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STUDIES ON THE PHYSIOLOGY AND PATHOGENICITY OF *SCLEROTIUM ROLFSII* SACC. CAUSING FRUIT - ROT OF TOMATO (*LYCOPERSICUM ESCULENTUM* MILL.)

A thesis presented by

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A B S T R A C T

Sclerotium rolfsii Sacc. grew best at 27 to 32°C, and maximmm infection of tomato fruits occurred at 32°C. *S. rolfsii* showed a double maximum of growth in relation to pH: a major peak at pH 4.8 and a minor one at pH 9.31 with a minimum of pH 8.2 in between. Light had no effect on growth. Growth occurred only at 100% R. H. whereas infection of tomato fruit occurred at a relative humidity of 95% and above. A moisture level of 55% W. H. C. of soil caused the greatest infection, and degree of infection diminished with a deviation in % W. H. C. in either direction from this level.

Even though externally supplied thiamine was not essential for initiation of growth, subsequent development depended on it. Thiamine at 25 µg and 50 µg supported the greatest growth. *S. rolfsii* was capable of using simple as well as complex carbohydrates as sources of carbon. Starch supported the best growth, followed by Maltose, Fructose and Glucose, whilst galactose and cellulose gave the poorest vegetative growth. Of the nitrogen sourc[illegible] Peptone and Asparagine were best used by the fungus, and the least suitable was Alanine.

S. rolfsii can enter the host through the intact fruit skin. Rate of infection increased almost directly proportional to increase in size of inoculum. Prior to infection, *S. rolfsii* formed infection cushions on the fruit wall. Penetration was achieved by hyphae, emerging from the infection cushions, mechanically piercing through the cuticle and epidermis. Growth within the fruit was both intercellular and intracellular. Pericarp cells were ultimately dissolved by substances produced by the fungus leaving the dead epidermis and cuticle intact. The disease was not seed borne.

Tomato fruits of all ages were infected, and Molokai was the only Ghanaian tomato variety whose fruits were completely resistant to *S. rolfsii*.

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I. INTRODUCTION

Sclerotium rolfsii Sacc. is one of the most destructive soil inhabiting pathogenic fungi. The widespread distribution of this fungus and its extensive and varied host range have stimulated excessive studies. Indeed, the history of this fungus and the diseases it causes has become quite voluminous, and there are indications that interest in it will still continue to grow. S. rolfsii occurs mostly in the warmer parts of the temperate zone and in the tropics. It has an unusually wide distribution in the tropics, and has been found in every tropical country (West, 1961).

The original description of this fungus, giving it an official, scientific name was published by Saccardo (1911) in 1911. Its history, however began at least 20 years earlier, when Rolfs, (1893) found it to be the cause of tomato blight in Florida in 1892. Since then S. rolfsii has been isolated from innumerable host plants. Rolfs (West 1961) in his early writings, listed 15 host plants. Weber (1931) reporting on the geographical distribution and host range of the fungus listed 189 host species from many different plant families covering a fern family and 8 monocotyledonous and 42 dicotyledonous families. Many new host plants have since been recorded after the compilation of Weber's list.

Many of these hosts are crop plants of great economic importance, but many other groups, such as garden ornamentals and weeds, are susceptible. A great majority of species on the host list are annuals or herbaceous perennials, but some woody plants are attacked as young plants.

S. rolfsii typically causes in its hosts a disease, generally designated southern blight. The fungus attacks the collar and stems of mature host species with a subsequent wilting of the infected plants. When seedlings are attacked, the usual damping-off symptoms are manifest. Very often, the fungus destroys planted seeds. Fruits touching the ground, as well as storage organs of herbaceous perennials and of biennials seem particularly subject to this disease. Southern blight in some specific hosts has been the subject of special studies by some workers. (Taubenhaus, 1919; Edson and Shapovalov, 1923; Higgins, 1927; Paintin, 1928).

In Ghana, several host species of *S. rolfsii* have been reported (Leather, 1959; Wills, 1962). Of particular economical interest, however are the serious diseases of groundnuts (*Arachis hypogea*) and tomato (*Lycopersicum esculentum*) caused by this fungus. It causes both collar-

rot and fruit-rot of tomato and a blight of groundnuts.

Although there are reports from various countries of tomato fruit-rot caused by S. rolfsii (Atienza, 1927 ; Del Prado, 1956 etc.), these are casual accounts of field observations made, and no special studies have been carried out on this particular disease. The present studies were, therefore, undertaken to examine the influence of some major environmental factors on the growth of the fungus and to investigate, more particularly some aspects of the pathogenicity of the fungus in relation to tomato fruit-rot.

II. LITERATURE REVIEW

The literature on the physiology of such an important pathogen is surprisingly very limited. There is a general agreement among various workers, on the optimum and maximum temperatures for the vegetative growth of the fungus. Higgins (1927), Nakata (1927), Treggi (1956), Abeygunawardena and Wood (1957) and Townsend (1957) found the optimum temperature for growth to be between 30° - 35°C, and a maximum temperature of 40°C. Higgins obtained a minimum temperature of 8°C and stated that, the vegetative hyphae did not stand freezing, being killed within 24 hours at - 2°C.

There are no reports in the literature on the effect of humidity on the vegetative growth of the fungus. A few observations have rather been made on the effect of humidity on the survival of the vegetative hyphae or the survival and germination of sclerotia. (Epps *et al.* 1951; Abeygunawardena and Wood, 1957).

Higgins (1927) found the minimum, optimum and maximum pH values for mycelial growth to be 1.4, 4.0 and 8.8, respectively, after 6 weeks of mycelial growth. Rosen and Shaw (1929) observed the fungus growing well at all the pH values from 3 to 6.5. Beginning with pH 7

and extending into the alkaline range, there was a noticeable falling off in growth of mycelium and growth ceased altogether at pH 8. Abeygunawardena and Wood (1957) found the optimum pH to be 2.0 to 3.5. Johnson and Joham (1954) found the optimum pH to be 2.5 to 3.0 within 6 days of mycelial growth, when glucose was used as the carbon source, but pH 4.9 within the same period when lactose was used as the carbon source. Coupled with this cultural characteristic, it has been observed that the fungus occurs more abundantly in acid soils (Nakata, 1927; Plant Pathology and Physiology, 1927; Labrousse and Sarejanni, 1930; Johnson 1953).

Abeygunawardena and Wood (1957) found that the differences in growth rate between cultures incubated in light and those incubated in the dark were not statistically significant.

Robbins and Kavanagh (1938) reported that _S. rolfsii_ needs an externally supplied thiamine to promote normal growth. Robbins and Kavanagh (1942) later showed that the fungus synthesizes thiazole which it combines with externally supplied pyrimidine to form thiamine. Joham (1943) attempted to determine the level at which thiamine was required, but found the lowest level at which

he incorporated the vitamin in the medium, 0.1 ppm., gave as abundant growth as did any of the higher concentrations. Townsend (1957) found that mycelial growth needed an externally supplied aneurin.

Higgins (1927) found maltose to be the best carbon source and glycerine and lactose to be the poorest, among several carbohydrates and few organic acids tested, for growth of the fungus. Johnson and Joham (1954) found xylose to be the best and rhamnose to be the poorest carbon sources among the pentoses they tried. They further found that, among the hexoses, lactose was the best carbon source followed by glucose and galactose, maltose, and fructose. Abeygunawardena and Wood (1957) found fructose to be best used. Townsend (1957) found sucrose to be the best at low concentrations but glucose to be the best at high concentrations, whilst lactose gave a poor growth. Treggi (1956) reported that 5% soluble starch favoured the growth of S. rolfsii.

Some of the carbon compounds on their own, do not support good growth of the fungus. However, in the presence of other compounds, S. rolfsii is able to utilize them. Johnson and Joham (1954) reported that, the pre-

sence of glucose in the culture medium stimulated the utilization of all the pentoses employed, especially noticeable in the case of rhamnose where there was no growth in the absence of glucose. However, as the carbohydrates become more complex, the mixture produced less growth than expected, except in the inulin-glucose mixtures where a marked increse in growth was noted. It appears that inulin is utilized more efficiently in the presence of glucose. Townsend (1957) also found growth on mannitol and inulin to be very poor, but in the presence of 0.5% glucose growth was very good. Glucose and sucrose at 5% were found to support the greatest amount of growth.

Higgins (1927) observed that S. rolfsii did not appear to utilise inorganic nitrogen readily when supplied either as nitrates or ammonium salts. Abeygunawardena and Wood (1957) found the orgaric nitrogen, asparagine and peptone, to be more readily utilised than ammonium salts and sodium nitrate. Townsend (1957) also found asparagine and peptone, in addition to potassium nitrate to support good growth. Ammonium tartrate and oxalate gave better growth than ammonium sulphate and chloride. Urea, however, was

found to give unsatisfactory growth. Following the lead suggested by the outcome of field experiments with nitrogenous fertilizers reported by Leach and Davey (1942) and confirmed by Hudgins (1952), in which some reduction in losses from southern blight occurred, Johnson (1953) investigated the effects of high concentrations of nitrite, nitrate and ammonium compounds on the growth of the fungus. Nitrites at 20 ppm. inhibited growth and at 400 ppm, growth was completely suppressed. Growth was found to be maximum at 16,000 ppm. for ammonium and sodium nitrates. Ammonium sulphate was found to inhibit growth beyond 36,000 ppm.

Growth of S. rolfsii in nature is almost always prominent at or near the soil surface where host organs are most commonly attacked by the mycelium in that zone. This has been interpreted as higher demands for oxygen than do most soil-borne micro-organisms. (Taubenhaus, 1919). Flados (1958), however, found that S. rolfsii grew equally well in a series of oxygen-nitrogen mixtures, ranging from pure oxygen to pure nitrogen. Data presented by Flados suggest that, the conspicuous concentration of the growth and pathogenic activities of S. rolfsii near the surface of the soil, is not

related primarily to the concentration of oxygen, but to the distribution of antagonists among the soil microflora.

S. rolfsii has been found to be subject to certain biotic factors even though it is capable of extensive saprophytic development in the presence of adequate undecayed plant debris and suitable physical environment. Weindling (1932) demonstrated that Trichoderma lignorum (Tode) Hartz parasitized and inhibited the mycelial development of S. rolfsii. Morton and Stroube (1955) found that only 0.2%, 1.7%, and 3.5% of bacteria, actinomycetes, and fungi, respectively, were antagonistic to S. rolfsii in culture, when antagonistic activity of the micro-organisms isolated from Louisiana soils was investigated. Hansen and Curl (1959) indicated a possible role of Trichoderma sp. and Geotrichum sp. on the disease development in white clover because of the frequent isolation of these organisms from the tissue of white clover infected with S. rolfsii.

In contrast, certain fungi grown with S. rolfsii stimulated its mycelial growth. Rosen and Shaw (1929) found Fusarium f vasinfectum (Atk) Synd. and Hans. to reduce the pH of alkaline media to levels favourable

for _S. rolfsii_ development. Morton and Stroube (1955) found that 81%, 68%, and 66% of the bacteria, fungi, and actinomycetes, respectively, stimulated mycelial development on a thiamine-deficient medium. No increase in disease severity, however, was observed by the addition of the stimulating organism to the soil with _S. rolfsii_ inoculum.

A number of workers have studied the mechanics of the infection by _S. rolfsii._ There is reasonably close agreement on the mode of infection. Taubenhaus (1919) described the accummulation of masses of hyphae on the surface of the host and the death of host cells near these hyphal masses. He noted the principally chemical invasion that exemplifies the "action in advance" first described by de Bary (1886) for _Sclerotinia sp._ on stems of _Vicia faba_ and found by many subsequent workers to be typical of the parasitism of most facultative parasites. Similar phenomena of destruction of the host cells in advance of the invading hyphae were observed and mentioned in the respective accounts of Edson and Shapovalov (1923), Higgins (1927) and Paintin (1928), studying invasion of Irish Potato, Soybean stem and Cowpea stem by _S. rolfsii_. Opinion, however, on the

active principle involved, is varied, Taubenhaus (1919), Edson and Shapovalov (1923), Paintin (1928), Rosen and Shaw (1929), and Husain (1958) all interpreted the parasitic action of the fungus to be primarily by enzyme, because of the characteristic details of cellular degradation shown in stained sections of lesions. Higgins (1927) on the other hand believes that oxalic acid produced by the fungus is the primary factor in the killing of host tissue. Watkins (1961), however, does not consider either oxalic acid or enzymes to be exclusive agents in this connection, since certain definable aspects of the microscopic picture of "action in advance", could be attributed to substances in each category.

Varietal resistance has been demonstrated in a number of hosts, including tomato plant. (Fulton, 1908; McClintock, 1917; Mendiola and Ocfemia, 1926; Reyes, 1937; Streets, 1948. Decker, 1950; Mekhaimer, 1950; Orian, 1952; Mehta, Singh and Mathur, 1953; and Garren, 1960). It has been attributed to impervious cuticle, thick-walled cortical cells and cork cambium activities in the resistant varieties. (Higgins, 1927; Mohr, 1955).

Higgins (1927), Ezekiel and Taubenhaus, (1931), Ritsma and Sloof (1950) noted that resistance of some plants increased with age. Epps et al (1951) stated that woody plants generally become more resistant with age whereas herbaceous and fleshy plants remained susceptible. Varietal immunity has not been shown in any susceptible species.

III: MATERIALS AND GENERAL METHODS

MATERIALS:-

The isolate of *Sclerotium rolfsii* Sacc. used in these studies, was isolated from naturally infected tomato fruits collected by Dr. G. C. Clerk and Mr. P. K. Mante from the vegetable garden of the University of Ghana Botanical Garden, at Legon. Infection studies were made on local variety tomato (*Lycopersicum esculentum* L.) fruits. The varieties used in the varietal resistance experiments were obtained from the Plant Breeder, Ministry of Agriculture, Tamale. Northern Region. The fruits of both the local and other varieties employed, were obtained from plants raised on a plot in the Botanical Garden at Legon.

GENERAL METHODS

(a) METHODS OF STERILIZATION:-

All media, conical flasks, McCartney tubes and pipettes were sterilised by autoclaving for 20 minutes at 15 pounds per square inch steam pressure. Cotton wool plugs were temperarily covered with grease paper to prevent the penetration of any condensed water during autoclaving. Pipettes were wrapped in brown paper.

Slides (7.5 x 2.5 cm) and cover slips were first soaked in cleansing solution of potassium dichromate ($K_2Cr_2O_7$, 100 gms; conc. H_2SO_4, 1000 ml; and water

1000 ml) for 24 hours followed by immersion in a dilute solution of Sodium Hydroxide (NaOH) and were finally rinsed several times in distilled water. They were stored in 90% ethyl alcohol and flame sterilised just before use.

Petri (9cm diameter) dishes were sterilised by heating at 165°C for 6 hours in an electrically heated oven.

Tomato fruits were surface sterilised by immersing the fruits for 10 minutes in a 5% Sodium hypochlorite solution and then rinsing them three times with sterile distilled water.

The inoculation room was sterilised by spraying with dettol solution (Dettol 5 ml; water 95 ml) for 10 minutes just before use.

(b) METHODS OF INOCULATION:

All agar plates were inoculated with single 3-mm. agar discs (diameter) removed from the growing edge of 3 days' old cultures. The agar discs were transferred to the centre of the medium in each petridish.

Liquid cultures were inoculated with single Sclerotia.

Sterilised tomato fruits were inoculated by placing 3-mm. agar discs upside down at about 2 cm. from the

stylar end of the fruit.

(c) HUMIDITY CHAMBERS :

Rectangular plastic containers (24.5cm x 12cm x 8cm) with tightly fitting lids were used as humidity chambers through out the investigations.

(d) INCUBATION:

Incubation conditions varied with the experiment and are described at the appropriate places in the text.

(e) METHODS OF ASSESSMENT:

Colony diameter, in the agar plates, were measured, at the end of the required period, along two pre-determined diameters at right angles, marked at the bottom of the petri dishes.

Growth in the liquid cultures was measured by weighing (dry weight) harvested mycelium at the end of the required period. The mycelia were filtered through a weighed Whatman No. 1 filter paper and dried at, between 60° and 70°C for 24 hours, and filter paper with dried mycelium re-weighed.

In the pathogenicity studies, both colony growth and infection were measured by stretching a piece of thread across the diameters of either the infection (discoloured area) or the colony as the case may be.

(f) MAINTENANCE OF STOCK CULTURES:

Stock cultures of *S. rolfsii* Sacc. were maintained

on slopes of Potato Dextrose agar (P.D.A.) medium in McCartney tubes. They were incubated at room temperature (26 ± 2°C); and sub-cultured fortnightly.

(g) CONSTANT HUMIDITY SOLUTIONS:

To obtain and maintain the different relative humidities aqueous saturated potassium hydroxide solution was prepared according to the data provided by Solomon (1952) (see table 1).

TABLE 1

KOH solution for the control of Atmospheric Humidity

% Relative Humidity at 20°C	% (Wt/Wt) GM, KOH/100 gm Soln.
100	0.00 (H_2O)
95	7.00
90	11.75
85	15.80
80	19.25
75	22.25
70	25.00
65	27.30
60	29.50
55	31.60
50	33.70

(h) BUFFER SOLUTIONS:

Adjustment of the pH of media, when required was obtained by adding Buffer solution. Buffer solutions

were prepared according to McIlvaine's standard Buffer solutions (see table 2) and Glycine: Na OH (Sørensen, Walbum's values). (see Table 3).

TABLE 2

McIlvaine's Standard Buffer Solutions

Stock solution A : 0.1M Citric acid ($C_6H_8O_7$) solution.

Stock solution B : 0.2M diSodium phosphate ($Na_2 PHO_4$) solution.

pH	Soln A (cc)	Soln B (cc)
3.0	15.89	4.11
4.0	12.29	7.71
5.0	9.70	10.30
6.0	7.37	12.63
7.0	3.53	16.47
8.0	0.55	19.45

TABLE 3

Glycine : NaOH (Sørensen, Walbum's Values) pH 8.3 - 12.90 at 20°C. 7.505 gm Glycine + 5.85 gm Na Cl/litre O.IN NaOH. To X ml of Glycine - Na Cl solution, add (10-X) ml O.In NaOH.

pH	Glycine Na Cl ml (X)
9.31	8.0
10.42	5.5

(i) CULTURE MEDIA :

All chemicals used in the preparation of media were either of the "Analar" grade or of the "B.D.H." (British Drug House) grade. Standard 10 oz. medicine bottles (approx. 13 x 6 x 3.5 cm) containing 30 ml media were used for liquid cultures. 9 cm petri-dishes with 25 ml media were used for agar cultures.

(j) EXPERIMENTAL PRECAUTIONS :

Except at the required period for taking results, the experiments were not disturbed. The times for taking measurements were standardized so as to eliminate any possible error due to time. In the liquid media experiments, the same kind of filter paper, Whatman No. 1, was used in all dry-weight measurements. In the patho-

genicity experiments, except in the humidity experiments, the lids of the plastic containers were removed daily, for a few seconds to let off any gases accummulated in the closed chambers. Care was taken to avoid water getting into the small miniature petri-dishes (4 cm diameter) in which rested the inoculated fruits, (see page 61). Cultures of the same age were used in all inoculations. Glassware was kept scrupulously clean. All contaminated cultures were removed immediately they were detected. As far as could be determined, fruits of the same age were used in each particular experiment.

(k) STATISTICAL ANALYSIS OF RESULTS :

All experimental results, except where specifically otherwise indicated in the text, were tested statistically. Details of statistical calculations will not be given. Results quoted as statistically significant were at the 5% level of Significance ($P=0.05$).

(l) HISTOLOGICAL METHODS :

i. WHOLE MOUNTS (EPIDERMIS)

At the end of the required period, the infected fruit was left in the deep-freeze, at a temperature of $-10^{\circ}C$ for 24 hours, a treatment which allowed the epi-

dermis to be easily removed free from sub-epidermal tissues. This was stained in 0.1% aqueous methylene blue solution.

ii. PARAFFIN EMBEDDED SECTIONS.

Inoculated and subsequently infected tomato fruits were killed and fixed in Formalin-aceto-alcohol (FAA) (90 cc : 5 cc : 5 cc) for 48 hours. Dehydration, infiltration, and embedding of fixed materials were carried out in the manner described by Johansen (1940). Microtome sections, 12 μ, were cut with the Cambridge Rocking Microtome. Sections were fixed using Haupt's adhesive. The sections were passed through a regular xylol-alcohol series down to water mordanted in aqueous iron alum solution for 1 hour and stained with aqueous solution of Delafield's Haematoxylin (3 : 1) for 24 hours. All sections were mounted in Canada Balsam. Photomicrographs were taken on Microfile film using Exa-35 mm. camera.

IV: RESULTS

1. PHYSIOLOGY OF GROWTH (EFFECT OF ENVIRONMENTAL FACTORS ON GROWTH)

A knowledge of the physiology of a fungus parasite is a pre-requisite to the understanding of its parasitic relation with its host. It is moreover useful in predicting the behaviour of the parasite under any existing environmental conditions and essential for the intelligent control of the disease it causes. The effect of external factors on growth, has always been one of the important aspects of the physiology of fungal parasite, to which much attention is devoted. Experiments were, therefore, carried out in this work to study the effect of some major environmental factors on the growth of *Sclerotium rolfsii*.

A: EFFECT OF TEMPERATURE.

One of the most important environmental factors that influence the growth of fungi is temperature, probably owing to its effect on the physical and chemical processes involved in growth (Hawker, 1950; Lilly and Barnett, 1951; Cochrane, 1958). Generally, there is a minimum temperature below which a fungus ceases to grow, an optimum temperature where the best growth takes place, and a maximum temperature above which it cannot grow. The range of temperature over

which fungi will grow varies to some extent with the various species. Most fungi, however, either do not grow or grow very slowly at freezing-point and, at the other extreme, are unable to grow at temperatures above 35°C. The optimum temperature for growth is usually between 20° and 30°C (Hawker, 1950). There are, however, certain very striking exceptions to this generalisation.

The effects of selected temperatures, (see Table 4), quite near to that obtained in the tropics, on the growth of the fungus, were investigated. Potato-dextrose-agar plates were inoculated with 3-mm agar discs from the growing edge of 3 days' old S. rolfsii cultures. There were 4 replicates per each temperature level. Daily measurements of diameters of the colonies were taken over a maximum period of 4 days. The results are presented in Table 4 and graphically in Fig 1.

TABLE 4

Effect of different temperature on growth of Sclerotium rolfsii on potato-dextrose-agar.

Temperature of Incubation in °C.	Mean Diameter of colony, in mm, on indicated day after incubation.			
	1	2	3	4
17	5.0	11.8	16.3	29.3
22	10.0	20.6	33.5	58.0
27	20.0	48.3	81.5	89.5
32	21.3	48.5	78.5	87.3
37	5.6	6.5	7.0	7.3

The results show that S. rolfsii was capable of growing at all the temperatures studied. Maximum growth occured at 27° and 32°C. Growth at 27°C was slightly higher than that at 32°C on the third and fourth days. At 22°C, growth was approximately twice as much as that at 17°C. Poor growth occured at 37°C. When, however, cultures incubated at 37°C were transferred to 22°C, vigorous growth resumed, showing that the colony retained its viability after 4 days continuous exposure to 37°C. Growth rate, as depicted by the graph, at 27°C and 32°C, for the first 3 days, was quite fast but fell off rapidly at the end of the third day. The reverse was

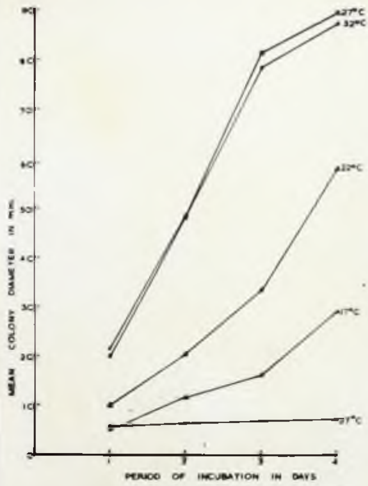


FIG 1 EFFECT OF TEMPERATURE ON GROWTH OF S.ROLFSII

true at $17^{\circ}C$ and $22^{\circ}C$. Practically, no significant increase, on the other hand, occurred after the day's growth in cultures, incubated at $37^{\circ}C$. The maximum temperature for growth could not be established with the temperature range employed in this experiment. There are indications, however, that this must be very close to $37^{\circ}C$. This value, therefore, agrees with that reported by Higgins (1927), and Abeygunawardena and Wood (1957). Abeygunawardena and Wood (1957) found that the maximum temperature lay between 35° and $40^{\circ}C$, and Higgins (1927) observed that $37^{\circ}C$ appeared to be the maximum for continuous normal growth. The minimum temperature is evidently below $17^{\circ}C$, the lowest temperature used in this experiment. Abeygunawardena and Wood (1957) reported ~~of~~ good growth even at $10^{\circ}C$. Higgins (1927) found that <u>S. rolfsii</u> grew slowly at $8^{\circ}C$ and stopped growth completely at $5^{\circ}C$. This could not be verified in this investigation because temperatures below $17^{\circ}C$ were not available.

The optimum temperature 27° to $32^{\circ}C$, obtained here is slightly lower than that reported by Higgins (1927). He found an optimum of 30° to $35^{\circ}C$, but it is similar to that reported ($30^{\circ}C$) by Abeygunawardena and Wood (1957) for one of the two isolates they studied. The other isolate however, gave an optimum of $20^{\circ}C$, a value far

below that found in this work.

B: EFFECT OF LIGHT:

Most reports on the effect of light on fungi have been concerned with reproduction rather than vegetative growth. From data available in the literature, no generalizations are possible concerning the influence of light on the growth of fungi. Although most fungi grow about equally well in semi-darkness, diffuse light or direct light, several observations agree that light retards mycelial growth; dry weight measurements confirm this for *Sclerotinia fructigena* and *Karlingia rosea* (Cochrane, 1958). The growth of *Botrytis squamosus* on agar is completely inhibited by light. It must be recalled that exposure to "white light" may, depending on the source, involve either exposure to some ultraviolet or heating effect. On the other hand, light stimulation of growth, infrequently reported, has been most carefully analysed in *Blastocladiella emersonii* (Lilly and Barnett, 1951). In this organism, dry weight increases of up to 141 per cent at constant temperature are induced by illumination. The effect of light on the growth of *S. rolfsii* was investigated on potato-dextrose-agar.

Inoculated petri dishes for the dark experiments were wrapped in black paper and placed in slide boxes,

26.5 cm long, 11.5 cm broad and 5 cm high. These boxes were in turn, wrapped in black paper and incubated at 27°C. These remained in the dark throughout the period of investigation, except for a brief period each day when the diameters of the colonies were measured in the dark-room in red light. The remaining inoculated dishes were exposed to continuous light of 45 foot-candles, provided by electric bulb of strength of 25 Watts, placed at 2 feet away from the petri dishes to avoid heating effect. This was verified by placing a thermometer by the petri dishes and the temperature noted at intervals. Measurements were taken daily for a maximum of 3 days and stopped because the hyphae started to grow into the lid of the petri dishes; thus making measurements impossible. There were 4 replicates per each treatment. The results are presented in Table 5.

TABLE 5

Effect of light on growth of *S. rolfsii* raised on potato-dextrose-agar.

Incubation period in Days	Mean Diameter of Colony, in mm. under indicated light conditions.	
	Light	Dark
1	17.6	15.9
2	43.8	42.0
3	80.0	80.5

The data in Table 5 show that there was negligible differences between colonies exposed to light and those incubated in darkness. This finding agrees with that reported by Abeygunawardena and Wood (1957).

C: THE EFFECT OF RELATIVE HUMIDITY ON GROWTH OF S. ROLFSII.

Fungi, in general, are particularly dependent upon a moist environment for growth. They require relatively high moisture levels, but most can grow in the absence of liquid water. (Cochrane, 1958). Certain fungi, such as Schizophyllum commune, and more particularly the grain deteriorating fungi, can tolerate such low humidity as 85 to 90% R.H; otherwise most fungi are limited to much higher relative humidities of 95% or more (Hawker, 1950, Lilly and Barnett, 1951; Cochrane, 1958). There is no report dealing exclusively, with the water relations of such an important fungus as S. rolfsii. An experiment was carried out to investigate the effects of different relative humidities on the mycelial growth of S. rolfsii. Constant relative humidities were maintained with saturated aqueous salt solutions, prepared according to the data provided by Solomon (1952) (see page 19).

Saturated aqueous salt solutions were prepared in oblong plastic containers (24.5 x 12 x 8cm) which served as the humidity chambers. Sterile, open small miniature petri dishes (4 cm diameter) were placed at the bottom of the humidity chambers. 3-mm agar discs from the advancing edge of 3 days' old cultures were then placed,

inverted, in the miniature petri dishes with the fungus coming directly in contact with the petri dishes. The humidity chambers were then covered with the lid and sealed with sellotape. These were incubated at 27°C for 7 days. There were 4 replicates per each relative humidity level. The humidity range used is presented in Table 6. Since the colony was formed by strands of mycelia - the usual characteristic growth of S. rolfsii - from the agar discs, the length of each strand for each agar disc in a particular relative humidity level was measured. The sum total for each agar disc was obtained and the average was taken to be the radius of each colony; and the diameter estimated from the radius. The results are presented in Table 6.

TABLE 6

Effect of Humidity on the growth of *S. rolfsii* at 27°C.

(Observation after 7 days' Incubation).

% Relative Humidity	Mean Colony Diameter in mm.
100	20.6
95	0.0
90	0.0
85	0.0
80	0.0
75	0.0
70	0.0

The data in Table 6 show that S. rolfsii required very high relative humidity for vegetative growth. Growth occurred only at 100 % R. H.

D: THE EFFECT OF pH ON GROWTH OF S. ROLFSII.

Under given conditions, a fungus will only grow over a certain range of pH of the medium. Most fungi will tolerate a wide range of hydrogen-ion concentration of the medium. Inhibition of growth is usually rather sharply defined at the limits of this range. Most fungi grow best at neutral reactions, that is, at pH 7 or slightly on the acid side of neutral. Growth is usually stopped on the acid side at pH 3 and on the alkaline side at pH 8 - 9. (Hawker, 1950; Lilly and Barnett, 1951; Cochrane, 1958). There are, however, exceptions to these generalisations. The effect of hydrogen-ion concentration on the growth of S. rolfsii was investigated in this section of the work. The range (see Table 7) was obtained by using different buffer mixtures prepared according to the data by Clark (1925) and Hale (1958), for different sections of the pH range. 15 ml of buffer solution of desired pH was added unto 15 ml of culture medium (see below) of double strength. The culture medium consisted of :

Glucose	2.5 gm
Asparagine	0.5 "
KH_2 PO4	0.25 "
MgSO4	0.25 "
FeSO4	atrace
Vit.B1 (Thiamine)	50 mg.
Deionised water	250 ml.

The adjusted medium was therefore diluted to the normal concentration employed in other experiments in this work. The adjusted media were then autoclaved. The pH of the media were determined after autoclaving and also after harvesting the mycelia. Since there was no pH-meter available, accurate pH determinations were not possible. Approximate determinations were measured with the "pH Testing Kit" based on the Lovibond Comparator, and "B.D.H. Wide Range Test Paper". The results are presented in Table 7 and Fig. 2. The results were analysed statistically.

The data in Table 7 show that S. rolfsii grew best in acid media as has been found by various workers (Higgins, 1927; Rosen and Shaw, 1929; Labrousse and Sarejanni, 1930; Treggi, 1956; Abeygunawardena and Wood, 1957 etc). S. rolfsii showed a double maximum of growth in relation to pH; a major peak at pH 4.8 and a minor one at pH 9.31 with minimum of pH 8.2 in between. The fungus grew at all pH levels tested. The minimum and maximum could, therefore, not be determined. The ability of S. rolfsii to change, to a great degree, the pH of the medium is worthy of note. There is a remarkable drift of the hydrogen-ion concentration at all pH levels supporting good growth to pH 2 - 3. This was also observed by Higgins (1927), and Abeygunawardena and Wood (1957).

TABLE 7

Effect of pH on growth of S. rolfsii at 27°C.

(Observation after 7 days' Incubation).

pH and Buffer solutions used	Mean Dry Weight of Mycelium* in mgm.	Approx. pH after autoclaving	Approx pH after Incubation
McIlvaine's Standards.			
3	402.9	3 - 3.6	2 - 3
4	481.6	4.8	2 - 3
5	350.0	5.2	2 - 3
6	362.0	6.8	2 - 3
7	59.6	7.6	7 - 8
8	28.9	8.2	7.0
Sørensen Walbum's Value			
9.31	142.1	9.4	2 - 3
10.42	87.8	10-11	3 - 4

* Least Significant difference between means of total dry weight (at p = 0.05,) = 4.27 mg.

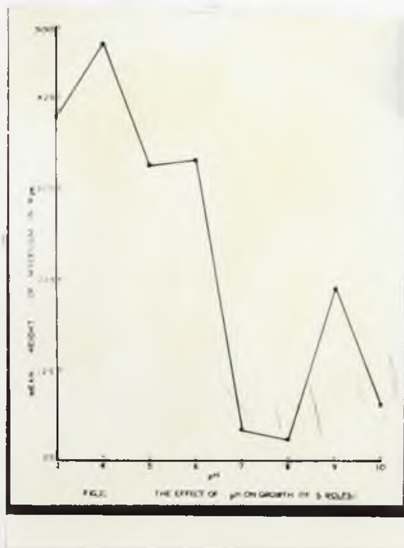


FIG.2. THE EFFECT OF pH ON GROWTH OF S. ROLFSII

E: THE EFFECT OF THIAMINE ON GROWTH OF S. ROLFSII.

Work on a number of fungi has revealed that many of them require an external supply of growth substances (Vitamins) to promote good growth. (Hawker, 1950; Lilly and Barnett, 1951; Cochrane, 1958). Robbins and Kavanagh (1942) in a comprehensive review on vitamin deficiencies of the filamintous fungi, reported of several fungus species which need an external supply of v vitamins to develop satisfactorily, Several reports indicate that thiamine deficiency is the most frequently occuring vitamin deficiency in fungi.

Robbins and Kavangh (1938) working on vitamin deficiency in some selected fungal species, observed that *S. rolfsii.* among other species, would only grow well in a purely synthetic medium when thiamine has been added. In experiments so far described above, *S. rolfsii* was grown on potato-dextrose agar. Natural media, such as potato-dextrose-agar, usually contain all the necessary vitamins and growth-subtances, and so strains deficient in the power of synthesis grow well on these and do not reveal their deficiency, unless they are grown on synthetic media lacking these substances. The observation of Robbins and Kavanagh was, therefore, verified by growing *S. rolfsii* in a purely synthetic

liquid medium with and without a supply of thiamine.

The fungus was grown in 10 oz - medicine bottles (approx 13 x 6 x 3.5 cm) containing 30 ml of liquid medium. Two lots of the basic synthetic liquid medium (see page 35) were prepared. One lot was used to grow the fungus without any further treatment, whilst thiamine was added to the second lot at rate of 50 μg per litre of medium. Since thiamine is unstable when heated in an alkaline medium (Lilly and Barnett, 1951) an aqueous solution of the vitamin was prepared separately and the pH adjusted to pH 3 and autoclaved. This was then added to the sterile basic medium aseptically immediately before inoculation. The concentration of the thiamine solution was such that, 5 ml of the solution added unto 25 ml of the basic liquid medium brought the concentration of the growth substance to an equivalent of 50 μg in a litre of medium. Medium in each medicine bottle was inoculated with a single, 10 days' old, sclerotium. There were 4 replicates for each treatment. The inoculated bottles were incubated at 27°C for 7 days and arranged to lie flat on the side, to increase the exposed surface of the medium.

At the end of the incubation period, the contents of each bottle was poured on to a previously weighed

and labelled Whatman No. 1 filter paper and filtered under pressure. The filter paper with its load of deposited mycelium was dried in an electric oven set at 65°C for 24 hours, and thereafter weighed. The dry weight of the mycelium was then estimated. The results are presented in Table 8.

TABLE 8

Effect of Thiamine on growth of *S. rolfsii* at 27°C.
(Observation after 7 days' Incubation)

Treatment	Mean Dry Wt of Mycelium in mgm.
No thiamine	118.6
Thiamine	158.9

The data in Table 8 show that the isolate of *S. rolfsii* did not require an extracellular supply of thiamine to initiate growth. Subsequent development was, however, poor and stunted. Addition of thiamine at the rate of 50 µg per litre of medium was enough to stimulate good growth, resulting in an increase of approximately 34 per

cent. S. rolfsii in the solution containing thiamine formed a heavy mat covering the surface of the liquid and formed numerous sclerotia (Plate 1).

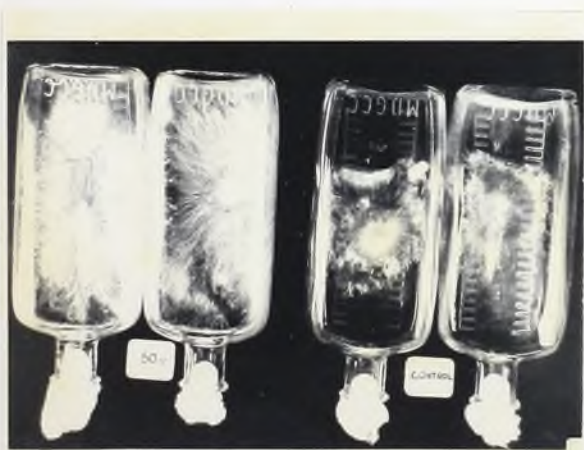


PLATE 1 : EFFECT OF THAMINE ON GROWTH OF S. ROLFSII (x 1/4)

F: THE EFFECT OF DIFFERENT CONCENTRATIONS OF THIAMINE ON GROWTH OF S. ROLFSII

In certain cases, particularly when present in excessive concentrations, vitamins may have inhibitory effect on growth of the fungus. It has been found for most fungi that 100 µg of thiamine per litre of medium is near optimum for growth. However, the optimum varies with the amount of sugar in the medium (Lilly and Barnett, 1951). Schopfer, (1934) also found that the optimal concentration of thiamine for <u>Phycomyces</u> <u>blakesleeanus</u> varied with concentration of asparagine. Joham (1943) attempted to determine the best level at which thiamine was required, but found that the lowest level at which he incorporated the vitamin in the medium, 0.1 ppm, gave as abundant growth as did any of the higher concentrations. n experiment was set up to find out whether there is an optimal concentration of thiamine for growth for this isolate of <u>S. rolfsii</u>. The materials and methods used were the same as followed in the previous experiment (see pages 40 - 41). Thiamine was incorporated into the basal liquid medium (see page 35) to give respective concentrations of 25, 50, 100, 150 and 200 µg per litre of medium. The results are presented in Table 9 and Fig. 2. The results were analysed statistically.

TABLE 9

Effect of different concentrations of thiamine on growth of *S. rolfsii* at 27°C.

(Observation after 7 days' Incubation)

Concentration of Thiamine used in µg	Mean Dry Weight of Mycelium in mgm.
No Thiamine	78.1
25	139.2
50	139.7
100	99.4
150	99.6
200	86.0

The data in Table 9 and Fig. 2 showed that optimum growth occured at 50 µg thiamine concentration. The lower concentrations of 25 µg and 50 µg highly stimulated growth, producing a heavy felt of mycelium almost covering the surface of the medium; whereas at higher concentrations (100, 150, and 200 µg) increase in growth was relatively less (Plate 2). The observation made in the previous experiment that this isolate of *S. rolfsii* appeared, not to require an extracellular supply of thiamine to initiate growth was confirmed. Sclerotia, in the control medium, with no thiamine, gave a measurable growth of 78.1 mgm. of dry weight of mycelium.

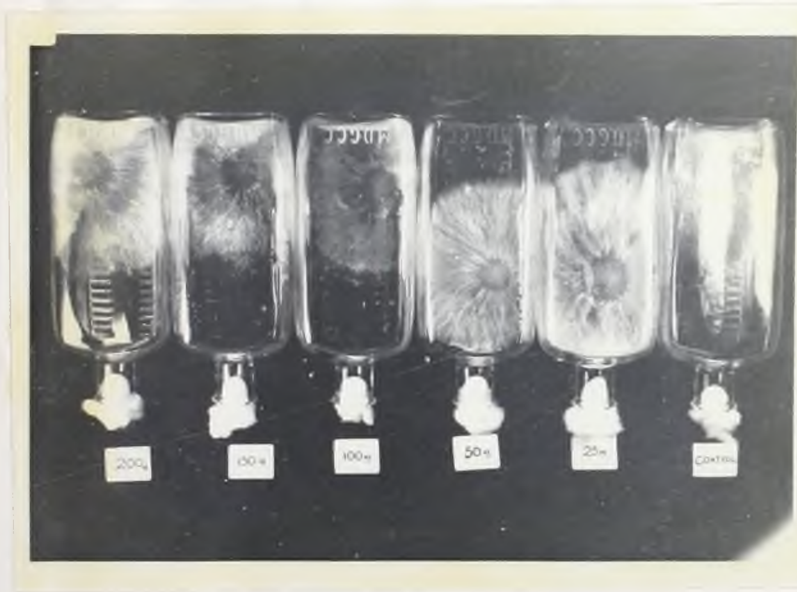


PLATE 2 : EFFECT OF DIFFERENT CONCENTRATIONS OF THPAMINE ON GROWTH OF <u>S</u>. <u>ROLFSII</u> (x 1/4)

FIG. 3. EFFECT OF DIFFERENT CONCENTRATIONS OF THIAMINE ON GROWTH OF S. ROLFSII

G: THE EFFECT OF DIFFERENT CARBON SOURCES ON GROWTH OF S. ROLFSII

Ingestigations in the nutrition of pathogenic fungi have been carried out on a number of species in the laboratory in pure culture. These have obvious practical importance. Such studies have shown that a change in substrate may result in a change in both the metabolism and morphology of the organism. For example, differences in the effect of the substrate on pectinase secretion or on the activity of the enzyme secreted by various fungi are readily demonstrated.

It is possible that the carbohydrates and nitrogenous compounds in the host tissues and exudates may play an important role in the behaviour of S. rolfsii. before and after penetration. This experiment was therefore designed to examine the effect of some carbon compounds on vegetative growth of S. rolfsii.

To determine which organic compounds could be utilised as sources of carbon by S. rolfsii. a number (Table 10) were separately incorporated in a basal liquid medium containing 50 µg. thiamine per litre of medium (see Page 35) less Glucose. Each was added at a concentration of 1 per cent. W/V. Since a low level of soluble carbohydrate might have been necessary to initiate growth on media containing insoluble polysaccharides.

starch and cellulose were each tested with and without 0.1 per cent glucose. The cellulose used in this experiment was mechanically macerated and well-washed filter paper. There were 5 replicates for each carbon source, and the inoculated media were incubated at 27°C for 7 days. The results are presented in Table 10.

The tabulated results show that S. rolfsii was capable of using all the carbon compounds tested to varying degrees. The sources of carbon employed fall into three main categories, namely :

(i) those which were used readily (starch, maltose and fructose) and gave goodvegetative growth.

(ii) those moderately used (glucose)

(ii) those which were used to a very limited extent (cellulose, lactose, glycerol and galactose).

There was no growth whatsoever in the control. Germination of the sclerotia, however, occurred in the control, but no subsequent measurable growth ensued.

The ready utilizationof starch was confirmed by repeating part of the above experiment.

The poor growth of <u>S. rolfsii</u> obtained here when supplied with galactose as the carbon source agreed with Abeygunawardena and Wood's (1957) finding that growth on this compound was poor. Johnson and Joham (1954) surprisingly observed that galactose was the best carbon

source among the hexoses studied. Higgins (1927) reported that glycerine was unsuitable for growth. This was confirmed in this work.

TABLE 10

Effect of different carbon sources on growth of *S. rolfsii* at 27°C.

(Observation after 7 days' Incubation).

Carbon source	Mean Dry Weight of Mycelium (mg.) *
Starch + 0.1% Glucose	214.5
Starch	137.4
Maltose	124.3
Fructose	110.6
Glucose	89.9
Cellulose	34.0
Lactose	34.0
Glycerol	33.3
Cellulose + 0.1% Glucose	30.1
Galactose	8.0
Control (No carbon compound)	---

* Least Significant difference between means of total fungus dry weight (P = 0.05). = 1.8 mgm.

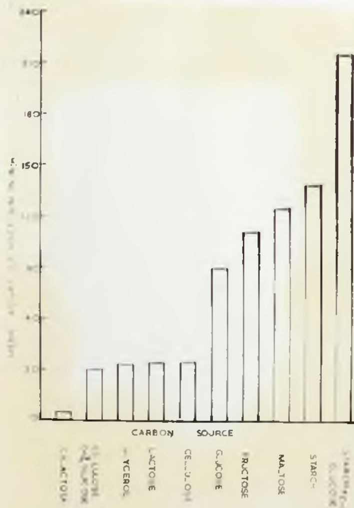


FIG.4.[illegible] EFFECT OF DIFFERENT CARBON SOURCES ON GROWTH OF [illegible]

H. THE EFFECT OF DIFFERENT GLUCOSE CONCENTRATIONS ON GROWTH OF S. ROLFSII

Glucose is, biologically, the most important of all the simple sugars. It is utilised for growth by virtually all cultivable fungi, and most frequently used carbon-source in culture media. It has indeed been used in this work so far. The previous experiment, however, indicated that at least, at a concentration of 1 per cent (Weight/volume), starch, maltose and fructose were superior to glucose as a carbon-source for growth for the fungus, the amount of growth in the previous experiments was then relatively lower than would have been obtained if any of the superior carbon sources were used. This experiment was designed to examine, the effect of different glucose concentrations on growth of *S. rolfsii* and to find out how far the concentration employed in earlier experiments deviated from the optimum.

The different glucose concentrations were incorporated into synthetic liquid media (see page 35) with a thiamine supply (50 µg. per litre of medium). These concentrations are shown in Table 11. There were 5 replicates for each glucose concentration level. The inoculated media were incubated at the optimum temperature for growth for *S. rolfsii* for a maximum period of

7 days, after which the dry weight of the mycelium was estimated in the usual way (see page 18) and the mean dry weight calculated for each glucose concentration level. The results are presented in Table 11 and Fig 5. These were analysed statistically.

TABLE 11

Effect of different glucose concentrations on growth of S. rolfsii at 27°C.

(Observations after 7 days' Incubation)

Glucose Percentage (Wt./wt.)	Mean Dry weight of Mycelium in mgm. *
Control (No glucose)	0.0
0.125	3.0
0.25	6.2
0.5	65.2
1.0	95.7
2.0	247.1

* Least Significant difference between means (P = 0.05) = 2.1 mgm.

The results in Table 11 show that the mean dry weight of the mycelium increased with an increase in glucose concentration over the range used. There was no growth in the absence of glucose. (The highest glucose concentration of 2.0% (wt./wt.) in this experiment, gave the greatest amount of growth of 247.1 mg. dry weight). Whether this is the optimal concentrations

for S. rolfsii could not be determined with the range employed. Reasons why the range was not extended beyond 2% concentration are fully discussed at page 127. The results have however showed that this concentration was enough to support maximal or near maximal growth in S. rolfsii.

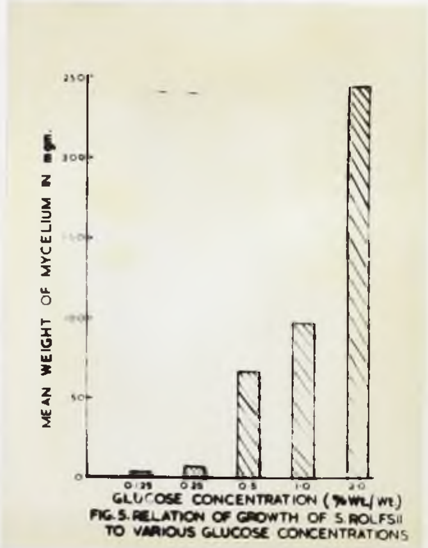


FIG. 5. RELATION OF GROWTH OF S. ROLFSII TO VARIOUS GLUCOSE CONCENTRATIONS

I: THE EFFECT OF DIFFERENT NITROGEN SOURCES ON GROWTH OF S. ROLFSII

The ability of S. rolfsii to utilise different nitrogenous compounds was next examined. Various nitrogenous materials were added to the liquid medium (see page 35) less Asparagine; the final concentration of nitrogen in each treatment was the same and equivalent to 0.05 per cent. W/V. There were 5 replicates for each nitrogen source used. The inoculated media were incubated at the optimum temperature for growth of S. rolfsii for 7 days. The results are presented in Table 12 and in Fig 6. Differences between means were found to be significant at $p = 0.05$.

Adopting the total yield of fungus as a measure of the efficiency with which the nitrogen source was used, it is evident that S. rolfsii was capable of utilizing all the nitrogen sources employed. Growth was very good on peptone and asparagine; was moderately good on ammonium nitrate and urea and ammonium chloride but poor on glycine, sodium nitrate and alamine. Aerial mycelium on cultures containing ammonium chloride and peptone was thick but restricted in diameter, whereas those on asparagine, urea and ammonium chloride appeared light and cottony and very extensive in diameter. Sclerotium formation was initiated very early on cultures containing sodium nitrate. The results agree with what Abeyguna-

wardena and Wood (1957) found. They found that good growth was in the order organic nitrogen, ammonium salts and sodium nitrogen. Higgins (1927) found that the fungus did not appear to utilize inorganic nitrogen readily when supplied either as nitrates or as ammonium salts. In this experiment, ammonium nitrate was found to be quite geadily utilized. Townsend (1957) found that in the presence of 2% glucose, urea as a sole source of nitrogen, gave an unsatisfactory growth. It was however observed in this investigation that in the presence of 1% glucose, urea was extensively used by S. rolfsii.

Growth in the control was almost identical to that on alanine medium. S. rolfsii made very little or no use of alanine.

TABLE 12

Effect of different nitrogen sources on the growth of S. rolfsii at 27°C.

(Observation after 7 days' Incubation)

Nitrogen Source	Mean Dry Wt. of Mycelium in mgm *
Peptone	143.8
Asparagine	142.1
Ammonium Nitrate	117.8
Urea	113.4
Ammonium Chloride	93.7
Glycine	41.2
Sodium Nitrate	35.8
Alanine	15.2
Control	13.9

* Least Significant different between means ($p = 0.05$) 3.6 mgm.

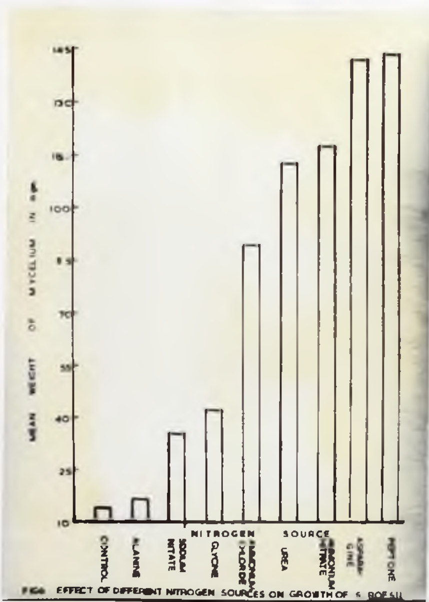


FIG 6 EFFECT OF DIFFERENT NITROGEN SOURCES ON GROWTH OF S. ROFSII

II: PATHOGENICITY STUDIES

A: MODE OF INFECTION OF TOMATO FRUIT BY S. ROLFSII

Pathogenic fungi may enter their respective hosts through wounds, through natural openings such as stomata, lenticels and hydathodes or by penetrating directly through the host's external tissues (Stakman and Harrar, 1957; Walker, 1957). Many plant pathogens such as *Penicillium glaucum* and *Penicillium digitatum* known to enter their hosts only through wounds, are even then controlled by certain factors, such as the depth of the wound. In similar manner, parasites penetrating directly through the host surface tissues are controlled by various factors, among which are thickness of cuticle, presence of suberised cells, presence of cork etc.

Problems of host penetration remain of utmost importance, and inspite of the large number of studies that have been devoted to this phenomenon, they should continue to receive unstinted attention because of their bearing on matters of tolerance or resistance to disease, on studies involving the causes of natural immunity, and on production of races of disease-resistant crop plants. The manner by which penetration of the tomato fruit is accomplished by *S. rolfsii* was investigated.

Since the "skin" of the tomato fruit does not possess any natural openings, there are only two possi-

bilities of the method of penetration by which the fungus can invade the fruit. This is either by a direct penetration or through wounds. To find out which alternative phenomenon is operative, in the process, the following experiment was set up.

Twenty eight tomato fruits were surface sterilised with a solution of sodium hypo-chlorite (see page 17) and divided into 2 lots of 14. A pyramid of pericarp and skin of the fruit, 5 mm in length along all edges was incised at a spot 2 cm away from the stylal scar with a flame-sterilised scapel, from each of the 14 fruits in one set. The pyramid was then lifted, in case of 10 of the fruits of this set and a 3 mm agar disc from the growing edge of a 3 days' old culture of *S. rolfsii* was placed in the wound and the pyramid of tomato tissue was replaced. The wound was finally sealed with sterile vaseline to prevent contamination. The remaining four fruits of the set formed the control. In their case, the 3 mm potato-dextrose agar disc inserted into the wound contained no mycelium.

The other set of fruits were similarly surface sterilised (see page 17) and each of ten of the fruits, this time, without any wounds (intact skin) were inoculated, on the exterior with single 3 mm agar discs bearing mycelium of *S. rolfsii*.The remaining four fruits again

served as control and were each inoculated with potato-dextrose agar discs bearing no mycelium.

The inoculated fruits in both sets were each placed on a miniature petridish (4 cm in diameter), to serve as support. The petridishes with their loads of fruits were then placed in humidity chambers (24.5 cm long, 12 cm broad and 8 cm high) and the chambers closed and sealed. The humidity in the chambers were maintained at 100% R.H. with pure water. These were incubated at 32°C for 7 days. Quick daily measurements of external mycelial growth on the fruit and spread of infection - indicated by colour change and texture (firmness) change - were made by stretching a piece of thread along the diameters and the lengths of thread coinciding with the respective diameters measured on a rule. This procedure was found to be more reliable for measurements on the curved surface of the fruit. The results are presented in Table 13 and graphically in Fig 7.

The tabulated results in Table 13 show that S. rolfsii is not exclusively a wound parasite, for it easily penetrated the intact skin of the fruit. Reasons for the difference in the rate of infection between the two methods are easy to find. The fungus placed on the outside naturally took sometime to penetrate the "skin"

PLATE 3 : INFECTION OF TOMATO FRUITS
Photograph showing infection in tomato fruits.
A - Inoculated exteriorly on intact skin. B. - Inoculated through wound.

before subsequent spread within the host tissue followed. On the intact fruit wall, therefore, external growth of the mycelium on the fruit skin superseded the rate of infection. On the other hand, where inoculum was introduced into the wound, infection started readily and rotting was observed sometime before the mycelium emerged from the fruit with subsequent growth and spread over the fruit exteriorly.

All the inoculated fruits in each category were subsequently and uniformly infected. All fruits in either of the controls remained healthy.

Incubation Period in Days	Fruit inoculated by placing mycelium on intact skin.		Fruit inoculated by placing mycelium in Wound.	
	Mean Diameter of Colony on fruit in mm.	Mean Diameter of Area of infection in mm.	Mean Diameter of Colony on fruit in mm.	Mean Diameter of Area of infection in mm.
1	0.0	0.0	0.0	0.0
2	5.6	0.0	0.0	10.1
3	9.0	0.0	15.6	16.1
4	13.3	8.0	22.5	20.1
5	18.6	16.8	24.7	25.2
6	30.4	20.5	40.1	44.3
7	31.9	22.5	45.2	49.4

TABLE 13. Comparative rate of Infection of Tomato fruits, inoculated externallyon intact "skin" and wound inoculated.

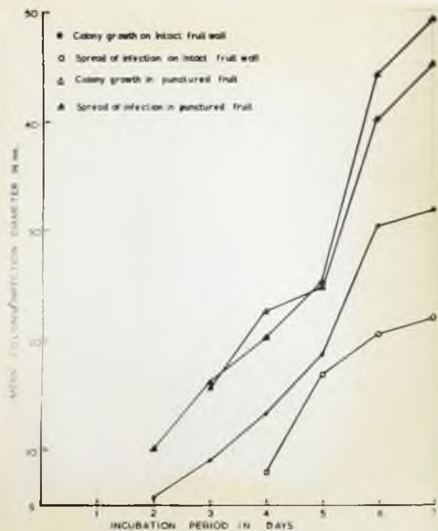


FIG.7: Comparative rates of infection in Tomato fruits inoculated exteriorly on intact skin and through wound

B: SIZE OF INOCULUM IN RELATION TO TOMATO FRUIT INFECTION BY S. ROLFSII

Inoculum, in plant pathology, signifies an indefinite quantity of a parasitic organism that meets or may be placed upon or near the surface of a potential host plant. This is usually referred to as an "effective inoculum", when it is adequate to produce a successful infection. The degree of infectivity of an inoculum was described by Garret, (1956) as its inoculum potential. The effectiveness of an inoculum may be increased either by increase in the size or number of infecting unit or by increase in the nutritional status of such units. In the previous experiment, an inoculum of mycelium on a 3 mm diameter agar disc was used and the rate of infection was found satisfactory to allow a study of progress of the disease. In nature, however, the size of the inoculum is bound to vary and this will bear a direct relation to the rate of infection. This experiment was therefore set up to find out how the size of inoculum will affect the rate of disease development in tomato fruits. Healthy, ripened surface-sterilised tomato fruits were inoculated by placing on the intact skin, agar discs of varying diameters removed from the advancing edge of 3 days' old S. *rolfsii* culture. The diameters employed were 3, 6, 9, and 12 mm respect-

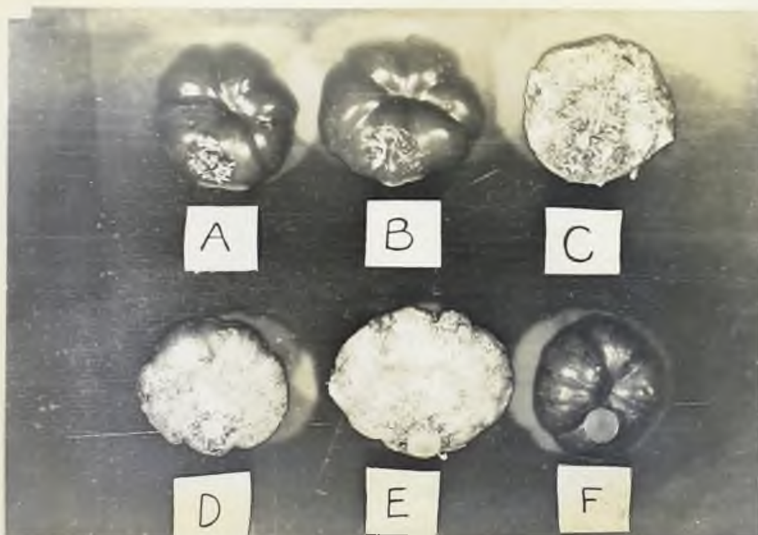


PLATE 4 : RELATION OF SIZE OF INOCULUM TO INFECTION RATE OF TOMATO FRUITS BY S. ROLFSII
Photograph of tomato fruits showing degree of infection at different sizes of inoculum.

A - SCLEROTIUM
B - 3mm
C - 6mm
D - 9mm
E - 12mm
F - CONTROL

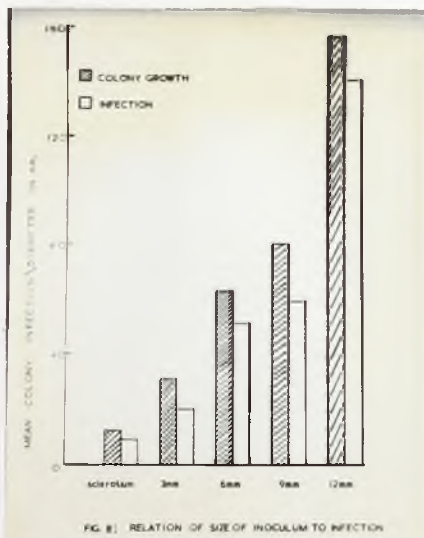


FIG. 8 | RELATION OF SIZE OF INOCULUM TO INFECTION

ively. In addition to these, a single sclerotium was used as an inoculum. The inoculated fruits were placed in humid chambers and incubated at 32°C for 7 days. There were 4 replicates per each inoculum diameter. The results are presented in Table 14 and in Fig. 8.

TABLE 14

Relation of size of inoculum to infection rate of tomato fruits by *S. rolfsii* at 32°C
(Observation after 7 days' Incubation)

Size of Inoculum used in mm.	Mean Colony Diameter in mm.	Mean Infection Diameter in mm.
F – Control	0.0	0.0
A – Sclerotium	12.0	9.3
B – 3	31.3	20.0
C – 6	73.0	51.0
D – 9	80.0	58.5
E – 12	156.0	140.0

The data show that the rate of infection increased with increase in size of inoculum. The rate of infection increased almost directly proportional to the increase in size of the inoculum. The maximum inoculum used (12 mm) caused the entire fruit to rot within 7 days. The rate of colony growth was always ahead of the rate of infection in all the inoculum sizes employed (Fig. 8).

The feeble rate of infection caused by the sclerotium is worthy of note. Whilst the mycelium can readily initiate attack, the sclerotium will need a period of time to germinate before invading the host. Besides, it is likely that the rate of germination of the sclerotium may depend on nutrients present in the soil or available in the exudate of the host.

C: HISTOLOGY OF INFECTION

Although some workers have conducted histopathological studies of S. rolfsii attacking various hosts, there is no information on the relationship of this fungus with tomato fruits. This section of the work was set up to gain information concerning the pathlogical anatomy of this tomato-rot disease.

It is known that many fungi penetrate the unbroken surfaces of various parts of host plants. This has also been established in this investigation. S. rolfsii does penetrate the tomato fruit through the intact "skin" (see page 64). Various studies have established the fact that, before some of these fungi penetrate the external tissues of their respective hosts, they exhibit a characteristic organisation or hyphae at certain loci, in the form of compact hyphal aggregations on the surface of the host. These hyphal aggregations are normally called "infection cushions". For example, Christou (1962) reported that Rhizoctonia solani formed infection cushions on the hypocotyl of bean seedlings. Gonzalez and Owen (1963) observed similar phenomenon in their work on fruit-rot of tomato caused by Rhizoctonia solani. Khadge, Sinclair and Exner (1963) also found that infection cushion

formation always preceded penetration of hypocotyl of cotton seedlings by Rhizoctonia solani. It is from these infection cushions that hyphae are produced to penetrate the host. The present investigations have revealed that S. rolfsii forms infection cushions on the tomato fruits prior to actual penetration. The manner in which these infection cushions are formed were investigated.

PRE-PENETRATION PHASE.

Twenty four surface sterilised tomato fruits were inoculated in the usual manner (see page 17) and incubated at 32°C. in an atmosphere of 100% R. H. At 2, 4, 6, 8, 10 and 12 hours after inoculation, samples of the tomato fruits were removed and kept in the deep freeze (- 10°C.) for 24 hours (see page 22). Four fruits were withdrawn at each stage for observation. After the chilling treatment, the epidermis with the spreading mycelium was stripped off from the pericarp and stained in 0.1% aqueous methylene blue solution for 5 minutes, and mounted in water. The edge of the coverslip was then sealed with nail varnish. The slides could thus be kept for several weeks. The mycelium stained deep blue, whereas the epidermis of the pericarp stained green.

Observations under the microscope showed that growth

of the mycelium from the inoculum, under optimal environmental conditions started very early, between 2 and 4 hours after inoculation. Infection cushions started to appear after 6 hours' incubation. Two forms of hyphae were observed: slender hyphae which seldom branched and of very limited extent were the normal form in which the fungus initiated growth from the inoculum. These, sooner or later, grew out into profusely branching hyphae of larger width, which formed the bulk of the mycelium. The hyphae ran irregularly over the epidermis of the fruit without bearing any special relation to the epidermal cells (Fig. 9, plate 5). It has been observed in certain instances that invading hyphae of some pathogenic fungi run along the surface of the host in a characteristic pattern imposed by the architecture of the host surface. Christou (1962) found that hyphae of <u>Rhizoctonia solani</u> infecting the hypocotyl of bean ran strictly in a lonitudinal direction along the joints of the cylindrical epidermal cells. Lateral branches normally coincided with the end wall of an epidermal cell and immediately assumed the longitudinal course once again along the next joint.

An infection cushion of <u>S. rolfsii</u> is formed from either a single hypha or from two or more hyphal branches.

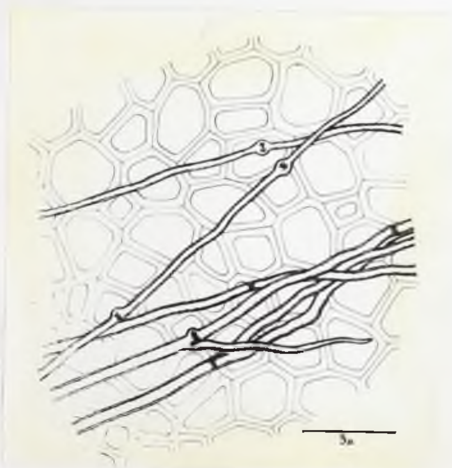


FIG.9: RELATION OF HYPHAE OF S. ROLFSII TO EPIDERMIS OF TOMATO FRUIT.

PLATE 5 : RELATION OF HYPHAE OF *S. ROLFSII.* TO EPIDERMIS OF TOMATO FRUIT
Photograph showing hyphae running irregularly over the epidermis of tomato fruit without bearing any special relation to the epidermal cells. (× 250)

In the case of a single hypha, the distal end branched repeatedly, the resultant branches bunching up to form a cushion. When two or more hyphal branches are involved the precess is exactly the same except that constituent branches of different origin, converge at a locus, and twist around each other to initiate a cushion formation (Fig. 10). In both cases, these initials continue to branch to form intricately woven and fused mass of short cells, firmly adhering to the fruit surface. Fully formed infection cushions showed wide variations in size and shape. Most of them, however, are flattened and circular in outline and range from 150 to 200 μ in diameter. (Plates 4 - 9).

PENETRATION & INVASION.

Subsequent activities of the fungus, after the development of the infection cushion were carefully followed with the aid of paraffin embedded sections. Surface sterilised tomato fruits were inoculated in the usual way and incubated at 32°C. and at 100% R.H. Two fruits were removed from the lot at daily intervals after inoculation for a maximum period of 10 days. In each case, the infected fruit was killed and fixed as previously described (see pages 22 - 23) in preparation for embedding and sectioning. Dehydration, infiltration and

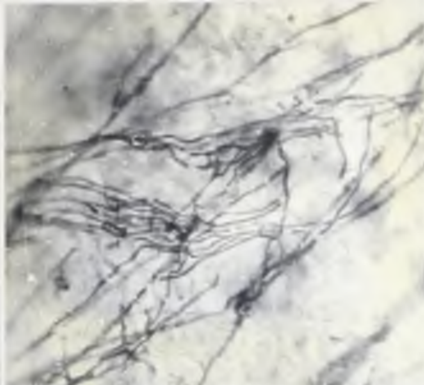


PLATE 6 : FORMATION OF INFECTION CUSHION OF S. ROLFSII
Photograph showing the aggregation of hyphae into an infection cushion

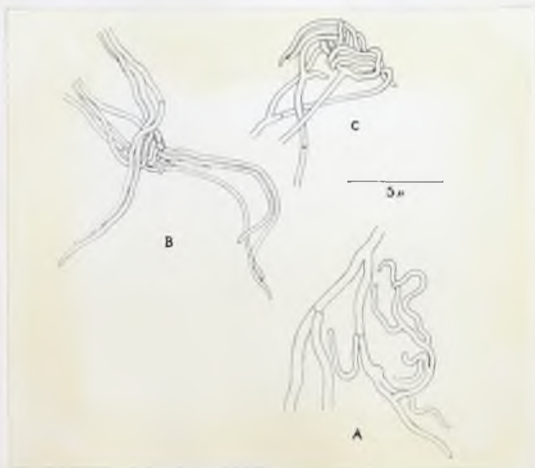


FIG 10 : FORMATION OF INFECTION CUSHION OF S. ROLFSII
Camera Lucida drawing showing the formation of infection cushion from : A. - a single hypha; B. - more than one hypha; C. - advanced stage on the infection cushion formation.

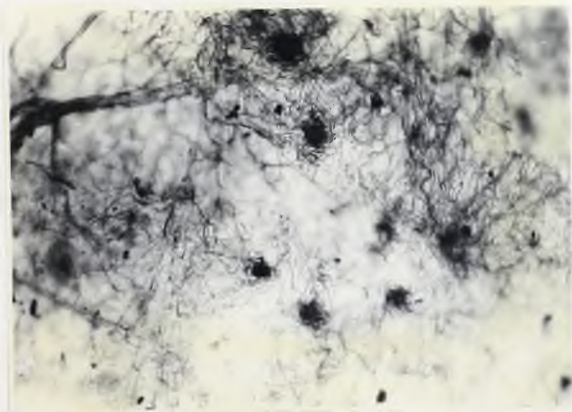


PLATE 7 : Photograph showing fully formed unfection cushions, formed from small hyphae. Note differenøt sizes of cushions.

(x 200)

PLATE 8 : Photograph showing fully formed infection cushions from large hyphae.

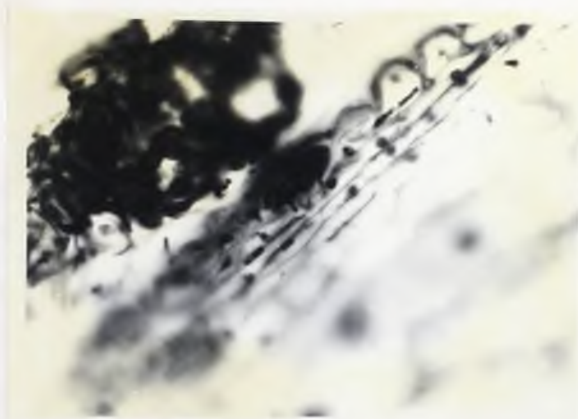
embedding of fixed materials were carried out in the manner described by Johansen (1940). Sections, 12 μ thick, were cut with a Cambridge Rocking Microtome. In preliminary staining trials, the following staining procedures were tried in an attempt to find the most suitable :

i. Iron alum - haematoxylin
ii. Methylene blue - clove oil - erythrosin.
iii. Safranin - fast green.
iv. Phloxine - fast green.
v. Delafield's haematoxylin - Erythrosin.

Iron alum - haematoxylin was found to be the most suitable among these and was accordingly employed throughout this work. It stained the cuticle of the epidermis yellow, the parenchyma cells of the pericarp brownish and the mycelium deep blue. All sections were mounted in Canada Balsam.

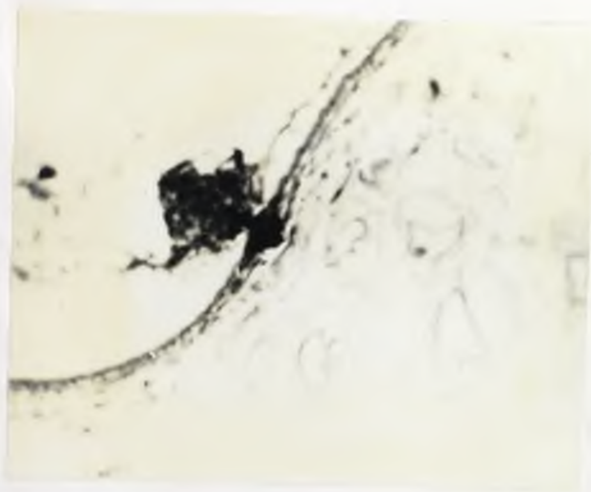
After the formation of the infection cushions, the fungus penetrated the host cuticle (average thickness, 10 μ) and the epidermal wall directly, invaded the epidermal cells, and finally emerged in the subepidermal layer. No infection pegs were observed. Repeated observations of the early phase of penetration indicated that hyphae of the cushion, peculiarly converged at a point and penetrated the thick cuticle into the epidermal cell. (Fig. 11, plates 9, 10).

The subepidermal cells were first to be attacked by



(x 600)

PLATE 9 : Photograph showing the hyphae of infection cushion aggregated in an epidermal cell of tomato fruit. Note lateral as well as vertical invasion of pericarp cells from this aggregation.



(x 400)

PLATE 10 : Photograph showing the hyphae of an infection cushion aggregated in an epidermal cell after penetrating through the cuticle.

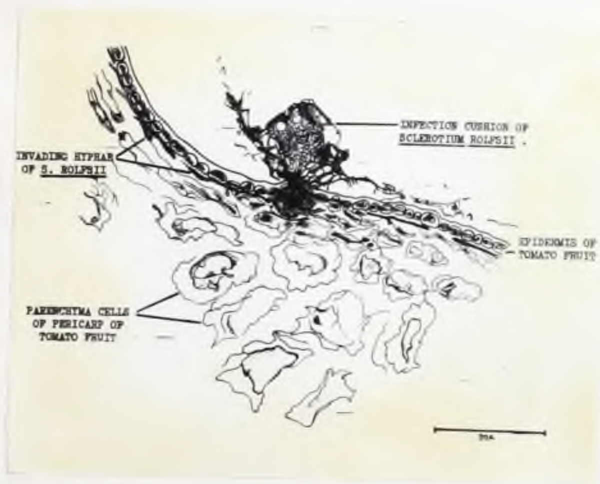
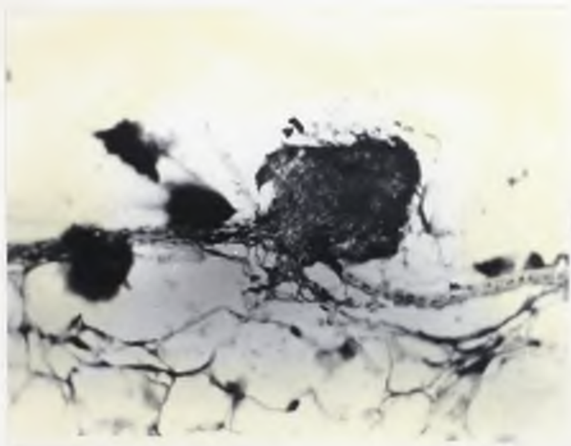


FIG 11 : Camera Lucida drawing showing the hyphae of an infection cushion ~~xxxxx~~ converging at a point and penetrating the cuticle into the epidermal cell of tomato fruit

the fungus after traversing the epidermis. These cells immediately below the infection cushion were destroyed. The fungus then grew out to fill the space created by the disintegration of the cells, forming an aggregated mass in the cavity within the subepidermal layer (Fig. 12, Plates 11,12). As the hyphal aggregations increased in size the epidermis and cuticle above them often gave way under pressure. It is from these collected mass that hyphae subsequently grew out in the next phase of invasion. The hyphae almost always grew out laterally into the sub-epidermal layer immediately underlying the epidermis for varying distances from the locus of penetration (Plate 13). Hyphae growing downwards towards the centre of the fruit were subsequently produced from these horizontal hyphal strands (Plates 4, 14). The fungus grew intercellularly in the first few layers along the middle lamella. In certain cases, the pericarp cells were pushed apart by thick strands of several hyphae growing together. Several contiguous cells were thus separated. There was always in the process of invasion, a zone of disorganised cells in advance of the spreading hyphae. There was recognisable dissolution of the middle lamella of these cells, thus loosening the cells from each other. (Fig.11, plate 10). Numerous hyphae could later be seen penetrating these apparently moribund

(x 400)

PLATE 11 : Photograph showing strands of hyphae filling the space created by the disintergration of pericarp cells.



(x 400)

PLATE 12 : Photograph showing strands of hyphae filling the space created by the disintergration of pericarp cells.

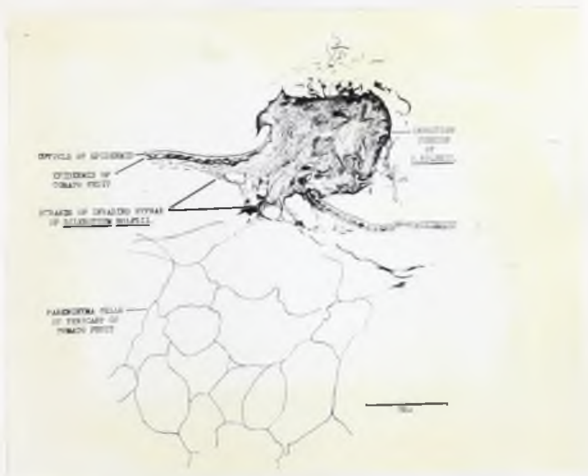


FIG 12: Camera lucida drawing showing strands of hyphae of S. rolfsii filling the space created by the disintegration of pericarp cells of tomato fruit.

(x 600)

PLATE 13 : Photograph showing the hyphae growing laterally into the subepidermal layer immediately underlying the epidermis

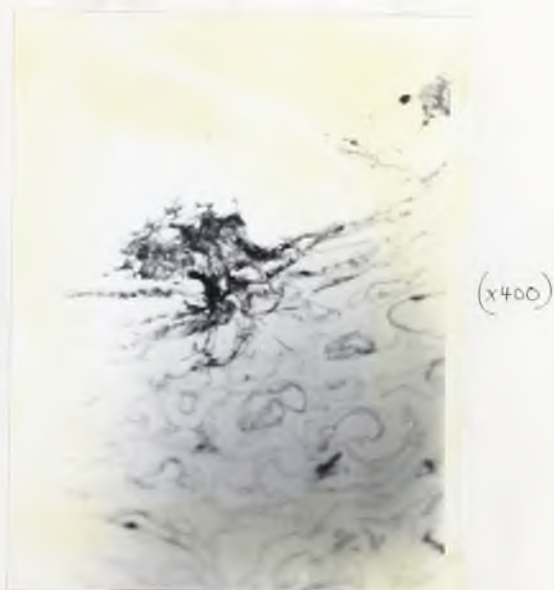
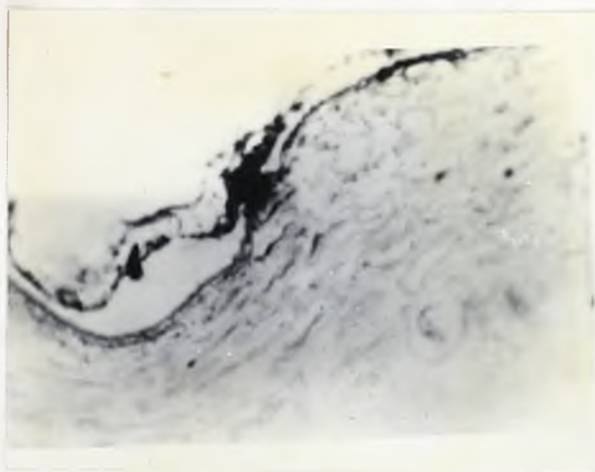


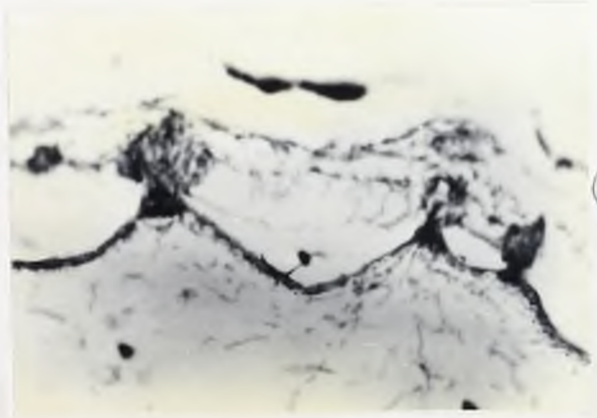
PLATE 14 : Photograph showing the hyphae growing downwards from the horizontal strands.



(x 400)

PLATE 15 : Photograph showing the scanty remains of almost completely destroyed parenchyma cells of the pericarp of the fruit.

FIG 13 : Camera lucida drawing showing the scanty remains of almost completely destroyed parenchyma cells of the pericarp of the fruit.



(x 400)

PLATE 16 : Photograph showing the parenchyma of the pericarp of the fruit completely disintegrated

PLATE 15 : Camera lucida drawing showing the parenchyma of the pericarp of the fruit campletely disintergrated.

or dead cells. In the event, no constrictions or swellings on either side of the cell wall appeared in the hyphae.

In the last stages of rot, all the pericarp cells eventually lost coherence and cell dissolution set in. The cell disintegration was almost complete by the 7th day. Naturally, if a larger inoculum were used, the rate of rotting will be immensely hastened (see page 68). In the end the entire pericarp is destroyed, leaving an empty shell of dead epidermal cells with a downy coat of mycelium. The fungus was unable to invade the seeds. The testa might obviously have formed a mechanical barrier. The following examinations were made to verify whether the fungus attacked the seeds or not.

i. A hundred washed and dried seeds extracted from completely rotten tomato fruits were paraffin embedded, sectioned and stained. No hyphae could be found in the seeds.

ii. A hundred washed and dried seeds from rotten fruits were later soaked and crushed on slides and stained with 0.1% cotton blue in lactophenol. There were no observable hyphae in the endosperm and embryo.

iii. A hundred seeds from rotten fruits were surface sterilised for 2 minutes with 1% mercuric

chloride in 70% alcohol. They were then rinsed several times with sterile distilled water and plated on potato dextrose agar in petri dishes and incubated at 27°C for 10 days. *S. rolfsii* was not recovered from any of the seeds tested.

Sclerotia were ultimately formed within the fruit and also on the outside by the external hyphae on the fruit surface.

Evidence from these studies show that *S. rolfsii* penetrated tomato fruits by mechanically piercing through the cuticle and the epidermis. This observation agrees with what Higgins (1927) and Paintin (1928) found during the invasion of stems of tomato and cowpea respectively by *S. rolfsii.* Edson and Shapovalov (1923) surprisingly reported that the mycelium of *S. rolfsii* failed to penetrate Irish potato blocks, and that enzymes secreted by the fungus dissolved the host tissue. *S. rolfsii* is convincingly a 'necrophytic' parasite from evidence gathered in this investigation. Some substances might have been produced which dissolved the pericarp cells well in advance of the invading hyphae, and nutrients subsequently obtained by the fungus from these dead cells. There is a positive indication that the cuticle was never dissolved by the fungus.

D: EFFECT OF ENVIRONMENTAL FACTORS ON RATE OF INFECTION OF TOMATO FRUIT BY S. ROLFSII

The influence of environmental factors on the degree of incidence of diseases of plants is very well known. But interaction of the different factors, leading to the outbreak of disease, or enabling a parasite to enter and to spread within its host, makes it difficult to determine the exact effect each exerts in the presence of the others in the field. Environmental factors exert their influence on parasite, or on the host or on both at the same time. However, they more often exert a greater influence on the parasite, and except in isolated cases less so on the host. Experimental studies of disease development in relation to various environmental factors have now become a necessity if attempts to control plant diseases are to succeed. The effects of selected environmental factors on the incidence of tomato fruit rot caused by S. rolfsii and on the rate of infection were studied in this work.

1. THE EFFECT OF TEMPERATURE

Temperature is one of the most important environmental factors affecting disease development. The incubation period of the disease may be affected by temperature. Temperature effects may render the host more susceptible to fungal diseases (Butler and Jones,

1949; Stakman and Harrar, 1957; Walker, 1957). The influence of temperature upon onset and subsequent spread of disease, may not necessarily be the same as that upon the behaviour of either the pathogen or the host when living alone (Butler and Jones, 1949). Temperature range favourable to the pathogen may be equally or less favourable to the host. The effect of selected temperatures, (see Table 15) very near to that obtained in the tropics, on the infection of tomato fruit by S. rolfsii was studied. Surface sterilized. tomato fruits were inoculated with 3-mm agar discs from the growing edge of 3 days' old cultures of S. rolfsii raised on potato-dextrose-agar. The inoculated fruits were placed on small miniature petri dishes (4 cm diameter) for support. The miniature petri dishes with their loads of inoculated fruits were then placed in humidity chambers (24.5 x 12 x 8 cm) and incubated at the respective temperatures at 100% R.H. There were 4 replicates per each temperature level. Measurements of infection diameter at 2 days' intervals were taken for a maximum period of 6 days. The results are presented in Table 15.

The data in Table 15 show that the infection of tomato fruit by S. rolfsii occurred to varying degrees

and at different rates at 17, 22, 27 and 32°C. There was no infection at 37°C. The minimum temperature for infection could not be established in this work, since temperatures below 17°C were not available.

The optimum temperature for infection was found to be 32°C. The maximmm temperature for infection was found to be between 32 and 37°C.

TABLE 15

Effect of temperature on infection of tomato fruit by *S. rolfsii* at 32°C.

Temperature Incubation in °C.	Mean Diameter of Infection, in mm, on indicated day after inoculation		
	2	4	6
17	0.0	0.0	6.0
22	0.0	11.5	25.5
27	10.2	32.1	85.1
32	18.0	44.0	117.5
37	0.0	0.0	0.0

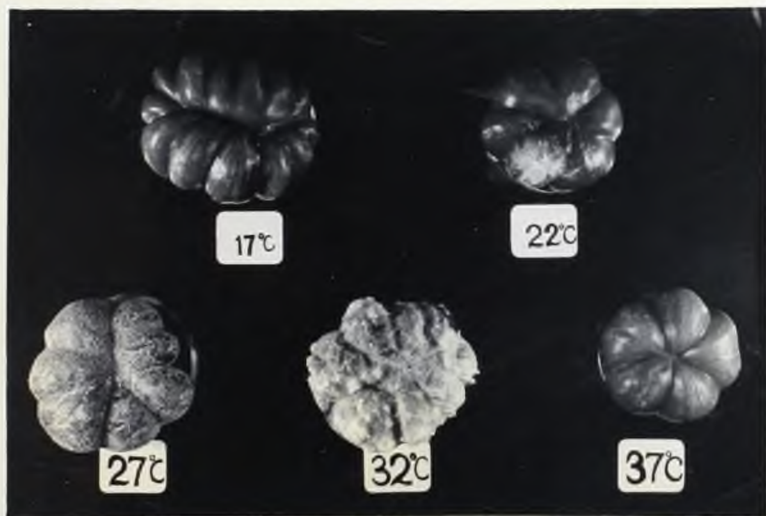


PLATE 17 : EFFECT OF TEMPERATURE ON INFECTION OF TOMATO FRUIT BY S. ROLFSII. Photograph of tomato fruits showing degree of infection at different temperature levels.

2. THE EFFECT OF RELATIVE HUMIDITY

Atmospheric humidity and precipitation in the form of rain, fog and dew often determine the fluctuations and distributions of diseases in a locality. The infective process requires a degree of humidity that is generally high in most cases of fungal diseases. Infection of carrot by *Sclerotinia sclerotium* (Lib) D By, for example, is favoured by 95% R.H. At a favourable temperature (23°C), optimum infection of sweet potato by *Rhizoctonia sp.* occurred at 75 to 84% R.H. (Walker, 1957). *Erysiphe graminis* on the other hand, may require relative humidity below 75% (Butler and Jones, 1949). The effects of different relative humidities on the infection of tomato fruits by *S. rolfsii* were investigated. Constant relative humidities were maintained with saturated aqueous salt solutions, prepared according to the data provided by Solomon (1952) (see page 19).

Saturated aqueous salt solutions were prepared in oblong plastic containers (24.5 x 12 x 8 cm) which served as humidity chambers. Surface sterilised tomato fruits were placed on sterile open small miniature petridishes (4 cm diameter). These were then placed at the bottom of the humidity chambers. The tomato fruits were inoculated with 3 mm agar disc from the growing edge of 3 days' old cultures. The humidity chambers were then

covered with their lids and sealed with sellotape. These were incubated at 32°C for 7 days. There were 4 replicates per each relative humidity level. The humidity range used and the results are presented in Table 16.

TABLE 16

Effect of humidity on the infection of tomato fruit by *S. rolfsii* at 32°C.

(Observation after 7 days' Infection).

% Relative Humidity	Mean Infection Diameter in mm.
100	99.2
95	99.0
90	0.0
85	0.0
80	0.0
75	0.0
70	0.0

The tabulated results show that a high relative humidity was essential for infection. *S. rolfsii* attacked the fruits at 95 and 100% R. H. only, where respective lesions of 99 mm and 99.2 diameter occurred within 7 days.

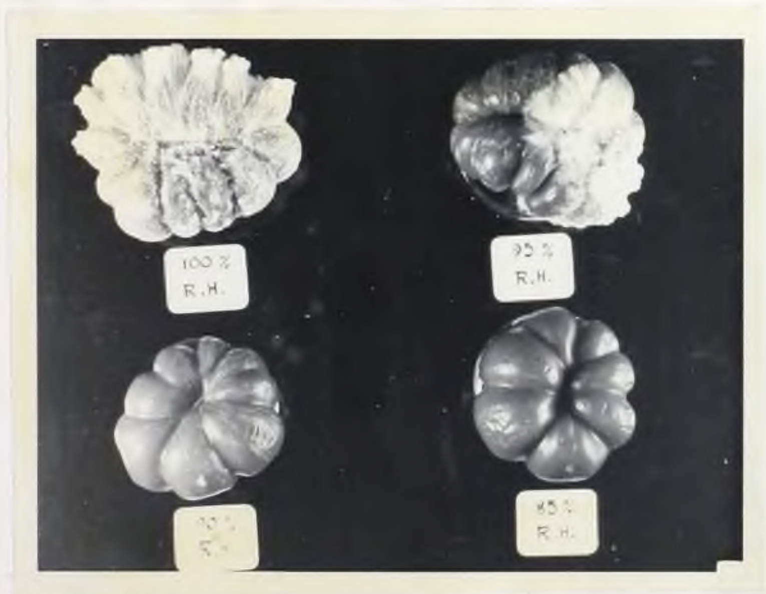


PLATE 18 : EFFECT OF HUMIDITY ON THE INFECTION OF TOMATO FRUIT BY <u>S. ROLFSII</u>
Photograph showing tomato fruits infected at various relative humidities.

3. EFFECT OF WATER HOLDING CAPACITY

Soil moisture is an important factor, affecting the spread of a parasitic fungus through the soil and the occurrence of root diseases. Soil moisture may affect, among other things, the concentration and availability of salts, degree of aeration and the level of population of other micro-organisms in the soil etc. (Stakman and Harrar, 1957). It is often a decisive factor in the prevalence and severity of diseases caused by soil pathogenic fungi. Some diseases, such as the root rot of garden pea, caused by Aphanomyces euteiches increase in prevalence and severity almost to the maximum water-holding capacity of the soil, while others, such as Sclerotium cepivorum the causative agent of white rot of onion, disappear with rise in percentage of water-holding capacity. An attempt was made to determine the effect of soil moisture on soil rot of tomato fruits caused by S. rolfsii.

The maximum water-holding capacity of a sample of the soil from which the isolate of S. rolfsii was obtained, was determined. Water was added to a quantity of the air-dried soil of known weight in a shallow metal pan with perforated bottom until the water started to drip through the pores at the bottom of the pan. By weighing,

the amount of water added was estimated and the maximum water-holding capacity of the soil sample calculated. Lots of 400 gms. of the sieved (using 3-mm mesh) unsterilised, and air-dried (sandy loam) soil were put into each of previously weighed plastic containers (24.5 x 12 x 8 cm). Mycelium of _S. rolfsii_ raised on liquid medium (see page 35) in 250-ml conical flasks at 27°C for 7 days was weighed, washed three times with distilled water, and macerated in a Waring Blendor. Aliquots of the macerated mycelium, were added to individual soil samples in the plastic containers. The soil and the mycelium were thoroughly mixed and air-dried at room temperature (26±2°C) for one to two hours. Distilled water was then added proportionately to give a range of 15, 25, 35, 45, 55, 65, 75 and 85% of the maximum water-holding capacity of the soil. Previously weighed surface sterilised tomato fruits were placed directly on the soil-fungus mixture and incubated in the open plastic containers at 32°C for 7 days. These were weighed daily and water was added when necessary, in order to maintain the respective percentage water-holding capacity level. Readings were taken in terms of (a) the number of fruits infected and (b) the mean diameter of infection. Results were analysed statis-

tically. These are presented in Table 17.

TABLE 17

Effect of Water-Holding Capacity of Soil on infection of tomato fruit by S. rolfsii at 32°C.

(Observation after 7 days' Incubation)

% W.H.C	Wt of mycelium, in mgms per 400 gms of soil	No of fruits infected.	Mean Diameter of Infection in mm. *
A - 85	11.6	0	0.0
B - 75	12.7	4	38.1
C - 65	11.9	4	69.8
D - 55	9.8	4	100.4
E - 45	13.2	4	93.3
F - 35	11.1	4	91.9
G - 25	11.7	4	92.2
H - 15	11.4	3	82.6

* Least significant difference between means ($p = 0.05$) = 12.3 mm.

S. rolfsii tolerates a wide range of soil moisture level for the infection and disease development in tomato fruits. There was a gradual rise in infection rate as the percentage water-holding capacity increased attaining a peak at 55% W.H.C. after which the rate of infection fell with no infection at 85% W.H.C. Sclerotia were produced abundantly on the fruits on soils maintained at 25 to 55% Water-holding capacity (Plate 18).

PLATE 19 : EFFECT OF SOIL MOISTURE ON TOMATO FRUIT ROT CAUSED BY S. ROLFSII
Photograph of tomato fruits showing degree of infection at various soil moisture levels

A. - 85%; B. - 75%; C. - 65%; D. - 55%; E. - 45%; F. - 35%; G. - 25%; and H. - 15% of the WATER HOLDING CAPACITY.

E: RELATION OF AGE OF TOMATO FRUIT TO INFECTION BY S. ROLFSII

It is normally observed in the field that soil rot of tomato fruits by *S. rolfsii* is very common among the mature and ripe fruits. The small green and immature fruits are hardly attacked. Since the tomato plants usually fall over under the weights of the fruits, especially that of the mature and heavy ones, there is the possibility that the greater percentage of infection among the mature and ripe fruits may be purely happening by chance, since they are more likely to come into contact with the soil - and hence the fungus in the soil- than the young and immature fruits. The possibility of other factors being responsible cannot, however, be ruled out. The composition and concentration of carbohydrates and proteins etc. in tomato fruits change with age (Gustafon, 1927, 1929; Houghtaling, 1935; Gustafon and Houghtaling, 1939; Glendenning, 1942). This experiment was, therefore, designed to find out whether fruits respond to the attack of S. rolfsii with age; and if they do, to find out the underlying factors involved. The exact age of the fruits was obtained by carefully labelling and noting the date on which each young fruit's petals abscissed. The fruits of the respective known ages of 10, 20, 25, 30, 35, 40 and 45 days were used in this experiment. The surface sterilised tomato fruits

were inoculated in the usual way with *S. rolfsii* and incubated at 32°C and 100% R. H. for 7 days. There were four replicated per each age level. The results could not be analysed statistically because of the different sizes of fruits involved. The results are presented in Table 18.

TABLE 18

Relation of age of tomato fruits to infection by *S. rolfsii* at 32°C.

(Observation after 7 days' Incubation)

Age of fruit in Days	Mean Diameter of Infection in mm.
A - 45	89.3
B - 40	93.6
C - 35	81.7
D - 30	82.1
E - 25	86.5
F - 20	85.1
G - 10	74.9

The data in Table 18 show that infection of tomato fruits occured at all the ages of the fruits used. The 10 day old fruit was completely covered by the fungus by the 7th day and entirely rotted, and the fungus had overgrown unto the petri dish. The figure of 74.9 mm indicated the circumference of the fruit. (Plate 19).

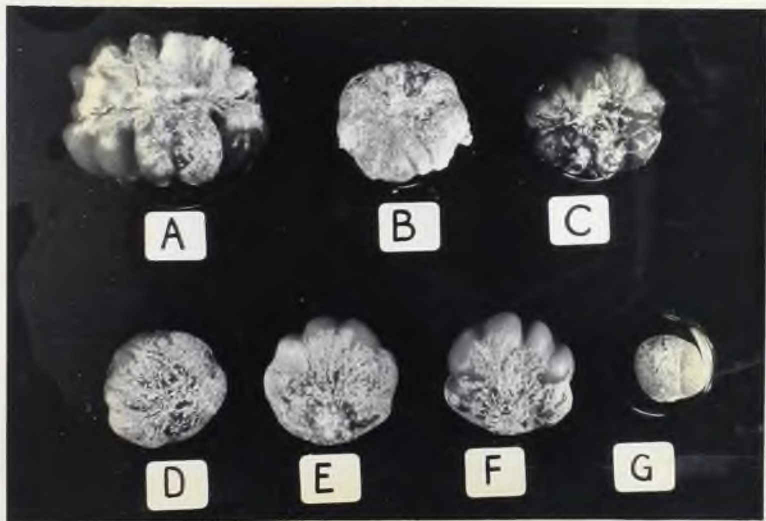


PLATE 20 : EFFECT OF AGE OF TOMATO FRUIT ON DISEASE DEVELOPMENT. Photograph showing infection in tomato fruit of different ages : A. - 45 days old; B. - 40 days old C. - 35 days old; D. - 30 days old; E. -25 days old; F. - 20 days old; G. -10 days old.

F: SUSCEPTIBILITY OF TOMATO VARIETIES TO S. ROLFSII

The breeding or selection of resistant varieties of the host is one of the most important and most succes-ful methods for combatting diseases. Varietal resistance to *S. rolfsii* has been demonstrated in a number of hosts, including tomato plants (Fulton, 1908; McClintock, 1917, 1918; Mendiola and Ocfemia, 1926; Reyes, 1937; Streets, 1942; Mekhaimer, 1950; Orian, 1952; Mehta, Singh and Mathur, 1953; and Mohr, 1955). The possibility of the existence of a tomato variety in Ghana whose fruits are resistant to this fungus was investigated.

Six Ghanaian varieties - the Local, Improved Zuarungu, Maui, Molokai, Hybrid (MM86/8) and Hybrid (MM84/17) - were tested. The surface sterilised tomato fruits were inoculated in the usual way and incubated at 32°C and 100% R. H. for 7 days. There were 4 replicates per each variety. The results which were analysed statistica-lly, are presented in Table 19.

TABLE 19

Variety	Mean Diameter of Infection in mm *
A - LOCAL	101.0
B - IMPROVED ZUARUNGU	20.0
C - MAUI	55.5
D - MOLOKAI	0.0
E - HYBRID (MM86/8)	60.7
F - HYBRID (MM84/17)	139.5

* Least significant difference between means ($p = 0.05$) $= 6.7$ mm.

Observations showed that in cases where the fruits were susceptible, the degree of infection was almost identical in fruits of that particular variety. All, except the Molokai variety, were susceptible to *S. rolfsii* (Plate 20). The Local and Hybrid (MM84/17) were heavily infected; the improved Zuarungu, Maui, and Hybrid (MM86/8) showed moderate infection, whilst all the Molokai fruits tested remained healthy. A second confirmatory test gave exactly the same trend.

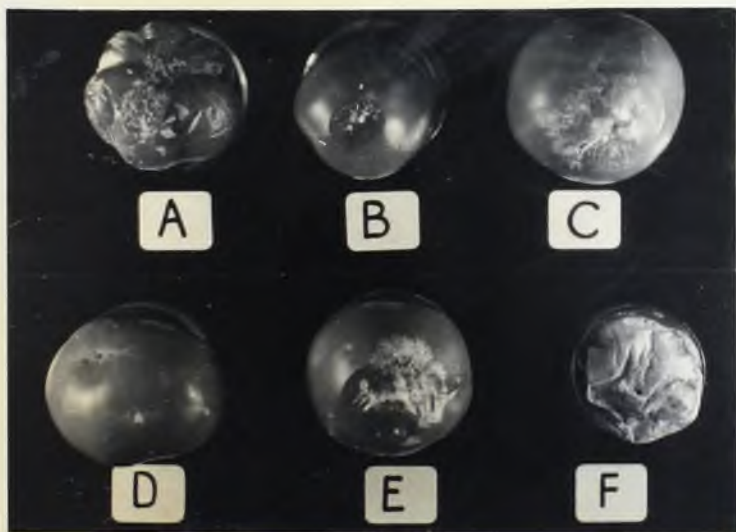


PLATE 21 : SUSCEPTIBILITY OF TOMATO VARIETIES TO S. ROLFSII
Photograph showing the infection of different tomato variety fruits.

A. - LOCAL; B. - Improved Zuarungu; C.- Maui
D.- Molokai; E.- Hybrid (MM86/8); F.- Hybrid (MM84/17).

V: GENERAL DISCUSSION

The economic importance of Sclerotium rolfsii lies in its ability to destroy many useful plants, including ornamentals and vegetables. The infected plants are killed outright. In Ghana, the disease caused by this fungus is particularly serious in groundnut and tomato farms where heavy losses occur. Even though several attempts, both chemical and cultural, have been employed in the field to control S. rolfsii in different susceptible hosts, these often give rather inconclusive and poor results. These results suggest that the control methods employed were unable to upset the conditions which favour parasitism of the fungus. Parasitism is almost always associated with vegetative growth in pathogenic fungi, this in turn is influenced by a multitude of factors. The object of the present study was to determine more precisely the influence of some of the major environmental factors, pertinent to Ghana, on the vegetative growth of the fungus and to investigate, more particularly some aspects of the pathogenicity of the fungus in relation to tomato fruit rot, as a contribution, chiefly to the understanding of the fungus and the disease it causes, and also as a guide in the choice of control measures to be adopted to combat the disease.

The results of the present work showed that S. rolfsii grew over the range 17 to 37°C with an optimum at 27 to 32°C. (see Table 4). The optimum temperature obtained was slightly lower than that reported by Higgins (1927) but similar to that reported by Abeygunawardena and Wood (1957) for one of the two isolates they studied. Higgins (1927) claimed an optimum of 30 to 35°C, whereas Abeygunawardena and Wood (1957) found an optimum of 30°C. The latter, however, observed a lower optimum temperature for the other isolate. This isolate, originally obtained from Ceylon, grew best at 20°C. This might possibly be a reflection of the origins of the isolates. The existence of different physiological activities among strains of the same species of fungus is not unknown (Wolf and Wolf, 1947). The maximum and minimum temperatures for vegetative growth could not be established in this work, as temperatures below and above the range employed were not available. Higgins (1927) found 37°C to be the maximum for continuous normal growth. Abeygunawardena and Wood (1957) found the maximum temperature to lie between 35 and 40°C. There were indications from results presented here that the maximum temperature for this particular isolate must be very close to 37°C., the highest temperature used. The minimum temperature was evidently below 17°C., the lowest temperature used in

this investigation. Abeygunawardena and Wood (1957) found the fungus to grow at 10°C and Higgins (1927) observed growth at 8°C, but failed to obtain any at 5°C.

S. rolfsii infected tomato fruits to varying degrees and at different rates over the temperature range of 17° to 32°C, with an optimum at 32°C. (see Table 15). The maximum temperature for infection was between 32 and 37°C. The minimum temperature for infection could not be established, as there was growth at the lowest temperature (17°C.) used. It is interesting to note that the optimum temperature for infection (32°C) lay within the optimal temperature range (27 to 32°C.) for growth of the fungus. When growth curves of a pathogen coincide with disease curves, or when the two curves have the same trend, as has been found in this work, it is fair to assume that the effect of temperature is principally on the activities of the pathogen. The possibility of a deleterious effect by temperature on the tomato fruit cannot, however, be ruled out altogether. The temperature (32°C) might have affected the fruit adversely in a way, to render it more susceptible to attack. The inability of the fungus to cause any infection at temperatures above 32°C. probably is a direct result of the

poor growth of the fungus.

Temperature does not appear to be an important factor which can possibly limit the growth of the fungus in the field in the tropics. The average daily temperature range in Ghana, extends from 26.1 to 34.4°C, (Boateng, 1960), which perfectly coincides with the optimal range for infection. Fruit-rot of tomato by S. rolfsii can therefore be effected at any period of the year, provided that other environmental conditions are ideal. The growth rate of the fungus as depicted graphically (see Fig. 1) was slow at 17 and 22°C for the first three days, but rose sharply on the third and fourth days. This means that if the temperature in the field, for any reason, should fall within this range for a period exceeding three days, and all other environmental factors are favourable, the fungus can infect any susceptible host.

Light of the visible range, 400 - 800 $m\mu$, exerts undoubted but ill-defined influences on mycelial growth of fungi (Cochrane, 1957). Different fungi vary considerably in their response to light. Light of the intensity of diffuse daylight usually has little influence on growth-rate of vegetative hyphae, but light of high intensity, comparable to that of sunlight, particularly

when blue or ultra-violet rays pre-ponderate, may retard or inhibit growth or may have a lethal effect (Hawker, 1950). There was no indication from the present investigation that light had any effect, favourable or otherwise, on the vegetative growth of S. rolfsii. (see Table 5). The ability of S. rolfsii to grow at the same rate in both light and dark has also been observed by Abeygunawardena and Wood (1957). This fungus will therefore be equally effective in nature in either light or dark.

S. rolfsii needed a very high humidity for mycelial growth (see Table 6). Over the range of 70 to 100% R.H., employed growth occurred only at 100% R.H. The ability of the fungus to grow only at 100% R.H. and probably very close to that level of humidity, indicates that the humidity of the atmosphere in the field will be a potent factor limiting the activity of the fungus. Successful establishment on any susceptible host will take place only under sufficiently protracted periods of high humidity.

The same relation between infection rate and humidity was observed in the subsequent experiments on infection (see pages 100 - 101). High relative humidities were essential for infection, which occurred at humidities between 95 and 100% R.H. only. The infection at 95% R.H. was not surprising, because the 'micro-atmosphere'

between the agar disc and the fruit surface probably had a higher humidity than 95% for, at least, a short period. Growth from the agar discs and subsequent infection usually occurred within 6 hours after inoculation (see page 73). If this "micro-atmosphere" of high humidity were to prevail for such a period or a little more, penetration into the fruit could be achieved. Once the fungus had penetrated the fruit, the external humidity would not be so critical. Within the host, the necessary moisture for growth is available and that, together with abundant nutrient supply, could account for infection even at 95% R.H. These results can be easily translated into field conditions. Although very high humidities are essential for infection, such humidities are always likely to be obtained beneath the fruit with a congenial saturated atmosphere for infection.

Evidence from this work showed that infection of tomato fruits occurred over a wide range of soil moisture level (see Table 17). Highest level of infection occurred at 55% Water Holding Capacity necessary for infection could not be established from the results. There was a gradual rise in infection rate as the percentage water-holding capacity increased from 15%, attaining a peak at

55% W.H.C., after which the infection rate fell. The upper limit of the moisture range permitting infection occurred between 75 and 85% W.H.C. Median soil moisture level is no doubt the most favourable for infection. The inability of the fungus to infect fruits at moisture levels above 75% W.H.C. could be possibly attributed to two main factors. Degree of aeration in a soil is inversely proportional to the soil moisture content. Aeration is therefore greatly reduced at high moisture levels. Watkins (1961) in a review on the physiology of S. rolfsii remarked that the characteristic occurrence of the fungus on the soil surface is a reflection of its inability to grow in atmospheres with low oxygen concentration. Probably the fungus failed to grow and hence infect the fruits in soils with moisture level above 75% W.H.C. due to lack of oxygen. On the other hand, the higher moisture level of the soil might have resulted in excessive growth of other moisture-loving soil micro-organisms. Most likely, some of these might have been either parasitic on the mycelium of S. rolfsii or might have produced metabolites antagonistic to it. For, indeed, experiments by Weindling (1932) have indicated that Trichoderma lignorum (Tode) Hartz, parasitised as well as inhibited the development of S. rolfsii mycelium.

These possibilities have however not been investigated further.

Variations in one environmental factor may alter the effects of the others. Thus is has been found that no single temperature level could be considered to be optimum for growth of any of some eight species of wood-rotting Basi-diomycetes when tested on different nutrient solutions (Stakman and Harrar, 1957). Similarly, the range of humidity over which a fungus will grow is greater the more nearly the fungus is kept to its optimum temperature for growth, and its temperature range is greatest when the humidity is near its most favourable level. Indeed, it seems that when any one of the factors that influences growth rate is altered the response of the fungus to other factors is also affected. The interactions between temperature and humidity and between the former and water-holding capacity of the soil were not investigated in this work, even though such interactions occur in the field. The effects of temperature, humidity and soil moisture have been examined individually here. The effects of interactions among these on the behaviour of the fungus and disease development must be studied in further investigations.

The fungus grew over a wide range of hydrogen-ion

concentration, from pH 3 to pH 10.42 (see Table 7). It grew best in acid media, as has been found by other workers (Higgins, 1927; Rosen and Shaw, 1929; Abeygunawardena and Wood, 1957). Since the fungus grew at all the pH levels used in this work, the minimum and maximum pH could not be determined. The optimum was found to be pH 4.8. It is most unlikely that pH could be a limiting factor in the activity of the fungus, under natural environmental conditions, for example as a factor of ecological significance. The limits of pH sustaining growth in the fungus significantly extend well beyond those for practical production of most crops.

An interesting feature was the double maximum showed by the fungus in relation to pH. There was a major peak at pH 4.8 and a minor one at pH 9,31 with a minimum of pH 8.2 in between. Since reports of earlier workers (Higgins, 1927; Rosen and Shaw, 1929; Abeygunawardena and Wood. 1957) indicated that the highest pH used in their various studies was pH 8, this might explain their failure to observe this phenomenon in *S. rolfsii*. This sort of phenomenon has been observed in various physiological functions of plants in general in relation to pH. Webb (1919) was apparently the first to call attention to this phenomenon. He found that, when spores of *Penicillium cyclopium* were germinated in buffer mixtures of H_3PO_4 and NaOH containing mannite,

two maxima appeared, with a minimum between, when percentage of spore germination was plotted against the pH of the solutions. He found similar curves for Fusarium sp. and, under certain temperature conditions, for Aspergillus niger. Slater and McIlvaine (1920) found a double maximum curve for the growth of wheat seedlings in solution cultures, the reaction of which was adjusted with citric acid and NaOH. Hixon (1920) found a double-maximum curve for the germination and seedling growth (in length) of corn, wheat and oats with the minimum at pH 6.0 between the two maxima. Clerk (1963) found similar curve for the germination of conidia of Isaria farinosa germinating at different pHs on pyridine-washed agar buffered with a mixture of Na_2HPO_4 and citric acid. Hixon (1920) quotes a suggestion by E. J. Cohn that the minimum point is the isoelectric point of a protein of the cell membrane of the seed. The cause of this double maximum is suggested by Arrhenius (1922) as being due to changed intensity of permeability for the different nutrients, or to the fact that the solubility of salt differs at different hydrogen-ion concentrations. Robbins (1923) from results of experiments on the absorption of water by potato-tuber tissue in solutions of different hydrogen-ion concentrations

and experiments on the absorption of dyes by plant tissue which had been in contact with solutions of different hydrogen-ion concentration concluded that the cytoplasm acts, in water absorption and absorption of solutes, like an ampholyte with an iso-electric point. He obtained a double maximum curve witha minimum between for the absorption of water by plant tissues in solutions of different hydrogen-ion concentration. This offered a satisfactory explanation for the double-maximum curves found in the germination of fungal spores, seed germination and growth of seedlings - physiological mechanisms closely associated with water absorption. Possibly, a similar phenomenon was operating in mycelium of *S. rolfsii*: pH 8.2 being the iso-electric point in water absorption for the fungus.

The fact that vitamins are essential for the growth and development of fungi has long been recognised. That improved growth and development usually followed the addition of small amounts to the medium has been shown by results of numerous experiments (Schopfer, 1934; Robbins and Kavanagh, 1938, 1942). The vitamin requirement may be partial or complete. Data from this study showed that *S. rolfsii* was partially deficient in thiamine (see Table 8). It did not require an extrace-

llular supply of thiamine to initiate growth. Satisfactory subsequent development, however, depended on externally supplied thiamine. It can, apparently synthesise some of the vitamin, but not enough for maximum growth. Robbins and Kavanagh (1942) also showed that S. rolfsii requires an external supply of thiamine for growth. They found that of the two thiamine intermedites, pyrimidine and thiazole, the former is effective in promoting normal growth, but the latter is not. Kavanagh (1942) showed later that the fungus synthesises thiazole, which it combines with externally supplied pyrimidine to form thiamine. It has been reported that several species of bacteria, fungi and actinomycetes produce large quantities of thiamine in the soil (Morton and Stroube, 1955). In the field therefore, thiamine is not likely to be a limiting factor in the growth of the fungus, since the soil microflora will probably supply the necessary thiamin required for normal development. Another likely source of vitamin for the fungus will either be plant debris, on which it subsists saprophytically or exudates and emanations from the host tissues.

The amount of thiamine needed to promote maximum growth of a deficient fungus is usually very small

In natural media, it is normally assumed that near-optimum amounts of the vitamins are present and sufficient to support growth. In purely synthetic liquid medium, thiamine at a concentration of 25 to 50 /ug in a litre of medium supported an optimum growth of S. rolfsii in this work (see Table 9). This is in contrast to the report of Joham (1943) who indicated that all levels of a wide range of thiamine concentrations sustained identical amount of growth. The optimum concentration of a vitamin may vary with changes in other conditions such as pH, carbon and nitrogen sources. The Effects of these changes on the thiamine requirements of the fungus was not investigated. It will be of interest to know how these factors will influence the thiamine needs of the fungus, since inthe field the different carbon and nitrogen sources occur. Thiamine, at excessive concentrations, has been reported to depress the growth of several fungi (Mathur et al. 1950). This was not observed over the range used. It is however less likely that in nature such high concentrations would occur to depress the growth of the fungus.

The isolate of S. rolfsii used in this work was able to utilize a large number of carbon compounds including the simpler carbohydrates and polysaccharides.

(see Table 10). This is a positive indication that the fungus produces a variety of enzymes in nature. Indeed the wide host range of the fungus can be attributed to its capacity to produce various types of enzymes in sufficiently large quantities to act on the different hosts. Starch, maltose and fructose were used readily whereas glucose was moderately used. Treggi (1956) also reported that 5% soluble starch favoured growth of S. rolfsii. Cellulose, lactose, glycerol and galactose were used to limited extent. When starch is enzymatically hydrolysed, maltose is produced. Further hydrolysis produces glucose. All these hydrolysis products are used by the fungus. This could account for the ready utilisation of starch. In the presence of glucose, starch was used even more than when it was the sole source of carbon, due obviously to the presence of the readily usable glucose to initiate growth. The poor growth on cellulose, even in the presence of 0.1% glucose, indicated that either the cellulolytic activities of the fungus is quite poor or it is unable to utilise the hydrolytic products of cellulose. The concentration of glucose added to the cellulose was too low to sustain any further development beyond the initial growth. Johnson and Joham (1954) could not also obtain any growth on cellulose.

The poor utilization of galactose is not easily explained, though this is not unusual in the fungi. Horr's (1936) observations show that both galactose and mannose constitute poor sources of carbon for *Aspergillus niger* and *Penicillium glaucum*. Higgins (1927) found maltose to be the best carbon source, whilst Abeygunawardena and Wood (1957) indicated that fructose was most suitable.

The dry weight of the fungus increased with an increase in glucose concentration over the range used (see Table 11). This range could not allow the optimum concentration to be established. It was not found advisable to extent the range beyond 2% concentration (the maximum employed), for fear of introducing other variables. It has been observed that at higher concentrations of glucose or other soluble substances, osmotic pressure effects among other things might interfere with accurate interpretation of effect of the glucose *per se* on growth (Cochrane, 1957).

The forms of nitrogen utilized included those of organic, ammonium and nitrate. (see Table 12). With the exception of alanine, the organic nitrogens were superior to the ammonium and nitrate ones. This agrees with what has been found by Higgins (1927), Abeygunawardena and Wood (1957) andTownsend (1957). The sources of

nitrogen used fell into three main categories, namely (i) those which were readily used (Peptone, Asparagine, Ammonium nitrate, urea). (ii) those moderately used (Ammonium chloride), and, (iii) those which were used to limited extent (Glycine, Sodium nitrate, Alanine). The effects of different concentrations of any of the nitrogenous compounds was not studied, as the nitrogen concentration requirements alter so largely with the source and concentration of the carbon source.

S. rolfsii cannot be regarded as a wound parasite, for it penetrated the intact skin of the tomato fruit (see pages 59 - 66). Even though abrasion or wounding of the host was not necessary, they naturally accelerated infection and subsequent rotting of the fruits. (see plate 3).

As the amount of inoculum increased, rate of infection also rose almost directly proportionally (see Table 14). Larger inocula did not only increase the magnitude of infection but also accelerated the onset of attack. This may have quite important implications in the field, especially where plant debris abounds in sufficient quantities to form a food base. This will encourage rapid growth with subsequent build up of inoculum.

It has been established in this work that S. rolfsii forms infection cushions on the surface of the fuits (see plates 6 - 8). These cushions were formed at randomly scattered loci in the mycelium. This ensured a concerted effort by the hyphae to puncture the fruit wall mechanically (see plate 9). The aggregation of hyphae into cushions might also increase the nutritional status of the invading hyphae. Surprisingly, Higgins (1927) made no mention of cushion formation in his infection studies. It has not been possible to investigate the specific stimulus or stimuli, determining the sites of these cushions and initiating their formation. Although infection cushions have been observed during studies on infection by some soil-borne pathogens by several workers (Abdel-Salem, 1933; Gonzalez and Owen, 1963; Matsumoto, 1921; Nakayama, 1940; Schaal, 1939; Ullstrup, 1936), they have been little studied from the point of view, as to whether or not plants secrete substances which in some way stimulate the hyphae to begin the process of infection. Recently, the stimuli affecting production of infection cushions by Rhizoctonia solani have been investigated. Kerr (1956) enclosed roots of radish, lettuce, and tomato seedlings in cellophane bags in soil infested with R. solani and

found that structures resembling infection cushions were formed on the surface of the cellophane enclosing radish or lettuce, but not tomato roots. Kerr and Flentje (1957) also found that the formation of infection cushions on radish roots is stimulated by substances diffusing to the surface from the underlying cells, and that only the radish strain responds to these substances. This is by no means the only stimulus because cushions are not formed when hyphae were in contact with cortical cells exposed by removal of the epidermis. On the living host it was postulated that contact with the cuticle as well as specific substances condition the formation of cushions. In contrast, Shepherd (1957) found no evidence that crucifer and lettuce strains are stimulated chemically, and considered that the stimulus is connected with the structure of the cuticle, citing the findings of Mueller _et al_ (1954) that the cuticular surface differs greatly in different species.

Clearly, the responses leading to cushion formation, may differ for different host-parasite Combination even for a single species of pathogen. Evidently, further investigation is required to elucidate the type of stimulation involved here.

In penetrating the host, many hyphae were involved

(see plates 9 & 10). The cuticle was mechanically pierced without showing any signs of dissolution at all. According to Brown (1948) direct penetration through cutinised walls is believed to be entirely by mechanical pressure, since no cutin-dissolving enzyme has been demonstrated in fungi. The fungus first invaded the epidermal cells at the point of penetration (see plate 9 & 10) and then proceeded to attack and destroy the parenchymatous cells immediately below the epidermis. A cavity was subsequently created, which was filled by the invading hyphae. (see plates 11 - 14). From this collection, hyphae travelled extensively laterally beneath the epidermis before invading the inner tissues of the pericarp (see plate 11). The hyphae coursed through the tissue both inter - and intracellularly. All the pericarp cells were ultimately destroyed. Manifestly, typical symptoms of soft-rot. The disease was not seed-borne.

There is sufficient evidence that some substances were produced by the fungus which killed and dissolved the pericarp tissue. These active substances could not, however, dissolve the cuticle. The process of pathogenesis has been followed in a number of studies and known to involve quite a complex mechanism - maceration

and killing of the tissues. By enlarge, two main enzyme systems, pectinase and cellulase are involved. Demonstration of the formation of pectinolytic enzymes in host tissues invaded by soft-rotting fungi is one essential fact in the ultimate proof that these enzymes are involved in tissue maceration. (Husain and Kelman, 1951). Conclusive evidence is now also available that certain of the soft rot fungi, including B. cinerea. produce cellulase (Reese and Levinson, 1952; Kohlmeyer,1956). S. rolfsii has been found to be a strongly cellulolytic fungus. In culture it produces an extracellular cellulase that can also be detected in invaded tomato stem tissues (Husain,1957); It is now evident that the degradation of cellulose in addition to that of pectic materials is an essential phase of tissue disintegration by many soft rot fungi. It can be presumed that S. rolfsii decomposed the tissues of the tomato fruit ny means of both pectinolytic and cellulolytic enzymes. The poor growth of the fungus on a synthetic medium with cellulose as the sole carbon source reported earlier (see pages 48 - 50) may not be due to the inability of the enzyme to hydrolyse cellulose, but may be more probably due to its inability to use the hydrolytic products - cellobiose - of cellulose.

The exact manner in which cells are killed by fungal parasites causing soft rots is still unknown . The concept that both killing and macerating can be attributed to pectic enzymes has a number of supporters (Brown, 1955; Tribe, 1955; Fushtey, 1957). Evidence for this viewpoint exists in the fact that heat treatment of culture filtrates of certain soft rot organisms destroys both macerating and killing activity. Furthermore, all attempts to separate macerating from toxic effects by chemical and other procedures have been unsuccessful. However, it is difficult to visualize how pectic enzymes alone can act as poisons to living protoplasm. Pectic enzymes may possibly make plant cells more susceptible to other toxic metabolic products by rendering cells accessible to toxic molecules that do not enter intact cells freely. Another possibility for which there is no direct evidence is that the cells are killed before the enzymes act, in which case pectic enzymes would not be involved in death.

If the pectic enzymes are not accepted as the direct cause of death, the nature of the toxic entity still remains to be determined. Several investigators adhere to the concept that _S. rolfsii_ (Higgins, 1927), _Sclerotinia sclerotiorum_ (Overell, 1952), _Botrytis cinerea_

(Smith, 1902; Peltier, 1912) and Sclerotinia cinerea (Cooley, 1914) kill cells of their respective hosts by the oxalic acid they produce. Brown (1936), however, concluded that oxalic acid was definitely not involved in the case of Botrytis cinerea. There is no doubt that oxalic acid can damage plant cells if concentrations are high enough. Until more experimental evidence is provided, the possibility still exists that oxalic acid takes part in the collapse of cells invaded by some fungal pathogens.

Although the nutrient level and internal environment - e.g. pH, of the tomato fruit varies with age, (Gustafon, 1927; Gustafon and Houghtalling, 1939; Glendenning 1941, 1942) this did not alter the susceptibility of the fruit to attack by S. rolfsii. Infection occurred in fruits of all ages. (see plate 19). If fruits of different ages therefore happen to come in contact with an infested soil, infection will develop in all of them.

Plant diseases are ably controlled by the use of resistant varieties. Of all the Ghanaian tomato varieties tested, Molokai was the only variety which showed resistance (see plate 20). It was completely resistant to S. rolfsii. The practical implication of this find-

ing is obvious. Orian (1952) demonstrated resistance to S. rolfsii by some varieties of groundnuts. Mehta, Singh and Mathur (1953) also found that certain varieties of groundnuts were slightly susceptible whereas others were completely resistant to S. rolfsii.

If the aim of developing the hybrids, especially MM84/17, is to increase yield, the ready susceptibility of these fruits to soil-rot by S. rolfsii is indeed worthy of note.

VI: S U M M A R Y

1. The optimum temperature for vegetative growth of S. rolfsii was from 27 to 32°C. Growth occurred over the range 17 to 37°C.
2. Light had no effect on the vegetative growth of S. rolfsii.
3. S. rolfsii grew only at 100% R.H. at the optimum temperature (27 to 32°C).
4. S. rolfsii grew well in acid media. It showed a double maximum of growth in relation to pH : there was a major peak at pH 4.8 and a minor one at pH 9.31 with a minimum of pH 8.2 in between.
5. Extracellular supply of thiamine was not necessary for S. rolfsii to initiate growth from sclerotia, but subsequent development critically depended on it.
6. Thiamine at concentrations of 25 and 50 μg, supported the maximum growth in S. rolfsii.
7. Of 10 carbon compounds tested at a concentration of 1% (Weight/volume) S. rolfsii readily utilized starch, maltose, and fructose whereas glucose was used moderately. Cellulose, lactose, glycerol and galactose were used to limited extent.
8. The mean dry weight of the mycelium increased with an increase in glucose concetration of 0.125 to 2%.

9. S. rolfsii was able to use organic, ammonium and nitrate nitrogen sources. Except alanine, the organic nitrogen were superior to all others.
10. The infection of tomato fruits can occur through wounds or through the intact fruit wall.
11. The rate of infection increased almost directly proportional to the increase in size of inoculum.
12. Prior to penetration, S. rolfsii ran irregularly over the fruit surface and formed infection cushions at scattered loci, on the fruit surface.
13. Infection cushions could be formed from by either repeated branching, of a single hypha, or of two or more intertwined hyphae.
14. The fungus entered the host by mechanically piercing through the cuticle and epidermis.
15. Growth within the fruit was both inter - and intra - cellular.
16. No constrictions nor swellings were observed in the hyphae either before or after cell wall penetration.
17. A substance produced by the fungus dissolved all the pericarp cells except the epidermis and the cuticle.
18. The disease was not seed borne.
19. Infection occurred over the temperature range 17 to 32°C., with an optimum at 32°C. There was no infection at 37°C.
20. S. rolfsii infected tomato fruits at 95 and 100% R.H. at the optimum temperature (32°C).
21. Maximum infection of tomato fruits occurred at 55% W.H.C., though infection occurred over the range 15% to 75% W.H.C.. There was no infection at 85% W.H.C.

22. Infection occurred at all ages of the fruit.
23. Molokai was the only Ghanaian tomato variety resistant to _S. rolfsii_.

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TABLE 6

Effect of Humidity on the growth of *S. rolfsii* at 27°C.
(Observation after 7 days' Incubation)

% Relative Humidity	Colony Diameter in mm. of each indicated Replicate				Total Diameter of Colony in mm.	Mean of Colony Diameter in mm.
	Replicate 1	2	3	4		
100	9.5	10.2	8.3	13.3	41.3	10.3
95	0.0	0.0	0.0	0.0		
90	0.0	0.0	0.0	0.0		
85	0.0	0.0	0.0	0.0		
80	0.0	0.0	0.0	0.0		
75	0.0	0.0	0.0	0.0		
70	0.0	0.0	0.0	0.0		

iv 137.7
v 328.8

TABLE 7

Effect of pH on growth of *S. rolfsii* at 27°C.

(Observation after 7 days' Incubation)

pH used	Dry Wt. of mycelium in mgm. of each indicated Replicate		Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of Mycelium in mgm.
	Replicate:			
3	i	488.2	1,611.7	
	ii	491.6		
	iii	460.0		402.9
	iv	171.9		
4	i	565.3		
	ii	416.5	1,926.5	481.6
	iii	419.2		
	iv	525.5		
5	i	362.0		
	ii	287.1	1,400.1	350.0
	iii	433.1		
	iv	317.9		
6	i	323.2		
	ii	262.4	1,447.9	362.0
	iii	339.8		
	iv	322.5		
7	i	Contaminated		
	ii	Contaminated		
	iii	91.7	119.2	59.6
	iv	27.5		

.../2

iv 117.7

v 328.8

TABLE 7 (2)

pH used	Dry Wt. of mycelium in mgm. of each indicated Replicate	Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of Mycelium in mgm.
	Replicate;		
	i 27.7	115.6	
8	ii 28.9		28.9
	iii 27.6		
	iv 31.4		
	i 106.6		
9.31	ii 44.9	568.4	142.1
	iii 90.3		
	iv 326.7		
	i 142.9		
	ii 109.5	351.2	87.8
	iii 61.7		
	iv 37.2		

iv 117.7
v 328.8

TABLE 8

Effect of Thiamine on growth of S. rolfsii at 27°C.
(Observation after 7 days' Incubation)

Treatment	Dry Weight of mycelium in mgm. of each indicated Replicate	Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in mgm.
No Thiamine	Replicate: i 97.0 ii 152.8 iii 96.0 iv 128.5	474.3	118.6
Thiamine	i 155.7 ii 163.1 iii 170.0 iv 147.8	636.6	158.9

iv 117.7
v 328.8

TABLE 9

Effect of different concentrations of thiamine on growth of *S. rolfsii* at 27°C.

(Observation after 7 days' Incubation)

Concentration of Thiamine used in µg	Dry Weight of mycelium in mgm. of each indicated Replicate		Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in mgm.
	Replicate:			
	i	81.0		
	ii	95.4		
No Thiamine	iii	58.5	390.1	78.1
	iv	106.1		
	v	49.3		
	i	159.5		
	ii	129.8		
25	iii	115.0	695.8	139.2
	iv	107.7		
	v	183.8		

	iv	117.7		
	v	328.8		

TABLE 9 (2)

Concentration of Thiamine used in µg	Dry Weight of mycelium in mgm. of each indicated Replicate		Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in mgm.
	Replicate:			
50	i	110.4	698.4	139.7
	ii	142.4		
	iii	125.8		
	iv	178.3		
	v	141.5		
100	i	115.8	497.0	99.4
	ii	98.2		
	iii	76.5		
	iv	112.5		
	v	94.0		
150	i	111.7	498.0	99.6
	ii	40.9		
	iii	59.5		
	iv	135.4		
	v	150.5		
200	i	91.8	430.0	86.0
	ii	77.9		
	iii	96.0		
	iv	33.7		
	v	130.6		

	iv	117.7		
	v	328.8		

Effect of different carbon sources on growth of *S. rolfsii* at 27°C.

(Observation after 7 days' Incubation)

Carbon Source	Dry Wt. of mycelium in mgm. of each indicated Replicate		Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in. mgm.
	Replicate:			
STARCH + 0.1% GLUCOSE	i	225.5	1074.5	214.5
	ii	204.5		
	iii	210.0		
	iv	221.9		
	v	213.6		
	i	56.3	686.8	137.4
	ii	117.1		
	iii	157.3		
	iv	203.1		
	v	153.0		
MALTOSE	i	147.8	621.4	124.3
	ii	122.3		
	iii	136.7		
	iv	130.3		
	v	84.4		
FRUCTOSE	i	115.9	552.[illegible]	110.6
	ii	125.7		
	iii	99.7		
	iv	131.3		
	v	80.4		

	iv	117.7		
	v	328.8		

(2) TABLE 10

Carbon Source	Replicate	Dry Weight of mycelium in mgm. of each indicated Replicate	Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in mgm.
GLUCOSE	i	94.0	449.3	89.9
	ii	114.0		
	iii	95.3		
	iv	97.8		
	v	51.3		
CELLULOSE	i	34.0	170.4	34.0
	ii	35.3		
	iii	33.0		
	iv	32.0		
	v	36.1		
LACTOSE	i	33.6	170.2	34.0
	ii	4.0		
	iii	91.0		
	iv	32.1		
	v	7.6		
GLYCEROL	i	28.4	166.5	33.3
	ii	25.0		
	iii	12.3		
	iv	32.9		
	v	67.9		

	iv 117.7		
	v 328.8		

TABLE 11

Effect of different glucose concentrations on growth of S. rolfsii at 27°C.

(Observation after 7 days' Incubation)

Percentage (Wt/Wt)	Dry Weight of mycelium in mgm. of each indicated Replicate	Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in mgm.
CONTROL	0.0	0.0	0.0
0.125	Replicate: i 6.1 ii 2.2 iii 1.0 iv 4.1 v 2.0	15.4	3.0
0.25	i 7.3 ii 5.2 iii 6.2 iv 8.3 v 4.2	31.0	6.2
0.5	i 70.7 ii 76.5 iii 50.5 iv 61.9 v 66.3	327.9	65.2
1.0	i 99.3 ii 90.9 iii 98.6 iv 90.0 v 100.0	478.8	95.7
2.0	i 285.6 ii 193.8 iii 309.0 iv 117.7 v 328.8	1,235.9	247.1

Effect of different nitrogen sources on the growth of S. rolfsii at 27°C.

(Observation after 7 days' Incubation)

Nitrogen Source	Dry Weight of mycelium in mgm. of each indicated Replicate		Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in mgm.
	Replicate:			
PEPTONE	i	167.8	718.8	143.8
	ii	93.9		
	iii	132.5		
	iv	144.0		
	v	180.7		
ASPARAGINE	i	162.5	710.4	142.1
	ii	135.5		
	iii	121.0		
	iv	161.5		
	v	130.0		
Ammonium Nitrate	i	104.3	588.9	117.8
	ii	100.0		
	iii	114.2		
	iv	137.0		
	v	133.4		
UREA	i	113.5	569.8	113.4
	ii	155.8		
	iii	119.1		
	iv	88.6		
	v	112.9		
Ammonium Chloride	i	44.9	468.6	93.7
	ii	75.4		
	iii	125.3		
	iv	95.0		
	v	128.0		
GLYCINE	i	55.0	206.2	41.2
	ii	30.1		
	iii	28.7		
	iv	91.0		
	v	1.4		

TABLE 12 (2)

Nitrogen Source	Dry Weight of mycelium in mgm. of each indicated Replicate	Total Dry Wt. of mycelium in mgm.	Mean Dry Wt. of mycelium in mgm.
	Replicate:		
	i 15.8		
	ii 88.6		
Sodium Nitrate	iii 32.4	179.0	35.8
	iv 33.8		
	v 8.6		
	i 28.4		
	ii 18.3		
ALANINE	iii 16.2	75.8	15.2
	iv 8.0		
	v 0.3		
	i 13.4		
	ii 5.3		
	iii 13.5	55.6	13.9
	iv 23.5		
	v Contaminated and discarded		

Contaminated and Discarded

TABLE 15

Effect of temperature on infection of tomato fruit by *S. rolfsii* at 27°C.

Temperature of Incubation in °C.		Diameter of infection in mm on indicated day after inoculation			Total Diameter of infection in mm on indicated day after inoculation			Mean Diameter of infection in mm on indicated day after inoculation		
		2	4	6						
	i	0	0.0	5.0						
17	ii	0	0	7.0			12.0			6.0
	iii	0	0		0	0		0	0	
	iv	0	0							
	i	0.0	5.0	15.0						
	ii	0.0	16.0	33.5	0.0	34.5	76.5	0.0	11.5	25.5
22	iii	0.0	13.5	27.5						
	iv	0.0	0.0							
	i	9.5	30.5	80.2				10.2		
	ii	10.0	42.5	140.0	30.5	128.5	340.2		32.1	
27	iii	11.0	36.0	70.0						
	iv	0.0	19.5	50.0						
	i	19.0	46.5	92.5			352.5			
	ii	17.0	46.0	170.5	0	44.0		18	44.0	117.5
32	iii	18.0	39.0	90.0		132.0				
	iv	Contaminated and discarded								
37		0.0			0.0			0.0		

	121.0 Contaminated and Discarded	182.0	60.7

TABLE 16

Effect of humidity on the infection of tomato fruit
by S. rolfsii at 32°C.

(Observation after 7 days' Incubation)

% Relative Humidity	Diameter of Infection in mm. of indicated Replicate		Total Infection Diameter in mm.	Mean Infection Diameter in mm.
100	Replicate:			
	i	58.5		
	ii	92.0	297.5	99.2
	iii	147.0		
	iv	Contaminated and discarded		
	i	75.0		
	ii	85.0	297.0	99.2
	iii	137.0		
		Contaminated and discarded		

	121.0 Contaminated and Discarded	182.0	60.7

Effect of Water-Holding Capacity of Soil on infection of tomato fruit by S. rolfsii at 32

(Observation after 7 days' Incubation)

	Diameter of infection in mm of indicated Replicate	Total Diameter of infection in mm.	Mean Diameter of infection in mm.
A - 85	0	0	0
B - 75	Replicate: i 28.0 ii 40.0 iii 35.0 iv 50.0	153.0	38.1
C - 65	i 57.5 ii 65.0 iii 72.5 iv 84.0	279.0	69.8
D - 55	i 86.0 ii 64.0 iii 57.5 iv 194.0	401.5	100.4
E - 45	i 92.0 ii 76.0 iii 85.0 iv 10.0	373.0	93.3
F - 35	i 51.0 ii 47.5 iii 65.5 iv 203.5	367.5	91.9
G - 25	i 58.5 ii 61.0 iii 64.0 iv 185.0	368.5	92.2
H - 15	i 96.8 ii 80.0 iii 71.0 iv Contaminated and discarded	247.8	82.6

	121.0 Contaminated and Discarded	182.0	60.7

TABLE 19

Susceptibility of Tomato varieties to *S. rolfsii*

Variety		Range of Diameter of Infection in mm	Total Diameter of Infection in mm	Mean Diameter of Infection in mm.
A - LOCAL	i	66.5		
	ii	61.5	303.0	101.0
	iii	175.0		
	iv	Contaminated and Discarded		
B - IMPROVED ZUARUNGU		27.0		
		20.0		
		22.0	80.0	20.0
		11.0		
C - MAWI		86.5		
		40.0		55.5
		50.0		
		45.5	222.0	
D - MOLOKAI		0.0	0.0	0.0
E - HYBRID (MM86/8)		25.0		
		36.0		
		121.0	182.0	60.7
		Contaminated and Discarded		