0.0	70 ± 12.9	0.20 ± 0.0
SrXA1	a. 152 ± 13.8 b. 32 ± 6.9	120 ± 7.8	0.15 ± 0.0	a. 150 ± 6.0 b. 24 ± 0.0	157 ± 16.1	0.15 ± 0.0
SrXA2	a. 132 ± 6.9 b. 24 ± 0.0	137 ± 22.8	0.15 ± 0.0	a. 138 ± 11.4 b. 24 ± 0.0	128 ± 7.2	0.18 ± 0.0
SrEL1	a. 150 ± 11.5 b. 30 ± 6.0	159 ± 14.2	0.15 ± 0.0	a. 138 ± 11.4 b. 24 ± 0.0	160 ± 10.6	0.15 ± 0.0
SrEL2	a. 144 ± 9.8 b. 36 ± 6.9	181 ± 11.3	0.18 ± 0.0	a. 138 ± 6.0 b. 24 ± 0.0	240 ± 20.5	0.20 ± 0.0
S. rolfsii strain	'Amankani kyirepe'			'Amankani pa'		
SrXLL	a. 156 ± 6.9 b. 24 ± 0.0	100 ± 31.7	0.20 ± 0.0	a. 132 ± 6.9 b. 24 ± 0.0	105 ± 12.2	0.20 ± 0.0

Table 4 con't

SrXA1	a. 168 ± 12.0 b. 24 ± 0.0	152 ± 23.1	0.18 ± 0.0	a. 126 ± 6.0 b. 24 ± 0.0	107 ± 7.9	0.15 ± 0.0
SrXA2	a. 128 ± 6.9 b. 24 ± 0.0	183 ± 15.9	0.15 ± 0.0	a. 126 ± 0.0 b. 24 ± 0.0	141 ± 21.1	0.18 ± 0.0
SrEL1	a. 156 ± 6.9 b. 24 ± 0.0	172 ± 11.5	0.15 ± 0.0	a. 132 ± 6.9 b. 24 ± 0.0	311 ± 9.5	0.15 ± 0.0
SrEL2	a. 156 ± 6.9 b. 36 ± 6.9	185 ± 15.7	0.20 ± 0.0	a. 132 ± 6.9 b. 24 ± 0.0	251 ± 6.9	0.20 ± 0.0

Table 4 con't

S. rolfsii strain	*Colocasia esculenta*		
	a. Mean time (hrs) of first appearance of sclerotia **b.** Mean time (hrs) after which melanin first developed	Mean number of sclerotia per block after 12 days	Mean volume (cm^3) of 100 mature sclerotia
SrXLL	a. 144 ± 9.8 b. 24 ± 0.0	280 ± 20.2	0.20 ± 0.0
SrXA1	a. 120 ± 0.0 b. 24 ± 0.0	870 ± 82.3	0.15 ± 0.0
SrXA2	a. 138 ± 11.4 b. 24 ± 0.0	633 ± 50.4	0.15 ± 0.0
SrEL1	a. 120 ± 0.0 b. 24 ± 0.0	976 ± 67.1	0.15 ± 0.0
SrEL2	a. 126 ± 6.0 b. 24 ± 0.0	1023 ± 92.8	0.18 ± 0.0

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