UTILIZATION OF NUTRITIONAL COMPOUNDS AND SCLEROTIAL DEVELOPMENT OF *SCLEROTIUM ROLFSII* ISOLATED FROM *ELAEIS GUINEENSIS* AND *XANTHOSOMA MAFAFFA* IN GHANA

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ABSTRACT

Sclerotium rolfsii is a facultative soil parasite of economic importance causing disease of several economic crop plants. Bacterial and fungal parasites are themselves an assemblage of strains differing genetically and liable to variation. The possible effect of external nutrients on different S. rolfsii strains in Ghana has not been studied. In this study the utilization of twelve different nutritional compounds and sclerotia formation by five strains of Sclerotium rolfsii, SrXLL, SrXA1, SrXA2, SrEL1 and SrEL2 was investigated. In broth media with different carbon compounds - fructose, glucose, maltose, starch and sucrose - and with different nitrogen compounds - Ammonium chloride, Ammonium nitrate, Asparagine, Peptone and Sodium nitrate - a clearly discernible order of ability to utilize the two groups of compounds was noticed. The order of the strains in descending order of efficiency was SrEL2 > SrEL1 > SrXLL > SrXA1 > SrXA2. Among the carbon compounds the least utilized was starch. The best carbon compound varied with the strains. Peptone was universally a good nitrogen source. Over a concentration range of 0.5 to 2.0%, the greatest amount of pectolytic enzymes was produced in pectin media (20.0 per minute). Filtrates of strains SrXLL, SrXA1 and SrEL2 showed greater pectolytic enzymes activity than those of strains SrXA2 and SrEL1. But strains SrXA1 and SrXA2 showed greater ability to break down cellulose than the other three strains. In all the media studied the pH of the culture filtrate decreased to acidic levels at the end of incubation period.

INTRODUCTION

Sclerotium rolfsii has been identified as causing damping-off of seedlings and soft rot at the collar region, stem or root rot of many economic crop plants as a result of its fast growth rate and ability to produce oxalic acid and cell wall degrading enzymes (Aycock, 1966; Punja, 1985). The fungus has successfully established itself as the main facultative soil parasite on varieties of *Xanthosoma mafaffa* and *Colocasia esculenta* in Ghana. The invasion of host plants by *S. rolfsii* both through wounds, natural openings and by direct penetration of intact surface tissue of the host has been well presented in literature (Aycock, 1966; Bowen *et al.*, 1992; Boyle, 1961; Cilliers *et al.*, 2000; 2003; Punja, 1985).

The formation of sclerotia by *S. rolfsii* is influenced by various environmental factors. For example Abeygunawardena and Wood (1957) found that the mean number of sclerotia per Petri plate on agar medium of initial pH 1.9, 2.4, 2.8, 4.2, 5.1, 7.8 and 8.0 was 0, 480, 1810, 1320, 1230, 1100 and 0, respectively. Out of a number of carbon sources tested, abundant sclerotia were formed on galactose, mannitol and sodium carboxymethyl - cellulose media of the same concentration. Wheeler and Sharan (1965) obtained the greatest sclerotium formation with 0.1% Glucose and the sclerotium number decreased with increasing concentration of glucose to zero at 3.0% glucose. Increasing the concentration of KH_2PO_4, during the same investigation, reduced the amount of sclerotia formed. Initiation of sclerotia was significantly greater at 0.2% KH_2PO_4 than at 0.0001% KH_2PO_4. In contrast sclerotium formation was significantly greater at 0.5% $NaNO_3$ than at either 0.25 or 1.0% $NaNO_3$.

Furthermore, Wheeler and Waller (1965) found that sclerotium initiation appeared to be regulated by the growing hyphae at the periphery of the mycelial mat. In Petri dishes of 5, 7, 9 and 13cm diameter, initials did not appear until the lateral extension of the mycelium was restricted. Also faster growing mycelia produced greater number of sclerotia than slower growing ones. Thus, cultures kept continuously at the favourable temperature of 25°C produced a maximum sclerotium initials of 900 per Petri plate at day 12 while those kept initially at 15°C for 5 days and then transferred to 25°C produced maximum initials of 600 at day 18.

Johnson and Joham (1954) found that *S. rolfsii* used fructose, galactose, glucose, lactose, maltose, raffinose and starch among other carbohydrates to more or less the same degree.

However, cellulose agar, gummastic, gum guaiac, gum arabic and rhamnose were not used by the fungus. In studies by Abeygunawardena and Wood (1957), fructose was found to be the best carbon source for growth while Townsend (1957) found sucrose to be the best at low concentrations but glucose to be the best at high concentrations with lactose supporting poor growth. Darkwa (1965) found quite a different order of preference. Of the carbohydrates he tested, the order of utilization was starch, maltose, fructose, glucose, cellulose, lactose, glycerol and galactose. Galactose was barely used. Some differences were also found in results of investigations on the use of nitrogen compounds for growth by *S. rolfsii*. Abeygunawardena and Wood (1957) also found the organic nitrogen asparagine and peptone to be more readily utilized than ammonium salts and sodium nitrate. Townsend (1957) found asparagine, peptone and potassium nitrate to support good growth while Darkwa (1965) reported that asparagine, peptone, urea, ammmonium chloride and ammonium nitrate were by far better nitrogen sources than alanine and glycine.

It is well-known that many bacterial and fungal parasites are themselves an assemblage of strains differing genetically and liable to variation. The effect of external nutrients on different *S. rolfsii* strains in the country has not been studied. This study was to investigate the possible effect of external nutrients on different *S. rolfsii* strains in the country.

MATERIALS AND METHODS

Isolation of *S. rolfsii* strains, maintenance of stock cultures and preparation of inocula

Sclerotia, which are mature brown-coloured were carefully removed with a pair of sterile fine forceps from the host plant and rinsed in distilled water to wash off any adhering soil particles. The sclerotia were then surface-sterilized in 1.0% Sodium hypochlorite for three minutes and then rinsed in three changes of sterile distilled water and inoculated onto sterile PDA in sterile Petri dishes. After 4 days the growing mycelium was subcultured. A second sub-culturing was done to ensure a completely pure culture.

Five different strains of *S. rolfsii* were obtained at different areas in Legon and Achimota from two host plants and given designation according to their

sources as follows: Strain SrXLL from *X. mafaffa* Schott at Legon campus; strain SrXA1 from *X. mafaffa* Schott at West Legon; strain SrXA2 from *X. mafaffa* at Achimota; strain SrEL1 from *Elaeis guineensis* Jacq at Legon campus; strain SrEL2 from *E. guineensis* Jacq at West Legon. Stock cultures were maintained on potato dextrose agar (PDA) slants in MaCartney tubes and stored in a refrigerator. They were sub-cultured fortnightly. The fungus was raised on PDA in sterile Petri dishes for 4 days and the mycelium then used as the inoculum whenever an experiment was to be carried out. The inoculum was always taken from the advancing edge of the culture. The five *S. rolfsii* strains were identified as different strains in earlier studies by Tortoe and Clerk (2008). All chemicals used in the investigation were from Oxoid Limited, Basingstoke, Hampshire, England, United Kingdom.

Fresh fruits of cucumber (*Cucumis sativus* L.) used in 'Pectolytic Enzyme Test' were purchased from Makola market in Accra on the day they were to be used.

Media preparation

The different *S. rolfsii* strains were raised in several synthetic liquid media and the amount of pectolytic enzymes produced in them assessed by the maceration method. The standard medium consisted of Peptone, Magnesium sulphate ($MgSO_4.7H_2O$), Potassium dihydrogen phosphate (KH_2PO_4), Thiamine and distilled water at 1.0%, 0.5%, 0.1%, 0.005% and 1 litre, respectively. The various media were made by adding different concentration of either Glucose or Pectin, referred to as Glucose and Pectin media, respectively. The Glucose or Pectin media contained the following glucose or pectin concentrations: 0.5%, 1.0%, 1.5% and 2.0%. Other media, Glucose-Pectin media, contained both glucose and pectin in different ratios as follows: 0.75% Glucose plus 0.25% Pectin, 0.50% Glucose plus 0.50% Pectin, 0.25% Glucose plus 0.75% Pectin.

The relative amounts of cellulolytic enzymes produced by the different *S. rolfsii* strains were assessed using the following medium: 5.0g Potassium nitrate ($KN0_3$), 1.0g Potassium dihydrogen phosphate (KH_2P0_4), 0.5g Magnesium sulphate ($MgS0_4.7H_20$), 0.5mg Thiamine, 1.0g Yeast extract, 10ml *Micronutrient solution in 1 litre distilled water. *Micronutrient Solution consisted of 0.10mg Copper sulphate ($CuSO_4.5H_20$), 0.01mg Sodium borate ($NaB_40_7.2H_20$), 0.20mg Ferrous phosphate ($FeP0_4.2H_20$), 0.02mg Magnesium sulphate ($MnS0_4.5H_20$), 0.02mg Sodium molybdate ($Na_2MO_4.2H_20$), 0.15mg Zinc sulphate ($ZnSO_4.7H_20$) and 1 litre distilled water.

Utilization of different carbon and nitrogen sources by *S. rolfsii* strains

The ability of the different *S. rolfsii* strains to utilize different carbon sources was investigated. The common quantity of carbon source in laboratory media, 1.0%, was used in this test. Five separate preparations of a basal medium containing 1% Peptone, 0.1% $MgSO_4.7H_20$, 0.1% KH_2PO_4, 0.005% Thiamine and 1 litre distilled water were made and to which was added either 1.0% fructose, glucose, maltose, starch or sucrose. Each preparation was then divided into five lots and allocated to the five *S. rolfsii* strains. The medium for each was then dispensed in quantities of 30ml into four 250ml Erlenmeyer flasks. All the flasks were then autoclaved, cooled and each inoculated with 3mm - mycelium disc of the test *S. rolfsii* strain. The inoculated flasks were incubated at 30°C for 6 days and the mycelium harvested separately with filter paper funnels and dried at 60°C for 24 hours. The dry weight of the mycelium was then determined as described above. The pH of the culture filtrate was then measured. A reserve medium in each case was autoclaved, cooled and the pH measured to provide the initial pH of the medium. 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 ability of the different *S. rolfsii* strains to utilize Ammonium chloride, Ammonium nitrate, Asparagine, Peptone and Sodium nitrate was determined. Each of these nitrogen sources was incorporated at

a concentration of 0.1% into a basal medium of 1.0% Glucose, 0.1% $MgSO_4.7H_20$; 0.1% KH_2PO_4, 0.005% Thiamine and 1 litre distilled water. Erlenmeyer flasks (250ml) each containing 30ml of the test medium were inoculated with 3mm - mycelium disc of the test *S. rolfsii* strain and incubated at 30°C for 6 days. The dry weight of the mycelium produced, the initial pH of the medium and the final pH of the culture filtrate were measured.

Determination of relative concentration of pectolytic enzymes in culture filtrates of the *S. rolfsii* strains

The experiment studied the duration for maceration of thin discs of *Solanum tuberosum* and pericarp of *Cucumis sativus* immersed in the culture filtrates to occur following the method used by Cole and Wood (1961). Plugs, 1.0cm in diameter were removed with a No. 8 cork borer from either the *S. tuberosum* tuber or *C. sativus* pericarp and placed separately in distilled water in Petri dishes. Thin (1 mm) discs, 10mm in diameter, of either *S. tuberosum* tuber or *Cucumis sativus* pericarp were carefully cut with a surgical blade from the plug and put in separate filtrates of 6-day-old cultures of the test fungi grown in different types of glucose, pectin and glucose-pectin media at 30°C. The discs were washed in sterile distilled water and the water drained on filter paper. Test filtrate (2ml) in a mini-Petri dish (6cm diameter) was adjusted to pH 5.0 by adding 0.5ml of 0.1M citrate buffer of pH 5.0, and six discs were then introduced. The duration taken by the constituent pectolytic enzymes of the filtrate to macerate a total of six discs was recorded and the mean calculated. There were four replicates for each test, and the mean enzyme activity (1/t x 100) calculated, where t = mean time of maceration. The mycelium dry weight and the pH of filtrate were measured at the end of the growth period.

Utilization of cellulose as carbon source by *S. rolfsii* strains

Cellulose is a major component of plant cell walls. The ability of *S. rolfsii* strains to use cellulose as carbon source was investigated. The experiment measured indirectly the relative level activity of cellulolytic enzymes in the culture filtrate of the various *S. rolfsii* strains. Whatman's No. 3 filter paper discs of 7cm-diameter were oven dried at 60° C for 24 hours and weighed singly after cooling in a desiccator. The filter paper was placed in a 250ml Erlenmeyer flask containing 30ml of the synthetic medium for assessment of production of cellulolytic enzymes. Two different experiments were carried out. In one experiment cellulose, in the form of filter paper, was added to the medium as the carbon source. In the other experiment, cellulose in the form of filter paper was supplemented with 0.1% glucose as a booster. The flasks were autoclaved and the medium in each was inoculated with a 3mm mycelial disc. Before autoclaving non-absorbent cotton wool plugs were covered with cellophane to prevent excessive water loss. The flasks were incubated at 30°C for 7 weeks. There were four replicates for each treatment. The remaining fragments of the decomposed filter paper from each flask were carefully collected into a pre-weighed aluminium foil cap, dried at 60°C for 24 hours, and weighed. The pH of the filtrate was measured after harvesting the remains of the filter paper. The weight of the constituent minerals of the media for cellulolytic enzymes was subtracted from the dry weight of the filter paper used by the fungus (Garrett, 1962; Forbes and Dickson, 1977).

Determination of the dry weight of mycelium

Using a funnel-shaped previously dried and weighed Whatman's No. 1 filter paper the mycelium of cultures in liquid medium was collected. The harvested mycelium with filter paper 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 after being allowed to cool in desiccators. The dry weight of the mycelium was then calculated.

Data analysis

Results were analyzed by employing one-way and multifactor ANOVA using LSD test with Confi-

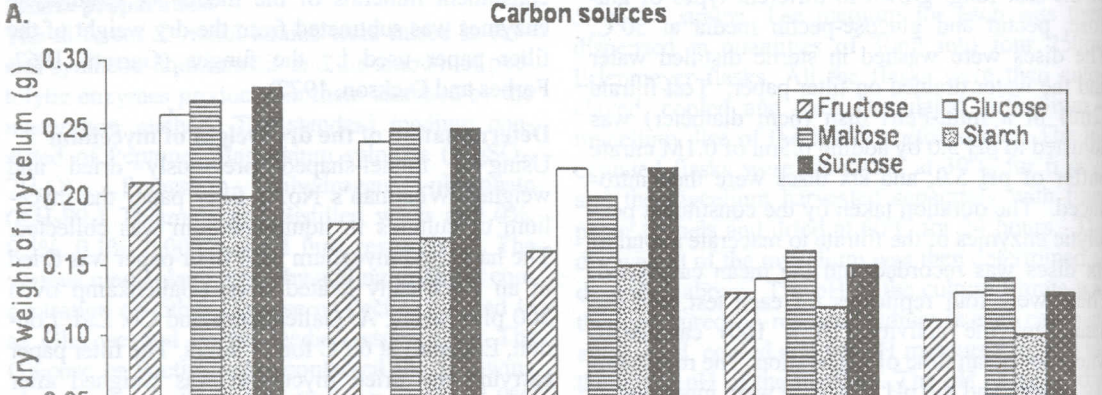
dence Limits set at 95% performed for the comparison of the test means.

RESULTS

Growth of the different strains of *S. rolfsii* in liquid medium with different carbon and nitrogen sources

The results on utilization of the different carbon sources by the five *S. rolfsii* strains are presented in Fig 1A. The utilization of the different carbon sources could be arranged in the following descending order SrEL2 > SrEL1 > SrXLL > SrXA1 > SrXA2 according to their mean dry weight (Fig 1A). Starch was least used by all the strains, followed by fructose. However, glucose, maltose and sucrose were superior to fructose and starch and the strains used them to almost the same degree. The best two carbon sources for growth of strains SrXA1, SrEL1 and SrEL2 were maltose and sucrose and those for strain SrXLL were glucose and sucrose while the best carbon source for strain SrXA2 was maltose followed by glucose and sucrose which supported the production of the same mean dry weight of mycelium. The pH of the culture filtrates turned more acidic during 6 days growth of the strains in the carbon medium. The initial pH was in the range of 4.6 - 4.8, whereas the finial pH range was 1.8 - 2.4. Evidently, the five carbon sources were utilized differently by the five *S. rolfsii* strains.

Similar pattern as was observed in the utilization of the nitrogen sources (Fig 1B). Strains SrEL2 grew best in all the different media, followed by strains SrEL1, SrXLL, SrXA1, SrXA2 in that order. Strains SrXLL, SrXA1, SrXA2 and SrEL2 grew best in the peptone-medium, while ammonium chloride, ammonium nitrate, sodium nitrate and peptone supported practically the same extent of growth of strain SrEL1 (Fig 1B). Different nitrogen sources were found to support the poorest growth in different strains. Ammonium chloride and sodium nitrate were least used by strains SrXLL, SrXA1, and SrXA2. The smallest mean mycelium dry weight of strain SrEL1 occurred in the asparagine medium. However, the value of 0.10g was close to those of the other media of 0.12g and 0.13g. With Strain SrEL2, the smallest mean mycelium dry weight occurred in the sodium nitrate medium. The pH of the culture filtrates turned more acidic during the growth of the strains in the nitrogen medium.



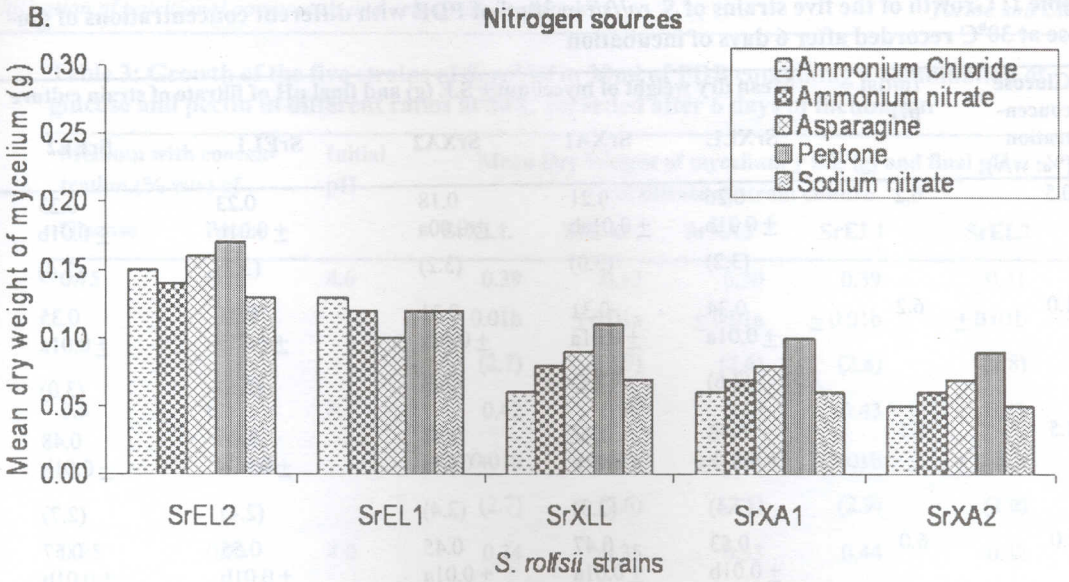


Fig. 1: Mean dry weight of mycelium of different *S. rolfsii* strains grown in 30ml liquid medium with different sources of (A) carbon and (B) nitrogen at 30°C for 6 days.

The culture filtrates showed an initial pH in the range of 4.6 - 4.9 and final pH range of 2.0 - 2.9 after 6 days growth.

Relative concentration of pectolytic enzymes in culture filtrates of the *S. rolfsii* strains

The maceration of plant tissues by pectolytic enzymes of facultative parasites and the degradation of pure pectin by pectolytic enzymes in viscometers are equally reliable and the former was used to compare the amount of pectolytic enzymes produced by the different *S. rolfsii* strains.

The mean dry weight of mycelium formed in 6 days by each *S. rolfsii* strain increased with increasing glucose concentration over the range of 0.5 - 2.0 per cent (Table 1). At glucose concentrations of 1.0, 1.5 and 2.0 per cent, strain SrEL2 produced the highest mean mycelial dry weight, although not statistically different from the amount produced by some of the other strains. At all glucose concentrations, Strain SrXA2 produced the least mean mycelial dry weight, although the difference between that value and those of some of the strains was not statistically significant. The pH of all the media drifted from an initial pH 6.0 - 6.2 to a final pH 2.0 - 3.4.

Growth of *S. rolfsii* strains in different pectin media of 0.5, 1.0, 1.5 and 2.0 per cent concentration showed similar pattern as with the glucose media. The mean dry weight of mycelium formed in 6 days by each strain increased with increasing pectin concentration from 0.5 to 2.0% (Table 2). Strains SrXA1 and SrXA2 produced the lowest mean mycelial dry weights at all pectin concentrations. The remaining three strains produced practically comparable mean mycelial dry weight in each concentration of pectin. The pH of all the media drifted from an initial pH 4.0 - 5.0 to a final pH 2.6 - 3.5.

The growth of *S. rolfsii* strains in media with different Glucose-Pectin combinations shows that the combination of 0.5% Glucose and 0.5% Pectin supported the greatest growth in strains SrXLL, SrXA1, SrXA2 and SrEL2 (Table 3). The combi-

Table 1: Growth of the five strains of *S. rolfsii* in 30ml of PDB with different concentrations of glucose at 30°C recorded after 6 days of incubation

Glucose concentration (%: w/v)	Initial pH	Mean dry weight of mycelium ± S.E (g) and final pH of filtrate of strain culture				
		SrXLL	SrXA1	SrXA2	SrEL1	SrEL2
0.5	6.2	0.26 ± 0.01b (3.2)	0.21 ± 0.01ab (3.0)	0.18 ± 0.00a (3.2)	0.23 ± 0.01b (3.2)	0.23 ± 0.01b (3.4)
1.0	6.2	0.34 ± 0.01a (2.6)	0.31 ± 0.01a (2.5)	0.31 ± 0.01a (2.6)	0.31 ± 0.01a (2.6)	0.35 ± 0.01a (3.0)
1.5	6.1	0.46 ± 0.01b (2.4)	0.43 ± 0.01b (2.3)	0.38 ± 0.01a (2.4)	0.46 ± 0.01b (2.4)	0.48 ± 0.01b (2.7)
2.0	6.0	0.53 ± 0.01b (2.0)	0.47 ± 0.01a (2.1)	0.45 ± 0.01a (2.1)	0.55 ± 0.01b (2.1)	0.57 ± 0.01b (2.3)

Values in parenthesis are final pH of filtrate of strain culture. Means in horizontal rows bearing the same letters are not significantly different (P > 0.05)

Table 2: Growth of the five strains of *S. rolfsii* in 30ml of PDB with different concentrations of pectin at 30°C recorded after 6 days of incubation

Pectin concentration (%: w/v)	Initial pH	Mean dry weight of mycelium ± S.E (g) and final pH of filtrate of strain culture				
		SrXLL	SrXA1	SrXA2	SrEL1	SrEL2
0.5	5.0	0.24 ± 0.01b (3.4)	0.17 ± 0.01a (3.4)	0.16 ± 0.00a (3.4)	0.25 ± 0.00b (3.4)	0.25 ± 0.00b (3.5)
1.0	4.6	0.25 ± 0.01c (3.3)	0.22 ± 0.00b (3.3)	0.19 ± 0.01a (3.4)	0.23 ± 0.00c (3.4)	0.28 ± 0.00d (3.5)
1.5	4.2	0.27 ± 0.00b (3.0)	0.25 ± 0.01ab (3.0)	0.23 ± 0.01a (3.1)	0.28 ± 0.00c (3.3)	0.28 ± 0.00c (3.2)
2.0	4.0	0.39 ± 0.00c (2.7)	0.30 ± 0.00a (2.6)	0.28 ± 0.01a (2.8)	0.35 ± 0.01b (3.0)	0.38 ± 0.01bc (2.9)

Values in parenthesis are final pH of filtrate of strain culture. Means in horizontal rows bearing the same letters are not significantly different (P > 0.05).

Table 3: Growth of the five strains of *S. rolfsii* in 30ml of PDB containing a combination of glucose and pectin in different ratios at 30°C recorded after 6 days of incubation

Medium with concentration (% w/v) of		Initial pH	Mean Dry Weight of mycelium ± S.E (g) and final pH of filtrate of strain culture				
Glucose	Pectin		SrXLL	SrXA1	SrXA2	SrEL1	SrEL2
0.75	0.25	4.6	0.39 ± 0.01b (2.7)	0.32 ± 0.01a (2.7)	0.30 ± 0.01a (2.6)	0.39 ± 0.01b (2.8)	0.41 ± 0.01b (2.8)
0.5	0.5	4.1	0.42 ± 0.01b (2.7)	0.37 ± 0.01a (2.6)	0.36 ± 0.01a (2.5)	0.43 ± 0.01b (2.9)	0.43 ± 0.01b (2.9)
0.25	0.75	4.0	0.34 ± 0.02a (3.0)	0.35 ± 0.01a (2.8)	0.33 ± 0.01a (2.9)	0.44 ± 0.02b (3.2)	0.42 ± 0.01b (3.2)

Values in parenthesis are final pH of filtrate of strain culture. Means in horizontal rows bearing the same letters are not significantly different (P > 0.05).

nations of 0.25% Glucose and 0.75% Pectin supported the greatest growth in strains SrEL1. However, the highest value in all cases was not statistically different from the other values. The pH of the media became strongly acidic during growth of the fungi drifting from an initial pH 4.0 - 4.6 to a final pH 2.5 - 3.2.

The enzyme activity determined by maceration of discs of tuber of *S. tuberosum* in culture filtrates of all the *S. rolfsii* strains was far greater, ranging from 6.8 - 8.4 per minute in culture filtrates of the 2.0% glucose medium than in the remaining three media of lower glucose concentration (Fig 2A). Media of glucose concentrations 0.5, 1.0 and 1.5 showed comparatively low enzyme activities in the range of 1.6 - 2.2 per minute. Results show that 2.0% glucose medium tests for Strain EL2 produced the greatest enzyme activity of 8.4 per minute whereas Strains XA1 and EL1 produce the lowest enzyme activity of 6.8 per minute.

The greatest enzyme activity occurred in the culture filtrates of the 2.0% glucose medium showing a range of 11.7 - 13.3 when pericarp tissue of *C. sativus* was used (Fig 2A). The enzyme activities of filtrates of glucose concentrations of 0.5, 1.0 and 1.5% were from 2.9 to 6.4 per minute. The strains fell into two groups at any of the glucose concentration. Higher levels of enzyme activity were recorded for strains SrXLL, SrXA1 and SrEL2 and lower levels for strains SrXA2 and SrEL1.

In pectin media, the high pectolytic enzyme activity of 16.2 and 20.0 per minute occurred in filtrates of 2.0% pectin concentration for both *S. tuberosum* and *C. sativus* tissues, respectively (Fig. 2B). There was generally greater enzyme activity in filtrates of Strains SrEL1 and SrEL2 than those of strains SrXLL, SrXA1 and SrXA2 in the pectin media.

The enzyme activities of the culture filtrates of the five *S. rolfsii* strains growing in media containing different combinations of glucose and pectin are

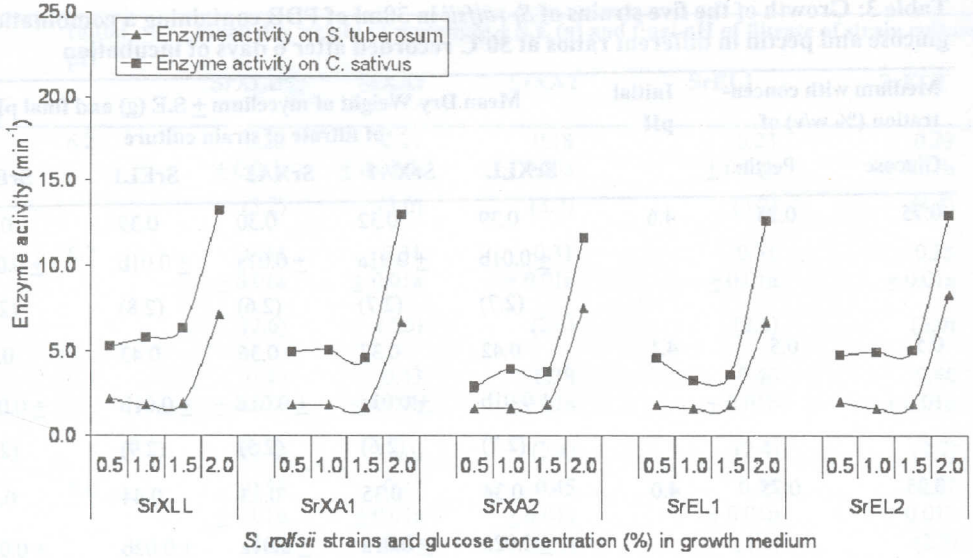


Fig. 2A: Enzyme activity of filtrate of 6 day-old cultures provided with glucose in the growth medium of the different *S. rolfsii* strains on *S. tuberosum* and *C. sativus*.

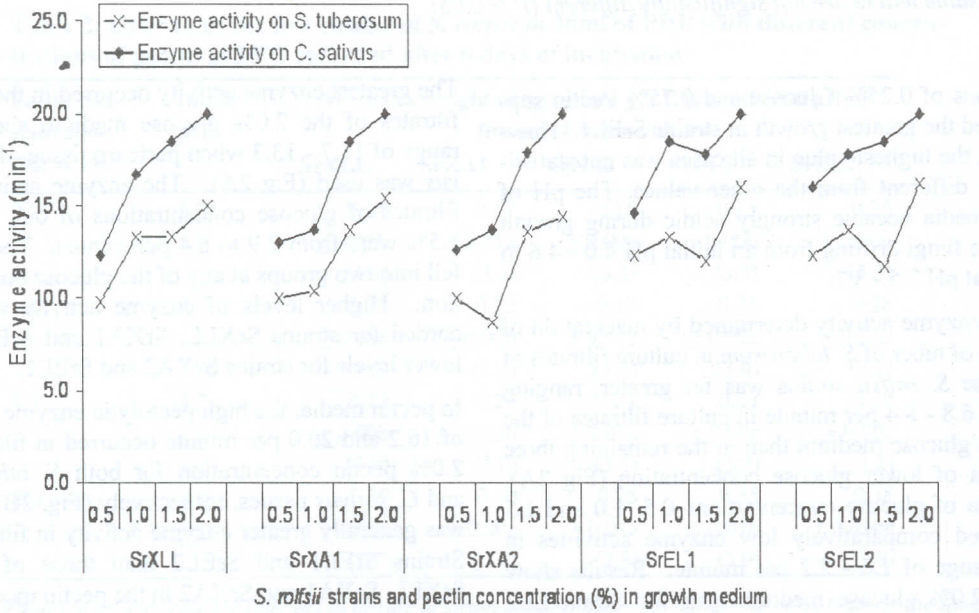


Fig. 2B: Enzyme activity of filtrate of 6 day-old cultures provided with pectin in the growth medium of the different *S. rolfsii* strains on *S. tuberosum* and *C. sativus*.

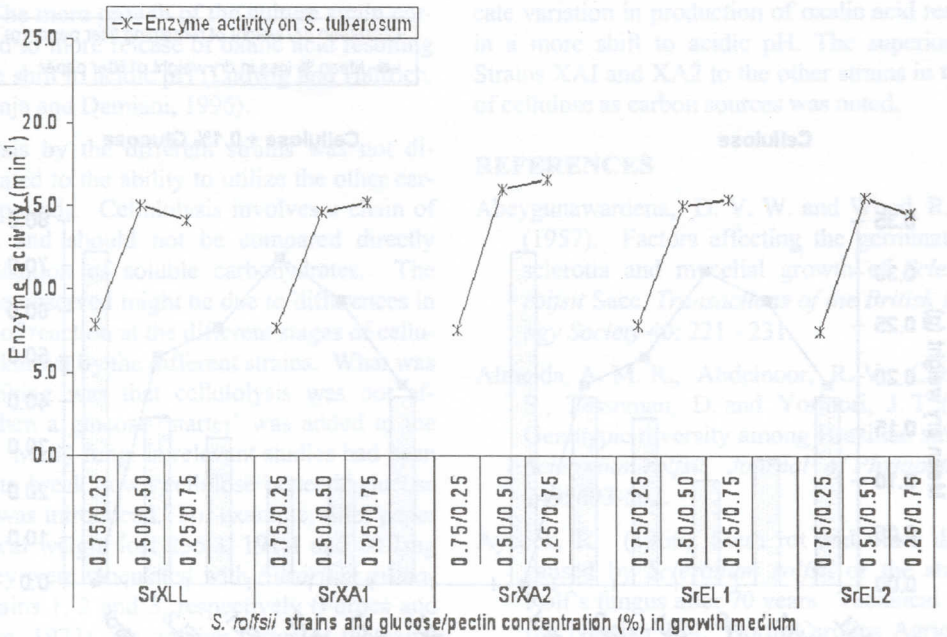


Fig. 2C: Enzyme activity of filtrate of 6 day-old cultures provided with glucose and pectin combination in the growth medium, of the different *S. rolfsii* strains on *S. tuberosum*.

shown in Fig. 2C. Higher values of enzyme activity, from 14.1 - 16.6 per minute were found in the 0.50% Glucose plus 0.50% Pectin and the 0.25% Glucose plus 0.75% Pectin than in the 0.75% Glucose plus 0.25% Pectin of 7.5-7.9 per minute enzyme activity. In all the media concentrations, pectin concentrations showed the highest enzyme activity in the range of 9.8 - 20.0 per minute followed by combination of glucose and pectin media in the range of 7.9 - 16.6 per minute and glucose media in the range 1.6 - 13.3 per minute.

Utilization of cellulose as carbon source by the five *S. rolfsii* strains

S. rolfsii strains SrXA1 and SrXA2 utilized cellulose to a greater extent than the remaining three strains, with strain SrXA2 superior to strain SrXA1 (Fig 3). The mean percentage loss in dry weight of the filter paper of 62.5 and 68.8 % were significantly different from values (43.8-50.0 %) for the remaining three strains. There was only a slight shift of pH of the media to the acidic side; that is, from pH 5.3 to pH 3.9 and pH 4.1. The results followed the same trend when 0.1% Glucose was added to the medium (Fig 3). Strains SrXA1 and SrXA2 used the carbon sources to a greater extent than the remaining three strains. Furthermore values for strain SrXA2 were superior to those of strain SrXA1 and the pH drifted slightly from 5.1 to a final pH of 3.8 - 4.0. Weight loss determinations of cellulolysis using filter paper showed that the lowest cellulose breakdown occurred in the SrXLL, SrEL1, and SrEL2 culture vessels (Fig 3).

DISCUSSION

Based on the study the five *S. rolfsii* strains were distinct by their ability to utilize carbon and nitrogen compounds. The five strains were distinguished by their different levels of enzyme activity, which indicates variation in production of oxalic acid and cause damping-off of seedlings and soft rot at the

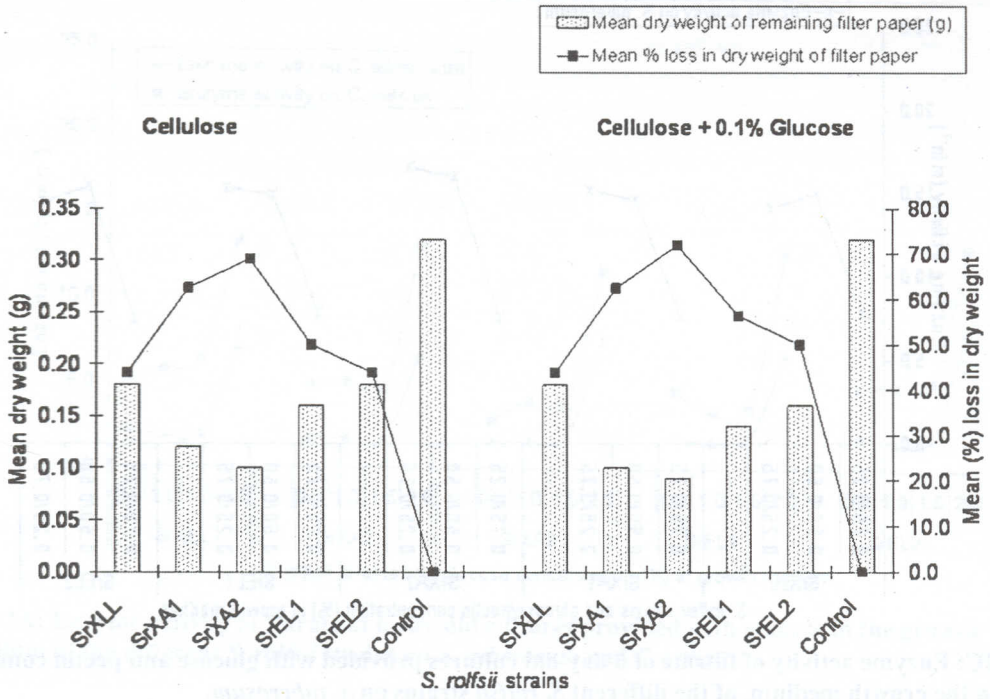


Fig. 3: Mean dry weight of remaining filter paper and mean loss in dry weight of filter paper (cellulose) in media inoculated with *S. rolfsii* strains

collar region, stem or root rot of many economic crop (Aycock, 1966; Punja, 1985). Clearly evident was the superiority of strains SrXAI and SrXA2 to the other strains in the use of cellulose as carbon sources. Relevant reports have confirmed that *S. rolfsii* exists in a number of distinct strains which differed sufficiently in morphology and physiology (Almeida *et al*., 2001; Aycock, 1966; Ludwig and Haltrich, 2002; Punja, 1985).

The host range of *Sclerotium rolfsii* is remarkable. In most pathogenic fungi, variation within species is common. Many parasites exist in various forms, strains and varieties associated with specific hosts thereby widening the host range and spectrum of host species. A typical example is *Fusarium oxysporum* in which the various forms are named after their specific host plants (Booth, 1971).

Comparing the utilization of carbon and nitrogen sources indicated that carbon sources were utilized to a greater extent than nitrogen sources by the different *S. rolfsii* strains which accounted for the decrease in pH caused by the secretion of large amounts of oxalic acid into the culture filtrates (Punja and Damiani, 1996). Ludwig and Haltrich (2002) reported a similar pattern in the decrease of pH from 5.0 to a range of 3.0 - 3.5 after 6 days of incubation for various strains of *S. coffeicola* and *S. delphinii*.

The maceration of plant tissues by pectolytic enzymes of *S. rolfsii* was enhanced in the pectin medium with increasing pectin concentration from 0.5 to 2.0 per cent than combination medium of glucose and pectin concentration followed by glucose concentration. In all cases the drift in pH is an indication of the release of oxalic acid into the culture

filtrate. The more growth of the culture strain corresponded to more release of oxalic acid resulting in a more shift to acidic pH (Ludwig and Haltrich, 2002; Punja and Damiani, 1996).

Cellulolysis by the different strains was not directly related to the ability to utilize the other carbon compounds. Cellulolysis involves a chain of reactions and should not be compared directly with utilization of soluble carbohydrates. The difference observed might be due to differences in the rates of reaction at the different stages of cellulose breakdown by the different strains. What was more striking was that cellulolysis was not affected when a glucose 'starter' was added to the medium. Many fungi in relevant studies had been induced to break down cellulose better if glucose 'starter' was introduced. For example, filter paper of identical weight lost 235.3, 191.4 and 80.1mg when they were inoculated with *Fusarium avenaceum* strains 1, 2 and 3, respectively (Forbes and Dickinson, 1977). In another series of the same media, containing a glucose 'starter' the loss in weight of the filter paper rose to 603.1, 570.0 and 606.7mg, respectively (Forbes and Dickinson, 1977).

The implication of the observation on cellulose utilization by the *S. rolfsii* strains is that *S. rolfsii* would use cellulose in plant tissues economically, and the cellulose would not be exhausted too quickly, even if soluble carbon compounds were present. This suggests that the slowly-used cellulose would contribute to the survival of the strains. However, an extended survival would only be possible if there were no cellulose decomposing organisms growing in the same habitat.

CONCLUSION

The utilization of carbon, pectin and nitrogen by fungus and the production of pectolytic and cellulolytic enzymes into culture filtrates were parameters by which a fungus species or strain can be characterized. The five *S. rolfsii* strains were distinguished by their ability to utilize carbon and nitrogen compounds to different extent. The different levels of enzyme activity of the strains indicate variation in production of oxalic acid resulting in a more shift to acidic pH. The superiority of Strains XAI and XA2 to the other strains in the use of cellulose as carbon sources was noted.

REFERENCES

Abeygunawardena, D. V. W. and Wood, R. K. S. (1957). Factors affecting the germination of sclerotia and mycelial growth of *Sclerotium rolfsii* Sacc. *Transactions of the British Mycology Society* 40: 221 - 231.

Almeida, A. M. R., Abdelnoor, R. V., Calvo, E. S., Tessnman, D. and Yorinori, J. T. (2001). Genotypic diversity among Brazilian strains of *Sclerotium rolfsii*. *Journal of Phytopathology* 149: 493-502.

Aycock, R. (1966). Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. Technical Bulletin Number 174, North Carolina Agricultural Experimental Station, Raleigh, NC, USA. pp. 202.

Booth, C. (1971). The genus *Fusarium*. Kew, Commonwealth Mycological Institute. pp. 237.

Bowen, K. L., Hagan, A. K. and Weeks, R. (1992). Seven years of *Sclerotium rolfsii* in peanut fields: yield losses and means of minimization. *Plant Disease* 76: 982-985.

Boyle, L. W. (1961). The ecology of *Sclerotium rolfsii* with emphasis on the role of saprophytic media. *Phytopathology* 51: 117-119.

Cilliers, A. J., Herselman, L. and Pretorius, Z. A. (2000). Genetic variability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. *Phytopathology* 90: 1026-1031.

Cilliers, A J., Pretorius, Z. A. and Wyk Van, P. S. (2003). Integrated control of *Sclerotium rolfsii* on groundnuts in South Africa. *Journal of Phytopathology* 151: 249-258.

Cole, M. and Wood, R. K. S. (1961). Pectic enzymes and phenolic substances in apples rotted by fungi. *Annals of Botany* 25: 100-110.

Darkwa, J. T. (1965). Studies on the physiology and pathogenicity of *Sclerotium rolfsii* Sacc. causing fruit-rot of tomato (*Lycopersicum esculentum* Mill). M.Sc. Thesis, University of Ghana, Legon, Ghana.

Forbes, R. S. and Dickinson, C. H. (1977). Effect of temperature, pH and nitrogen on cellulolytic activity of *Fusarium avenaceum*. *Transactions of the British Mycology Society* 68: 229-235.

Garett, S. D. (1962). Decomposition of cellulose in soil by *Rhizotonia solani* Kuhn. *Transactions of the British Mycology Society* 45: 115-120.

Johnson, S. P. and Joham, E. (1954). Some physiological notes on *Sclerotium rolfsii*. *Plant Disease Report* 38: 602-606.

Ludwig, R. and Haltrich, D. (2002). Cellobiose dehydrogenase production by *Sclerotium* species pathogenic to plants. *Letters of Applied Microbiology* 35: 261-266.

Punja, Z. K. (1985). The biology, ecology and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology* 23: 97-127.

Punja, Z. K. and Damiani, A. (1996). Comparative growth, morphology, and physiology of three *Sclerotium* species. *Mycologia* 88: 694-706.

Tortoe, C. and Clerk, G. C. (2008). Pathogenicity of *Sclerotium rolfsii* on cocoyam varieties in Ghana. *Ghana Journal of Agriculture Science* (In press).

Townsend, B. B. (1957). Nutritional factors influencing the production of sclerotia by certain fungi. *Annual Review of Botany* 21: 153-166.

Wheeler, B. E. J. and Sharan, N. (1965). The production of sclerotia by *Sclerotium rolfsii*. I. Effects of varying the supply of nutrients in an agar medium. *Transactions of the British Mycology Society* 48: 391-401.

Wheeler, B. E. J. and Waller, J. M. (1965). The production of sclerotia by *Sclerotium rolfsii*. II. The relationship between mycelial growth and initiation of sclerotia. *Transactions of the British Mycology Society* 48: 303-314.