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STUDIES ON SOME PHYSIOLOGICAL CHARACTERISTICS AND PATHOGENICITY OF *SCLEROTIUM ROLFSII* SACC. CAUSING COLLAR ROT AND CORMEL ROT OF COCOYAMS (*COLOCASIA* AND *XANTHOSOMA* SPECIES) IN GHANA.

A THESIS PRESENTED BY CHARLES TORTOE BSc. (HONS)

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IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE M. PHIL. DEGREE OF THE UNIVERSITY OF GHANA, THE DEPARTMENT OF BOTANY, UNIVERSITY OF GHANA, LEGON.

INTEGRI PROCEDAMUS

SEPTEMBER, 1997.

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DECLARATION

I hereby declare that, except for reference to other peoples' work which have been duly cited, this work is the result of my own original research work and that this thesis has neither in whole nor in part been presented for another degree elsewhere.

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DATE: 25 SEPT. 1997.

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DEDICATION

Dedicated to the Almighty God

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ABSTRACT

The physiological and morphological characteristics of five strains of *Sclerotium rolfsii*, namely, XLL, XA1, XA2, EL1 and EL2, which were identified as strains by the aversion test, were studied. The susceptibility of five varieties of cocoyam, 'Amankani fitaa', 'Amankani fufuo', 'Amankani kyirepe' and 'Amankani pa' of *Xanthosoma sagittifolium* and a single variety of *Colocasia antiquorum* to these strains, was also investigated. The appearance of the strains on Potato Dextrose Agar plates was similar and mycelial dry weights in Potato Dextrose Broth after 6 days' incubation were statistically similar. However, extensional growth on PDA was fastest in Strains EL1 and EL2, moderate in Strains XLL and XA1 and slowest in Strain XA2. Cultures of Strain XLL produced a mean number of 185 larger and heavier sclerotia per Petri plate whereas the remaining four strains formed 324 to 364 smaller and lighter sclerotia per Petri plate.

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 EL2 > EL1 > XLL > XA1 > XA2. Among the carbon compounds the least utilized was starch. The best carbon compound varied with the strains. Peptone was universally a good nitrogen source.

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Over a concentration range of 0.5 to 2.0%, the greatest amount of pectolytic enzymes was produced in both the glucose and pectin media. Filtrates of Strains XLL, XA1 and EL2 showed greater pectolytic Enzymes Activity than those of Strains XA2 and EL1. But Strains XA1 and XA2 showed greater ability to break down cellulose than the other three strains.

The pathogenicity tests did not show a direct relationship between ability to produce pectolytic enzymes and cellulose-degrading enzymes and infection of the host plants. Thus, Strains XA1 and XA2 caused the greatest rot in wound-inoculated cormels, while infection of 3-month old plants proceeded fastest in soils inoculated with Strain EL1 and EL2.

None of the cocoyam varieties showed resistance to the *S. rolfsii* strains, and they only showed varying degrees of vulnerability. However, *Colocasia antiquorum* which supported more than 2-4 times the number of sclerotia formed by the strains than *Xanthosoma sagittifolium* would play a greater role in the survival of *S. rolfsii* in the field.

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I
INTRODUCTION AND LITERATURE REVIEW

Sclerotium rolfsii Sacc. is a widely occurring facultative soil parasite of economic importance, principally in tropical and subtropical regions that are subjected to both abundant rainfall and high temperatures (Weber, 1931). It has a wide host range. It causes damping - off of seedlings and collar, stem or root rot of older plants. The fungus has since been isolated from innumerable host plants. As far back as 1931 Weber (1931) listed 189 host species from different plant families covering a fern family and 8 monocotyledonous and 42 dicotyledonous families. Several new host plants have since been recorded. Majority of the hosts are crop plants of great economic importance, and most of the host plants are annuals or herbaceous perennials.

In the United States nearly every southern state from Florida to California has recorded its presence, and it has even been reported from Canada (Erdman, 1961).
According to Erdman (1961) the fungus has been recorded in South America from Argentina, Brazil, Colombia, British Guyana, Surinam and Trinidad. There are many reports from the West Indies, including Barbados, Bermuda, Cuba, Dominican Republic, Jamaica, Puerto Rico and St. Vincent (Erdman, 1961). The few records from Europe include those from Germany, Italy and Russia (Erdman, 1961), and it occurs, as expected, in numerous warm countries in Africa including Benin, Congo, Egypt, The Gambia, Ghana, Malagasy Republic, Nigeria, Sierra Leone, South Africa, Togo, Uganda and Zimbabwe (Erdman, 1961).

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In the Pacific area it has been found in Australia, Indonesia, Java, Malaysia, the Philippines and Sumatra (Erdman, 1961).

In Ghana it has been recorded on several plants of economic importance including *Albemuschus esculentus* L., *Allium cepa* L., *Arachis hypogea* L., *Elaeis guineensis* Jacq, *Nicotiana tabacum* L., *Phaseolus* Spp, and *Xanthosoma sagittifolium* Schott (Leather, 1959). It has been estimated that the damage caused by the fungus in Ghana ranges from 5 to 30 per cent (Addison and Chona, 1971).

S. rolfsii invades the host both through wounds, natural openings and by direct penetration of intact surface tissue of the host as, for example, recorded by Darkwa (1965). 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 (Hawker, 1950) 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* (Darkwa, 1965; Flentje, 1957; Khadga, Sinclair and Exner 1963; Ullstrup, 1936). 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.

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S. rolfsii is able to thrive in the soil for a long time in the absence of a host as a saprophyte provided organic matter is present in the soil. It also freely forms tiny sclerotia which are capable of withstanding adverse conditions. The fungus, thus, 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 (Boyle, 1961). Formation of sclerotia by *S. rolfsii* is influenced by various environmental factors. Abeygunawardena and Wood (1957) found that the mean number of sclerotia per Petri plate on agar medium of initial pH of 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 per cent 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% $NaNO_3$.

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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.

Studies by Boswell (1958) on the sclerotia of *S. rolfsii* is related to the continunity of the rind of these bodies. An intact rind was associated with slow, depauperate germination. According to Boswell (1958) the sclerotia became wrinkled during drying and the surface layers showed considerable cracking. He found that sclerotial dormancy could be broken by any of the several types of mechanical or chemical methods of scarification.

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 an external source of thiamine or at least one of the moieties which form thiamine, namely pyrimidine and thiazole, it is encouraged to grow at or near the soil surface which is rich in organic matter.

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One of the valuable crops attacked by *S. rolfsii* in Ghana is cocoyam, *Xanthosoma sagittifolium* Schott and *Colocasia antiquorum* Schott. The fungus causes both cormel and root rot. The disease takes the form of a wet root rot, yellowing and wilting of leaves and failure to form cormels, followed in severe cases by death. It affects plants of all ages. It is also the cause of post-harvest rot of the cormels. Decaying cormlets are usually coated with a typical mycelial felt of the fungus.

Cocoyam, Cassava (*Manihot esculenta* Crantz), yam (*Dioscorea* spp Linn) and sweet potato (*Ipomea batatas* Poir) provide the main source of carbohydrate for a large proportion of people of the tropics, especially in the wetter tropics areas, including Ghana (Doku, 1966). When cereals are in short supply, as they occasionally are, these root crops supply the sole source of carbohydrates.

Generally, cocoyams are not grown on a large scale. However, they are of immense importance and are grown in every farm, garden, or small holding. The total annual production must, therefore, be considerable (Doku, 1966). Among root crops, cocoyams can be regarded as important because they keep well, both in the field and in store and are, therefore, available throughout the year.

Due to its excellent storage quality, cocoyam is most often used as travelling food by local people. When famine threatens, cocoyams, *Xanthosoma sagittifolium* and *Colocasia antiquorum*, are the crop which are most frequently sought for to alleviate the situation.

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Infact when people talk of famine in this country they refer primarily to a shortage of cocoyams (Doku, 1966).

The uses to which cocoyam can be put are numerous. The tender leaves are the main if not the only source of spinach for the inhabitants of the humid forest of Ghana and in fact, most Ghanaians. The protein content per 100g dry weight of the leaf of cocoyam is 22.17g (Liefstingh, 1963). The cormels supply easily digestible starch and are known to contain substantial amount of protein, vitamin C, thiamine, riboflavin and niacin (Cobley and Steele, 1976; Maduewesi and Onyike, 1980). The leaves and tender parts of the stem are relished by livestock. The peelings of the cormels are also fed dried or fresh to goats, sheep, cattle and pigs.

The cormel is used in different forms as described by Karikari (1971). It is peeled and boiled and pounded into 'fufu' that is taken with soup. The boiled cormel is often also eaten directly with stew. The unpeeled cormel is roasted and the skin removed when ready for eating. The boiled or roasted form may also be mashed and palm oil added and taken with either fish or roasted groundnut.

The Akan name 'Okumkom' meaning, killer of all hunger, which is applied to cocoyam no doubt portrays its importance to the whole country and to the Akans in particular who form the major tribe of this country (Karikari, 1971).

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Karikari (1971) listed seven cocoyam varieties found in Ghana and described them as follows:

'Amankani pa' (proper cocoyam) or 'Amankani kokoo' (red cocoyam). Large plant 182.9 - 243.8cm high; leaf sagitate, dark green lighter on the under surface, prominent venation, petiole reddish-purple, tubers pink. The plant flowers in the wetter districts. This is the commonest variety and is said to possess all the desirable properties of cooking, texture and taste.

'Amankani fufuo' (Light-coloured cocoyam). This is similar to 'Amankani pa'. Leaf not quite so dark, petiole purple, tubers white.

'Amankani fitaa' (White cocoyam). Above ground parts resemble ''Amankani fufuo' but the plant is much more delicate. Tubers are white and the cormels have one or several constrictions. They are smaller than in the preceding two varieties. 'Amankani serwah' (Serwah was the name of a very beautiful Ashanti Queen). This resembles the 'Amankani fitaa' except that the cormel are very white and the skin splits and become peelable.

'Amankani Kyirepe' (hard cocoyam). This resembles the 'Amankani antwibo' variety. It is, however, rich in sugar and is therefore used in sweeting foods. It contains some poisonous properties and therefore needs to be boiled for 12 hours before been eaten.

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'Amankani antwibo' (cocoyam which can choke one). Similar to 'Amankani fitaa'. Petioles are very pale green and cormels pink and unconstricted. The central stem of the plant is edible.

Colocasia antiquorum is known as 'Kooko' in Twi. Petioles are pale green. The tubers are white and smaller than the 'Amankani' varieties. The central stem is edible. Before the advent of the 'Amankani' varieties it was the favoured variety but is seldom eaten now except in cases of scarcity. However, because of its softness, it is still preferred by old people who find it easier to eat.

For such a valuable crop any disease that could decimate the produce should, naturally be a matter for concern.

Nakata (1925) stated that *S. rolfsii* is a group species comprising numerous biological forms which can be distinguished by the phenomenon of aversion. Aversion was reported to occur between different strains of the fungus but not between two cultures of the same strain. When two colonies of the same strain are started at opposite sides of an agar plate, the colonies grow towards each other and meet. Sclerotia are then produced along the meeting zone due to obstruction to extensional growth. However, when two different strains are raised in similar manner, their colonies never meet. Their advancing edges eventually come to a stop as they get near to each other and become separated by a distinct inhibition zone. Each colony thereby forms its own row of

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sclerotia along the boundary resulting in two rows of sclerotia separated by the zone of inhibition.

The presence of distinct strains of *S. rolfsii* with different physiological characteristics must be the underlying cause for the differences in reports on the physiology of the fungus by different workers. Taking the response to nutrients as an example.

Higgins (1927) found maltose and lactose among several carbohydrates and a few organic acid tested, to support the poorest growth. In contrast, 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.

Abeygunawardena and Wood (1957) found fructose 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 negative 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 are also found in results of investigations on the use of Nitrogen compounds for growth by *S. rolfsii*. Higgins (1927) observed that *S. rolfsii* did not

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appear to utilize readily inorganic nitrogen either as nitrates or ammonium salts. Abeygunawardena and Wood (1957) also found the organic nitrogen asparagine and peptone to be more readily utilized than ammonium salts and sodium nitrate. But Townsend (1957) found asparagine, peptone and potassium nitrate to support good growth and Darkwa (1965) reported that asparagine, peptone, urea, ammonium chloride and ammonium nitrate were by far better Nitrogen sources than alanine and glycine.

This important knowledge of presence of distinct strains should be considered in any *S. rolfsii* - host relationship. It is well-known that many bacterial and fungal parasites are themselves an assemblage of strains differing genetically and liable to variation. The existence of distinguishable *S. rolfsii* strains in Ghana has hitherto not been examined. The effect of different strains on crops has, therefore, also not been studied. The aim of the project was, first, to investigate the possible existence of *S. rolfsii* strains in Ghana. If they do exit, it will be important to find out whether the seven cocoyam varieties in Ghana are equally susceptible to the different *S. rolfsii* strains. A particular variety that would exhibit tolerance or resistance to as many *S. rolfsii* strains as possible would be a good choice for cultivation in Ghana, as the existing cultural and chemical methods of control either have not made any impact on the disease in Ghana so far or have never been tried because of the cost involved. Posnette (1945) could not reduce the incidence of the disease by application of woodashe.

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There are many other suggested ways of combating the fungus. The most recommended cultural practices are burning of crop thrash with the inoculum, and digging in the top soil to depths which would deprive the fungus of oxygen.

Chemical control is also possible. The growth of *S. rolfsii* was inhibited by atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-S-triazine), (Curl and Funderburk, 1966) and by paraquat (1, 1-dimethyl-4, 4-bipyridium salt) (Rodriquez - Kabana *et al.*, 1967).

Curl and Funderbunk (1966) found that sclerotia production by *S. rolfsii* was reduced in atrazine - treated soil. Clerk and Bimpong (1969) showed that prometryne (4, 6-bis (isoproylamino-2-methylthio - 1,3,5 - triazine) and ramrod (2 - chloro - N - isopropylacetanilide) were more toxic than atrazine.

This thesis contains mainly results of experiments set out to study the

a. physiology of different isolates of *S. rolfsii* from the Legon-Achimota area,

b. identify strains among the isolates, and

c. study pathogenicity of the identified strains with special reference to collar rot and cormel rot of cocoyam in cocoyam varieties, 'Amankani fitaa' (white cocoyam); 'Amankani fufuo' (light coloured cocoyam); 'Amankani Kyirepe' (hard cocoyam); 'Amankani pa' (proper cocoyam) of *Xanthosoma sagittifolium* and *Colocasia antiquorum* (kooko).

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II MATERIALS AND GENERAL METHODS

I MATERIALS

a Strains of *Sclerotium rolfsii* Sacc

The five different strains of *Sclerotium rolfsii* used were obtained at different areas in Legon and Achimota and given code numbers according to their sources.

Strain XLL from *Xanthosoma sagittifolium* Schott at University of Ghana, Legon.

Strain XA1 from *Xanthosoma sagittifolium* at Achimota, 20km west of Legon.

Strain XA2 from *Xanthosoma sagittifolium* at Achimota.

Strain EL1 from *Elaeis guineensis* Jacq at Sinna's Garden, Faculty of Agriculture, University of Ghana, Legon.

Strain EL2 from *Elaeis guineensis* at Forecourt, Faculty of Agriculture, University of Ghana, Legon.

Stock cultures were maintained on Potato Dextrose Agar Slants in MaCartney tubes and stored in the refrigerator. They were sub-cultured at fortnightly intervals.

b. Cocoyam varieties

Five varieties of local cocoyams were used in this investigation. Four of these, namely 'Amankani fitaa', 'Amankani fufuo', 'Amankani Kyirepe', 'Amankani pa' are varieties of *Xanthosoma sagittifolium* Schott and the remaining one *Colocasia antiquorum*.

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They were supplied by the Agriculture Research Station of the University of Ghana at Kade, Ghana. Both the cormels and petioles were used in some of the experiments.

i. Peptiole of the Cocoyam

The cocoyam varieties were raised in garden loam soil in black polythene bags (36.5 x 22.5cm) with drainage holes at the bottom for 12 weeks and then used. The ages of the leaves and, therefore, the petioles were related to the positions of the leaves on the plant.

ii. Cormel of the Cocoyam

The cormels of the different cocoyam varieties were purchased when needed from a particular cocoyam seller at the Central Market, Tema with whom a standing order had been placed.

c. Potato Tubers of Irish Potato (*Solanum tuberosum* L.)

Tubers of Irish Potato (*Solanum tuberosum* L.) used in the preparation of Potato Dextrose Agar and Potato Dextrose Broth and in 'Pectolytic Enzyme Tests' were purchased from the Makola Market in Accra when needed and used the same day.

d. Fruits of Cucumber (*Cucumis sativus* L.)

Fresh Fruits of Cucumber (*Cucumis sativus* L) used in 'Pectolylic Enzyme Test' were purchased from Makola Market in Accra on the day they were to be used.

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e. **Fruits of Tomato (*Lycopersicum esculentum* Mill)**

Fruits of tomato (*Lycopersicum esculentum* Mill) were used in studying the formation of infection cushions of the different *S. rolfsii* strains. Firm unblemished ripe fruits were purchased from Makola Market in Accra on the day they were to be used.

f. **Chemicals**

All chemicals used in the investigation were from British Drug House, Poole, England and OXOID Limited, Basingstoke, Hampshire, England.

2. GENERAL METHODS

a. Isolation of the *S. rolfsii* Strains

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 Sodium hypochlorite (1.0%) for three minutes, rinsed after that in three changes of sterile distilled water and inoculated onto sterile Potato Dextrose Agar 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.

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b. Maintenance of stock Cultures and preparation of inocula

Stock culture of each *S. rolfsii* strain was maintained on Potato Dextrose Agar slants in MaCartney's tubes in the refrigerator, and subcultured fortnightly. Whenever an experiment was to be carried out, the fungus was raised on Potato Dextrose Agar 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.

C. Culture Media

i. Potato Dextrose Agar

An amount of 200g of peeled Irish potato was thoroughly washed, cut into small pieces and boiled in 500ml of distilled water for 15 minutes. The extract was then strained with muslin cloth and made up to one litre with distilled water. Fifteen grams of Agar and 10g of Dextrose were added. The mixture was stirred and warmed in a water bath at 80°C to melt the agar before the medium was autoclaved.

ii Potato Dextrose Broth

Prepared in the same way as Potato Dextrose Agar without the addition of Agar.

iii Synthetic Media for production of Pectolytic Enzymes

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.

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The standard medium consisted of:

Peptone	1.0%
$MgSO_4 . 7H_2O$	0.5%
KH_2PO_4	0.1%
Thiamine	0.005%
Distilled water	1L

The various media were made by adding different concentrations of either Glucose or Pectin, referred to as Glucose and Pectin media, respectively.

The Glucose media contained the following Glucose concentrations:

Medium 1	0.5%
Medium 2	1.0%
Medium 3	1.5%
Medium 4	2.0%

The Pectin media contained the following Pectin concentrations:

Medium 1	0.5%
Medium 2	1.0%
Medium 3	1.5%
Medium 4	2.0%

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Other media, Glucose-Pectin Media, contained both Glucose and Pectin in different ratios as follows:

Medium 1	Glucose 0.75%, Pectin 0.25%
Medium 2	Glucose 0.50%, Pectin 0.50%
Medium 3	Glucose 0.25%, Pectin 0.75%

Several 250ml Erlenmeyer flasks each containing 30ml of the test medium were plugged with non-absorbent cotton wool covered with aluminium foil and then autoclaved. The aluminium foil prevented entry of condensed water into the medium and it was removed after the medium had been inoculated. This procedure was followed throughout this investigation. Each flask was inoculated after cooling with a 3mm - disc of the mycelium of the fungus.

iv. Synthetic Medium for assessment of production of Cellulolytic Enzymes

The relative amounts of cellulolytic enzymes produced by the different *S. rolfsii* strains were assessed using the following medium used in cellulolytic enzyme studies by Ryan, Beadle and Tatum (1943)

Potassium nitrate ($KN0_3$)	5.0g
Potassium dihydrogen phosphate (KH_2P0_4)	1.0g
Magnesium sulphate ($MgS0_4$. $7H_20$)	0.5g
Thiamine	0.5mg
Yeast extract	1.0g
*Micronutrient	10ml
Distilled water	1L

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*Micronutrient Solution

Copper sulphate ($CuSO_4.5H_20$)	0.10mg
Sodium borate ($NaB_40_7.2H_20$)	0.01mg
Ferrous phosphate ($FeP0_4.2H_20$)	0.20mg
Manganesium sulphate ($MnS0_4.5H_20$)	0.02mg
Sodium molybdate ($Na_2MO_4.2H_20$)	0.02mg
Zinc sulphate ($ZnSO_4.7H_20$)	0.15mg
Distilled water	1L

Aliquots of 20ml were dispensed into several 250ml Erlenmyer flasks and one 7cm - diameter Whatman No.3 filter paper put into each. The flasks were plugged with non-absorbent cotton wool and the medium autoclaved.

d. Methods of Sterilization

All media and distilled water used were sterilized by autoclaving at 1.1kg/cm^2 steam pressure at 121°C for 15minutes. All glassware were sterilized by heating at 160°C for 18 hours in an electrically heated oven (Gallenkamp Model, Town and Mercer Ltd., Croydon, England).

Inoculation was done in the inoculating room under the Laminar Microflow Chamber. The air conditioner and the Laminar Flow Cabinet were switched on for about 30minutes to reduce air microflora in the room before inoculation was done. Inoculating pins,

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inoculating loops and cork borers were flame - sterilized prior to use. Surface sterilization of tomato fruits, cocoyam cormels and blocks of tissue of cocoyam cormels was done by immersing the materials in 5.0% Sodium hypochlorite for five minutes. They were then rinsed in three changes of sterile distilled water.

e. Replication

Four replicates per treatment were used in all the various experiments performed.

f. Determination of Utilization of different Carbon Sources by the different *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 Peptone, 1%; $MgSO_4.7H_20$, 0.1%; $KH_2\ PO_4$, 0.1%; Thiamine, 0.005% and 1L 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 with filter paper funnels. The dry weight of the mycelium was then determined as described in section 2(i).

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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.

g. Determination of Utilization of different Nitrogen sources

The preceding procedure was followed to determine the ability of the different *S. rolfsii* strains to utilize Ammonium chloride, Ammonium nitrate, Asparagine, Peptone and Sodium nitrate. Each of these Nitrogen sources was incorporated at a concentration of 0.1% into a basal medium of Glucose, 1.0%; $MgSO_4.7H_20$, 0.1%; KH_2PO_4, 0.1%; Thiamine, 0.005% and 1L distilled water.

The inoculated 250ml - Erlenmeyer flasks each containing 30ml of the test medium were also 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 determined as in the previous experiment.

h. Assessment of Radial Growth on Solid Agar Medium

About 20ml of the medium was poured into each of 9.0cm - diameter sterile Petri dishes 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 of the culture of the fungus.

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The diameter of each growing culture was measured along the two diameters and the mean for the replicates calculated.

i. Determination of the dry weight 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) at 60°C for 24 hours. The filter paper carrying the dried mycelium was weighed after being allowed to cool in a desiccator. The dry weight of the mycelium was then calculated.

j. Preparation of O.1M Citrate Buffer at pH 5.0

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From the Henderson - Hasselbach equation of buffers:

$$pH = pKa + \log \frac{[Salt]}{[Acid]}$$

pKa of citric acid at pH 5 is 4.7

(Segel, 1975).

Substituting pH = 5 and pKa = 4.7 into equation

$$5 = 4.7 + \log \frac{[Salt]}{[Acid]}$$

$$\frac{[Salt]}{[Acid]} = \log^{-1} 0.3$$

$$= 1.99$$

$$= 2.0$$

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Therefore 0.1M Acid should react with 0.2M salt. For the same molar concentration, therefore, the volume ratio will be 1:2 for the Salt and Acid, respectively.

$$\frac{[Salt]}{[Acid]} = \frac{b/10}{a/100} = \frac{b}{a} \quad \frac{\text{Molarity of Salt x Vol. Of salt (V-Salt)}}{\text{Molarity of Acid x Vol. Of Acid (V-Acid)}} = \frac{1}{2}$$

For same malority and a final buffer volume of 100ml

$$\frac{[Salt]}{[Acid]} = \frac{Vsalt}{Vacid} = \frac{1}{2}$$

$$2Vsalt = Vacid$$

(b = no. of moles of salt; a = no. of moles of acid). Therefore, 10ml of 1M Citric acid was mixed with 20ml of 1M disodium hydrogen phosphate (Na_2HPO_4) and the volume topped to 100ml with sterile distilled water to give 0.1M Citrate buffer of pH 5.0.

The pH of the prepared 100ml stock of 0.1M citrate buffer was confirmed with a pH meter.

k. Measurement of pH

The pH of all media, culture filtrates and the 0.1M citrate buffer was measured using a pH meter (TOA pH meter, HM-60s. OSK - 11478, OGAWA SEIKO CO. LTD., Japan).

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l. Method for accurate 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 felt 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.

m. Method for determining fresh weight of mature sclerotia

A reliable method of determing 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 ER - 108A, A & D Co. Ltd, Tokyo Japan.

n. Method for determining volume of mature sclerotia

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.

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o. Determination of Sclerotium diameter

The diameter of the sclerotium was measured under the microscope using the eye piece graticule (GRATICULE LTD., TONBRIDGE, KENT ENGLAND).

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

The experiment studied the time it took for maceration of thin discs of Irish potato tuber and pericarp of cucumber immersed in the culture filtrates to occur following the method used by Cole and Wood (1961). For each test, the replicate culture filtrates were pooled and used.

Plugs, 1.0cm in diameter were removed with a Number 8 cork borer from either the potato tuber or cucumber pericarp and placed separately in distilled water in Petrin dishes. In each case, 0.5mm - thin discs were carefully cut with a surgical blade from the plug. The discs were washed in sterile distilled water and drained on filter paper. An amount of 2ml of the test filtrate 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. Each disc was picked and gently pulled from two opposite ends at 5 minutes intervals using two pairs of blunt forceps. The occasion came when the disc broke apart when it had been macerated by the constituent pectolytic enzymes of the culture filtrate. The time was noted. The Enzyme Activity was calculated by the formula:

$$\frac{1}{\text{mean time of maceration of the six discs}} \times 100$$

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q. Determination of ability of the *S. rolfsii* strains to utilize Cellulose

The experiment measured indirectly the relative level of cellulolytic enzymes in the culture filtrate of the various *S. rolfsii* strains. Several 7 cm-diameter discs of Whatman's number 3 filter paper were oven dried at 60^{0}C for 24 hours and weighed singly. Each was placed in a 250ml Erlenmeyer flask containing 20ml of the medium described in Section 2(c)iv. to which 1.0% Glucose was added as a booster. The flasks were autoclaved and the medium in each was inoculated with a 3mm mycelial disc. The non-absorbent cotton wool plugs were covered with cellophane to prevent excessive water loss. The flasks were incubated at 30°C for 7 weeks. The remaining fragments of the decomposed filter paper were carefully collected into a pre-weighed aluminium foil cap, from each flask, dried at 60°C for 24 hours, and weighed. The weight of the constituent minerals of the medium was substracted from the computed dry weight of the filter paper used by the fungus (Garrett, 1962; Forbes and Dickson, 1977).

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

About 20ml of Potato Dextrose Agar was poured into each of 9.0cm diameter sterile Petri dishes and allowed to solidify. 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.

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The inocula belonged to two different strains or were of the same strain. The inoculated plates were incubated at 30°C until the plates were covered. They were then examined to see whether the two colonies grew into each other-which would occur where there was no aversion - or just stopped short when the growing edges come close to each other leaving a zone of bare agar between them indicating incidence of aversion.

s. Study of infection cushion formation by the *S. rolfsii* strains

Infection cushion formation by different *S. rolfsii* strains was compared using

i the mean number formed on unit tomato fruit surface area of 5x5mm and

ii the mean diameter, measured with the aid of an eye-piece graticule of 50 randomly selected infection cushions.

Surface-sterilized mature semi-ripe tomato fruits were placed in plastic boxes (24.0 x 12.0 x 6.0cm) with humid internal atmoshpere maintained with miostened filter paper. The fruits were surface-inoculated by placing mature sclerotia on the intact surface of each. Four sclerotia were placed equidistantly on each fruit. After four days, tiny squares of epidermal strips (5x5mm) were removed, stained with cotton blue in lactophenol and mounted on glass slides in drops of plain lactophenol, keeping the external surface uppermost. The infection cushions on the epidermal strips were observed under the microscope and the details mentioned above were recorded.

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t. Study of infection of cocoyam petioles by *S. rolfsii*

Three-month old cocoyam plants were inoculated by the different strains of *S. rolfsii*. Cultures of the test fungus were grown in Potato Dextrose broth in 250ml Erlenmeyer flasks each containing 30ml of the medium. The culture filtrate was poured off after 6 days' incubation. Sterile distilled water was then used to rinse the mycelium to remove traces of the acidic filtrate and poured off. The mycelium was used to inoculate the growing potted cocoyam plants in garden loam soil of pH 5.0 and 25.0% WHC.

The content of one flask was stirred into the soil of one pot (36.5 x 22.5cm) and the mouth of the pot covered with black polythene bag for two days to enable the fungus to become established in the soil. The cumulative number of petioles which had been infected were counted after 4, 8, 12, 16, 20 and 24 days.

u. Study of rotting of cocoyam cormel tissue by *S. rolfsii* strains by inoculating whole cormels

Mature cocoyam cormels were inoculated with the different strains of *S. rolfsii* and the rotted areas measured after 8 days.

Mycelium was raised in Potato Dextrose Broth at 30°C for 4 days and used as inoculum. The culture filtrate was poured off and the mycelium rinsed with sterile distilled water. The mycelium was then transferred into a sterile Petri dish containing 10ml sterile distilled water and macerated with a pair of blunt forceps.

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The cocoyam cormels were surface-sterilized and placed in 24.0 x 12.0 x 6.0cm plastic boxes with tight-fitting lids. A plug, 3mm deep, was then removed with a 5mm - cork borer from opposite sides, at both the apical and basal halves of the cormel. Into each of the four cavities was placed the same amount of macerated mycelium using a microspatula. The plug of cocoyam tissue removed was replaced and the wound sealed with vaseline. The inoculated cormels were incubated at 30°C for 8 days. Each cormel was then cut transversely with a sharp scapel along the plane of the two opposing plugs. The radii of the rot along the surface and depth of the rot from the inoculum were measured.

v. Estimation of degradation of cocoyam cormel tissue on the basis of loss in dry weight

Cormels of the cocoyam were peeled and each was cut transversely into two halves. Blocks, measuring 1.0 x 1.0 x 0.5cm, were cut from both halves and surface sterilized.

Five blocks were selected from each batch and dried at 80°C for 24 hours. They were then weighed individually and the mean dry weight per block for the two batches calculated. Blocks for the test were surface-sterilized with 5% Sodium hypochlorite for 5 minutes and rinsed in three changes of sterile distilled water and drained on sterile filter paper. The blocks were then placed on 4-day old PDA culture plates of *S. rolfsii*, five per Petri plate, and incubated at 30°C. One Petri plate of every treatment was withdrawn after 2, 4, 6 and 8 days. The adhering mycelium on the blocks was carefully removed with a pair of fine forceps and the blocks were oven dried at 80°C for 48 hours

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and weighed. The mean dry weight for each batch and percentage loss in dry weight were calculated.

w. Study of production of sclerotia on cocoyam cormel tissue

Flat blocks of peeled cormels, measuring 30 x 30 x 5mm, were cut and surface sterilized with 5% Sodium hypochlorite for 5 minutes and rinsed in three changes of sterile distilled water. Each block was placed in a sterile empty Petri dish and inoculated with a 3mm mycelial plug of a 4-day old *S. rolfsii* culture growing on PDA. The preparation was incubated at 30°C and inspected everyday.

Recordings were made of the following:

i time of first appearance of sclerotia.

ii time melanin deposition was observed in the earliest formed sclerotia.

iii number of sclerotia formed on each block in 12 days; and

iv volume of 100 mature brown sclerotia.

x. Sclerotium 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.

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y. Experimental Precautions

i. Glassware cleaned with detergents were rinsed several times with tap water to remove all traces of the detergent and then air-dried.

ii. Petri dishes were only half-opened when pouring media in order to avoid contamination.

iii. There were usually four replicates for the various treatments. Whenever two replicate cultures became contaminated the entire set of four was discarded and the experiment repeated.

iv. The Laminar Microflow Chamber in the inoculating room was cleaned with disinfectant and switched on for 30 minutes immediately before being used.

v. Mycelium serving as inoculum for all experiments was always obtained from the advancing edge of 4-day old cultures.

vi. Oven-dried filter paper usually lost some weight because of the heating. Filter paper used throughout the investigation for harvesting mycelium from broth media was, therefore, heated at 60°C for 24 hours prior to use.

vii. Filter paper with oven-dried mycelium was conveyed to the balance room in a closed desiccator to avoid absorption of moisture.

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z. Statistical Analysis

Standard error of means was calculated using the formulae:

$$\text{Variance} = \frac{\Sigma(X - X)^2}{n - 1}$$

$$\text{Standard Error of mean (S.E.M)} = \sqrt{v/n}$$

Significance of the difference between the means in the Tables of results was determined by means of Confidence Limits at 95%.

Confidence Limit = mean $\pm$(1.96 x Standard Error of Mean) (Kershaw, 1973).

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III

EXPERIMENTAL DETAILS

The results of the experiments described here have been presented in the same order and under the same heading in Chapter IV - RESULTS.

EXPERIMENT 1

COMPARATIVE RATE OF GROWTH OF THE DIFFERENT STRAINS OF *S. ROLFSII* SACC AND PRODUCTION OF SCLEROTIA ON PDA

Potato Dextrose Agar (PDA) was used in studying the radial growth of the five *S. rolfsii* strains : XLL, XA1, XA2, EL1 and EL2. Agar plates in 9cm diameter Petri dish were inoculated at the center with a 3mm - diameter mycelium of the test strain and incubated at 30°C. There were four replicates for each strain. The diameter of each culture along two predetermined diameters drawn at the bottom of the Petri dish was measured at 12hr intervals until either the plate was completely covered or growth stopped. The mean of the 8 diameter measurements for each treatment at each recording time was calculated (Table 1). The plates were then left at the end of the growth studies for a total of 12 days to enable sclerotia to develop. All the sclerotia, both brown mature and pale immature, on each plate were then counted and the mean per plate calculated (Table 2). A photograph was taken of the samples of the five treatments to show the formation of sclerotia by the five strains (Plate 1).

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One hundred of the mature sclerotia were then picked at random and weighed together to obtain the fresh weight. This was done for all the four replicate plates and the mean per hundred sclerotia was calculated (Table 2).

The volume of the hundred sclerotia selected from each plate was also determined by water displacement method. The mean per hundred sclerotia was then calculated (Table 2). Finally the diameter of 50 mature sclerotia of each isolate was measured and the mean calculated in each case (Table 2).

EXPERIMENT 2

COMPARATIVE RATE OF GROWTH OF THE DIFFERENT STRAINS OF *S. ROLFSII* IN POTATO DEXTROSE BROTH (PDB)

Since the surface growth method does not take into account mycelium growing in the agar medium, Potato Dextrose Broth was used in a subsequent experiment to study again growth of the five *S. rolfsii* strains. At desired intervals the mycelia were harvested, dried at 60°C for 24 hours and weighed. They were harvested on pre-weighed filter paper and the difference in weight represented the dry weight of the mycelium.

Twenty-one 250ml Erlenmeyer flasks each containing 30ml of the medium, were prepared for each strain. Twenty of the flasks were each inoculated with one 3mm - diameter culture disc. The remaining flask was set aside after autoclaving the media and used to determine the initial pH.

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The inoculated flasks were incubated at 30°C and four flasks of each strain were withdrawn after 4, 6, 8, 10 and 12 days in that order. The cultures were each harvested separately, dried and weighed and the mean dry weight calculated (Table 3)

The culture filtrates of the four replicates of each treatment were pooled and the pH measured with a pH meter. The values of the pH obtained are presented in Appendix A and the data used to plot a graph alongside the graph of the dry weight of the mycelium (Fig 1)

EXPERIMENT 3

GROWTH OF THE DIFFERENT STRAINS OF *S. ROLFSII* IN LIQUID MEDIUM WITH DIFFERENT CARBON SOURCES

The ability of the five strains to use different carbon sources was next investigated as they may most likely use the different compounds to varying degrees.

The dry weight of mycelium of each strain produced in six days was recorded. The carbon sources tested were fructose, glucose, maltose, starch and sucrose at a concentration of 1.0%. As in Experiment 2, the cultures were raised in 250ml Erlenmyer flasks each containing 30ml of the liquid medium and the inoculated flasks were incubated at 30°C. The mycelia were harvested separately after 6 days on pre-weighed filter paper and dried at 60°C for 24hours. The pH of the filtrate at the end of the incubation period and the pH of an autoclaved uninoculated medium representing the initial pH were measured with a pH meter.

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In addition to the dry weights obtained which are presented in a tabular form in Table 4, photographs were taken of the cultures. Photographs of growth of Strain EL2 and Strain XA1 in media containing the different Carbon sources have been presented as examples (Plates 2 and 3).

EXPERIMENT 4

GROWTH OF THE DIFFERENT STRAINS OF *S. ROLFSII* IN LIQUID MEDIUM WITH DIFFERENT NITROGEN SOURCES

The ability of the five strains to use different Nitrogen sources was also studied. The same procedure was followed using the same basal medium containing 1.0% Glucose as Carbon source and 0.1% of either ammonium chloride, ammonium nitrate, asparagine, peptone, or sodium nitrate as Nitrogen source. The dry weight of mycelium produced after 6 days of incubation at 30°C was determined as in Experiment 3. The initial pH of the autoclaved uninoculated medium in each case and the final pH of each filtrate were measured. Data of the dry weight and the pH obtained were put together in a composite table (Table 5). To illustrate the appearance of some of the cultures, photographs of cultures of Strains EL2 and XA1 in media containing the different Nitrogen sources have been presented (Plates 4 and 5).

It was observed that the strains showed the same order of ability to use both Carbon and Nitrogen sources. The results of both Chapter 3 and 4 were therefore put together in Fig 2 to show the corresponding order of the ability of the isolates to use the Carbon and Nitrogen sources.

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EXPERIMENT 5

MACERATION OF DIFFERENT PLANT TISSUES BY PECTOLYTIC ENZYMES IN CULTURE FILTRATES OF THE FIVE STRAINS

The production of pectolytic enzymes into culture filtrates is one of the parameters by which a fungus species or strain can be characterized. The amount of pectolytic enzymes secreted by the different *S. rolfsii* strains into the culture filtrate was examined. The procedure used has been described in General Methods Section 2 (p). Thin (1mm thin) discs, 10mm in diameter, of either Irish potato tuber or cucumber pericarp were put in separate filtrates of 6 day old cultures of the test fungi grown in different types of media at 30°C. The times taken by the constituent pectolytic enzymes of the filtrate to macerate a total of six discs were recorded and the mean calculated. The mean enzyme activity (1/t x 100) calculated, where t = mean time of maceration. The fungi were grown in media with either Glucose or Pectin as Carbon sources of different concentrations as follows:

a. media with Glucose concentration of 0.5, 1.0, 1.5, and 2.0 per cent.

b. media with Pectin concentration of 0.5, 1.0, 1.5 and 2.0 per cent.

c. media with a combination of Glucose and Pectin:

0.75% Glucose and 0.25% Pectin;

0.5% Glucose and 0.5% Pectin;

0.25% Glucose and 0.75% Pectin.

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The pH of the test media was standardized by adjusting with 0.1M citrate of pH 5.0. The mycelium in each case was harvested as in the preceding experiments and the dry weight recorded. The final filtrate pH was measured at the end of the growth period. The results of these various tests are recorded in Tables 6 to 13.

EXPERIMENT 6

USE OF CELLULOSE AS CARBON SOURCE BY THE FIVE STRAINS

Since cellulose is a major component of plant cell walls the ability of the five strains to use cellulose as carbon source was investigated. Filter paper was used as the cellulose. The basal medium described at Section 2 (q) of the General Methods was used. Several 250ml Erlenmyer's flasks each containing 30ml of the basal medium were autoclaved. Discs of 7cm - diameter filter paper were autoclaved separately and one was put in the autoclaved basal medium in each flask and then inoculated with 3mm - diameter culture discs.

At the end of the incubation period, the mycelium and sclerotia were carefully removed with a pair of forceps and the remains of the filter paper harvested by picking with a pair of fine forceps into previously weighed aluminium foil cups. The cups were put in an oven of 60°C for 24 hours to dry the fragmented filter paper and weighed to obtain the dry weight.

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Because of the way the fragmented filter paper was harvested it was possible that some pieces might have been left unharvested. It was therefore considered necessary to repeat the experiment to confirm the results.

The results of the first and the second experiments are shown in Table 14.

A third experiment on cellulose utilization was carried out. In that experiment an attempt was made to hasten growth of the fungi by providing a supplementary readily absorbable Carbon source to the cellulose.

The same experiment was designed but in this case 0.1% glucose was added to the medium as a soluble carbon source. The experiment was repeated as in the original cellulose test. The two sets of results are shown in Table 15. Histograms of all the results of the cellulose experiments appear in Fig 3.

As in the earlier experiments the pH of the filtrate was measured after harvesting the remains of the filter paper.

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EXPERIMENT 7

A TEST OF PHENOMENON OF AVERSION

It has already been mentioned in the Introduction and Literature Review that a reliable method of identifying strains of *S. rolfsii* is by the demonstration of the phenomenon of aversion. The results of the various preceding experiments have shown differences in the characteristics of isolates and this experiment was conducted to varify whether the isolates were indeed strains.

Several PDA plates were prepared and the five isolates were paired in all combinations as shown in Fig. 4.

For each test, two 2mm culture discs, of two different strains or of the same strain were placed near the edge of the agar plate diametrically opposite each other. The plates were incubated at 30°C for 12 days. They were then withdrawn and examined for the incidence of aversion. The observations are recorded in Fig. 4

Plates 6, 7, 8 and 9 are photographs of some of the preparations.

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EXPERIMENT 8

FORMATION OF INFECTION CUSHIONS ON TOMATO FRUITS BY THE FIVE STRAINS

Having established that the five isolates were indeed strians they were then used in various pathogenicity tests. In this first experiment, the formation of infection cushion by the different strians was studied.

Firm ripe fruits of tomato (local variety) were surface sterilized and dried with sterile filter paper. They were then placed in empty Petri dish lower halves which were standing in a shallow pool of water in plastic humidity chambers.

Four mature sclerotia were picked from 12 day old cultures and placed equidistantly on the upper hemisphere of each fruit. The lids of the humidity chambers were replaced and the preparations incubated at 30°C for four days.

The mycelium grew on the surface of the fruits and formed infection cushions. Infection cushion formation by the different strains was assessed by:

a) determining the number of infection cushions per unit area of 5x5mm, and

b) determining the mean diameter of the infection cushions.

At the end of the incubation period tiny strips (5x5mm) of the tomato epidermis were gently removed and placed in lactophenol cotton blue in watch glasses to stain the hyphae.

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They were then mounted on a slide in drops of plain lactophenol with the outer surface of the strips uppermost.

All the infection cushions on each strip were counted and the diameters of fifty randomly selected infection cushions were measured with the aid of an eye - piece graticule. The mean number of infection cushions per unit area of (5x5mm) and the mean diameter of the infection cushions in each case are shown in Table 16.

EXPERIMENT 9

INFECTION OF COCOYAM PLANTS GROWING IN *S. ROLFSII* - INOCULATED SOILS

Four varieties of *Xanthosoma sagittifolium*: 'Amankani fitaa', 'Amankani fufuo", 'Amankani kyirepe' and 'Amankani pa' and one variety of *Colocasia antiquorum* were grown in separate pots in garden loam soil. The plants were allowed to grow for three months and then inoculated. The inoculum for each pot consisted of mycelium raised in 30ml broth medium (PDB) (in a 250ml Erlenmeyer flasks) for six days at 30°C. Because of the drift of the culture filtrate to acidic pH during growth of *S. rolfsii*, the mycelium was first washed with sterile distilled water before using as inoculum.

The cumulative number of petioles infected was recorded after 4,8,12,16 and 20 days. The results are tabulated in Table 17 and the progress of petiole infection presented graphically in Fig 5.

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In the chapter of Results, Plates 10 and 11 show photographs of the three month old 'Amankani pa' and 'Amankani fufuo' plants respectively prior to inoculation. Plates 12 and 13 are photographs of 'Amankani pa' plants on the 4th and 20th day, respectively, after inoculation with *S. rolfsii* Strain EL2 and Plate 14 shows dead plants of 'Amankani fufuo' on the 20th day after inoculation with Strain XA2.

EXPERIMENT 10

ROTTING OF WOUND - INOCULATED CORMELS OF COCOYAM VARIETIES BY THE FIVE *S. ROLFSII* STRAINS

Using the procedure described at Section 2 (u) of Materials and General Methods, surface - sterilized cormels of the five cocoyam varieties were inoculated in a series of tests by the five *S. rolfsii* strains. With each *S. rolfsii* strian, five cormels of each of the varieties *Xanthosoma sagittifolium* - 'Amankani fitaa', 'Amankani fufuo', 'Amankani kyirepe' and 'Amankani pa' and cormels of *Colocasia antiquorum* were inoculated. Each cormel was inoculated at both the apical and basal regions. The inoculated cormels were put in plastic humidity chambers and incubated at 30°C for four days. Transverse sections of the cormels through the site of inoculation were made and the dept of rot and the diameter of rot immediately beneath the cormel covering were measured and the mean calculated for each treatment. The results obtained are tabulated in Tables 18 to 22 and also presented together as histograms in Fig. 6 for purposes of comparison.

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EXPERIMENT 11

DEGRADATION OF BLOCKS OF TISSUE OF CORMELS OF THE DIFFERENT COCOYAM VARIETIES BY THE FIVE STRAINS OF *S. ROLFSII*

Another way of assessing the ability of facultative parasites to attack host organs is to inoculate different organs in vitro. This experiment as in the preceding experiment used both the apical and basal regions of the cormels separately. Mini-blocks, measuring 10x10x5mm, were cut from the two regions, surface sterilized and placed on growing mycelium of the test *S. rolfsii* strain in petri dishes. Five mini-blocks were placed on the culture in each petri dish. The plates were incubated at 30°C for 8 days. Identical samples of the mini-blocks were earlier used to determine the original mean dry weight of the blocks.

After the incubation period the mycelium covering the blocks were carefully removed with a pair of fine forceps and the decomposing blocks were carefully lifted with a spatula and transferred to aluminium foil cups and dried in the oven at 80°C for 48 hours.

The mean dry weight and percentage loss in dry weight are shown in Table 23 and 24 and Fig. 7.

Plate 15 shows the mini-blocks of *Xanthosoma sagittifolium*, 'Amankani fufuo' variety over-grown by mycelia of *S. rolfsii* Strains XLL, XA1, XA2, EL1 and EL2.

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EXPERIMENT 12

FORMATION OF SCLEROTIA BY THE FIVE *S. ROLFSII* STRAINS GROWING ON CORMEL TISSUES OF THE DIFFERENT COCOYAM VARIETIES

In Section 2 (w) of Material and General Methods, the methods used in this experiment have been described. Large flat cocoyam blocks, measuring 3x3x0.5cm, taken from the two cormel regions (apical and basal) were surface sterilized and each placed in a sterilized Petri dish. Each block was inoculated with one 3mm- diameter culture and the inoculated blocks were incubated at 30°C over a total period of 12 days. The plates were examined daily so that the day sclerotia started to appear and the day melanin deposition in the sclerotia could be detected were recorded.

The results presented in Table 25 and 26 cover:

a) mean time (hours) of first appearance of sclerotia.

b) mean time (hours) after which browning of the first-developed sclerotia occurred.

c) the number of sclerotia formed on each block after 12 days,

d) the volume of 100 mature sclerotia in each treatment.

e) the germination capacity of the mature sclerotia of each treatment placed on PDA plates and incubated at 30°C for 36 hours as described in Section 2 (x) of Materials and General Methods.

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The photographs of plates 16 and 17 show blocks of Colocasia antiquorum and *Xanthosoma sagittifolium*, 'Amankani fitaa' inoculated with *S. rolfsii* Strain EL2 and Strain XA2, respectively, after incubation for 4 days and 8 days. Plate 18 also shows blocks of *Colocasia antiquorum* and *Xanthosoma sagittifolium*, 'Amankani pa' variety, 8 days after inoculation with *S. rolfsii* Strain EL1 and Strain XA1, respectively.

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IV RESULTS

EXPERIMENT I

COMPARATIVE RATE OF GROWTH OF THE DIFFERENT STRAINS OF *S. ROLFSII* SACC. AND PRODUCTION OF SCLEROTIA ON PDA

There were differences in the rates of growth of the five Strains as shown in Table 1. The five strains could be separated into three categories of fast-growing, slow-growing and intermediate. The fast-growing ones were Strains EL1 and EL2 which covered the agar medium of 9cm in diameter in 72 hours, while the slow-growing Strain XA2 took 96 hours to cover the plate. Strains XLL and XA1 formed the intermediate group which covered the PDA plate in 84 hours.

The mycelia of all the strains appeared similar in density (Plate 1). Also extensional growth was linear in all the strains. *S. rolfsii* is a fast-growing fungus (Darkwa, 1965) and it was noteworthy that within the first 12 hours of incubation the diameter of the culture increase from the initial 3mm of the disc of inoculum to 11-12mm.

The data in Table 2 and the photograph (Plate 1) show that the five *S. rolfsii* strains differed in the number of sclerotia they formed. The diameter, volume and weight of the sclerotia also differed with the strains. Strain XLL formed significantly fewer sclerotia of 185 per Petri plate than the rest where the number ranged from 324 to 364 per Petri plate: on the other hand, Strain XLL with the smaller number of sclerotia had the heavier sclerotial weight and the greatest sclerotia diameter and volume. The value

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of all three parameters were significantly different from those of the remaining four strains. However, unlike the number of sclerotia formed, these parameters differed among those four strains. By the calculated confidence limits at 95%, the four strains could be placed in descending order of magnitude of EL2, XA2, XA1 and EL1.

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TABLE 1 Growth of the five strains of *S. rolfsii* on Potato Dextrose Agar (PDA) at 30°C

Period of Incubation in Hours	Mean Diameter ± Standard Error (S.E.) cm of strain				
	XLL	XA1	XA2	EL1	EL2
12	1.1±0.02	1.1±0.02	1.1±0.02	1.1±0.03	1.2±0.02
24	2.0±0.04	1.9±0.02	1.8±0.02	2.4±0.02	2.3±0.02
36	3.3±0.04	2.7±0.03	2.6±0.03	3.9±0.03	3.9±0.02
48	4.5±0.03	4.0±0.03	3.8±0.02	5.4±0.02	5.3±0.01
60	5.7±0.04	5.3±0.03	5.1±0.04	7.2±0.03	6.7±0.02
72	6.8±0.04	7.0±0.04	6.5±0.04	9.0±0.00	9.0±0.00
84	9.0±0.03	9.0±0.03	8.8±0.02	-*	-
96	-	-	9.0±0.0	-	-

* Measurements stopped after plate had been covered.

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TABLE 2 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 No. of Sclerotia/ plate*	Weight of 100 Sclerotia ($X10^{-2}$mg)	Volume of 100 Sclerotia ($X10^{-2}$ cm^3)	Sclerotium Diameter (μm)
XLL	185±20.5a	27.8±0.002d	20.0±0.00d	84.6±0.3d
XA1	356±42.5b	23.6±0.002b	13.0±0.03a	65.3±0.3b
XA2	324±12.5b	25.5±0.001c	15.0±0.03b	75.9±0.3c
EL1	332±44.0b	21.7±0.002a	13.0±0.03a	58.3±0.3a
EL2	364±28.0b	26.0±0.002c	18.0±0.03c	76.9±0.3c

* Corrected to the nearest whole number.

By the calculated confidence limits at 95%, means in the vertical rows bearing the same letters are not significantly different.

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PLATE 1. Photograph showing sclerotia formed by cultures of the *S. rolfsii* strains growing on PDA and incubated at 30°C for 12 days (x 1/3)

(From left: Strains, XLL, XA1, XA2, EL1, EL2)

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EXPERIMENT 2

COMPARATIVE RATE OF GROWTH OF THE DIFFERENT STRAINS OF *S. ROLFSII* IN POTATO DEXTROSE BROTH (PDB)

In this experiment in which the entire mycelium produced was considered, the results of the growth test changed slightly. The data in Table 3 show that whereas *S. rolfsii* Strains EL1 and EL2 again had the greatest mean mycelium dry weight of 0.31g in 6 days, the mean dry weight of 0.26g of Strain XA1 also after 6 days was lower than the mean dry weight of the mycelium of 0.28g of Strain XA2 which in Experiment 1 grew slowest on the PDA plate. Anyway, all the mean mycelial dry weights after 6 days were not, by the calculated Confidence Limits at 95% R.H significantly different from each other. All the strains grew rapidly and attained the highest mycelial dry weight in 6 days. The dry weight declined, due to autolysis, after that, as is clearly shown in Fig. 1. The rate of autolysis, however, differed according to the species. By the graph (Fig. 1) the decline was gentle in Strian XLL and sharper in Strains EL1 and EL2. Using the data in Table 3, the calculated percentage loss in mean dry weight from the 6th to 12th day in Strain EL2, EL1 XA1, XA2 and XLL was 16.1, 12.9, 11.5, 10.7 and 3.5 per cent, respectively. The pH of the culture media from the 4th day to the end of the incubation period was very acidic, the range of the initial pH was pH 5.3 to 6.1 and range during growth of the cultures was 2.4 to 3.3. It was observed that, although the pH was still acidic, it rose slightly from the lowest by the 6th day (a range of pH 2.4 to 2.7, to a range of pH 2.6 to 3,2 by the 12th Day).

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TABLE 3 **Growth of the five strains of *S. rolfsii* in Potato Dextrose Broth (PDB) at 30°C**

Period of Incubation in Days	Mean Dry weight ± S.E (g) of mycelium in 30ml PDB of Strain				
	XLL	XA1	XA2	EL1	EL2
4	0.05±0.0b	0.04±0.0a	0.16±0.0c	0.18±0.01c	0.18±0.01c
6	0.29±0.01a	0.26±0.01a	0.28±0.02a	0.31±0.02a	0.31±0.01a
8	0.28±0.01ab	0.25±0.01a	0.28±0.02ab	0.30±0.01b	0.27±0.01ab
10	0.28±0.02a	0.25±0.01a	0.28±0.01a	0.28±0.01a	0.27±0.01a
12	0.28±0.01b	0.23±0.01a	0.25±0.01ab	0.27±0.02ab	0.26±0.01ab

By the calculated confidence limits at 95%, means in the horizontal rows bearing the same letters are not significantly different.

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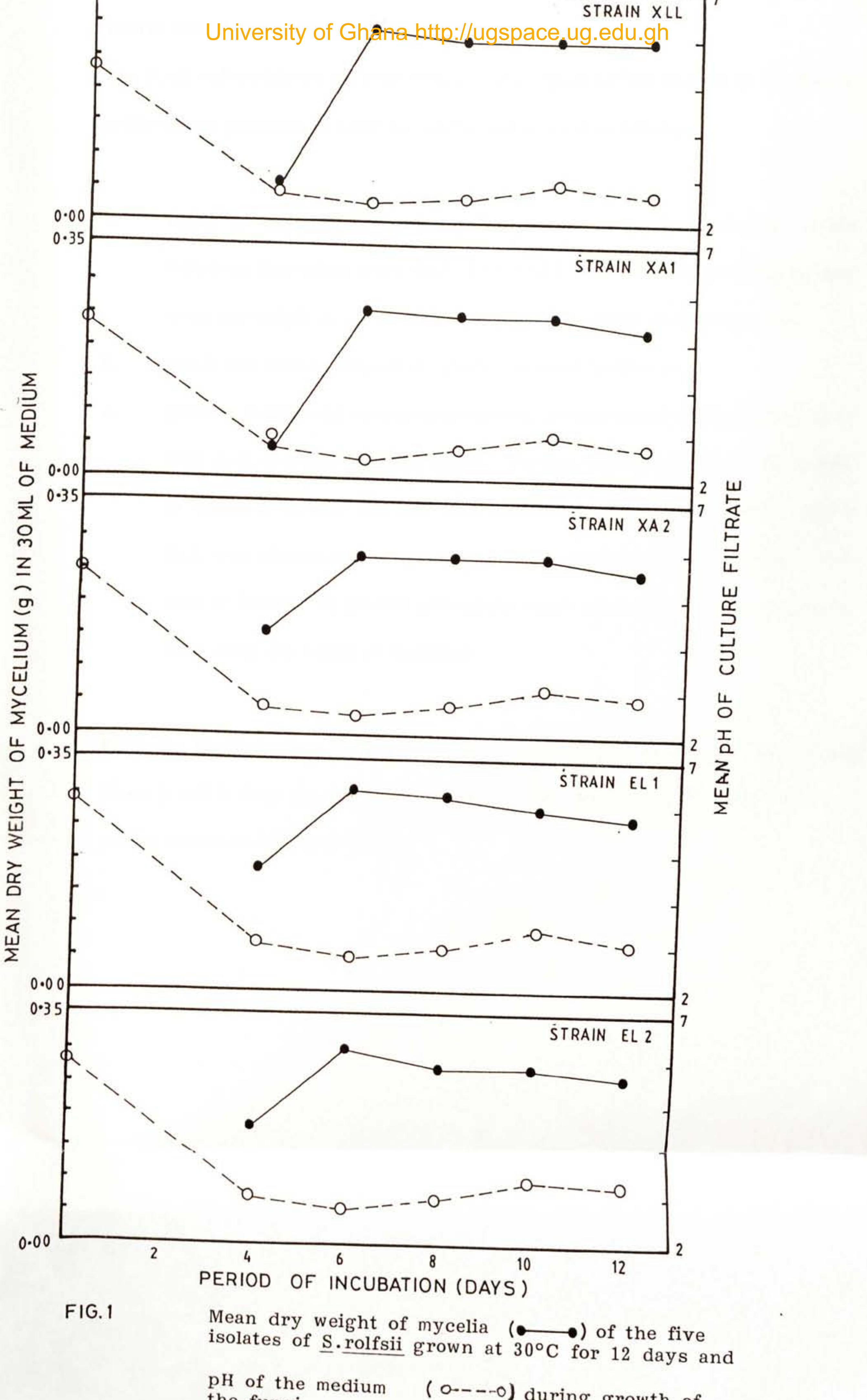

FIG. 1 Mean dry weight of mycelia (●——●) of the five isolates of S. rolfsii grown at 30°C for 12 days and pH of the medium (o----o) during growth of the fungi.

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EXPERIMENT 3

GROWTH OF THE DIFFERENT STRAINS OF *S. ROLFSII* IN LIQUID MEDIUM WITH DIFFERENT CARBON SOURCES

The results of studies on the utilization of the different carbon sources by the five *S. rolfsii* strains presented in Table 4 could be summarised as follows:

a. taking all the carbon sources together, the strains could be arranged in the following descending order EL2 > EL1 > XLL > XA1 > XA2 according to their mean dry weight as can be seen in Fig. 2 in the results of Experiment 4.

b. starch was least used by all the strains, followed by fructose.

c. 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 XA1, EL1 and EL2 were maltose and sucrose; and those for Strain XLL were glucose and sucrose while the best carbon source for Strain XA2 was maltose followed by glucose and sucrose which supported the production of the same mean dry weight of mycelium.

Evidently, the five carbon sources were utilized differently by the five *S. rolfsii* strains. Plates 2 and 3 show the growth of Strains XA2 and EL2 in media of the different carbon sources at 30^0C over 6 days.

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Table 4 Growth of different S. rolfsii strains in Liquid Med

Carbon Sources	Mean Initial pH of medium	Mean Dry Weight of myc pH of filtrate (sh	
		XLL	XA1
Fructose	4.6	0.16 ± 0.00b	0.13 ± 0.0C
		2.1	2.3
Glucose	4.8	0.22 ± 0.01b	0.14 ± 0.01
		2.0	1.8
Maltose	4.7	0.20 ± 0.01b	0.16 ± 0.01
		2.2	2.1
Starch	4.7	0.15 ± 0.01ab	0.12 ± 0.0C
		2.3	2.1
Sucrose	4.8	0.22 ± 0.01b	0.15 ± 0.01
		2.0	1.8

By the calculated confidence limits at 95%, means in horizontal rows

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ım with different carbon sources at 30°c for 6 days

ıum (g) ± S.E produced in 30ml of medium and final vn below the dry weight) of culture of strain			
	XA2	EL1	EL2
	0.11 ± 0.01a	0.18 ± 0.00c	0.21 ± 0.02c
	2.0	2.4	2.4
	0.13 ± 0.01a	0.24 ± 0.00b	0.26 ± 0.01b
	1.9	2.0	2.0
b	0.14 ± 0.00a	0.25 ± 0.01c	0.27 ± 0.01c
	2.2	2.2	2.0
	0.10 ± 0.02a	0.17 ± 0.10b	0.20 ± 0.01b
	2.3	2.4	2.2
	0.13 ± 0.00a	0.25 ± 0.01bc	0.28 ± 0.01c
	1.8	2.1	2.2

ıearing the same letters are not significantly different.

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Plate 2 Photograph of cultures of *S. rolfsii* Strain EL2 supplied with different Carbon sources and grown at 30°C for 6 days (x 1/3).

(Carbon source from left: Sucrose, Glucose, Fructose, Starch, Maltose).

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Plate 3 Photograph of cultures of *S. rolfsii* Strain XA1 supplied with different Carbon sources and grown at 30°C for 6 days (x 1/3).

(Carbon source from left: Sucrose, Glucose, Fructose, Starch, Maltose).

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EXPERIMENT 4

GROWTH OF DIFFERENT STRAINS OF *S. ROLFSII* IN LIQUID MEDIUM WITH DIFFERENT NITROGEN SOURCES

The results showed that the response of the five *S. rolfsii* strains to the five Nitrogen sources supplied showed practically the same pattern as was observed in Experiment 3. Strains EL2 grew best in all the different media, followed by Strains EL1, XLL, XA1, XA2 in that order. The results of tests of the use of both the carbon and nitrogen sources have therefore, been illustrated together by histograms in Fig. 2, for purposes of comparison.

The other salient points of results tabulated in Table 5 are:

a. Strains XLL, XA1, XA2 and EL2 grew best in the Peptone-medium, while Ammonium chloride, Ammonium nitrate, Sodium nitrate and Peptone supported practically the same extent of growth of Strain EL1.

b. different nitrogen sources were found to support the poorest growth in different strains. Ammonium chloride and Sodium nitrate were least used by Strains XLL, XA1, and XA2.

The smallest mean mycelium dry weight of Strain EL1 occurred in the Asparagine medium. However, the value of 0.10g was close to those of the other media of 0.12 and 0.13g. With Strain EL2, the smallest mean mycelium dry weight occurred in the Sodium nitrate medium. Plates 4 and 5 show growth of Strains XA1 and EL2 in media of the different nitrogen sources at 30°C over 6 days.

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Table 5 **Growth of different *S. rolfsii* strains ir**

Nitrogen Sources	Mean Initial pH of medium	Mean Dry Weight of pH of filtra	
		XLL	XA1
Ammonium Chloride	4.6	0.06 ± 0.00a	0.06 ±
		2.2	2.0
Ammonium nitrate	4.7	0.08 ± 0.01a	0.07 ±
		2.4	2.1
Asparagine	4.6	0.09 ± 0.00a	0.08 ±
		2.4	2.2
Peptone	4.9	0.11 ± 0.01ab	0.10 ±
		2.2	2.0
Sodium nitrate	4.6	0.07 ± 0.01a	0.06 ±
		2.9	2.6

By the calculated confidence limits at 95%, means in horizont

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ı liquid medium with different Nitrogen sources at 30°C for 6 days

˜ mycelium (g) ± S.E produced in 30ml of medium and final te (shown below the dry weight) of culture of strain			
	XA2	EL1	EL2
± 0.01a	0.05 ± 0.00a	0.13 ± 0.03ab	0.15 ± 0.01b
	2.1	2.3	2.3
± 0.01a	0.06 ± 0.01a	0.12 ± 0.00b	0.14 ± 0.01b
	2.2	2.2	2.3
± 0.01a	0.07 ± 0.01a	0.10 ± 0.01a	0.16 ± 0.01b
	2.2	2.5	2.7
± 0.00b	0.09 ± 0.00a	0.12 ± 0.01b	0.17 ± 0.00c
	2.1	2.2	2.3
± 0.01a	0.05 ± 0.01a	0.12 ± 0.01b	0.13 ± 0.00b
	2.5	2.7	2.9

tal rows bearing the same letters are not significantly different.

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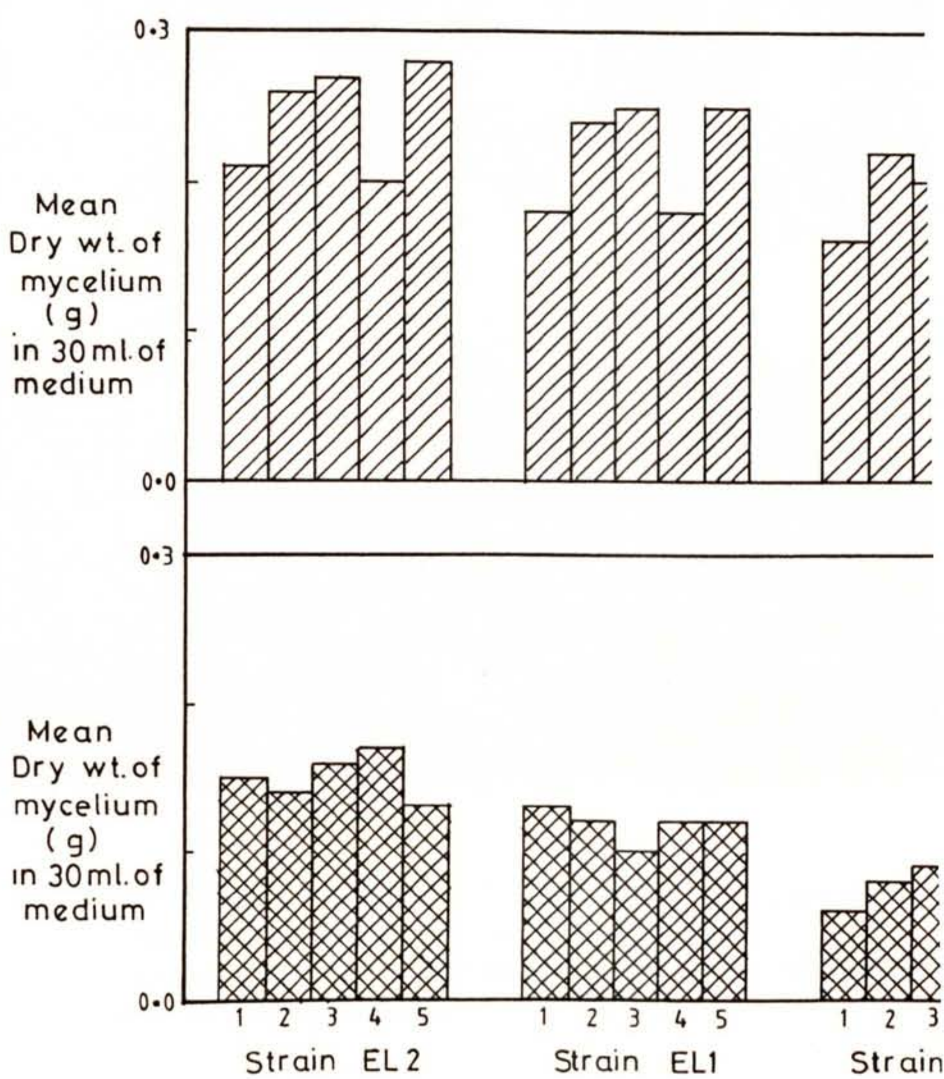

FIG. 2 Mean Dry wt. of mycelium of different
of response) grown in 30ml liquid medi
2, Glucose; 3, Maltose; 4, Starch and
2, Ammonium nitrate; 3, Asparagine; 4

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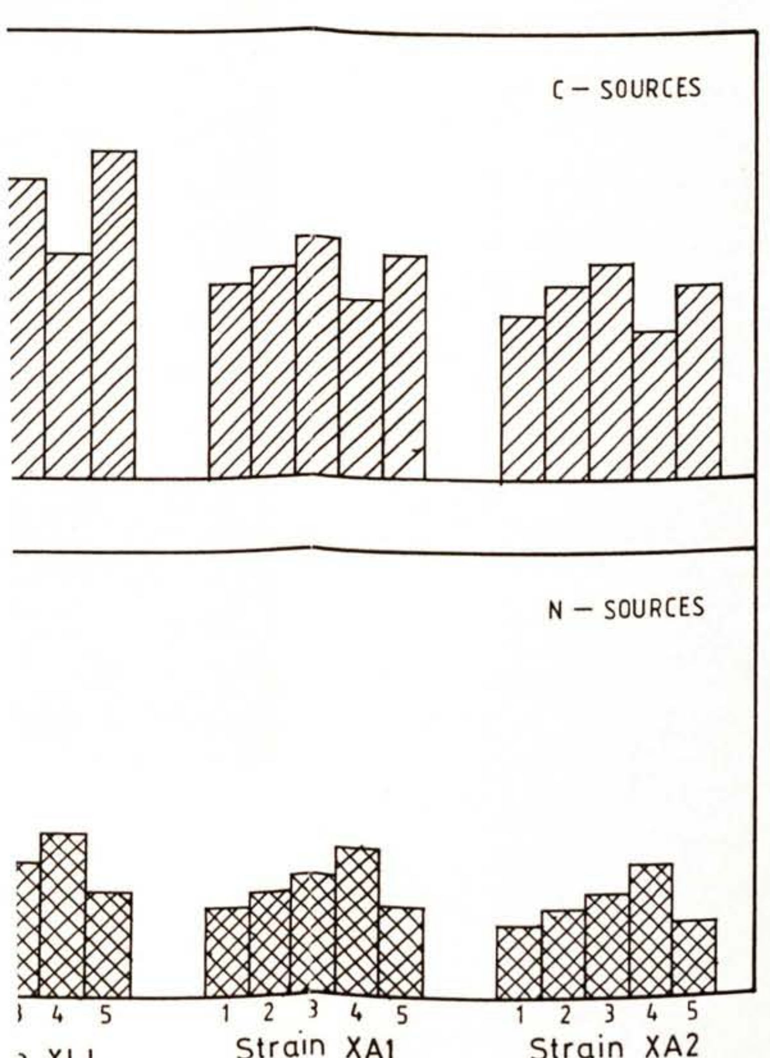

S.rolfsii strains (arranged in descending order
um with different sources of carbon (1, Fructose;
5, Sucrose) and Nitrogen (1, Ammonium chloride;
, Peptone; 5, Sodium nitrate) at 30°C for 6 days.

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Plate 4 Photograph of cultures of *S. rolfsii* Strain EL2 supplied with different Nitrogen sources and grown at 30°C for 6 days (x 1/3).
(Nitrogen source from left: Peptone, Asparagine, Ammonium nitrate, Ammonium chloride, Sodium nitrate).

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Plate 5. Photograph of cultures of *S. rolfsii* Strain XA1 supplied with different Nitrogen sources and grown at 30°C for 6 days (x 1/3)
(Nitrogen source from left: Peptone, Asparagine, Ammonium nitrate, Ammonium chloride, Sodium nitrate).

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EXPERIMENT 5

MACERATION OF DIFFERENT PLANT TISSUES BY PECTOLYTIC ENZYMES IN CULTURE FILTRATES OF THE FIVE 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 different *S. rolfsii* strains. The experiment examined at the same time the influence of the growth medium on enzyme production and the rate of maceration of tissues of different plant species. Tables 6 to 13 contain results of the various tests.

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Growth of the *S. rolfsii* strains in different Glucose media (Table 6)

a. In media with different glucose concentrations of 0.5, 1.0, 1.5 and 2.0 per cent, the mean dry weight of mycelium formed in 6 days by each *S. rolfsii* strain increased with increasing glucose concentration.

b. At glucose concentrations of 1.0, 1.5 and 2.0 per cent, Strain EL2 produced the highest mean mycelial dry weight, although not statistically different from the amount produced by some of the other strains.

c. At all glucose concentrations, Strain XA2 produced the least mean mycelial dry weight, although the difference between that value and those of some of the strains was not statistically significant.

d. The pH of all the media drifted from an initial pH 6.0 - 6.2 to a final pH 2.0 - 3.4.

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Table 6 **Growth of the five strains of *S. rolfsii* recorded after 6 days of incubation**

Glucose Concentration (%: w/v)	Initial pH	Mean Dry Weig (sh	
		XLL	XA1
0.5	6.2	0.26 ± 0.01b	0.21
		3.2	3.0
1.0	6.2	0.34 ± 0.01a	0.31
		2.6	2.5
1.5	6.1	0.46 ± 0.01b	0.43
		2.4	2.3
2.0	6.0	0.53 ± 0.01b	0.47
		2.0	2.1

By the calculated confidence limits at 95%, means in horizontal

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***sii* in 30ml of PDB with different concentrations of Glucose at 30°C**

eight of mycelium ± S.E (g) of strain and final pH of filtrate 'shown below the dry weight) of culture of strain			
ι1	XA2	EL1	EL2
1 ± 0.01ab	0.18 ± 0.00a	0.23 ± 0.01b	0.23 ± 0.01ab
	3.2	3.2	3.4
1 ± 0.01a	0.31 ± 0.01a	0.31 ± 0.01a	0.35 ± 0.01a
	2.6	2.6	3.0
3 ± 0.01b	0.38 ± 0.01a	0.46 ± 0.01b	0.48 ± 0.01b
	2.4	2.4	2.7
' ± 0.01a	0.45 ± 0.01a	0.55 ± 0.01b	0.57 ± 0.01b
	2.1	2.1	2.3

tal rows bearing the same letters are not significantly different.

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Growth of *S. rolfsii* strains in different Pectin media (Table 7)

a. 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 per cent.

b. Strains XA1 and XA2 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.

c. The pH of all the media drifted from an initial pH 4.0 - 5.0 to a final pH 2.6 - 3.5.

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Table 7 **Growth of the five strains of *S. rolfsii* in 3 recorded after 6 days of incubation**

Pectin Concentration (%: w/v)	Initial pH	Mean Dry Weight of (shown	
		XLL	XA1
0.5	5.0	0.24 ± 0.01b	0.17 ± 0.
		3.4	3.4
1.0	4.6	0.25 ± 0.01c	0.22 ± 0.
		3.3	3.3
1.5	4.2	0.27 ± 0.00b	0.25 ± 0.
		3.0	3.0
2.0	4.0	0.39 ± 0.00c	0.30 ± 0.
		2.7	2.6

By the calculated confidence limits at 95%, means in horizontal ro

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30ml of PDB with different concentrations of Pectin at 30°C

of mycelium ± S.E (g) of strain and final pH of filtrate n below the dry weight) of culture of strain			
	XA2	EL1	EL2
0.01a	0.16 ± 0.00a	0.25 ± 0.00b	0.25 ± 0.00b
	3.4	3.4	3.5
0.00b	0.19 ± 0.01a	0.23 ± 0.00c	0.28 ± 0.00d
	3.4	3.4	3.5
0.01ab	0.23 ± 0.01a	0.28 ± 0.00c	0.28 ± 0.00c
	3.1	3.3	3.2
0.00a	0.28 ± 0.01a	0.35 ± 0.01b	0.38 ± 0.01bc
	2.8	3.0	2.9

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Growth of *S. rolfsii* strains in media with different Glucose-Pectin combinations (Table 8)

The combination of glucose and pectin used were: 0.75% Glucose and 0.25% Pectin; 0.50% Glucose and 0.50% Pectin; and 0.25% Glucose and 0.75% Pectin. Growth of the *S. rolfsii* strains in the different media differed.

a. A combination of 0.5% Glucose and 0.5% Pectin supported the greatest growth in Strains XLL, XA1, XA2 and EL2.

b. Combinations of 0.25% Glucose and 0.75% Pectin supported the greatest growth in Strains EL1. However, the highest value in all cases was not statistically different from the value next to it.

c. The pH of the media became strongly acidic during growth of the fungi.

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Table 8 **Growth of the five strains of *S. rolfsii* in 30m in different ratios at 30°C recorded after 6 da**

Medium with Concentration (% w/v) of		Initial pH	Mean Dry Weigh (shov	
Glucose	Pectin		XLL	XA1
0.75	0.25	4.6	0.39 ± 0.01b	0.32 ±
			2.7	2.7
0.5	0.5	4.1	0.42 ± 0.01b	0.37 ±
			2.7	2.6
0.25	0.75	4.0	0.34 ± 0.02a	0.35 ±
			3.0	2.8

By the calculated confidence limits at 95%, means in horizonta

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nl of PDB containing a combination of Glucose and Pectin
lays of incubation

ht of mycelium ± S.E (g) of strain and final pH of filtrate own below the dry weight) of culture of strain			
	XA2	EL1	EL2
± 0.01a	0.30 ± 0.01a	0.39 ± 0.01b	0.41 ± 0.01b
	2.6	2.8	2.8
± 0.01a	0.36 ± 0.01a	0.43 ± 0.01b	0.43 ± 0.01b
	2.5	2.9	2.9
± 0.01a	0.33 ± 0.01a	0.44 ± 0.02b	0.42 ± 0.01b
	2.9	3.2	3.2

ıtal rows bearing the same letters are not significantly different.

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Enzyme activity of filtrates of *S. rolfsii* strains growing in Glucose and Pectin media (Table 9-12)

The Enzyme Activity determined by maceration of discs of tuber of Irish potato in culture filtrates of all the *S. rolfsii* strains was far greater, ranging from 6.8 to 8.4, in culture filtrates of the 2.0% Glucose medium than in the remaining three media of lower glucose concentrations as shown in Table 9. These showed comparatively low Enzyme Activities of 1.6 to 2.2. Using the results of the 2.0% Glucose medium tests to compare the *S. rolfsii* Strains, Strain EL2 produced the greatest Enzyme Activity of 8.4 and Strains XA1 and EL1 the lowest of 6.8.

The data in Table 10 showed that the same trends were also observed when discs of cucumber pericarp were used as the test tissue. The greatest Enzyme Activity in each case occurred in the culture filtrates of the 2.0% Glucose medium, showing a range of 11.7 to 13.3. The Enzyme Activities of filtrates of the other glucose concentrations were from 2.9 to 6.4.

The strains fell into two groups at any of the glucose concentrations. Higher levels of Enzyme Activity were recorded for Strains XLL, XA1 and EL2 and lower levels for Strains XA2 and EL1.

Results of experiments carried out with culture filtrates of fungi growing in pectin media using Irish Potato tuber and cucumber pericarp tissues are shown in Tables 11 and 12. The greatest Enzyme Activity occurred in filtrates of the 2.0% pectin media. Also, there was generally greater Enzyme Activity in filtrates of Strains EL1 and EL2 than those of Strains XLL, XA1 and XA2 in the 0.5, 1.0 and 2.0% pectin media.

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Table 9 Extent of maceration of discs of stem tuber of Irish I
of strains each provided with different concentratio

Glucose concentration (%) in growth medium	Mean time of maceration of 6 discs (min) by filtrate of strain				
	XLL	XA1	XA2	EL1	EL2
0.5	45.0	55.8	64.2	55.4	51.0
1.0	55.6	54.4	62.1	63.3	61.5
1.5	52.1	59.6	55.8	57.1	51.9
2.0	14.0	14.8	13.1	14.8	11.9

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Potato (*Solanum tuberosum*) by filtrate of 6 day-old cultures ons of Glucose

	Enzyme activity of strain				
	XLL	XA1	XA2	EL1	EL2
	2.2	1.8	1.6	1.8	2.0
	1.8	1.8	1.6	1.6	1.6
	1.9	1.7	1.8	1.8	1.9
	7.2	6.8	7.6	6.8	8.4

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Table 10 Extent of maceration of discs of pericarp of strains each provided with different concentr

Glucose concentration (%) in growth medium	Mean time of maceration of 6 discs (min) b filtrate of strain				
	XLL	XA1	XA2	EL1	E
0.5	18.7	20.2	33.9	21.9	2
1.0	17.3	19.8	24.8	30.4	2(
1.5	15.6	21.8	26.7	28.1	1
2.0	7.5	7.7	8.5	7.9	7.

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f cucumber (*Cucumis sativus*) by filtrate of 6 day-old cultures of
rations of Glucose

by	Enzyme activity of strain				
EL2	XLL	XA1	XA2	EL1	EL2
21.0	5.3	5.0	2.9	4.6	4.8
20.2	5.8	5.1	4.0	3.3	5.0
19.8	6.4	4.6	3.8	3.6	5.1
7.7	13.3	13.0	11.7	12.7	13.0

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Table 11 Extent of maceration of discs of stem tuber of Iris of strains each provided with different concentra

Pectin concentration (%) in growth medium	Mean time of maceration of 6 discs (min) by filtrate of strain				
	XLL	XA1	XA2	EL1	EL2
0.5	10.2	10.0	10.0	8.1	8.1
1.0	7.5	9.6	11.5	7.5	7.3
1.5	7.5	7.3	7.3	8.3	8.5
2.0	6.7	6.5	6.9	6.3	6.2

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·ish Potato (*Solanum tuberosum*) by filtrate of 6 day-old cultures rations of Pectin

?	Enzyme activity of strain				
	XLL	XA1	XA2	EL1	EL2
	9.8	10.0	10.0	12.3	12.3
	13.3	10.4	8.7	13.3	13.7
	13.3	13.7	13.6	12.0	11.7
	15.0	15.4	14.4	16.0	16.2

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Table 12 Extent of maceration of discs of pericarp o strains each provided with different concent

Pectin concentration (%) in growth medium	Mean time of maceration of 6 discs (min) filtrate of strain			
	XLL	XA1	XA2	EL1
0.5	8.1	7.7	7.9	6.9
1.0	6.0	7.3	7.3	5.4
1.5	5.4	5.4	5.6	5.6
2.0	5.0	5.0	5.0	5.0

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ıf cucumber (*Cucumis sativus*) by filtrate of 6 day-old cultures of
:rations of Pectin

ı by	Enzyme activity of strain				
EL2	XLL	XA1	XA2	EL1	EL2
6.3	12.3	13.0	12.6	14.6	16.0
5.6	16.7	13.7	13.7	18.5	17.8
5.4	18.5	18.5	17.9	17.8	18.5
5.0	20.0	20.0	20.0	20.0	20.0

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Enzyme activity of filtrates of *S. rolfsii* strains growing in Glucose - Pectin media

The Enzyme Activities of filtrates of the five *S. rolfsii* strains growing in media containing different combinations of glucose and pectin are shown in Table 13. The results indicated that the levels of pectolytic enzymes in any one particular medium was practically the same for all five *S. rolfsii* strains.

Secondly, with all the five strains, higher values of Enzyme Activity, from 14.1 to 16.6, were found in the 0.5% of Glucose + 0.5% Pectin, and the 0.25% Glucose + 0.75% Pectin media than in the 0.75% Glucose + 0.25% Pectin media of 7.5 to 7.9 Enzyme Activity.

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Table 13 Extent of maceration of discs of stem tuber of strains each provided with Glucose and

Medium with concentration (%) of		Mean time of maceration (by filtrate of strain		
Glucose	Pectin	XLL	XA1	XA2
0.75	0.25	12.7	12.9	13.1
0.5	0.5	6.7	6.9	6.3
0.25	0.75	7.1	6.6	6.0

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er of Irish Potato (*Solanum tuberosum*) by filtrate of 6 day-old cultures

d Pectin in different proportions

n of 6 discs (min)		Enzyme activity of strain				
EL1	EL2	XLL	XA1	XA2	EL1	EL2
12.7	13.3	7.9	7.7	7.6	7.9	7.5
6.7	6.5	15.0	14.6	16.0	15.0	15.5
6.5	6.9	14.1	15.2	16.6	15.4	14.5

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EXPERIMENT 6

USE OF CELLULOSE AS CARBON SOURCE BY THE FIVE STRAINS

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 was supplemented with 0.1% Glucose. Both experiments were repeated, but the results were kept apart. The tables of results (Tables 14 and 15) therefore contain results of the two tests.

In the experiments using cellulose only as carbon source (Table 14) the results were consistent. *S. rolfsii* Strains XA1 and XA2 utilized cellulose to a greater extent than the remaining three strains, with Strain XA2 superior, in both tests, to Strain XA1 but the difference between the two values was not statistically significant. However, the two values mean percentage loss in dry weight of the filter paper of 62.5 and 68.8 per cent in the original test and 62.5 and 71.9 per cent in the second test were significantly different from values for the remaining three strains.

Unlike the previous growth experiments, 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 4.1. The results followed the same trend when 0.1% (w/v) Glucose was added to the medium as shown in Table 15, and in Fig. 3. Strains XA1 and XA2 used the carbon sources to a greater extent than the remaining three strains.

Furthermore values for Strain XA2 were superior to those of Strain XA1 and the pH drifted slightly from the original pH 5.1 to a final pH of pH 3.8 to 4.0.

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Table 14 **Growth of the five strains of *S. rolfsii* ir for 7 weeks**

Test	Initial pH	Initial Dry wt. of filter paper (g)	*S. rolfsii* strain
1	5.3	0.32	XLL
			XA1
			XA2
			EL1
			EL2
			CONTROL
2	5.3	0.32	XLL
			XA1
			XA2
			EL1
			EL2
			CONTROL

By the calculated confidence limits at 95%, means bearir

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n medium with cellulose (filter paper) as carbon source incubated at 30°

Final pH of filtrate	Mean Dry wt. of remaining filter paper (g)	Mean loss in Dry wt. of filter paper (g)	Mean % loss in Dry wt. of filter paper
4.1	0.18±0.01b	0.14	43.8
4.1	0.12±0.01a	0.20	62.5
4.1	0.10±0.01a	0.22	68.8
4.1	0.16±0.01b	0.16	50.0
3.9	0.18±0.01b	0.14	43.8
5.2	0.32±0.01c	0.0	0.0
4.1	0.19±0.01c	0.13	40.6
4.1	0.12±0.01a	0.20	62.5
4.1	0.09±0.01a	0.23	71.9
4.1	0.16±0.0b	0.16	50.0
4.0	0.18±0.01bc	0.14	43.8
5.2	0.32±0.0d	0.0	0.0

ng the same letters are not significantly different.

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Table 15 **Growth of the five strains of *S. rolfsii* i source incubated at 30°C for 7 weeks**

Test	Initial pH	Initial Dry wt. of filter paper (g)	*S. rolfsii* strain
1	5.1	0.32	XLL
			XA1
			XA2
			EL1
			EL2
			CONTROL
2	5.1	0.32	XLL
			XA1
			XA2
			EL1
			EL2
			CONTROL

By the calculated confidence limits at 95%, means beari

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in medium with cellulose (filter paper) and 0.1% (w/v) Glucose as carbo

Final pH of filtrate	Mean Dry wt. of remaining filter paper (g)	Mean loss in Dry wt. of filter paper (g)	Mean % loss in Dry wt. of filter paper
4.0	0.18±0.00c	0.14	43.8
3.9	0.10±0.01a	0.20	62.5
4.0	0.09±0.01a	0.23	71.9
3.9	0.14±0.01b	0.18	56.3
3.8	0.16±0.01bc	0.16	50.0
5.1	0.32±0.01d	0.0	0.0
4.0	0.18±0.01c	0.14	43.8
3.9	0.11±0.0b	0.21	65.6
4.0	0.08±0.01a	0.24	75.0
3.8	0.14±0.01c	0.18	56.3
3.8	0.16±0.01c	0.16	50.0
5.1	0.32±0.01d	0.0	0.0

ing the same letters are not significantly different.

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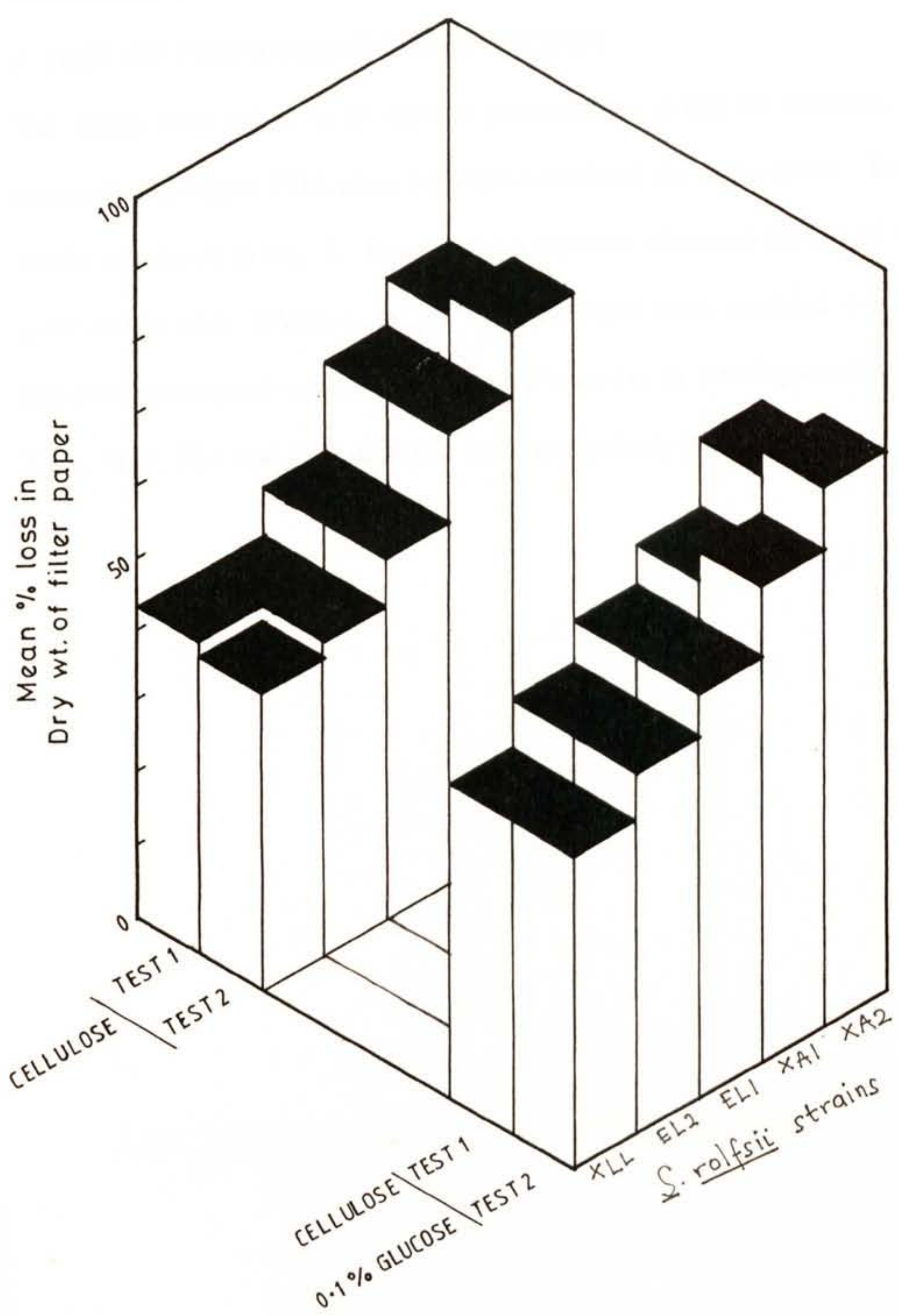

FIG. 3 Histograms of Mean % loss in dry weight of filter paper in media inoculated with S. rolfsii strains.
Note superiority of Strains XA1 and XA2 to the other strains in the use of cellulose as carbon source.

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EXPERIMENT 7

A TEST OF PHENOMENON OF AVERSION

The strains were paired in all sorts of permutations to test for aversion, in addition to inoculating the same PDA plate with two inocula of the same strain. The pairings and results are shown in Fig. 4. There was no aversion when two inocula of the same strain grew on the plate (Plates 6 and 7). On the other hand, aversion occurred in all the numerous pairings of two different strains (Plates 6 to 9), proving conclusively that XLL, XA1, XA2, EL1 and EL2 deserved to be recognized as true strains.

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Fig. 4 Checker-board showing results of pairing of the five strains of *S. rolfsii* on Potato-Dextrose Agar (PDA) at 30°C to test for aversion phenomenon.

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

S. rolfsii strains

S. rolfsii strains

	XLL	XA1	XA2	EL1	EL2
XLL	–	+	+	+	+
XA1	+	–	+	+	+
XA2	+	+	–	+	+
ELI	+	+	+	–	+
EL2	+	+	+	+	–

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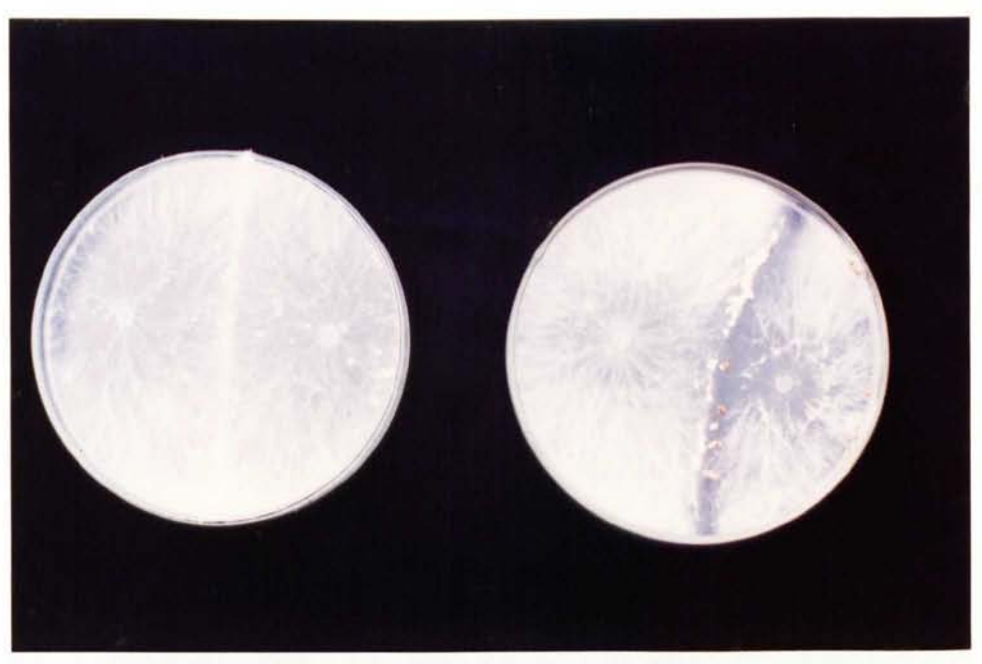

Plate 6. Photograph showing aversion between *S. rolfsii* Strains EL1 and XA2 growing on PDA at 30°C (RIGHT) and the absence of aversion between two inocula of Strain EL1 (LEFT) (x 5/9)

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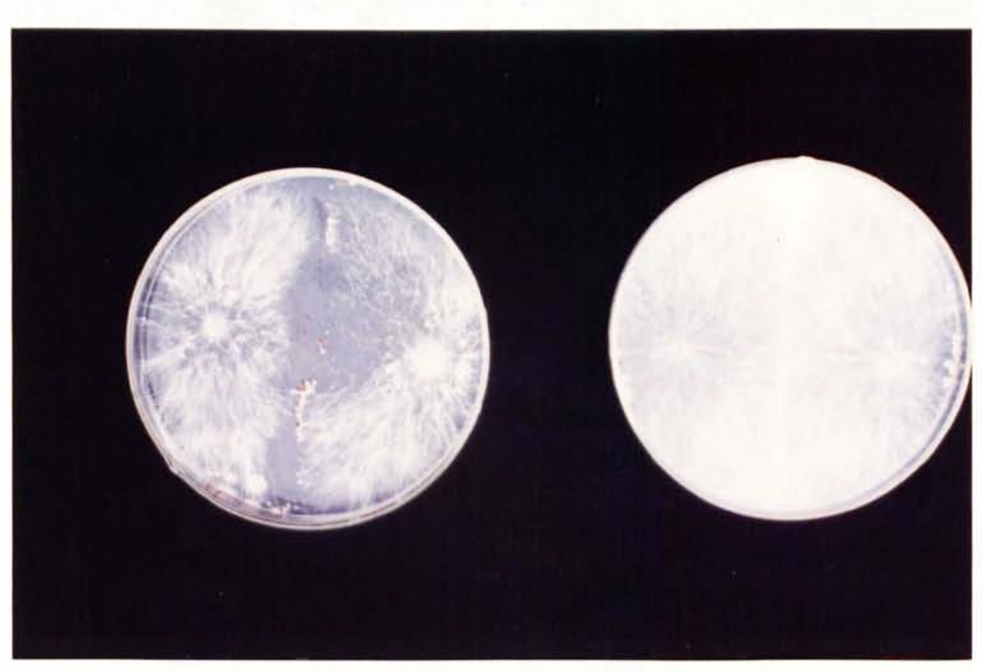

Plate 7. Photograph showing aversion between *S. rolfsii* Strains EL1 and XLL growing on PDA at 30°C (LEFT) and the absence of aversion between two inocula of Strain EL1 (RIGHT) (x 5/9)

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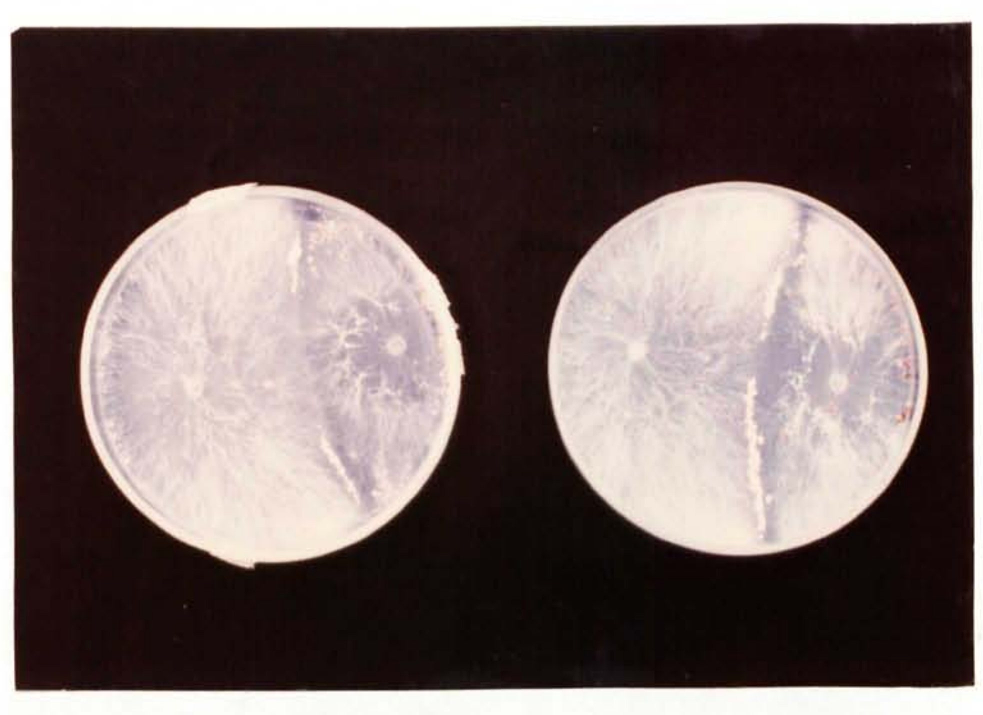

Plate 8 Photograph showing aversion between *S. rolfsii* Strains XLL and XA1 (LEFT) and XLL and XA2 (RIGHT) growing on PDA at 30°C (x 5/9).

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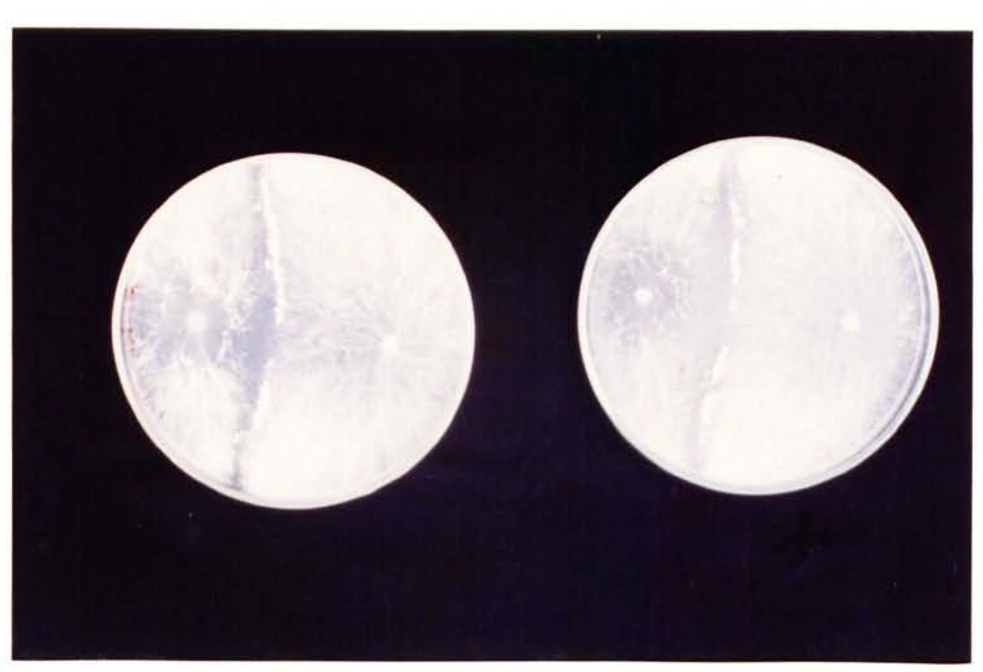

Plate 9 Photograph of two Petri plates showing similar patterns of aversion between *S. rolfsii* Strains XA1 and XA2 growing on PDA at 30°C (x 5/9).

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EXPERIMENT 8

FORMATION OF INFECTION CUSHIONS ON TOMATO FRUITS BY THE FIVE STRAINS

There was a remarkable variation in the pre-penetration habits of the five *S. rolfsii* strains as indicated by the data in Table 16. That involved both the number of infection cushions formed per unit area and the mean diameters of the infection cushions.

Strains EL1 and EL2 formed a mean number of 99 and 102 infection cushions per unit area of 5x5mm respectively, which were vastly greater than the mean numbers of 34-39 by Strains XLL, XA1 and XA2. It was observed that there was an inverse relationship between mean number of infection cushions and mean diameters of the infection cushions. Thus, the infection cushions of Strains XLL, XA1 and XA2 had larger mean diameters of 12.7 - 18.0μm which were significantly larger than the mean diameter of 11.3μm of strains EL1 and EL2.

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TABLE 16 Formation of infection cushion on surface of Local Variety Tomato Fruits by the five strains of *S. rolfsii* incubated at 30°C for 4 days

S. rolfsii Strain	Mean Total Number of infection cushions per unit surface area of 25mm² ± S.E	Mean Diameter (μm) of infection cushions ± S.E
XLL	34 ± 1.12a	18.0 ± 0.15c
XA1	37 ± 1.96a	12.7 ± 0.13b
XA2	39 ± 2.25a	13.3 ± 0.12b
EL1	99 ± 3.03b	11.3 ± 0.14a
EL2	102 ± 2.64b	11.3 ± 0.11a

By the calculated confidence limits at 95%, means in vertical rows bearing the same letters are not significantly different.

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EXPERIMENT 9

INFECTION OF *XANTHOSOMA SAGITTIFOLIUM* AND *COLOCASIA ANTIQUORUM* PLANTS GROWING IN *S.ROLFSII*-INOCULATED SOILS

Three-month old plants of cocoyam varieties, *X. sagittifolium* var 'Amankani fitaa', *X. sagittifolium* var 'Amankani fufuo' (Plate 10), *X. sagittifolium* var. 'Amankani kyirepe', *X. sagittifolium* var. 'Amankani pa' (Plate 11) and *C. antiquorum* were separately inoculated by the soil-inoculation method, with the different *S. rolfsii* strains.

In all cases, all the replicates were infected and the leaves were dead by the 20th day after inoculation as shown in Plates 13 and 14. The plants were dug out at the end of the experiment and bissected longitudinally and examined. The cormels of all the treatments were heavily rotted, the rot progressing from the top downwards.

Apparently, the fungus entered the plant through the base of the outermost petiole and migrated into the inner petioles of the plant. Also,the direction of infection of the petioles was centripetal. The fungus moved from the outermost petiole into the cormel and then moved both downwards into the cormel and transversely inwards to infect the inner petioles. Plate 12 shows the progressive invasion and death of the petioles of *X. sagittifolium* var. 'Amankani pa' from the outside inwards.

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The plants were not infected at the same rate by the different *S. rolfsii* strains according to the data in Table 17. Infection proceeded slowest in *X. sagittifolium* var. 'Amankani fufuo', in which some of the leaves still remained uninfected by the 16th day after inoculation. On the other hand, Strains EL1 and EL2 had infected all the leaves of *X. sagittifolium* var. 'Amankani fitaa' by the 12th day. This variety could be considered the most susceptible. All the leaves of the remaining three varieties had been infected by the 16th day by all the *S. rolfsii* strains. The graphs in Fig. 5 show that infection progress fastest in the first 8 days after inoculation. None of the control plants showed any signs of infection during the investigation.

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Table 17 **Infection of varieties of *Xanthosoma sagittifolium* different *S. rolfsii* strains**

Cocoyam Variety	Strain	No. of petioles at time of inoculation	No. of
			4
X. sagittifolium 'Amankani fitaa'	XLL	13	6
	XA1	13	5
	XA2	14	6
	EL1	13	6
	EL2	14	7
	CONTROL	12	0
X. sagittifolium 'Amankani fufuo'	XLL	13	6
	XA1	13	6
	XA2	14	6
	EL1	15	7
	EL2	13	6
	CONTROL	12	0

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ttifolium and *Colocasia antiquorum* growing in soil inoculated with

No. of petioles rotted in indicated number of days				
4	8	12	16	20
6	10	11	13	-
5	9	10	13	-
6	11	12	14	-
6	10	13	-	-
7	11	14	-	-
0	0	0	0	0
6	8	10	12	13
6	8	9	12	13
6	9	10	13	13
7	10	12	14	14
6	9	10	11	13
0	0	0	0	0

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Table 17 (Cont.'d)

Cocoyam Variety	Strain	No. of petioles at time of inoculation	No
			4
X. sagittifolium 'Amankani Kyirepe'	XLL	14	6
	XA1	13	4
	XA2	12	5
	EL1	13	6
	EL2	13	5
	CONTROL	12	0
X. sagittifolium 'Amankani pa'	XLL	12	6
	XA1	12	5
	XA2	13	6
	EL1	14	5
	EL2	13	7
	CONTROL	12	0

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n	No. of petioles rotted in indicated number of days				
	4	8	12	16	20
	6	10	11	12	12
	4	9	11	13	-
	5	9	10	12	-
	6	10	12	13	-
	5	10	12	13	-
	0	0	0	0	0
	6	9	10	12	-
	5	8	9	11	11
	6	8	9	12	12
	5	10	11	13	13
	7	9	10	12	12
	0	0	0	0	0

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Table 17 (Cont.'d)

Cocoyam Variety	Strain	No. of petioles at time of inoculation	No
			4
Colocasia antiquorum	XLL	11	5
	XA1	14	7
	XA2	14	6
	EL1	14	6
	EL2	13	6
	CONTROL	12	0

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No. of petioles rotted in indicated number of days				
4	8	12	16	20
5	7	8	11	-
7	10	11	14	-
6	9	11	14	-
6	9	10	13	13
6	9	11	12	12
0	0	0	0	0

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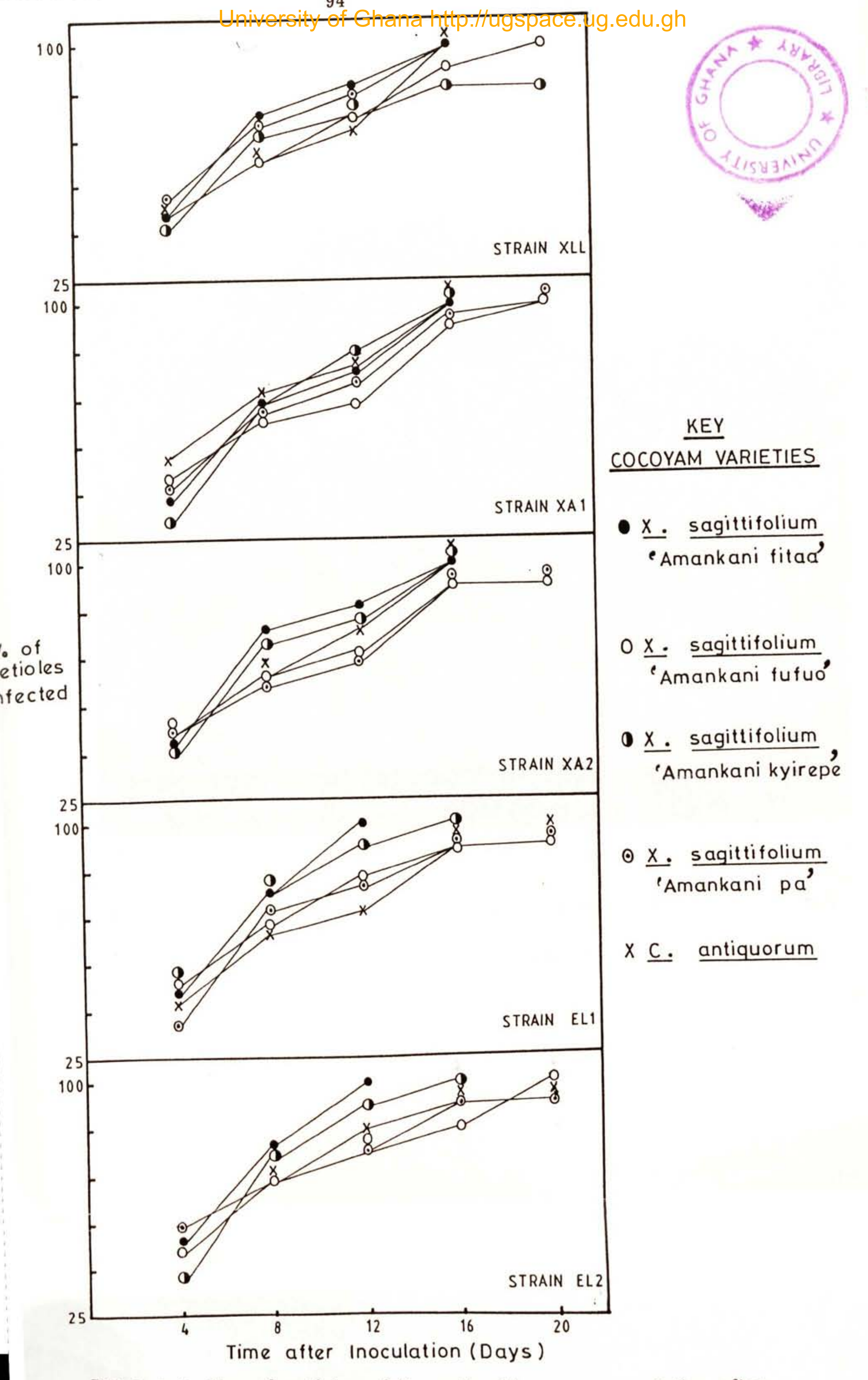

FIG.5 Infection of petioles of 3 month-old cocoyam varieties after

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Plate 10. Photograph showing 3 month-old plants of *Xanthosoma sagittifolium* varieity 'Amankani fufuo' at the time of the pathogenicity test with the different *S. rolfsii* strains (x 1/4).

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Plate 11. Photograph showing 3 month-old plant of *Xanthosoma sagittifolium* varieity 'Amankani pa' at the time of the pathogenicity test with the different *S. rolfsii* strains (x 5/9).

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Plate 12. Photograph showing infection of leaves of 3 month-old plant of *Xanthosoma sagittifolium* varieity 'Amankani pa' 4 days after introduction of the soil with *S. rolfsii* Strain EL2 (x 5/9).

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Plate 13. Photograph showing total destruction of leaves of 3 month-old plant of *Xanthosoma sagittifolium* varieity 'Amankani pa' 20 days after inoculation of the soil with *S. rolfsii* Strain EL2 (x 5/9).

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Plate 14 Photograph showing total destruction of the leaves of 3 month-old plants of *Xanthosoma sagittifolium* varieity 'Amankani fufuo' 20 days after inoculation of the soil with *S. rolfsii* Strain XA2 (x 1/6).

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EXPERIMENT 10

ROTTING OF WOUND - INOCULATED CORMELS OF COCOYAM VARIETIES BY THE FIVE *S. ROLFSII* STRAINS

The histograms in Fig. 6 bring together the results contained in Tables 18 to 22 of the various tests which examined rotting of cormels of the five cocoyam varieties which were wound - inoculated with the different *S. rolfsii* strains. Information was obtained on the extent of rotting at the apical and the basal ends of the cormels, on the depth of rot and on the diameter of rot.

The data in all the tables (Table 18-22) indicated that in all the cases (a) the basal region of the cormels was rotted to a greater extent than the apical region and (b) the diameter of the rot measured immediately beneath the cormel covering was greater than the depth of the rot.

The diameter and depth of the rot both at the apical and basal regions were greatest in all the cocoyam varieties inoculated with *S. rolfsii* Strains XA1 and XA2. These two strains were therefore, the most potent. Strain EL1, on the other hand, was the least active, in all the varieties except *X. sagittifolium* var. 'Amankani fitaa', followed by Strains EL2 and XLL. The diameter of the rot caused by Strain EL1 was in some cases markedly and significantly smaller statistically than those of rots caused by the rest (Table 19 and 20).

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The cormels of the different cocoyam varieties were rotted to varying degrees by the different *S. rolfsii* strains. As clearly shown in Fig.6, the broadest rot diameter occurred in cormels of *Colocasia antiquorum* inoculated with any of the *S. rolfsii* strains. While the deepest rot was observed in *Xanthosoma sagittifolium* 'Amankani pa' cormels. None of the control cormels showed any signs of rotting.

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TABLE 18. ROTTING OF WOUND - INOCULATED CORMELS OF 'AMANKANI FITAA' BY THE FIVE STRAINS OF *S. ROLFSII* AT 30°C IN 8 DAYS

S. rolfsii strain	Rotting at inoculated Apical region of cormel		Rotting at inoculated Basal region of cormel	
	Extent of Rot (mm)±S.E		Extent of Rot (mm)±S.E	
	Diameter	Depth	Diameter	Depth
XLL	8.4±0.05a	3.4±0.03b	12.8±0.09a	4.0±0.03a
XA1	9.3±0.08c	3.0±0.03a	17.7±0.07c	4.9±0.03b
XA2	13.3±0.06d	5.0±0.04d	18.8±0.09d	6.5±0.04c
EL1	8.8±0.06b	3.7±0.03c	15.1±0.08b	4.8±0.03b
EL2	8.7±0.05b	3.0±0.03a	15.3±0.11b	4.9±0.03b
Control	0.0±0.0e	0.0±0.0e	0.0±0.0e	0.0±0.0d

By the calculated confidence limits at 95%, means in vertical rows bearing the same letters are not significantly different.

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TABLE 19 ROTTING OF WOUND - INOCULATED CORMELS OF 'AMANKANI FUFUO' BY THE FIVE STRAINS OF *S. ROLFSII* AT 30°C IN 8 DAYS.

S. rolfsii strain	Rotting at inoculated Apical region of cormel		Rotting at inoculated Basal region of cormel	
	Extent of Rot (mm)±S.E		Extent of Rot (mm)±S.E	
	Diameter	Depth	Diameter	Depth
XLL	11.5±0.05b	3.8±0.03b	17.1±0.11c	4.6±0.03bc
XA1	12.9±0.09d	4.1±0.03c	17.2±0.09c	4.8±0.04c
XA2	14.2±0.44e	4.4±0.02d	18.0±0.08d	5.8±0.04d
EL1	11.0±0.07a	3.9±0.03b	12.7±0.10a	4.8±0.03a
EL2	12.4±0.07c	3.0±0.02a	14.6±0.08b	4.4±0.03b
Control	0.0±0.0f	0.0±0.0e	0.0±0.0e	0.0±0.0e

By the calculated confidence limits at 95%, means in vertical rows bearing the same letters are not significantly different.

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TABLE 20 ROTTING OF WOUND - INOCULATED CORMELS OF 'AMANKANI KYIREPE' BY THE FIVE STRAINS OF *S. ROLFSII* AT 30°C IN 8 DAYS.

S. rolfsii strain	Rotting at inoculated Apical region of cormel		Rotting at inoculated Basal region of cormel	
	Extent of Rot (mm)±S.E		Extent of Rot (mm)±S.E	
	Diameter	Depth	Diameter	Depth
XLL	9.8±0.06a	3.4±0.03a	13.4±0.06a	4.8±0.04c
XA1	10.8±0.06c	4.1±0.04b	16.4±0.09c	5.0±0.03c
XA2	11.8±0.08d	3.9±0.02b	15.6±0.09b	4.5±0.03b
EL1	10.1±0.08ab	3.9±0.03b	15.3±0.07b	4.5±0.03b
EL2	10.4±0.07b	3.9±0.03b	13.6±0.08a	4.9±0.04a
Control	0.0±0.0e	0.0±0.0c	0.0±0.0d	0.0±0.0d

By the calculated confidence limits at 95%, means in vertical rows bearing the same letters are not significantly different.

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TABLE 21 ROTTING OF WOUND - INOCULATED CORMELS OF 'AMANKANI PA' BY THE FIVE STRAINS OF *S. ROLFSII* AT 30°C IN 8 DAYS.

S. rolfsii strain	Rotting at inoculated Apical region of cormel		Rotting at inoculated Basal region of cormel	
	Extent of Rot (mm)±S.E		Extent of Rot (mm)±S.E	
	Diameter	Depth	Diameter	Depth
XLL	10.4±0.03a	4.3±0.04c	12.7±0.20b	6.0±0.10c
XA1	14.7±0.20d	6.0±0.10d	18.1±0.12d	6.8±0.07c
XA2	13.0±0.07c	4.3±0.04c	18.1±0.20d	6.8±0.10d
EL1	10.9±0.05b	4.0±0.03b	14.4±0.07c	5.4±0.04b
EL2	10.7±0.20ab	3.4±0.03a	11.8±0.08a	4.1±0.04a
Control	0.0±0.0e	0.0±0.0e	0.0±0.0e	0.0±0.0e

By the calculated confidence limits at 95%, means in vertical rows bearing the same letters are not significantly different.

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TABLE 22 ROTTING OF WOUND - INOCULATED CORMELS OF *COLOCASIA ANTIQUORUM* BY THE FIVE STRAINS OF *S. ROLFSII* AT 30°C IN 8 DAYS.

S. rolfsii strain	Rotting at inoculated Apical region of cormel		Rotting at inoculated Basal region of cormel	
	Extent of Rot (mm)±S.E		Extent of Rot (mm)±S.E	
	Diameter	Depth	Diameter	Depth
XLL	11.2±0.07b	3.5±0.03a	17.0±0.07b	4.9±0.03c
XA1	13.0±0.07d	3.4±0.04a	17.5±0.09c	4.4±0.05b
XA2	17.2±0.07e	4.4±0.03b	21.0±0.11d	6.0±0.03d
EL1	10.5±0.06a	3.6±0.04a	16.2±0.11a	4.6±0.05a
EL2	12.6±0.09c	3.5±0.03a	17.1±0.06b	4.6±0.04a
Control	0.0±0.0f	0.0±0.0c	0.0±0.0e	0.0±0.0e

By the calculated confidence limits at 95%, means in vertical rows bearing the same letters are not significantly different.

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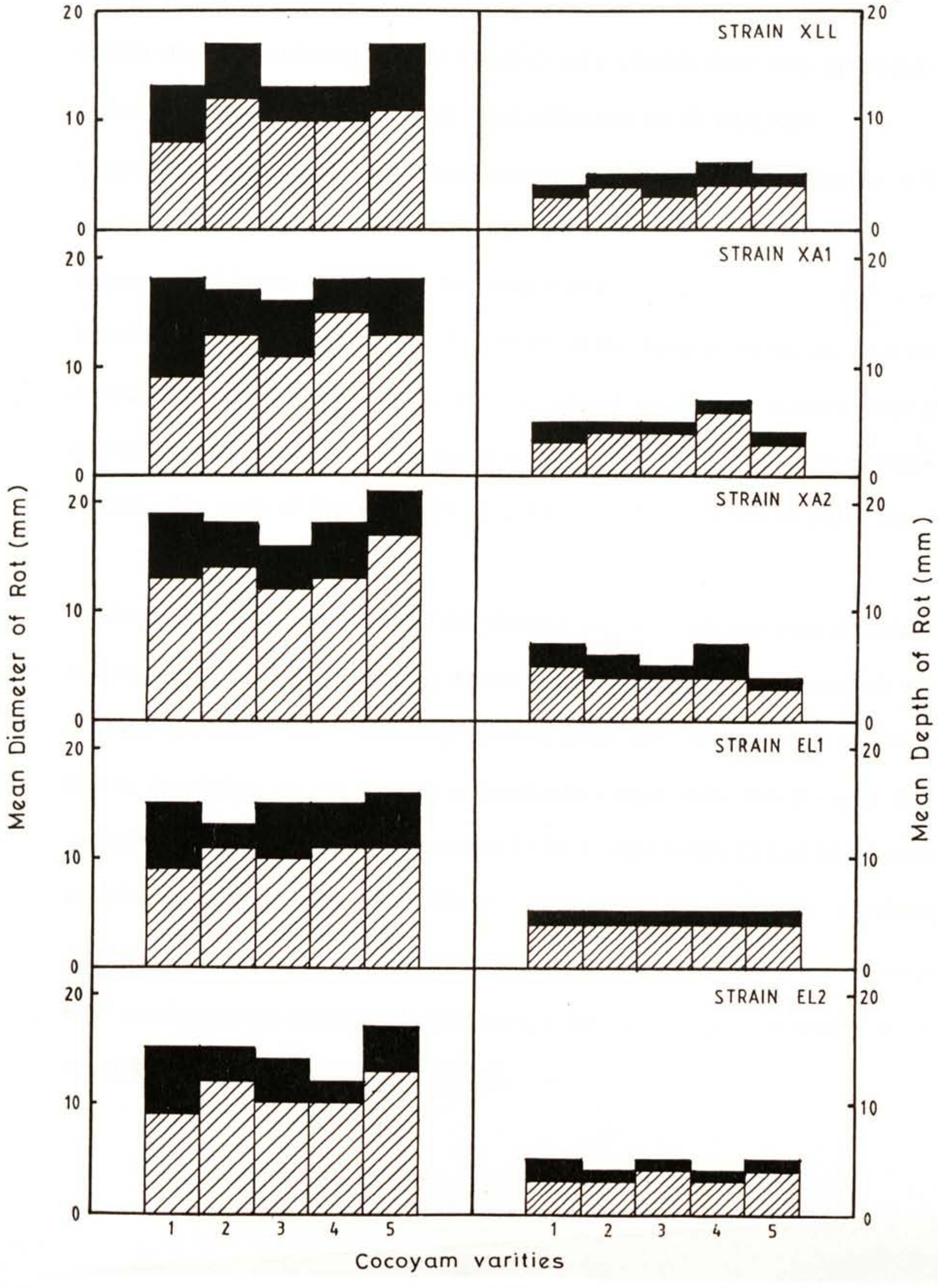

FIG.6 Rotting at apical region(▨)and basal region (■) of wound - inoculated cormels of cocoyam varieties* by the five strains of S. rolfsii at 30°C for 8 days;
*1, X. sagittifolium var. 'Amankani fitaa'
2, X. sagittifolium var. 'Amankani fufuo'
3, X. sagittifolium var. 'Amankani kyirepe'
4, X. sagittifolium var. 'Amankani pa'
5, Colocasia antiquorum.

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EXPERIMENT 11

DEGRADATION OF BLOCKS OF TISSUES OF CORMELS OF THE DIFFERENT COCOYAM VARIETIES BY THE FIVE STRAINS OF *S. ROLFSII*

Experiment 10 measured both diameter and depth of rots caused by the different *S. rolfsii* strains in wound-inoculated cormels. Experiment 11 which determined the extent of degradation of blocks of tissues of the cormels embodied the two activities - that is - lateral extent and depth of the rot. The results of the two experiments are, therefore, comparable to some extent. Blocks of tissues placed, on *S. rolfsii* cultures (Plate 15) provided greater surface area to volume ratio to the hyphae than in the usual inoculation method. The result of Experiment 11 are presented in Table 23 and 24 and in Fig. 7.

Blocks of tissues of the cormels of the different cocoyam varieties were degraded at different rates. As in Experiment 10, the blocks from the basal half of the cormels were degraded to a greater extent than those from the apical half. Differences in percentage loss in dry weight by the 8th day of incubation ranged from 2.0 per cent in *X. sagittifolium* var. 'Amankani fitaa' inoculated with *S. rolfsii* Strain XLL to 14.2 per cent in *Colocasia antiquorum* inoculated with Strain XLL (Table 24). Generally, considering degradation of both apical and basal regions, blocks of *X. sagittifolium* var. 'Amankani fitaa' lost the greatest dry weight and *X. sagittifolium* var 'Amankani Kyirepe' the least especially by the activity of Strains XA1, EL1 and EL2.

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Table 23 **DEGRADATION OF TISSUES OF THE APICAL AN**
COCOYAM VARIETIES BY THE DIFFERENT *S. RO*

S. rolfsii strains	Period of Incubation	Weight loss (g) out of original mea the two regions of cormels of *Xa*				
		'Amankani fitaa'		'Amankani fufuo'		'
		Apical (0.52)	Basal (0.46)	Apical (0.52)	Basal (0.47)	A (
XLL	2	0.06±0.01	0.07±0.01	0.06±0.01	0.06±0.01	0
	4	0.15±0.01	0.16±0.01	0.08±0.02	0.08±0.01	0
	6	0.20±0.01	0.21±0.01	0.09±0.01	0.11±0.01	0
	8	0.22±0.00	0.23±0.01	0.15±0.02	0.18±0.01	0
XA1	2	0.06±0.01	0.09±0.01	0.08±0.00	0.09±0.00	0.
	4	0.10±0.01	0.13±0.01	0.08±0.01	0.09±0.01	0.
	6	0.19±0.01	0.20±0.01	0.13±0.01	0.15±0.00	0.
	8	0.22±0.01	0.24±0.01	0.19±0.0	0.21±0.01	0.
XA2	2	0.05±0.01	0.08±0.00	0.09±0.01	0.10±0.01	0.
	4	0.07±0.01	0.12±0.01	0.15±0.01	0.14±0.01	0.
	6	0.17±0.01	0.19±0.01	0.16±0.01	0.18±0.00	0.
	8	0.22±0.01	0.25±0.00	0.19±0.00	0.21±0.00	0.
EL1	2	0.08±0.00	0.09±0.00	0.07±0.00	0.09±0.00	0.
	4	0.10±0.00	0.13±0.01	0.12±0.01	0.14±0.00	0.
	6	0.19±0.01	0.20±0.01	0.17±0.01	0.19±0.01	0.
	8	0.23±0.00	0.24±0.01	0.21±0.00	0.23±0.00	0.
EL2	2	0.07±0.00	0.08±0.01	0.08±0.01	0.09±0.01	0.
	4	0.08±0.00	0.10±0.01	0.14±0.01	0.13±0.00	0.
	6	0.16±0.01	0.18±0.01	0.19±0.01	0.21±0.00	0.
	8	0.23±0.02	0.24±0.01	0.23±0.02	0.24±0.01	0.

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BASAL REGIONS OF THE CORMELS OF THE DIFFERENT
.FSII STRAINS AT 30°C OVER 8 DAYS

dry weight (g) in parenthesis by *thosoma sagittifolium* variety				Weight loss(g) out of original mean dry weight (g) in parenthesis by the two regions of cormels of *Colocasia antiquorum*	
mankani Kyirepe'		'Amankani pa'			
oical	Basal	Apical	Basal	Apical	Basal
54)	(0.48)	(0.52)	(0.49)	(0.52)	(0.46)
)5±0.01	0.06±0.01	0.06±0.02	0.08±0.01	0.06±0.01	0.07±0.00
)7±0.01	0.09±0.01	0.10±0.00	0.12±0.01	0.09±0.01	0.12±0.00
3±0.01	0.14±0.02	0.16±0.01	0.18±0.01	0.17±0.00	0.21±0.01
6±0.01	0.18±0.01	0.18±0.02	0.19±0.02	0.18±0.01	0.22±0.01
)8±0.01	0.09±0.00	0.07±0.02	0.09±0.01	0.08±0.02	0.09±0.01
0±0.01	0.11±0.02	0.09±0.01	0.12±0.01	0.10±0.00	0.13±0.01
2±0.02	0.14±0.01	0.15±0.01	0.19±0.01	0.15±0.02	0.16±0.02
7±0.01	0.18±0.01	0.17±0.01	0.20±0.02	0.17±0.01	0.20±0.01
)7±0.01	0.08±0.01	0.08±0.01	0.09±0.01	0.08±0.01	0.10±0.01
2±0.01	0.14±0.02	0.13±0.02	0.13±0.03	0.12±0.01	0.16±0.01
4±0.02	0.17±0.01	0.15±0.03	0.17±0.01	0.19±0.01	0.22±0.02
9±0.01	0.21±0.01	0.18±0.01	0.20±0.01	0.23±0.01	0.26±0.01
4±0.01	0.05±0.01	0.06±0.01	0.08±0.01	0.07±0.01	0.09±0.01
0±0.02	0.10±0.01	0.09±0.01	0.11±0.02	0.10±0.01	0.12±0.02
0±0.00	0.12±0.00	0.14±0.01	0.16±0.01	0.12±0.01	0.18±0.02
8±0.02	0.19±0.02	0.19±0.01	0.23±0.01	0.22±0.01	0.23±0.01
8±0.03	0.07±0.01	0.06±0.01	0.07±0.01	0.05±0.01	0.08±0.01
9±0.02	0.10±0.03	0.08±0.01	0.10±0.01	0.08±0.01	0.10±0.02
2±0.03	0.11±0.01	0.16±0.02	0.19±0.02	0.10±0.01	0.14±0.02
5±0.01	0.16±0.01	0.19±0.01	0.21±0.01	0.16±0.01	0.18±0.01

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Table 24 **DEGRADATION OF TISSUES OF THE APICAL ANI COCOYAM VARIETIES BY THE DIFFERENT *S. R(***

S. rolfsii strains	Period of Incubation	% Weight loss (calculated from data in Table 23) of the			
		'Amankani fitaa'		'Amankani fufuo'	
		Apical	Basal	Apical	Basal
XLL	2	11.5	15.6	11.1	12.2
	4	28.3	34.8	14.8	17.4
	6	38.5	45.7	17.0	24.0
	8	44.0	46.0	30.0	40.0
XA1	2	11.3	19.6	14.8	18.8
	4	19.2	27.7	15.1	19.6
	6	35.8	44.4	26.0	30.6
	8	42.3	52.2	37.3	46.7
XA2	2	9.6	17.4	17.3	22.2
	4	13.2	26.7	28.8	31.1
	6	34.5	41.3	30.8	40.0
	8	43.1	54.3	38.0	47.7
EL1	2	15.1	20.0	13.0	19.6
	4	19.2	27.7	23.1	31.0
	6	35.8	43.5	32.7	41.3
	8	45.1	52.2	38.9	51.1
EL2	2	13.2	16.7	15.1	20.0
	4	26.7	22.2	26.4	28.3
	6	30.2	40.0	35.8	45.7
	8	42.6	51.1	42.6	52.2

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SAL REGIONS OF THE CORMELS OF THE DIFFERENT
***II* STRAINS AT 30°C OVER 8 DAYS**

regions of cormels of *Xanthosoma sagittifolium* variety				% Weight loss (calculated from data in Table 23) of the two regions of cormels of *Colocasia antiquorum*	
'Amankani Kyirepe'		'Amankani pa'			
Apical	Basal	Apical	Basal	Apical	Basal
9.3	12.5	11.1	16.3	11.8	14.6
12.5	19.1	19.2	25.5	17.3	25.5
23.6	29.2	30.2	37.5	33.3	44.6
32.0	40.0	34.6	38.8	34.6	48.8
15.7	19.1	13.5	18.0	15.4	18.8
18.2	23.9	17.0	25.0	18.9	28.3
21.8	30.0	28.8	39.6	28.8	35.6
30.9	37.5	33.3	40.0	32.1	42.6
12.7	16.7	15.7	18.0	15.1	22.2
21.4	29.2	25.0	27.7	23.1	33.3
25.9	33.3	29.4	34.7	35.8	48.9
34.5	44.7	34.6	40.8	43.4	56.5
7.5	10.4	11.8	16.7	13.2	18.8
18.2	20.8	17.6	23.4	18.9	24.5
18.5	25.0	28.0	34.0	23.1	38.3
34.0	41.3	37.3	47.9	42.3	47.9
14.5	15.2	11.3	17.5	9.8	16.7
17.3	21.3	15.4	22.2	15.4	20.8
22.6	23.4	30.2	41.3	19.2	31.1
30.2	34.0	36.5	45.7	30.8	37.5

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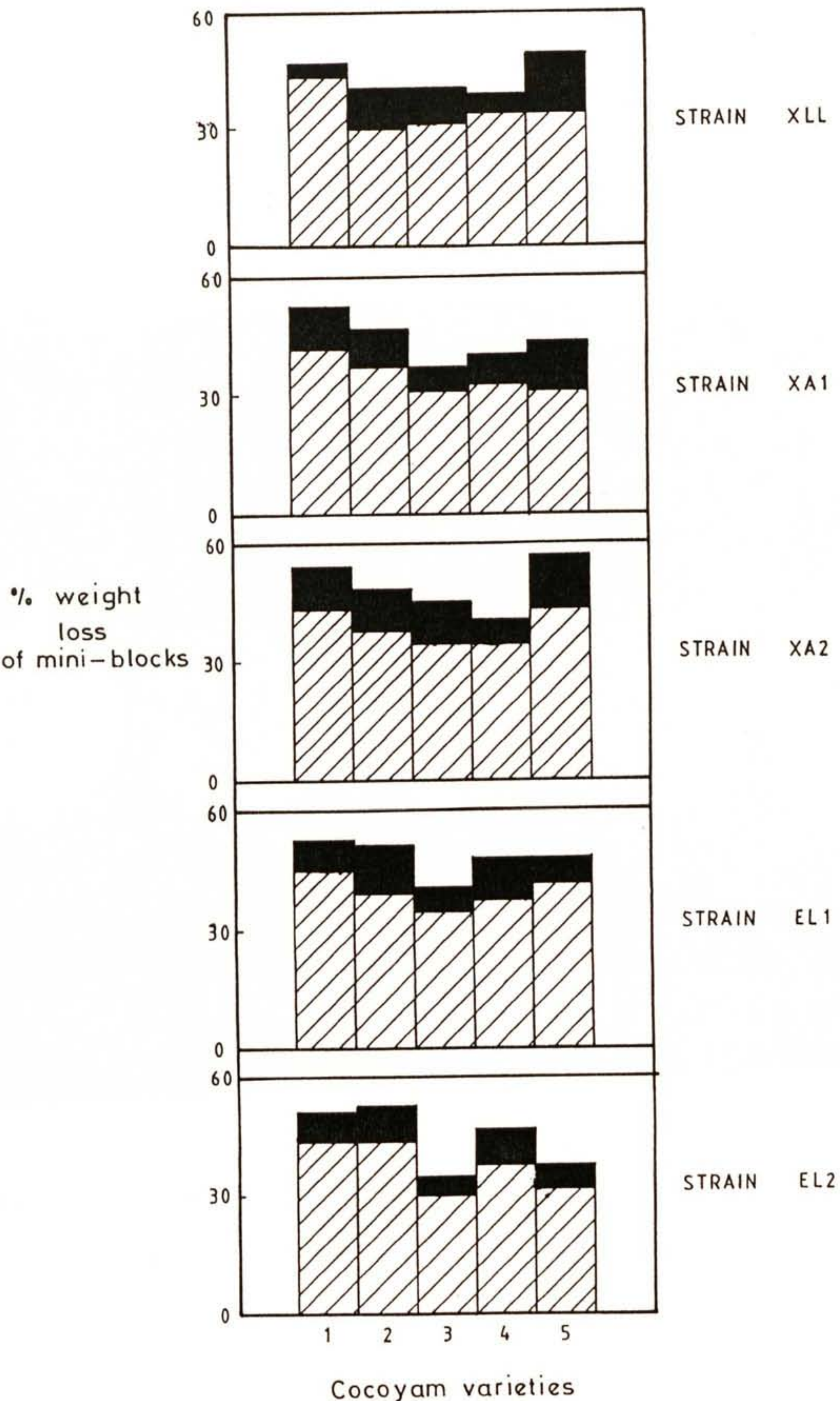

FIG. 7. Percentage weight loss of mini-blocks (10x10x5mm) of apical region (▨) and basal region (■) of cocoyam varieties* placed on cultures of the different S. rolfsii strains for 8 days.

*1, X. sagittifolium var. 'Amankani fitaa'
2, X. sagittifolium var. 'Amankani fufuo'
3, X. sagittifolium var. 'Amankani kyirepe'
4, X. sagittifolium var. ''

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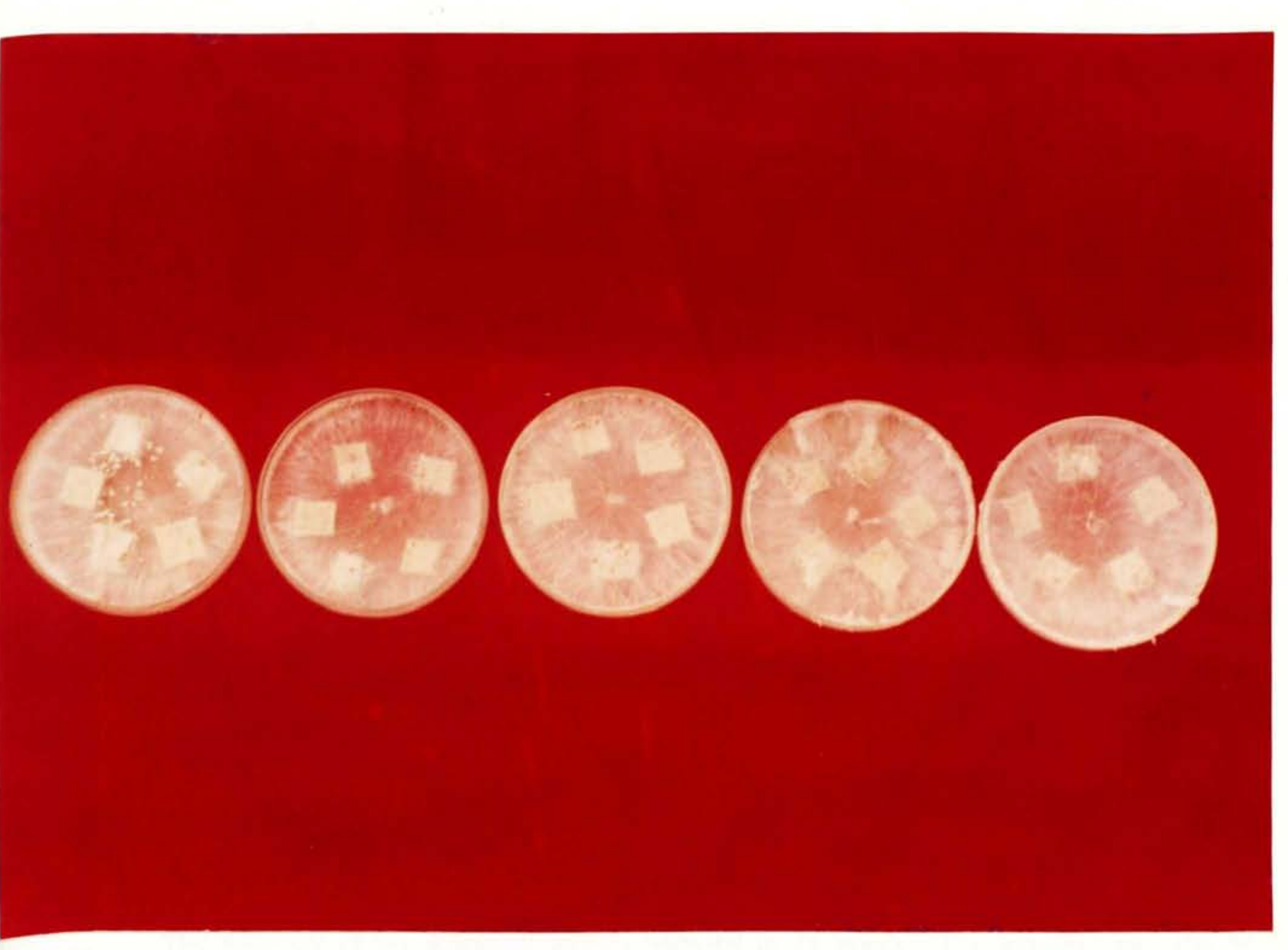

Plate. 15 Photograph showing mini-blocks of cormel of *Xanthosoma sagittifolium* variety 'Amankani fufuo' covered by mycelia of the different *S. rolfsii* strains after 8 days at 30°C (x 1/3).

(from left: Strains XLL, XA1, XA2, EL1, EL2)

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EXPERIMENT 12

FORMATION OF SCLEROTIA BY THE FIVE S. ROLFSII STRAINS GROWING ON CORMEL TISSUES OF THE DIFFERENT COCOYAM VARIETIES

The importance of the relationship between *S. rolfsii* strains and cocoyam varieties involves not only the susceptibility of the variety but also its role as substrate for sclerotium formation, and hence the survival of the strain. Plates 16, 17 and 18 show the sort of variation which was recorded in both growth and sclerotium formation in the *S. rolfsii* strain - cocoyam variety combinations. The various data collected are tabulated in Tables 25 and 26.

To summarise the major observations:

a. *S. rolfsii* Strains XLL and XA2 produced sclerotial initial earliest on the *X. sagittifolium* variety 'Amankani pa' blocks, while Strains XA1, EL1 and EL2 produced the sclerotial initials earliest on *Colocasia antiquorum*.

b. The longest time for the sclerotial initials of Strains XLL, XA1, EL1 and EL2 to appear were recorded in cultures on *X. sagittifolium* var. 'Amankani kyirepe'.

   The longest time taken by Strain XA2 to produce sclerotial initials was recorded on *X. sagittifolium* var. 'Amankani fufuo' and *Colocasia antiquorum*.

c. The majority of the sclerotial initials matured in 24 hours. The longest time was 36 hours by Strain XLL sclerotial initials on *X. sagittifoilium* var. 'Amankani fitaa' and Strain EL2 on *X. sagittifolium* var. 'Amankani fitaa' and *X. sagittifolium* var. 'Amankani kyirepe'.

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d. There was a significantly heavier sclerotium production on the *Colocasia antiquorum* blocks (plates 17 and 18).

The highest mean numbers of sclerotia on the blocks of *X. sagittifolium* varieties formed by Strains XLL, XA1, XA2, EL1 and EL2 were 105, 157, 183,, 311, and 251 respectively. The corresponding mean numbers on the blocks of *C. antiquorum* were 280, 870, 633, 976 and 1,023 respectively.

e. The mean volume of 100 mature sclerotia formed on *C. antiquorum* was either identical to that of sclerotia on the *X. sagittifolium* varieties (Strains XLL, XA1 and EL1) or smaller in some cases (Strains XA2 and EL2).

f. Sclerotia of Strains XLL and EL2 had greater germination capacity than those of Strains XA1, XA2 and EL1 (Table 26).

g. Sclerotia formed on the different cocoyam varieties did not germinate to the same degree. Sclerotia of Strains XLL, XA1 and EL2 formed on *X. sagittifolium* var. 'Amankani kyirepe' germinated better than those formed on the other cocoyam varieties, while the best substrate for Strains XA2 and EL1 was *X. sagittifolium* varieties 'Amankani fitaa' and 'Amankani fufuo' respectively.

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Table 25 **Formation of Sclerotia by the different *S. rolfsii* strains on**

S. rolfsii strain	*Xanthosoma* 'Amankani fitaa'			
		a. Mean Time (HRS) of first appearance of sclerotia b. Mean Time (HRS) after which melanin first developed	Mean No. of Sclerotia per block after 12 days	Mean volume (cm^3) of 100 mature scleroti:
XLL	a.	144±9.8	80±8.2	0.20±0.0
	b.	36±6.9		
XA1	a.	152±13.8	120±7.8	0.15±0.0
	b.	32±6.9		
XA2	a.	132±6.9	137±22.8	0.15±0.0
	b.	24±0.0		
EL1	a.	150±11.5	159±14.2	0.15±0.0
	b.	30±6.0		
EL2	a.	144±9.8	181±11.3	0.18±0.0
	b.	36±6.9		

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on blocks of tissue of cormels of the different cocoyam varieties at 30°C

na *sagittifolium* variety				
		'Amankani fufuo'		
ıe) ·otia	a. b.	Mean Time (HRS) of first appearance of sclerotia Mean Time (HRS) after which melanin first developed	Mean No. of Sclerotia per block after 12 days	Mean volume (cm³) of 100 mature sclerotia
	a.	150±6.0	70±12.9	0.20±0.0
	b.	24±0.0		
	a.	150±6.0	157±16.1	0.15±0.0
	b.	24±0.0		
	a.	138±11.4	128±7.2	0.18±0.0
	b.	24±0.0		
	a.	138±11.4	160±10.6	0.15±0.0
	b.	24±0.0		
	a.	138±6.0	240±20.5	0.20±0.0
	b.	24±0.0		

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°C

Xanthosoma sagittifolium v

'Amankani kyirepe'				'Amankani pa'	
a.	Mean Time (HRS) of first appearance of sclerotia	Mean No. 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			b.	Mean Time (HRS) after which melanin first developed
a.	156±6.9	100±31.7	0.20±0.0	a.	132±6.9
b.	24±0.0			b.	24±0.0
a.	168±12.0	152±23.1	0.18±0.0	a.	126±6.0
b.	24±0.0			b.	24±0.0
a.	128±6.9	183±15.9	0.15±0.0	a.	126±0.0
b.	24±0.0			b.	24±0.0
a.	156±6.9	172±11.5	0.15±0.0	a.	132±6.9
b.	24±0.0			b.	24±0.0
a.	156±6.9	185±15.7	0.20±0.0	a.	132±6.9
b.	36±6.9			b.	24±0.0

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riety

			Colocasia antiquorum		
Mean No. of Sclerotia per block after 12 days	Mean volume (cm^3) of 100 mature sclerotia	a. b.	Mean Time (Hrs) of first appearance of sclerotia Mean Time (HRS) after which melanin first developed	Mean No. of Sclerotia per block after 12 days	Mean volume (cm^3) of 100 mature sclerotia
105±12.2	0.20±0.0	a.	144±9.8	280±20.2	0.20±0.0
		b.	24±0.0		
107±7.9	0.15±0.0	a.	120±0.0	870±82.3	0.15±0.0
		b.	24±0.0		
141±21.1	0.18±0.0	a.	138±11.4	633±50.4	0.15±0.0
		b.	24±0.0		
311±9.5	0.15±0.0	a.	120±0.0	976±67.1	0.15±0.0
		b.	24±0.0		
251±6.9	0.20±0.0	a.	126±6.0	1023±92.8	0.18±0.0
		b.	24±0.0		

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Table 26 **Percentage germination of sclerotia f different cocoyam varieties at 30°C (l**

Cocoyam variety	Period of Incubation (Hrs)	Percentage gei the differ	
		XLL	XA1
'Amankani fitaa'	12	88.7	33.4
	24	100	42.5
	36	100	60.0
'Amankani fufuo'	12	80.0	52.5
	24	95.0	76.3
	36	100	92.5
'Amankani Kyirepe'	12	87.5	66.3
	24	100	100
	36	100	100
'Amankani pa'	12	77.5	38.8
	24	97.5	56.3
	36	100	63.8
Colocasia antiquorum	12	28.8	41.3
	24	73.8	96.3
	36	95.0	100

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formed by *S. rolfsii* strains on the (Percentage germination on PDA plates)

ermination out of a total of 40 Sclerotia formed on erent cocoyam varieties by the *S. rolfsii* strain			
\1	XA2	EL1	EL2
4	76.3	77.5	82.5
5	86.3	85.0	96.3
0	92.5	88.8	97.5
5	56.3	80.0	76.3
3	72.5	100	100
5	92.5	100	100
3	68.8	81.3	87.5
	86.3	88.8	100
	91.3	93.8	100
3	52.5	83.8	85.0
	75.5	96.3	100
	82.5	100	100
	76.3	58.8	67.5
	77.5	80.0	87.5
	77.5	80.0	95.0

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PLATE 16. Photograph showing growth of *S. rolfsii* Strain EL2 and Strain XA2 on 30x30x5mm blocks of *Colocasia antiquorum* (left) and *Xanthosoma sagittifolium* variety 'Amankani fitaa' (right), respectively (x 2/3).

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PLATE 17. Photograph showing extent of sclerotium formation at 30°C in 8 days by *S. rolfsii* Strain EL2 and Strain XA2, growing on 30x30x5mm blocks of *Colocasia antiquorum* (left) and *Xanthosoma sagittifolium* variety 'Amankani pa' (right), respectively (x 8/9).

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PLATE 18. Photograph showing extent of sclerotium formation at 30°C in 8 days by *S. rolfsii* Strain EL1 and Strain XA1 growing on 30x30x5mm blocks of *Colocasia antiquorum* (left) and *Xanthosoma sagittifolium* variety 'Amankani pa' (right), respectively (x 8/9)

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V. DISCUSSION

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). Even though *S. rolfsii* attacks not less than 189 plant species (Weber, 1931) the possible existence of strains with specific hosts has not received sufficient attention. This is surprising as the hosts of *S. rolfsii* include dicotyledonous and monocotyledonous plants and ferns. Relevant reports have indicated that the fungus exists in a number of distinct strains. Isolates studied by Nakata (1927), Takahashi (1927), Epps, Patterson and Freeman (1951), Weber (1931) and Abeygunawardena and Wood (1957) differed sufficiently in morphology and physiology. Epps, Patterson and Freeman (1951) used the phenomenon of aversion to identify strains. This method was used in this study and the five isolates of *S. rolfsii* obtained from Achimota and Legon were identified as strains (see Fig. 4 and Plates 6-9). This showed that some morphological and physiological differences noticed among the five strains were reliable indicators.

Because of the lack of sufficient number of incubators to provide a range of temperatures, the optimum temperature for growth of *S. rolfsii* of 30°C reported by Higgins (1927), Abeygunawardena and Wood (1957) and Darkwa (1965) was adopted for the present investigation. The descending order of magnitude of growth of the five

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strains XLL, XA1, XA2, EL1, and EL2 was the same for mycelial extensional growth on PDA plates and mycelial dry weight in PDB. Strains EL1 and EL2 grew fastest on the PDA plates and produced the heaviest mycelia in PDB (See Tables 1 and 3). The greatest dry weights of the mycelia of the strains, all recorded after incubation for 6 days, were, however, not statistically different (see Table 3). Furthermore, autolysis practically proceeded at the same rate in the broth medium and the amount of mycelia broken down by autolysis in 6 days after the peak growth had been achieved was less than 20 percent of the maximum mycelial weight in all strains (see Fig. 1). It has always been appreciated that the weight of mycelium gave the better measure of growth, and the results of the PDB experiment showing statistically similar peak dry weights, could be used to compare growth of the strains instead of growth on the PDA plates.

There were, however, significant differences in sclerotial production and the number, weight and size of sclerotia formed could be used to distinguish the strains. Strain XLL stood out clearly among the strains. It formed the smallest number of sclerotia, but the sclerotia were statistically the largest and heaviest (see Table 2). This relationship between the number of sclerotia on one hand and the size and weight on the other hand was noteworthy. If the larger sclerotia would produce more emerging hyphae on germination than the smaller ones, the greater number of hyphae would compensate the smaller number of sclerotia during establishment of Strain XLL in the habitat. Abeygunawardena and Wood (1957) noticed somewhat similar distinguishing sclerotial features in two strains they studied. Strain SR4 was isolated from tomato plants in Sri

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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.

There were also significant differences in mycelial dry weights when the fungi were supplied with different carbon and nitrogen compounds. Strain EL2 consistently grew better in all the media than the rest, with Strain EL1 a close second. All the values of Strain EL2 were significantly greater than the corresponding values of Strians XLL, XA1, and XA2 (see Tables 4-8 and Fig. 2). It seemed possible that the original hosts of the strains had an influence on their physiological characteristics. Those from *Elaeis guineensis* fruits grew better than strains isolated from *Xanthosoma sagittifolium*.

Cellulolysis by the different strains was not directly related to the ability to utilize the other carbon compounds. Weight loss determinations of cellulolysis using filter paper showed that the lowest cellulose breakdown occurred in the XLL, EL1, and EL2 culture vessels (see Table 14). 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 reactions 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 (see Table 15 and Fig.3). Many fungi in relevant studies had

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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* isolates 1, 2 and 3,respectively. 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.

Root crop is a term used by agriculturists for all sorts of plant products which are formed in the soil. Root crops include stem tubers such as Irish potato and yam (*Dioscorea* spp.), root tubers of cassava (*Manihot esculenta* Crantz), tiger nut (*Cyperus esculentus*), sugar beet (*Beta vulgaris*) and sweet potato (*Ipomoea batata* Poir.), rhizomes of ginger (*Zingiber officinale*) and cormels of cocoyam etc. They have very high moisture content like succulent fruits and vegetables and are together referred to as perishables. The internal high moisture content allows active enzymatic action which leads to tissue degradation. In addition, the high atmospheric temperatures and relative humidities support rapid growth and metabolism of fungi and bacteria which attack the products and cause rotting.

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Some of the organisms responsible for the rotting infect the products after harvest. Others, on the other hand, are carried over from the field, and referred to as field infection. Field infection is the more serious of the two as it is responsible for both pre-harvest and post-harvest losses. Cormels of cocoyam and their associate rot fungus, *S. rolfsii*, belong to this category. There has been hitherto, no studies on the susceptibility of the various cocoyam varieties cultivated in Ghana to this widespread soil facultative parasite. Relevant studies have been duly carried out on the pathogenicity of the five *S. rolfsii* strains on four varieties of *Xanthosoma sagittifolium* ('Amankani fitaa', 'Amankani fufuo', 'Amankani kyirepe' and 'Amankani pa') and *Colocasia antiquorum*.

Although *S. rolfsii* belongs to the sub-division Deuteromycotina, it does not form conidia in nature. In the absence of conidia, healthy cormels are normally attacked by actively growing mycelium in the soil. There are two ways by which cormels could be infected. The mycelium may penetrate the epidermis of the petioles and grow in the petiole and subsequently invade the cormel. The mycelium may also enter the cormels through wounds. Plates 12, 13 and 14 show the progressive invasion of the petioles of the growing cocoyam plants. Tables 18 to 22 also clearly indicate the rotting of wound - inoculated cormels of all the five cocoyam varieties by the five *S. rolfsii* strains.

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Strain XA2 caused the greatest rot followed by Strain XA1, and the least damage was caused by Strains XLL and EL2. The rate of growth of a strain in culture alone could not determine the extent of rot it could cause. For, in the experiments on growth of the strains, Strain EL2 gave the highest values of mean mycelial dry weight in Potato Dextrose Broth (see Table 3 and Fig. 1) and it utilized best the carbon and nitrogen sources (see Table 4 and 5, and Fig. 2). It also had the greatest mean mycelial dry weight in some of the Glucose-Pectin media (see Table 8). The activity of the fungi in the cormels was certainly influenced by a combination of factors which deserve future studies. Another supporting evidence for this view is that the culture filtrate of Strain XA2 which caused the greatest rot did not show high Enzyme Activity (see Tables 9-12).

The probability that factors in infected tissues could alter observations in pure cultures had been observed by many workers. Cole and Wood (1961), for example found that there were more compounds in apple fruits rotted by *Botrytis cinerea, Penicillium expansum*, *Pyrenochaeta furfuracea* and *Sclerotinia fructigena* than in sound apples. There were greater number of these compounds in extracts of apple rotted by *P. expansum* and *P. furfuracea* than in extracts of rots produced by *B. cinerea*. This shows that tissues rotted by different fungi, and also different strains, would contain different products from the break-down of polysaccharides which may have different effects on further activity of the rotting organisms.

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The course of events in the rotting process of plant tissues by facultative parasites is well known. The parasite begins to grow into healthy plant tissue and starts to produce pectinesterase and chain-splitting enzymes. The latter cause some degradation of cell wall pectic substances and the pectinesterase de-esterifies some of the high molecular-weight pectinases to form pectates. The cells of the host are killed, possibly as a result of the action of pectic enzymes on the cell walls, and phenols are brought together with host and parasite phenolases to form coloured oxidative products some of which inactivate the chain-splitting enzymes (Wood, 1967).

In this investigation the rot did not extend uniformly from the inoculated spot. The rot was wider near the exterior of the cormel and lesser penetration inwards as shown by the data in Tables 18 to 22 and in Fig. 6. Because of the nature of the rot, it was not possible to calculate the linear rate of advance of the strains through the cormels. Gregory and Horne (1928) working with apple fruits which are spherical derived the relation

$\underline{V} = (y/R)^3 (8-3y/\underline{R}) /16$

between $\underline{V}$ the volume of the rot as a proportion of the volume of the apple, $\underline{R}$ the radius of the apple and y the radius of the rot.

The slower growth of the fungi into the inner regions of the cormels might be due to decreasing oxygen concentration with increasing depth.

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Different amounts of oxidative products might have been formed in the rotting cormels of the different varieties resulting in difference in their susceptibility. Cormels of *Colocasia antiquorum* were the most susceptible and the greatest extent of rot occurred when they were inoculated especially with Strains XA2 and XA1 (see Table 22). Among the *X. sagittifolium* varieties, 'Amankani fitaa' and 'Amankani kyirepe' rotted to a lesser extent than 'Amankani fufuo' and 'Amankani pa' (see Tables 18-21). No variety could, however, be regarded as resistant. The cocoyam varieties could be considered as showing varying degrees of vulnerability.

Taubenhaus (1919) and Weber (1931) isolated cultures of *S. rolfsii* from a number of different crops, and cross-inoculated from one crop to another. Their results indicated that the original cultures varied only slightly in their virulence on any given crop regardless of the plant from which the cultures were originally isolated. No significant differences among isolates were indicated. In another study of Epps, Patterson and Freeman (1951), all four strains of *S. rolfsii* tested on 12 crops infected all the plants. The relative degree of susceptibility of the crops to the four strains varied only slightly. It was stated that all four were capable of killing essentially all the plants if vigorous inoculum was used. They furthermore reported that between two strains B and R, B strain was slightly more virulent than the R strain, but the difference was inconsistent and occasionally in the other direction. The results of the present investigation provide another instance of the nature of strains of *S. rolfsii*.

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This view was supported by studies on the degradation of mini-blocks, measuring 10x10x5mm of the different cocoyam varieties placed on mycelia of the different *S. rolfisii* strains. In that experiment the hyphae had greater access to the cormel tissues and oxygen concentrations was not restricted. The varieties did not exhibit the same order of susceptibility as was observed in the wound-inoculation experiments. *X. sagittifolium* variety 'Amankani kyirepe' again consistently showed the smallest loss in mean tissue dry weight (see Table 24). However, in contrast to the trend in the wound - inoculation experiments, *C. antiquorum* showed a lesser loss in mean dry weight than *X. sagittifolium* variety 'Amankani fitaa' - a less susceptible variety in the earlier experiment. Indeed, this variety showed the greatest loss in mean dry weight (see Table 24).

Infection of the 3-month old cocoyam plants also showed a different trend. This maybe due to the fact that the course of infection was different.
Mycelium from the soil would first form infection cushions on the petioles, followed by penetration and invasion of the tissues. The length of period for infection to occur will depend on the efficiency of breaching the epidermis and the rate of growth and activity in the petioles. These processes occurred fastest in *X. sagittifolium* variety, 'Amankani fitaa', and slowest in 'Amankani fufuo' and 'Amankani pa' varieties (see Table 17). This shows that an assessment of the degree of susceptibility of the different cocoyam varieties to *S. rolfsii* should be based on the various aspects of the disease.

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Although the five cocoyam varieties are close in susceptibility, some are likely to play a more significant role in the survival of *S. rolfsii* in the field than others. *Colocasia antiquorum* 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. sagittifolium* varieties ranged from 80 to 311, compared to a range of 280 to 1023 sclerotia per block of *C. antiquorum* (see Table 25 and Plates 17 and 18).

Also, among the *S. rolfsii* strains, Strain EL2 was the most prolific, confirming the results in Table 2, with a mean range of 181 to 1023 sclerotia per block, and Strain XLL the least productive with a range of 70 to 280 per block (see Table 23). Sclerotium production should naturally be influenced by compounds in the cormels, because glucose and other compounds have been found to influence sclerotium formation in *S. rolfsii* (Abeygundawardena and Wood, 1957; Wheeler and Sharan, 1965). It is likely that *C. antiquorum* cormel was a more favourable substrate. The constituents of the cormels of the varieties should be investigated in future studies to verify this hypothesis.

In conclusion, there are strains of *S. rolfsii* with distinctive characteristics in Ghana. Any studies concerning the species must involve as many strains as possible to provide a balanced view of events. The five strains were not widely different in their pathogenicity and it is reasonable to suggest that the five cocoyam varieties tested will be vulnerable to many *S. rolfsii* strains in Ghana. In order to safeguard the stability of

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the soil ecosystem it is proposed that cultural practices should be used and they could be relied upon to prevent catastrophic epidemics. The disease is easily detectable either by the death of the large leaves or by the presence of the conspicuous clusters of sclerotia on the infected plants. Infected plants should be removed and destroyed, the top soil in the area should be dug in, and after harvesting, the thrash should be burnt. Since most parts of the plant is eaten very little thrash is left behind and its disposal is less formidable than it would seem. In addition to these field practices only sound cormels should be stored so that the crop of the next planting season would be raised with clean materials.

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VI SUMMARY

1(a). Five strains of *Sclerotium rolfsii*: XLL, XA1, XA2, EL1 and EL2 were studied.

(b). The five Strains inoculated onto PDA plates could be separated into three categories as fast-growing, Strains EL1 and EL2, covering the plates in 72 hours; slow-growing, Strain XA2, covering the plates in 96 hours; and the intermediate group, Strains XLL and XA1, covering the plates in 84 hours. All the cultures had similar appearance.

2(a). In Potato Dextrose Broth peak mean mycelial dry weight was achieved by all the strains in 6 days. Although Strain EL2 had the highest mean mycelial dry weight after 6 days of incubation, the mean mycelial dry weights of all the strains were found not to be statistically different.

(b). Autolysis proceeded after the 6th day of incubation at different rates according to the strains in the descending order of EL2, EL1, XA1, XA2, and XLL.

(c). The pH of the media drifted from an initial of pH 5.3 - 6.1 to the acidic side of pH 2.4 - 3.3 during growth of the strains.

3(a). The five strains could be distinguished by the number of sclerotia formed, and by the size and weight of the sclerotia.

(b). Strain XLL produced significantly fewer sclerotia of 185 per Petri plate whereas Strains XA1, XA2, EL1, and EL2 formed from 324 to 364 sclerotia per Petri plate.

(c). On the other hand Strain XLL had the largest sclerotia (mean diameter of 84.6μm and the heaviest sclerotia (mean weight of 100 sclerotia of 27.8×10^{-2}mg). The corresponding values for the remaining four strains ranged from 58.3 to 76.9μm and 21.7×10^{-2} to 26.0×10^{-2}mg.

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4(a). The five strains could also be distinguished by their ability to utilize different carbon compound. Taking all the carbon sources - fructose, glucose, maltose, starch and sucrose - together, the strains could be arranged in the following descending order of utilization: EL2 > EL1 > XLL > XA1 > XA2, on the basis of the mean dry weights of the mycelia.

(b). Starch was least used by all the strains, followed by fructose.

(c). Glucose, maltose and sucrose were best used by each strain to almost the same degree.

(d). Maltose and sucrose were the best two carbon sources for growth of Strains XA1, EL1 and EL2.

(e). Glucose and sucrose were the best two carbon sources for growth of Strain XLL.

(f). Maltose followed by glucose and sucrose were the best carbon sources for growth of Strain XA2.

5(a). The order of response of the five *S. rolfsii* strains to the five Nitrogen sources - Ammonium chloride, Ammonium nitrate, Asparagine, Peptone and Sodium nitrate - was as observed with the carbon sources.

(b). Strains XLL, XA1, XA2 and EL2 grew best in the Peptone - medium, while Strain EL1 grew to the same extent in Ammonium chloride, Ammonium nitrate, Sodium nitrate and Peptone media.

(c). Ammonium chloride and Sodium nitrate were least used by Strains XLL, XA1, and XA2.

(d). Strain EL1 recorded smallest mean mycelium dry weight in the Asparagine medium.

(e). Strain EL2 recorded smallest mean mycelium dry weight in the Sodium nitrate medium.

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6(a). 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 to 2.0 per cent.

(b). At glucose concentrations of 1.0, 1.5 and 2.0 per cent, Strain EL2 produced the highest mean mycelial dry weight although not statistically different from the amount produced by some of the other strains.

(c). Strain XA2 produced the least mean mycelial dry weight, at all glucose concentrations, although the difference between that and those of some of the strains was not statistically significant.

7(a). The mean dry weight of mycelium formed in 6 days by each strain increased with increasing pectin concentration from 0.5 to 2.0 per cent.

(b). The lowest mean mycelial dry weights at all pectin concentrations were produced by Strains XA1 and XA2.

8(a). Glucose - Pectin combination of 0.5% Glucose and 0.5% Pectin supported the greatest growth in Strains XLL, XA1, XA2 and EL2.

(b). The greatest growth in Strain EL1 occurred in combination of 0.25% Glucose and 0.75% Pectin.

9(a). The 2.0% Glucose medium culture filtrates of all the strains produced in a tissue maceration method using tuber of Irish potato, the greatest Enzyme Activity ranging from 6.8 to 8.4 compared to the low values of 1.6 to 2.2 at the lower glucose concentrations.

(b). At 2.0% Glucose concentration, Strain EL2 produced the greatest pectolytic Enzyme Activity and Strains XA1 and EL1 the lowest.

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10. The 2.0% Glucose medium culture filtrates of all the strains also produced the highest pectolytic Enzyme Activity ranging from 11.7 to 13.3 when pericarp tissue of Cucumber was used. The pectolytic Enzyme Activity of filtrates of 0.5 to 1.5% glucose filtrates of 0.5 to 1.5% glucose concentration ranged from 2.9 to 6.4.

11. The strains could be separated into two group at any glucose concentration: (i) Strains XLL, XA1 and EL2 which showed higher pectolytic Enzymes Activity and (ii) Strains XA2 and EL1 which had lower pectolytic Enzyme Activity.

12. In pectin media, the greatest pectolytic Enzyme Activity occurred in filtrates of the 2.0% pectin concentration using both Irish potato tuber and cucumber pericarp tissues.

13(a) In Glucose - Pectin media the level of pectolytic enzymes in any one particular medium was practically the same for all the five *S. rolfsii* strains.

(b) In all the five strains, higher values of pectolytic Enzyme Activity, from 14.1 to 16.6, were recorded in the 0.5% Glucose + 0.5% Pectin, and the 0.25% Glucose + 0.75% Pectin media than in the 0.75% Glucose + 0.25% Pectin media of 7.5 to 7.9 pectolytic Enzyme Activity.

14. *S. rolfsii* Strains XA1 and XA2 utilized cellulose to a greater extent than the remaining three strains.

15. Addition of 0.1% (w/v) glucose as 'starter' to the medium did not improve the breakdown of the cellulose (filter paper).

16. No incidence of aversion occurred when two inocula of the same strain grew on the PDA plate but pairing of two different strains showed incidence of aversion proving conclusively that XLL, XA1, XA2, EL1 and EL2 deserved to be recognized as true strains.

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17a. Strains EL1 and EL2 formed a mean number of 99 and 102 infection cushions per unit area of 5x5mm, respectively, on tomato fruits which were vastly greater than the mean numbers of 34 to 39 by Strains XLL, XA1 and XA2.

b. There was an inverse relationship between mean number of infection cushions and mean diameters of the infection cushions. The infection cushions of Strains XLL, XA1 and XA2 had larger mean diameters of 12.7 - 18.0μm and Strains ELI and EL2 had mean diameter of 11.3μm.

18a. Three-month old plant of five cocoyam varieties individually innoculated, by soil-inoculation method, with the different *S. rolfsii* strains were all killed by the fungi in 20 days.

b. Infection proceeded slowest in *X. sagittifolium* var. 'Amankani fufuo' in which some of the leaves still remained uninfected by the 16th day after inoculation.

c. Strains EL1 and EL2 infected all the leaves of *X. sagittifolium* var. 'Amankani fitaa' by the 12th day and this variety was considered the most susceptible.

d. All the leaves of the remaining three varieties had been infected by the 16th day by all the *S. rolfsii* strains.

19a. Wound-inoculated cormels showed greater rot at the basal region of cormel than at the apical region.

b. The diameter of the rot measured immediately beneath the cormel covering was greater than the depth of the rot in all the cocoyam varieties.

c. Strains XA1 and XA2 caused the greatest diameter and depth of the rot both at the apical and basal regions in all the cocoyam varieties and were considered most potent.

d. Considering growth in cormels of all varieties, Strain EL1 caused the least rot followed by Strain EL2 and XLL.

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20. The cormels of the different cocoyam varieties were rotted to varying degrees by the different *S. rolfsii* strains. The broadest rot diameter occurred in cormels of *Colocasia antiquorum* inoculated with any of the *S. rolfsii* strains and *Xanthosoma sagittifolium* var. 'Amankani pa' cormels showed the deepest rot.

21a. Blocks of tissue of the cormels of the different cocoyam varieties were degraded at different rates. Blocks from the basal half were degraded to a greater extent than blocks of the apical half.

b. Blocks of *X. sagittifolium* var. 'Amankani fitaa' lost the greatest dry weight and *X. sagittifolium* var. 'Amankani kyirepe' the least.

22a. Strains XLL and XA2 produced sclerotial initials earliest on the *X. sagittifolium* var. 'Amankani pa' blocks while Strains XA1, EL1 and EL2 produced the sclerotial initials earliest on *Colocasia antiquorum*.

b. The majority of sclerotial initials matured in 24 hours.

c. A significantly heavier sclerotium production occurred on the *Colocasia antiquorum* blocks. The mean number of sclerotia produced by Strains XLL, XA1, XA2, EL1 and EL2 on this variety was 280, 870, 633, 976 and 1,023, respectively. The corresponding figures on blocks of *X. sagittifolium* were 105, 157, 183, 311 and 251

23. No cocoyam variety was found to be resistant to the *S. rolfsii* strains, at best the varieties showed different degrees of vulnerability.

24. For a crop which is planted in small holdings and in mixed farming systems, the best control against *S. rolfsii* would be achieved by cultural practices, as infected plants are easily detectable in the farms.

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VII

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APPENDIX A

Initial pH of Potato Dextrose Broth and pH of the medium during growth of the five strains of *S. rolfsii* at 30°C

Period of Incubation in Days	pH of culture filtrate strains				
	XLL	XA1	XA2	EL1	EL2
0	5.3	5.4	5.5	6.1	6.0
4	2.6	2.6	2.6	3.0	3.0
6	2.4	2.4	2.4	2.7	2.7
8	2.5	2.6	2.6	2.9	3.0
10	2.8	2.9	3.0	3.3	3.3
12	2.6	2.7	2.8	3.1	3.2