AN ABSTRACT OF THE THESIS OF

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Title Physiologic Specialization and Physiology of Sclerotinia sclerotiorum (Lib.) Masse

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SUMMARY

A series of experiments was conducted to determine the physiologic properties of Sclerotinia sclerotiorum and also to find out if there are physiologic differences between isolates of the fungus.

In laboratory tests little growth occured at 0° C. The rate of growth increased with each increase in temperature up to 20° C. Above 20° C. the rate of growth decreased, although all cultures grew fairly well at 30° C. Optimum temperature for growth of the fungus is between 20° and 25° C. Temperature also affects the size of sclerotia formed by S. sclerotiorum. When the temperature is near the minimum for the fungus the sclerotia have a tendency to be larger than those produced on the same medium at room temperature.

The tests indicated that S. sclerotiorum can grow over a very wide range of pH concentrations. The fungus grew well on media of pH 2.4 to 9.65. At pH 2.04 all cultures made some growth, but there were great differences in the rate and character of growth. Very slight growth occured at pH 10.20 and none at all at 10.95. Thus the extreme limits for growth of S. sclerotiorum are approximately pH 2 to pH 10.

The amount of Mg, Fe, S and K in the substrate has

no apparent affect on the rate or character of growth of S. sclerotiorum. It grew equally well when these elements were absent and when they were present in quantities much greater than those ordinarily used. Of the minerals tested, only phosphorous, nitrogen and carbohydrates appeared to be of any importance in the growth and formation of sclerotia by S. sclerotiorum.

The best growth was on media containing 5 to 10 grams of NaH_2PO_4 per liter. Concentrations greater than 10 grams per liter caused a reduction in the rate of growth and the formation of sclerotia.

Inorganic nitrogen appeared to have a greater effect on growth of S. sclerotiorum than any other single element. The optimum amount of $NaNO_3$ is 1-5 grams per liter. The presence of nitrogen caused much aerial mycelia to be formed by the fungus. High concentration of nitrates depressed the rate of growth.

Three organic forms of nitrogen had quite different effect on the growth of S. sclerotiorum. Growth of fungus on media containing 1 and 5 grams of cystine per liter was no more rapid than when no nitrogen was present. 5 and 10 grams of asparagine per liter was optimum amount for growth. Urea was toxic to the fungus at concentrations greater than 0.5 gram per liter.

Growth of S. sclerotiorum on media containing optimum concentration of nitrogen and phosphorous was most rapid, and all cultures completely covered the surface of the media in 3 to 4 days.

Addition of carbohydrates to the media had comparatively little effect upon the rate of radial growth of mycelium, but was essential for the formation of sclerotia. Addition of $NaNO_3$ to the various carbohydrates caused the growth to be more luxuriant.

Repeated host plant inoculations were mostly unsuccessful, few plants became infected and the results were inconclusive. The results of these experiments did not indicate the existence of pathogenically distinct physiologic forms of S. sclerotiorum.

PHYSIOLOGIC SPECIALIZATION AND PHYSIOLOGY OF *SCLEROTINIA SCLEROTIORUM* (LIB.) MASSE

by

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INTRODUCTION

The literature concerning Sclerotinia sclerotiorum (Lib.) Masse contains many references to etiology and control of the disease and to descriptions of the disease on its many host plants. Very little has been written concerning the physiology of the fungus. These investigations were undertaken in hope that they might clarify certain aspects of the physiology of the fungus and aid in understanding the complex factors which influence its spread and development in the field. Indirectly, such information as is presented may indicate lines of investigation which will result in the development of better control practices. For instance, from a market point of view, it is of extreme importance to determine the temperature relations of S. sclerotiorum and to determine whether there is any possibility of controlling decay by low temperature.

PHYSIOLOGIC SPECIALIZATION AND PHYSIOLOGY OF *SCLEROTINIA SCLEROTIORUM* (LIB.) MASSE.

NAME, HISTORY, AND GEOGRAPHIC DISTRIBUTION

Sclerotinia sclerotiorum has long been recognized as an important pathogen in Europe. As early as 1886 Debary (1) made a comprehensive study of *S. sclerotiorum* and showed it was able to produce disease in a variety of plants. Since that time it has been reported from Argentina, Bermuda, Canada, England, France, Germany, Italy and New Zealand. In America apparently no reference was made to this fungus until about 1890, when it was found associated with lettuce diseases of the type called "drop" (14).

Sclerotinia sclerotiorum has been known in Florida for many years, having been reported on celery as early as 1909 (18). Just why it did not spread to other crops or cause more than incidental losses in the beginning is not clearly known, although the kinds of crops planted and weather conditions during the growing seasons probably influenced its development to some degree. Other than intermittent appearances on celery, no serious outbreaks occurred until 1933. Between 1935 and 1940 its occurrence increased, infecting such major crops as beans, potatoes and tomatoes. The disease has increased in

severity in Florida since 1935 and is at present a disease of considerable importance to much of the vegetable growing industry of that state (10).

Sclerotinia sclerotiorum is widely distributed in the United States and may be found during some seasons in bean fields in practically every section where weather conditions are favorable. In the middle Atlantic states outbreaks of Sclerotinia wilt are more prevalent on the fall than on the spring crops, especially if warm, rainy weather occurs in September. In the Pacific Northwest the disease is severe on beans, especially during cool, rainy seasons.

This disease is known in this country by such common names as Sclerotinia wilt, "pink rot" of celery, "drop" of lettuce, Sclerotiniose, white mold, and watery soft rot of bean.

ECONOMIC IMPORTANCE

Sclerotinia sclerotiorum can affect practically all vegetable crops and has in recent years become serious on beans, potatoes, tomatoes, celery and lettuce.

This disease frequently occurs after a period of warm, humid weather and a few days of such weather may result in large crop losses. In general, the disease is more prevalent in the Southern States than elsewhere, and crops grown on low ground surrounded by woods, which reduce air circulation, suffer the greatest loss. The losses vary greatly from year to year; however, 50 to 25 per cent loss is not uncommon. In the Northern States, Sclerotinia wilt occurs usually in August and September and has been so severe that entire fields have been plowed under and planted to other crops.

Sclerotinia wilt causes heavy losses, not only in the field, but also during transportation. Losses in transit may be even greater than those in the field. Ramsey (14) in his extensive studies on some of the species of Sclerotinia causing decay of vegetable, found that, although S. sclerotiorum, S. intermedia Ramsey, S. minor Jagger, and S. ricini Godfrey are able to infect and decay beans, S. sclerotiorum is responsible for most of the decay occurring in transit, storage and market. Also, in their studies of the market diseases of vegetables, Link and

Gardner (9) adopted the term "watery soft rot" to describe the decay caused by S. libertiana and said "In the market it occurs on a wide variety of hosts. It is the prevalent rot at low temperatures and is the most important storage rot of root crops."

According to Ramsey (14) the average infection of green beans under transit and market conditions was 24 per cent. In addition to beans the following important crops were affected by Sclerotinia decay: asparagus, bean, broccoli, cabbage, carrot, cauliflower, celery, chicory, cucumber, endive, lettuce, parsnip, pea, rutabaga, salsify, sweet potato and turnip. He found that 54 per cent of the lettuce and 50 percent of celery products were affected by Sclerotinia decay. S. sclerotiorum occurs much less frequently on fruits than on vegetables.

HOST RANGE OF THE FUNGUS

The greatest economic losses from S. sclerotiorum occur on the cultivated species in the following families: Compositae, Cruciferae, Cucurbitaceae, Chenopodiaceae, Leguminosae, Solonaceae, and Umbelliferae.

Joshy (6) described Sclerotinia wilt of Safflower and reported that artificial inoculation with this fungus caused infection in wheat, oats and gram.

The sclerotinia of clover and alfalfa is usually referred to as S. trifoliorum Eric. It may be a distinct species upon alfalfa and clover, but S. sclerotiorum also may attack these plants and it is possible that S. trifoliorum may yet prove to be only a synonym of S. sclerotiorum.

In 1890 a Sclerotial disease of sunflowers was observed by Pammel (11) in Iowa. The plant disease surveys of the United States and Canada in 1921 both reported the common occurrence of the same disease, recording it in Oregon, Washington, Minnesota and Ontario.

According to Bisby (2) after one year without sunflowers the fungus was still present and produced stem rot upon sunflowers planted the second year. The farmer in Western Canada can greatly lessen the danger of loss from this disease by planting sunflowers in fields which have

grown grain crops, for the fungus does not attack grain or grasses under natural conditions.

The plant disease survey for Canada published in 1922 contains a note by Cunningham (4) who found S. libertiana Fckl. on sunflowers and also records a root rot on sunflowers seemingly distinct from the disease caused by Sclerotinia. Lowrens in Washington, Morris and Swingle in Montana and Bisby in Manitoba have studied the disease. In regard to the taxonomy of the causal fungus, the workers are agreed that the sclerotia resemble those of S. sclerotiorum.

A disease of Irish potatoes caused by S. sclerotiorum was reported first from Ireland in 1910. It has since been found in other parts of the world. In the United States the disease has been reported from New York, Florida, Washington and Montana (3).

SYNONYMY OF S. SCLEROTIORUM (LIB) MASSE

The fungus was first distributed in 1837 by Madame Libert in her Pl. Crypt. Orduennae, Fasc. IV No. 326 under the name Peziza sclerotiorum. Fuckel in his Symbolae Mycologicae (1869-70) transferred the species to his genus Sclerotinia, and at the same time changed the name to S. libertiana, presumably merely because he disliked the combination S. sclerotiorum.

Nearly all other European workers have adopted Madame Libert's specific name. Giller in 1879 called it Phialea sclerotiorum and Phillips in 1887 put it in Hymenoscypha (sub-genus Sclerotinia) sclerotiorum. The actual combination S. sclerotiorum was apparently first used by Masse in Vol. IV of his British Fungus Flora (1895) and has been retained in all English pathological literature since (16).

METHODS AND MATERIALS

Potato dextrose agar was used for all cultural studies except those concerned with nutrition. Media used in the nutrition work were modifications of Richards' Solution (13) solidified by the addition of 2% agar.

The formula of the Richards' Solution:

KNO_3	-	10.0	grams
KH_2PO_4	-	5.0	"
$MgSO_4$	-	2.5	"
$Fecl_3$	-	0.02	"
Sucrose	-	50.0	"
Dist. water	-	1000.0	c.c.

Culture plates were "seeded" with small pieces of mycelium, or with uniform sized discs of mycelium and agar from cultures one to two weeks old. All cultures except those in the temperature studies were incubated at room temperature (approximately 70° F.).

Hydrogen ion concentration of media was determined with the Beckman pH meter, using a calomel electrode. N/1 Hcl and N/2 NaOH were added to the agar after sterilization to adjust the reaction to the desired level, and no attempt was made to maintain the pH of the media after the plates were "seeded."

Plant inoculations were made with 4-6 day old cultures grown on potato dextrose broth. The broth containing the fungus was first homogenized in the Waring Blendor for 1½ minutes, then atomized onto the plant surfaces. After inoculation plants were kept in a moist chamber for 48 hours before being returned to the greenhouse bench.

Sources of cultures: The source of each culture used in these studies is shown in Table 1. All cultures were isolated by plating sclerotia, using the sodium hypochlorite technique.

Table 1. Origin of cultures used in these studies:

Number	Sclerotia from	Location	Isolated by	Date
27B	Bean	Jefferson, Oregon	E. K. Vaughan	April 1949
27C	Cabbage	Mt. Vernan, Wash.	"	" "
27S	Squash	Eugene, Ore.	"	" "
34	Cabbage	Corvallis, Ore.	"	July "
35	"	Portland, Ore.	"	" "
52	Bean	Corvallis, Ore.	S. Tanrikut	June 1950
F	"	Ft. Lauderdale, Fla.	"	" "
A	"	Corvallis, Ore.	"	" "

EFFECT OF AGE OF INOCULUM ON RATE OF GROWTH OF *S. SCLEROTIORUM*

As an aid in determining what age culture should be used as inoculum in order to obtain the most rapid resumption of growth, plates of potato dextrose agar were "seeded" with mycelium from cultures 8, 25 and 100 days old. The younger culture started growth much more rapidly than the older ones (Fig. 1). However, once growth had resumed, the older cultures grew as rapidly as the younger ones and there were no apparent differences in vigor or character of growth. In order to avoid the recovery period of older cultures, mycelium from 7-day old cultures was used in practically all of the tests.

THE INFLUENCE OF TEMPERATURE ON GROWTH OF S. SCLEROTIORUM

The influence of temperature on the growth of S. sclerotiorum and upon infection and development of the mycelium within the host has been noted by various workers. Lauritzen, Harter and Whitney (8) were able to obtain infection of snap beans at temperatures ranging from 0° to 28° C. These temperatures correspond almost exactly with those reported by Ramsey (14). Infection occurs very slowly at 0° to 2° C. The maximum number of infections occured at temperatures ranging from 19° C. to 24.3° C. in 4 or 5 experiments. It is believed that the optimum temperature lies between 19° C. and 24° C.

Ramsey (14) studied the effect of temperature upon the growth of several species of Sclerotinia. He noticed that temperature reactions of S. intermedia are quite distinct from those of S. minor and S. sclerotiorum. These differences are especially noticeable at temperatures just below and a few degrees above 0° C. S. intermedia grows about twice as fast on potato dextrose agar as either S. minor or S. sclerotiorum.

At temperatures around 7° C. each of the three named species grows at approximately the same rate, while at 20° C. S. sclerotiorum develops at about one half the rate of the two other species.

To determine the effect of different temperatures upon the growth of S. sclerotiorum two tests were conducted. In the first test 5 cultures of S. sclerotiorum (34, 35, 27B, 27C, 27S) were grown at temperatures of 0°, 5°, 10°, 15°, 20°, 25° and 30° C.

Little growth occured at 0° C. The rate of growth increased with each increase in temperature up to 20° C., at which most rapid growth occured. Above 20° C. the rate of growth decreased, although all cultures grew fairly well at 30° C. (Table 2) (Fig. 2).

A second test was made to determine the effect of temperature on the formation of sclerotia. The results of this test (Fig. 3) also indicated that the optimum temperature for growth of S. sclerotiorum was between 20° and 25° C.

Cultures of Sclerotinia grown at room temperature produce approximately the same relative size of sclerotia for a given species, provided a suitable medium is used. In cultural studies, however, Ramsey (14) has often noted that the relative size of sclerotia of different species does not remain the same when extremes of temperatures are involved. In general, when the temperature is near the minimum for the fungus, the sclerotia have a tendency to be larger than those produced on the same medium at room temperature.

The results of the second experiment were similar to those obtained by Ramsey (14). At 5° C. the sclerotia were almost twice as large as those grown at 20° C.

Table 2. Effect of temperature on the rate of growth of *S. sclerotiorum*

Isolate	Age of Culture	Temperature-Degree C. 0	5	10	15	20	25	30
		Average diameter of colonies in cm.						
27B	3 days	-	0.1	1.1	1.8	4.9	3.18	2.56
	6 days	-	0.3	3.7	6.27	9.0[a]	5.69	3.81
	9 days	-	0.8	8.0	9.0	9.0	7.0	3.9
27C	3 days	-	0.1	0.3	2.6	7.9	1.2	3.05
	6 days	T[b]	0.3	3.5	7.0	9.0	4.8	3.9
	9 days	-	0.9	9.0	9.0	9.0	8.0	4.1
27S	3 days	-	-	1.2	2.2	9.0	9.0	7.74
	6 days	T	0.2	7.8	9.0	9.0	9.0	9.0
	9 days	-	1.5	9.0	9.0	9.0	9.0	9.0
34	3 days	-	-	0.2	1.9	6.2	2.2	2.7
	6 days	-	0.1	1.3	4.7	9.0	9.0	3.8
	9 days	T	0.3	5.0	9.0	9.0	9.0	4.0
35	3 days	-	-	0.7	3.1	9.0	2.5	7.9
	6 days	T	0.1	6.8	9.0	9.0	9.0	9.0
	9 days	-	0.8	9.0	9.0	9.0	9.0	9.0

(a) Inside diameter of plate = 9 cm.
(b) T = Trace growth of fungus.

HYDROGEN ION CONCENTRATION

To determine the effect of Hydrogen ion concentration on the rate and character of growth of S. sclerotiorum in culture three experiments were carried out. In the first two potato dextrose agar was used and was "seeded" with bits of mycelium from seven day old cultures.

At pH 3.50, 5.10, 7.25 and 8.33 there were no differences in the rate or character of growth of five isolates (27B, 27C, 27S, 34 and 35), and with one exception (27C) all isolates grew well at pH 2.58. At pH 9.24 growth was considerably retarded, and at pH 10.95 no growth occured (Table 3) (Figs. 4, 5, 6).

In the second test, at pH 2.98, 3.45, 4.03, 4.45, 6.3, 8.35 and 8.95 there were no significant differences in the rate or character of growth of three isolates (27C, A and F). At pH 9.65 the rate of growth was retarded, but the type of growth was unchanged.

The results indicated that S. sclerotiorum can grow over a very wide range of pH concentrations, but they did not indicate the limits at which growth could occur.

In the third experiment the same three cultures were grown on Richards' Solution agar. All three made fair growth at pH 2.40 and grew normally at pH 3.14. At pH 2.04 all cultures made some growth, but there were great

differences in the rate and character of growth. Very slight growth occured at pH 10.20 and none at all at 10.95. Thus the extreme limits for growth of S. sclerotiorum are approximately pH 2 to pH 10 (Figs. 8, 9, 10).

Table 3. Effect of pH on rate of growth of three isolates of *S. sclerotiorum* and on production of sclerotia.

	Diameter of colonies in cm. on media of pH:									
	2.05	2.40	3.14	4.45	6.3	8.35	8.95	9.65	10.2	10
Isolate A										
3 days	-	5.0	9.0[a]	9.0	9.0	9.0	7.0	5.3	0.2	-
6 "	2.0	3.8	9.0	9.0s	9.0s	9.0s	9.0s	9.0s	0.9	-
9 "	5.0	7.6	9.0s	"	"	"	"	"	1.4	-
12 "	7.0s[b]	9.0s	"	"	"	"	"	"	1.6	-
Isolate C										
3 days	-	0.1	4.0	9.0	9.0	9.0	9.0	6.8	-	-
6 "	0.3	2.3	9.0	9.0s	9.0s	9.0s	9.0s	9.0s	-	-
9 "	1.2s	3.5	9.0s	"	"	"	"	"	0.1	-
12 "	1.7s	5.0s	"	"	"	"	"	"	0.3	-
Isolate F										
3 days	-	-	3.0	9.0	9.0	9.0	9.0	9.0	-	-
6 "	-	-	7.5	9.0s	9.0s	9.0s	9.0s	9.0s	-	-
9 "	2.4	-	9.0s	"	"	"	"	"	-	-
12 "	3.2s	-	"	"	"	"	"	"	-	-

(a) Inside diameter of plate = 9.0 cm.
(b) s = earliest appearance of sclerotia.

. MINERAL NUTRITION

A series of experiments was conducted to determine the effect of certain minerals upon growth of S. sclerotiorum in culture. Richards' solution (13) was used as a base medium with various amounts of each element being added (Table 4). In all tests several different cultures were used. All plates were run in triplicate and most tests were repeated 2 or 3 times. Growth was measured at 24-hour intervals from the time the plates were "seeded."

The amount of Mg, Fe, S and K in the substrate has no apparent effect on the rate or character of growth of S. sclerotiorum. Five isolates (F, S, 34, 35 and 52) grew equally well when these elements were absent and when they were present in quantities much greater than those recommended by Richards. Of the minerals tested, only Phosphorous, Nitrogen and carbohydrates appeared to be of any importance in the growth and formation of sclerotia by S. sclerotiorum.

Table 4. Modifications of Richards' Solution used in nutritional studies:

To study the influence of:	Omitted from Richards' formula		Added to the test medium		Gram added per liter
Potassium	KH_2PO_4	5 gm.	NaH_2PO_4	5 gm.	
	KNO_3	10 "	$NaNO_3$	10 "	
			Kcl		0, 10.0, 20.0.
Sulphur	$MgSO_4$	2.5 "	$Mgcl_2$	2.5 "	
			Na_2SO_4		0, 0.5, 2.5, 5.0.
Magnesium	$MgSO_4$	2.5 "	Na_2SO_4	2.5 "	
			$Mgcl_2$		0, 0.5, 2.5, 5.0.
Iron	$Fecl_3$	0.02 "	$Fecl_3$		0, 0.2, 1.0.
Phosphorous	KH_2PO_4	5 "	Kcl	10 "	
			NaH_2PO_4		0, 1.0, 5.0, 10.0, 20.0.
Nitrogen	KNO_3	10 "	Kcl	10 "	
	"	10 "	$NaNO_3$		0, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0.
	"	10 "	NH_4NO_3		0, 1.0, 2.0, 5.0.
	"	10 "	NH_4cl		0, 1.0, 5.0.
	"	10 "	Cystine		0, 1.0, 5.0.
	"	10 "	Asparagine		0, 1.0, 5.0, 10.0.
	"	10 "	Urea		0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0.
Carbohydrates	Sucrose	50 "	Dextrose		50
	"	50 "	Sucrose		0, 5.0, 2.50, 50.0.
	"	50 "	Roffinose		50.
	"	50 "	Starch		50.
	"	50 "	Inulin		50.

EFFECT OF PHOSPHOROUS

Even when no phosphorous was present in the medium six cultures (34, 35, 52, F, 27C, 27S) all completely covered the surface of the agar in petri dishes in 4 to 7 days and some (F, C and S) formed sclerotia. However, growth was more rapid and more luxuriant when phosphorous was present (Fig. 12). The best growth was on media containing 5 to 10 grams of NaH_2PO_4 per liter. Concentrations greater than 10 grams per liter caused a reduction in the rate of growth and the formation of sclerotia.

EFFECT OF NITROGEN

Inorganic nitrogen appeared to have a greater effect on growth of S. sclerotiorum than any other single element (Fig. 13). In these tests rapid growth occured on media containing 0.16 to 0.80 grams of nitrate nitrogen (1-5 grams $NaNO_3$) per liter (Table 5). Even when no nitrogen was present the fungus covered the surface of the agar in 3 to 5 days and formed sclerotia in 7 to 9 days. Growth, however, was weak, thin, and composed almost entirely of sessile mycelium, and few sclerotia were formed. When nitrogen was present a felt of aerial mycelia was formed, and numerous sclerotia were present. High concentration of nitrates depressed the rate of growth but did not interfere with the formation of aerial mycelium or sclerotia.

To test further the growth of S. sclerotiorum on media containing no nutrient other than $NaNO_3$, cultures 27C, 27S and 52 were grown on media composed of:

Agar	20	grams
$NaNO_3$	5	"
Water	1	liter.

Although growth was sparse with almost no aerial mycelium, all cultures completely covered the surface of the plates in 4 to 5 days and two formed a few small sclerotia. This test serves as an excellent example of

the ability of this fungus to maintain itself under extremely adverse conditions.

When ammonia nitrogen (0, 1 and 5 grams NH_4Cl per liter) was substituted for nitrate, cultures F and 52 grew well and formed abundant sclerotia. Culture 34, on the other hand, grew slowly but formed sclerotia even though the total area covered by the colonies was small (Table 6) (Fig. 14).

On a combination of nitrate and ammonia nitrogen (1, 2 and 5 grams NH_4NO_3 per liter) the same three cultures grew luxuriantly and formed numerous sclerotia. Growth was no better and no worse than when either form of nitrogen was used alone.

Three organic forms of nitrogen had quite different effects on the growth of S. sclerotiorum. Growth of cultures 34, 52 and F on media containing 1 and 5 grams of cystine ($C_6H_{12}O_4N_2S_2$) per liter was no more rapid than when no nitrogen was present. Growth on media containing 5 and 10 grams of asparagine ($C_4H_8N_2O_3$) per liter was luxuriant and the sclerotia formed were larger than those formed on any other media (Table 7) (Figs. 15, 16, 17 and 18).

Urea (NH_2CONH_2) was toxic to the fungus at concentrations greater than 0.5 gram per liter (Figs. 12 and 20). Rate of growth was slowed appreciably when only

1 gram of urea was used per liter. Isolate A made no growth when more than 1.5 grams was used per liter, isolates F when more than 3 grams was used and isolate 52 when more than 4 grams was used per liter (Table 8). At concentrations below these amounts all cultures eventually covered the surface of the agar and formed sclerotia. These latter were much larger than those formed on ordinary culture media and comparable with those formed on media containing asparagine.

Growth of S. sclerotiorum on media containing optimum concentration of nitrogen and phosphorous

It has been noted above that growth of S. sclerotiorum was most rapid on media containing high concentration of phosphorous and low concentration of inorganic nitrogen. When isolates F, 52 and 34 were grown on agar media in which the standard Richards' Solution had been modified by reducing the KNO_3 from 5 to 1 grams per liter and by adding 10 grams NaH_2PO_4, radial growth was very rapid and all cultures completely covered the surface of the media in 3 to 4 days. The mycelium at first was sparse but quickly became dense and covered with numerous sclerotia.

Utilization of carbohydrates

The relationships between utilization of carbon and nitrogen by higher plants have been reported by numerous investigators (4, 6 and 15). Because of the importance of nitrogen for the growth of S. sclerotiorum it seemed well to determine whether any similar relationships might apply to nutrition of this fungus. Isolate A was grown on water agar, alone, and with 5 grams $NaNO_3$ per liter and on water agar to which 50 grams per liter of the following carbohydrates had been added, with and without the addition of 5 grams $NaNO_3$ per liter:

Dextrose $C_6H_{12}O_6$, a monosaccharide.
Sucrose $C_{12}H_{22}O_{11}$, a disaccharide (dextrose, fructose).
Raffinose $C_{18}H_{32}O_{16}$, a trisaccharide (dextrose, fructose, galactose).
Inulin $(C_6H_{10}O_5)_6 + H_2O$, a polysaccharide (fructose).
Starch $(C_6H_{10}O_5)_x$, (dextrose).

Addition of carbohydrates had comparatively little effect upon the rate of radial growth of mycelium but was essential for the formation of sclerotia (Tables 9 and 10) (Figs. 21 and 22).

Addition of $NaNO_3$ to the various carbohydrates had little effect on rate of growth. On those plates which contained $NaNO_3$, however, the growth was much more luxuriant and greater numbers of sclerotia were formed.

On potato agar the rate and density of growth

increased with increased amounts of sucrose (Figs. 23, 24 and 25). Best mycelial growth occured when fairly large amounts (25-50 grams per liter) were used, but more sclerotia were formed at 25 grams per liter than at either lesser or greater concentrations.

Table 5. Effect of nitrogen (NO_3) on rate of growth of S. sclerotiorum and production of sclerotia (Richards' Solution agar containing 0, 1, 5, 10 and 20 grams of $NaNO_3$ per liter used as media).

Isolate	Age of culture	$NaNO_3$ grams per liter 0	1	5	10	20
		Diameter of colonies in cm.				
27C	2 days	1.5	1.2	0.5	0.2	-
	4 "	9.0[a]	9.0	9.0	9.0	2
	6 "	9.0	9.0s[b]	9.0s	9.0s	9.0
	8 "	9.0s	9.0s[b]	9.0s	9.0s	9.0s
34	2 days	-	0.5	0.2	-	-
	4 "	4	5.5	4.5	3	1
	6 "	9.0	9.0	9.0	9.0	4
	8 "	9.0	9.0s	9.0s	9.0s	9.0
35	2 days	3	3	2.5	1.2	2
	4 "	9.0	9.0	9.0	6	6
	6 "	9.0	9.0s	9.0	9.0	9.0
	8 "	9.0s	9.0s	9.0s	9.0s	9.0
27S	2 days	1.5	3	0.5	-	-
	4 "	9.0	9.0	4	3.5	2.5
	6 "	9.0	9.0s	9.0s	9.0	9.0
	8 "	9.0	9.0s	9.0s	9.0s	9.0s

(a) Inside diameter of plate = 9 cm.
(b) s = earliest appearance of sclerotia.

Table 6. Effect of (NH_4) on rate of growth and production of sclerotia of S. sclerotiorum (Richards' Solution agar containing 0, 1 and 5 grams of NH_4Cl per liter was used as media).

Isolate	Age of culture	NH_4Cl grams per liter 0	1	5
		Diameter of colonies in cm.		
F	2 days	-	-	-
	4 "	1	0.3	0.2
	6 "	2.5	2.4	2.5
	8 "	4.5	5.5	6
34	2 days	-	-	-
	4 "	0.3	0.5	0.3
	6 "	1.5	1.3	1.5
	8 "	2.5	4.s[a]	4.s
52	2 days	0.4	0.1	0.1
	4 "	2.3	2	2.5
	6 "	4	6.5	9.0[b]
	8 "	6.5	9.0	9.0s

(a) s = earliest appearance of sclerotia.
(b) Inside diameter of plate = 9.0 cm.

Table 7. Effect of asparagine on rate of growth and production of sclerotia of S. sclerotiorum (Richards' Solution agar containing 0, 1, 5 and 10 grams of asparagine per liter was used as media).

Isolate	Age of culture	Asparagine grams per liter 0	1	5	10
		Diameter of colonies in cm.			
A	2 days	0.1	1.1	1.0	0.8
	4 "	1.3	3.6	3.4	3.5
	6 "	2.9	7.6	6.2	6.4
	8 "	6.5	9.0s	9.0s	9.0s
F	2 days	1.8	1.2	2.0	2.2
	4 "	3.6	4.3	3.5	4.5
	6 "	5.3	7.8	7.2	8.0
	8 "	7.1	9.0s	9.0s	9.0s
52	2 days	0.9	1.9	1.6	2.2
	4 "	2.1	5.2	4.2	4.9
	6 "	4.5	8.	7.2	7.4
	8 "	7.4	9.0s	9.0s	9.0s

(a) Inside diameter of plate = 9.0 cm.
(b) s = earliest appearance of sclerotia.

Table 8. Effect of urea on rate of growth and production of sclerotia of S. sclerotiorum (Richards' Solution agar containing 0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4 grams of urea per liter was used as media).

Isolate	Age of culture	Urea grams per liter 0	0.5	1	1.5	2	3	4
		Diameter of colonies in cm.						
A	3 days	1.1	0.9	-	-	-	-	-
	6 "	6.4	4.2	1.8	-	-	-	-
	9 "	9.0[a]	9.0s[b]	5.8	1.8	-	-	-
	12 "	9.0	9.0s[b]	9.0s	7.0	-	-	-
	15 "	9.0	9.0s	9.0s	9.0s	-	-	-
F	3 days	1.9	1.7	0.1	-	-	-	-
	6 "	6.6	7.2	4.2	1.2	-	-	-
	9 "	9.0	9.0s	9.0s	6.6	3.5	-	-
	12 "	9.0	9.0s	9.0s	9.0s	9.0s	0.4	-
	15 "	9.0s	9.0s	9.0s	9.0s	9.0s	3.1	-
52	3 days	1.7	1.3	0.2	-	-	-	-
	6 "	7.1	7.4	5.8	2.2	1.0	-	-
	9 "	9.0	9.0s	9.0s	9.0	6.5	-	-
	12 "	9.0	9.0s	9.0s	9.0s	9.0s	3.5	-
	15 "	9.0s	9.0s	9.0s	9.0s	9.0s	9.0	1.0

(a) Inside diameter of plate = 9.0 cm.
(b) s = earliest appearance of sclerotia.

Table 9. Effect of (NO_3) nitrogen and carbohydrates upon rate of growth and formation of sclerotia (Isolate C).

Nutrient added to water agar	Days after "seeding" 1	2	3	4	5	6	7	8	9	10
None						C[a]				
$NaNO_3$					C					
Dextrose			C				S[b]			
Dextrose and $NaNO_3$			C				S			
Sucrose			C				S			
Sucrose and $NaNO_3$			C			S				
Raffinose			C				S			
Raffinose and $NaNO_3$			C			S				
Inulin					C		S			
Inulin and $NaNO_3$					C			S		
Potato starch					C			S		
Potato starch and $NaNO_3$				C		S				

(a) Surface of agar completely covered.
(b) Sclerotia formed.

Table 10. Effect of carbohydrates and nitrogen on rate of growth and production of sclerotia of S. sclerotiorum. Water agar with, and without nitrogen (5 grams $NaNO_3$ per liter) was used as a base medium to which 50 grams per liter of various kinds of carbohydrates was added:

Isolates and age of isolates	Water agar only	$NaNO_3$	Dextrose	Dextrose plus $NaNO_3$	Sucrose	Sucrose plus $NaNO_3$	Raffinose	Raffinose plus $NaNO_3$	Inulin	Inulin plus $NaNO_3$	Potato starch	Potato Starch plus $NaNO_3$
A												
2 days	1.4	3.4	3.9	5.2	4.7	5.3	3.1	5.4	1.1	1.3	1.9	3.2
4 days	3.9	9.0[a]	9.0	9.0	9.0	9.0	9.0	9.0	4.3	5.5	4.7	9.0
6 days	6.4	9.0[a]	9.0	9.0	9.0	9.0s[b]	9.0	9.0s	9.0	9.0	9.0	9.0
8 days	9.0	9.0[a]	9.0	9.0	9.0s	9.0s[b]	9.0s	9.0s	9.0	9.0s	9.0s	9.0s
10 days	9.0	9.0[a]	9.0	9.0	9.0s	9.0s[b]	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s
C												
2 days	2.7	3.4	4.7	4.8	4.7	4.8	4.2	5.8	2.6	1.3	3.3	3.9
4 days	4.9	7.3	9.0	9.0	9.0	9.0	9.0	9.0	6.2	9.0	7.2	9.0
6 days	9.0	9.0	9.0	9.0	9.0	9.0s	9.0	9.0s	9.0	9.0	9.0	9.0s
8 days	9.0	9.0	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s
10 days	9.0	9.0	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s	9.0s

(a) Inside diameter of plate = 9.0 cm.
(b) s = earliest appearance of sclerotia.

EVIDENCES OF PHYSIOLOGIC SPECIALIZATION IN *S. SCLEROTIORUM*

The discovery of the phenomenon of physiologic specialization was undoubtedly one of the most important developments in plant pathology. Various terms have been introduced to apply to what we now term physiologic forms, such as: "species sorores," "biologishe arter," "biologische rassen," "physiologic species," "formae speciales," "gewohnheitrassen" or "adapted rassen," "races special'ees," "biologic or biological forms." "Biologic form" has been the most commonly used in the United States. Recently a joint committee of phytopathologists, botanists, horticulturists and agronomists adopted the term physiologic forms (12).

The underlying conception formerly was that these forms could be distinguished only on the basis of their physiological behavior or by their ability to infect some host plants and not others. It is true that physiologic forms of certain presumably obligate parasites, like the rust fungi and the powdery mildews, can be differentiated readily only by their action on differential hosts.

At the present time, forms may also be recognized to a limited extent by morphology, by cultural characteristics on artificial media and by physiochemical reactions. The phenomenon may be defined as the existence of

physiologic races within morphologic species (12).

The concept of physiologic specialization was expressed by Stakman (12) briefly thus:

> Entities within a species which differ from each other primarily and consistently, physiologically should be considered as physiologic forms; whether the differences are in pathogenicity or other physiologic attributes.

Ramsey (14) made several cross-inoculation tests to determine by infection of the several hosts whether there were any physiological races or strains of Sclerotinia species. Twelve strains of the Sclerotinia libertiana type which were isolated from different hosts were used, together with one strain each of S. intermedia and S. ricini and two strains of S. minor. These cultures were used to inoculate nine different hosts that are commonly affected with Sclerotinia decay. In addition, potato tubers, beet roots, tomato and lemon fruits were inoculated.

All of the strains obtained from the various host plants were pathogenic to those hosts commonly found attacked by Sclerotinia.

Ramsey's experiments indicated that the host range of Sclerotinia spp. is so wide that it is impossible to separate the different species on the basis of host infection. All species produced definite infection and

decay. On most host plants the type of decay produced by the Sclerotinia species is quite similar, and therefore does not help much in distinguishing species.

Host inoculation with S. Sclerotiorum

Six series of plant inoculations were made. In each series 3 isolates were used. Two pots each of crimson clover, cabbage, tomato, turnip, sunflower, squash, radish, snap bean and barley were held in a moist chamber for 24 hours. One pot of each pair was retained as a check and the plants in the other were inoculated with 4-6 day old inoculum. After inoculation the plants were kept in the moist chamber for 48 hours before being returned to the greenhouse bench. Most of the inoculations were unsuccessful. So few plants became infected that the results were inconclusive.

It was noted earlier that at very low Hydrogen ion concentrations there were great differences in the rate and character of growth of the various isolates. At pH 2.04, for example, isolates A (from Corvallis, Oregon) grew well, producing a rather thin felt of mycelium with numerous sclerotia scattered over the surface (Fig. 8). Isolate 27C (from Mt. Vernon, Wash.) on the other hand produced very dense colonies more than .2 cm. in thickness and only 2 cm. in diameter after 13 days. Isolate F

(from Ft. Lauderdale, Fla.) was not as uniform or as consistent as 27C and A but occupied a position approximately midway between these two extremes.

At low temperatures all isolates grew equally well and there were no apparent differences between them. On the other hand they could be separated readily into two distinct groups when grown at high temperatures. The rate of growth of isolates such as 27B, 27C and 34 increased rapidly with rising temperature up to 20° C., at which they made most rapid growth. Above that temperature the rate of growth decreased rapidly (Fig. 26). The rate of growth of isolates such as 27S and 35 also increased rapidly up to 20° C. These isolates, however, grew well at temperatures of 25° and 30° C.

It has already been noted that none of the isolates was able to grow in media containing appreciable amounts of urea. Isolates F and 52 grew fairly well and produced abundant sclerotia when 3 and 4 grams of urea respectively were used per liter of media. Isolate A grew slowly when only 1.5 grams were used per liter and not at all when 2 grams per liter were used (Fig. 20).

Ramsey has noted that the host range of S. sclerotiorum is so wide that it is impossible to distinguish physiologic forms on the basis of host infection. There is nothing in the results of these

investigations to indicate the existence of such forms among the isolates studied. The meager evidence that has been obtained is based entirely on physical reactions of the isolates to differences in nutrient substrate, Hydrogen ion concentration and temperature.

The fact that the isolates studied showed no differences in their ability to utilize a wide range of nutrients perhaps explains their ability to thrive on such a wide variety of host plants. The differences in their reaction to temperature, Hydrogen ion concentration, and to certain toxic chemicals perhaps explains why the fungus is a serious parasite in certain localities, certain soil types, and in certain seasons, and entirely innocuous in others.

DISCUSSION OF RESULTS

Sclerotinia sclerotiorum attacks a wide variety of economic crops and weeds, and under certain conditions is able to live for long periods as a saprophyte. These facts indicate that its nutrient requirements are not specific. The results of these investigations have repeatedly shown that this is true. While the fungus did show preferences for certain elements and for certain types of carbohydrates, amino acids, etc., it also exhibited a bewildering ability to grow on almost any substrate, regardless of the presence or absence of any specific mineral or organic nutrient. Such freedom from rigid nutrient requirements makes it an almost ideal parasite.

Another puzzling fact was that Sclerotiniose is a serious disease in Florida during the winter and spring months, in the Pacific Northwest during late summer and fall and in the Middle Atlantic States in August and September. Although all cultures grew best at temperatures from 15° to 25° C., they were able to grow quite normally and to produce sclerotia at temperatures ranging from 0° to 30° C. Again, S. sclerotiorum is a practically ideal parasite, able to maintain itself at almost any temperature that is suitable for the growth of crop plants.

Sclerotinia sclerotiorum grew quite well on media of pH 2.4 to 9.65, a tremendous range from extremely acid to extremely alkaline. This also might have been predicted when it is recalled that the fungus thrives in green tomatoes, immature apricots and other acid fruits, that it is an important parasite of such neutral or slightly alkaline fruits as squash, bean and sweet potato, and that it can also thrive as a saprophyte in highly alkaline organic residues.

The extreme adaptability of S. sclerotiorum, plus its habits of producing tough, resistant sclerotia which withstand unfavorable periods, indicates that it will not be easy to find a simple control practise which will be effective under all conditions. This, of course, is amply borne out by the difficulties that have been experienced in attempting to control it. The most promising factor indicated by these investigations is the sensitivity of the fungus to certain chemicals such as urea. The use of such materials is already under investigation by a number of research workers in the United States and elsewhere.

SUMMARY

A series of experiments was conducted to determine the physiologic properties of Sclerotinia sclerotiorum and also to find out if there are physiologic differences between isolates of the fungus.

All cultures grew best at temperatures from 15° to 25° C. However, they were able to grow and to produce sclerotia at temperatures ranging from 0° to 30° C. Sclerotia formed at low temperatures were larger than those formed at high temperatures.

The fungus grew well on media of pH 2.4 to 9.65. At pH 2.04 and 10.20 growth was retarded and no growth occured at pH 10.95.

The amount of Mg, Fe, S and K in the substrate has no apparent effect on the rate or character of growth of S. sclerotiorum, while phosphorous, nitrogen and carbohydrates appeared to be of importance in determining the rate of growth and formation of sclerotia by the fungus.

Repeated host plant inoculations were mostly unsuccessful. The results of these experiments did not indicate the existence of pathogenically distinct physiologic forms.

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APPENDIX

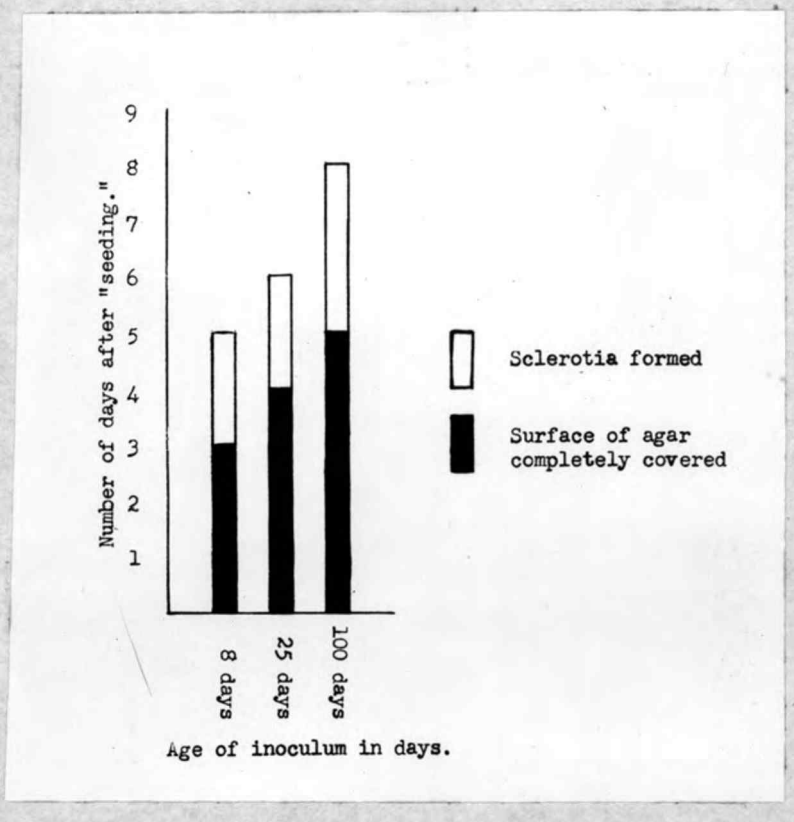

Fig. 1. Effect of age of inoculum on rate of growth and formation of sclerotia.

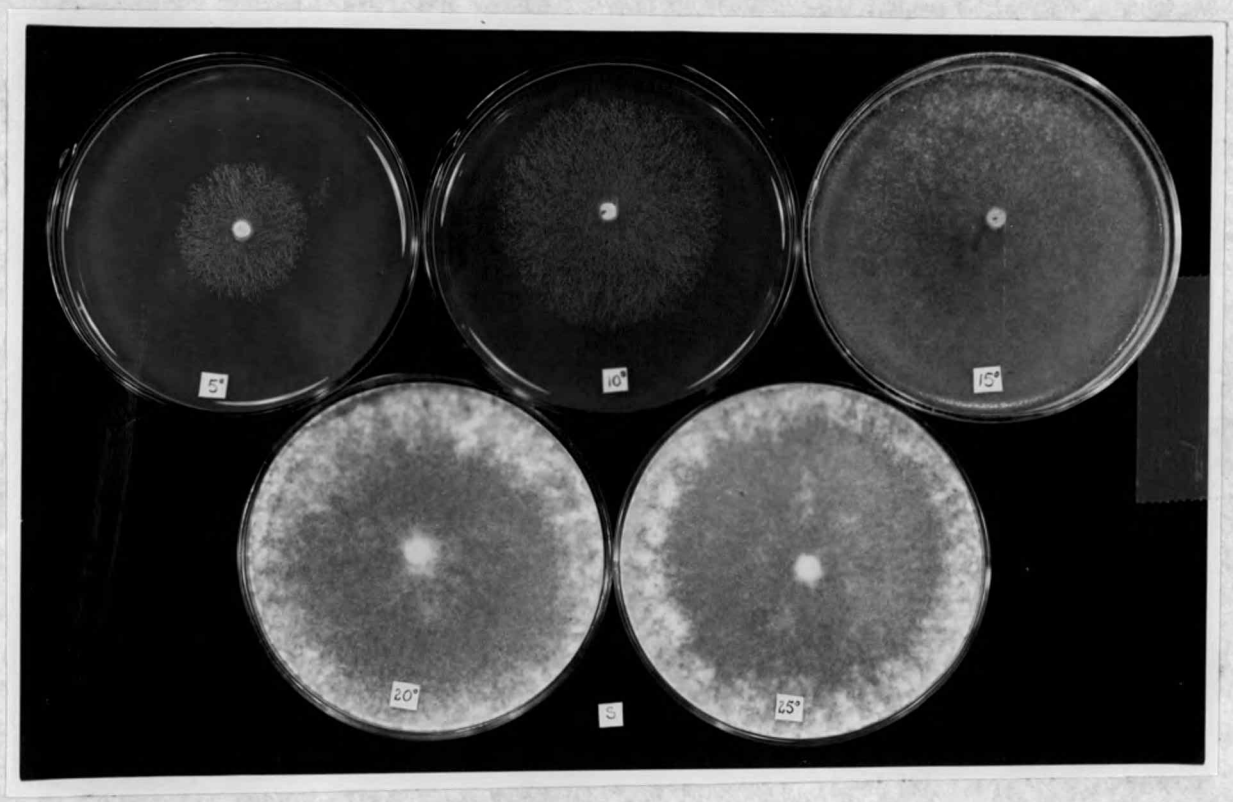

Fig. 2. Growth of S. sclerotiorum at temperatures from 5° to 25° C. (Isolate 27S).

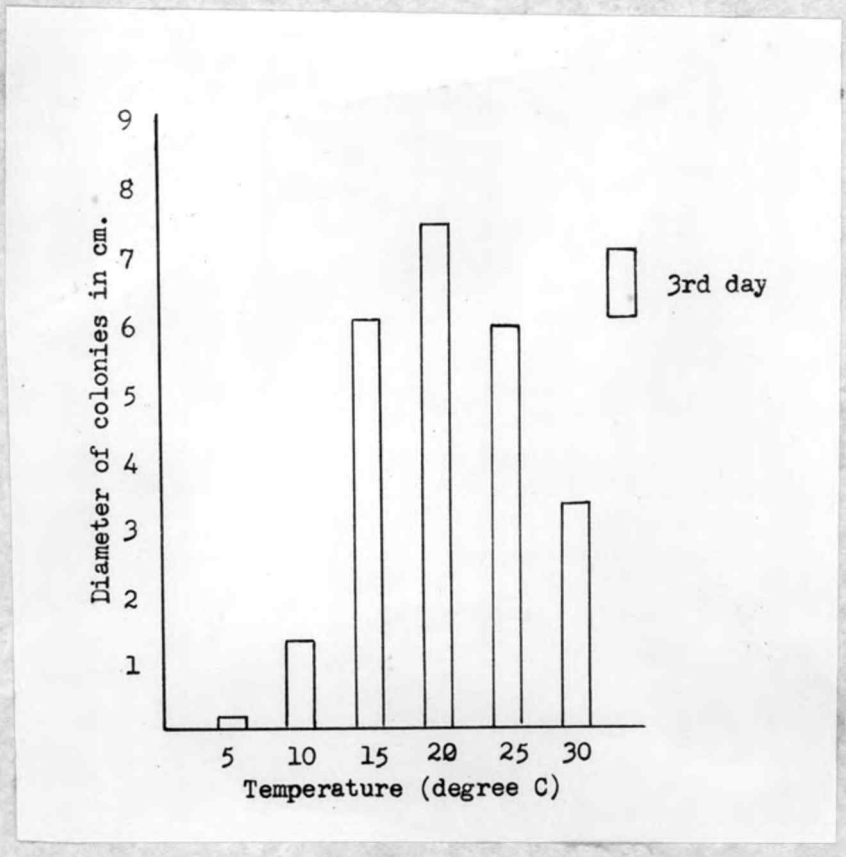

Fig. 3. The effect of temperature on the rate of growth of S. sclerotiorum (Columns indicate average diameter of colonies).

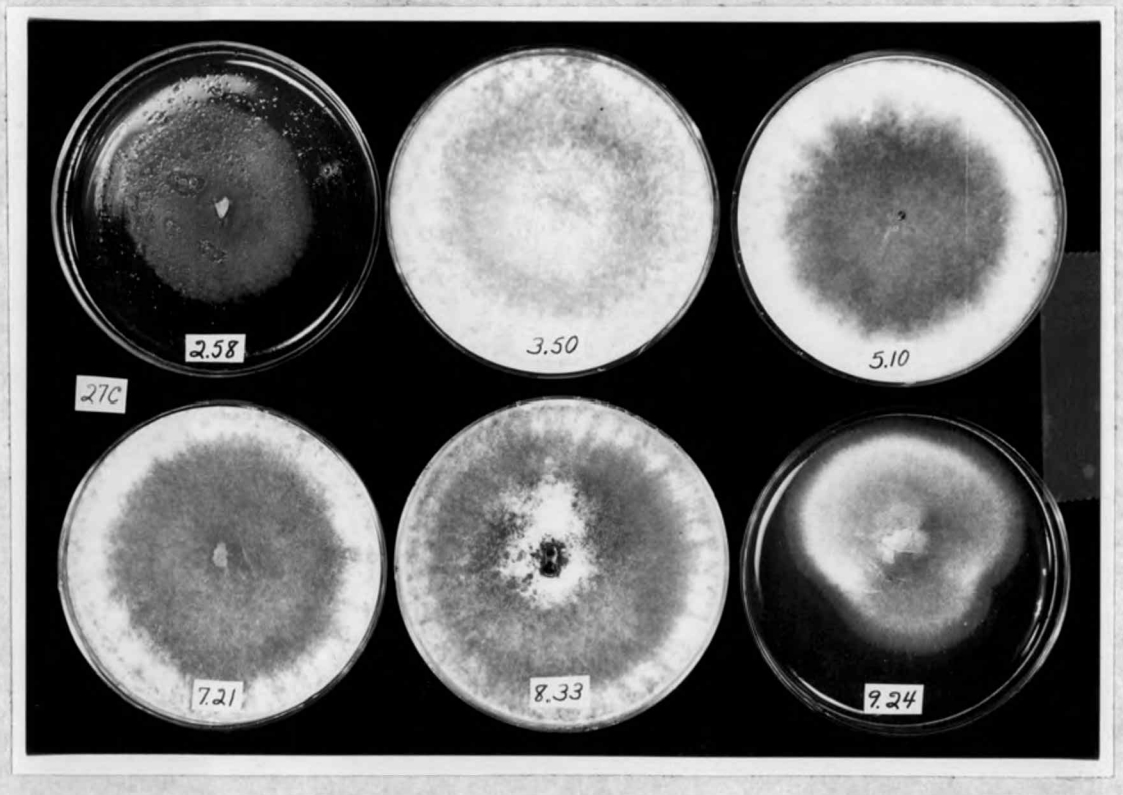

Fig. 4. Growth of S. sclerotiorum on potato dextrose agar at pH 2.58, 3.50, 5.10, 7.21, 8.33 and 9.24 (Isolate 27C).

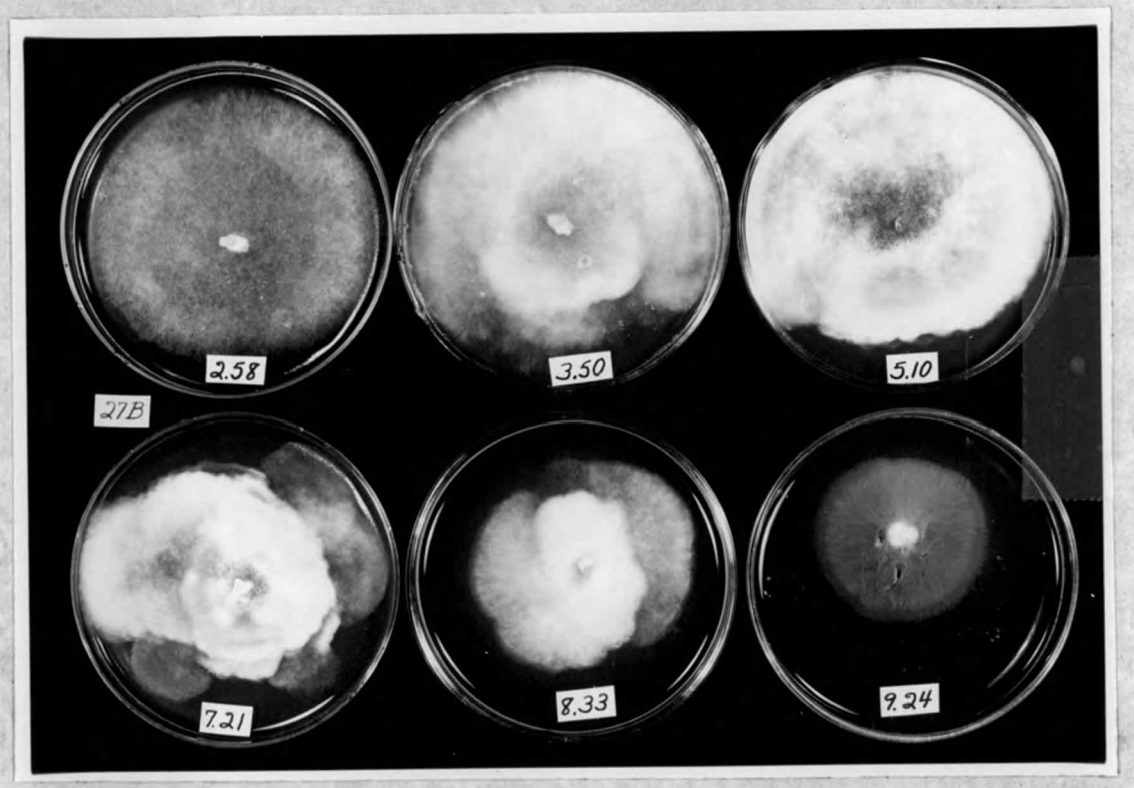

Fig. 5. Growth of S. sclerotiorum on potato dextrose agar at pH 2.58, 3.50, 5.10, 7.25, 8.33 and 9.24 (Isolate 27B).

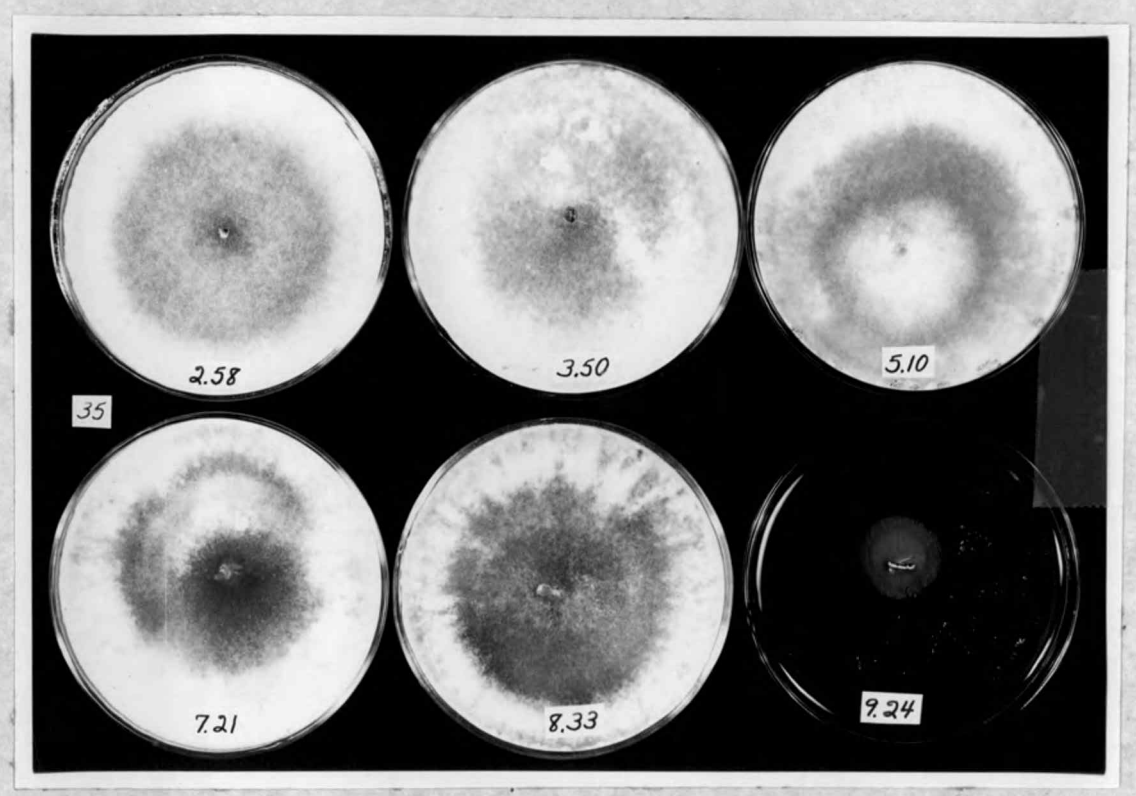

Figure 6. Growth of S. sclerotiorum on potato dextrose agar at pH 2.58, 3.50, 5.10, 7.21, 8.33 and 9.24 (Isolate 35).

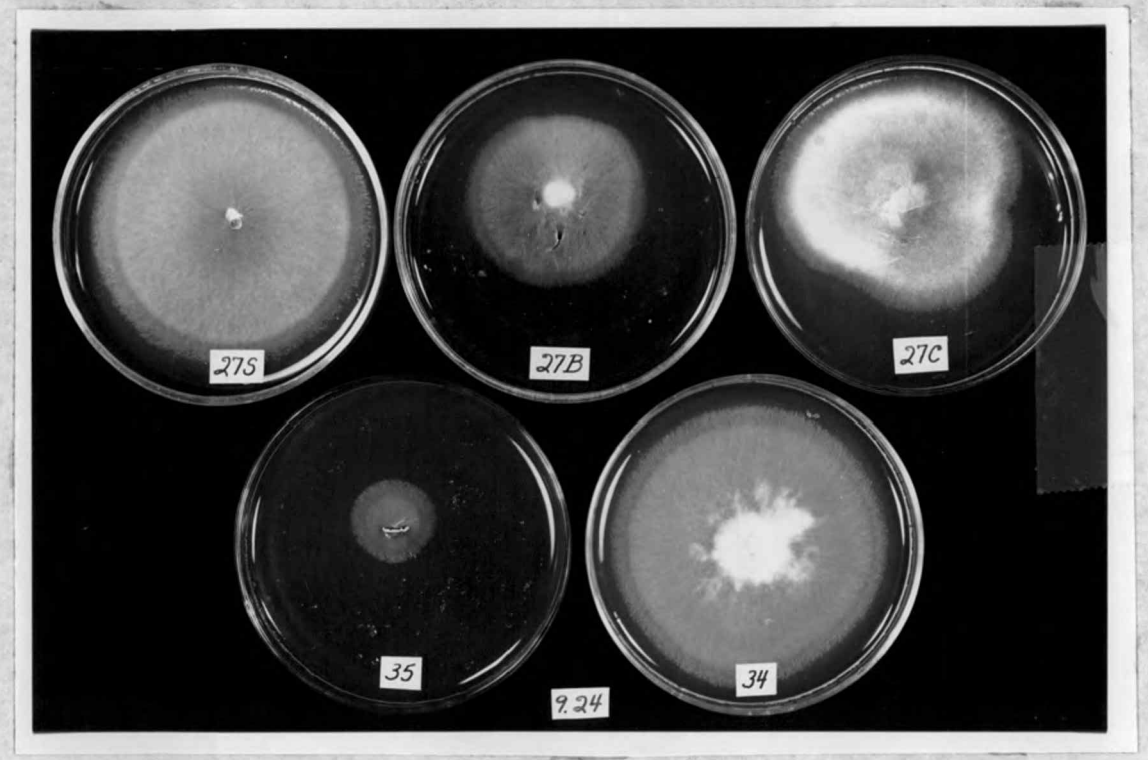

Fig. 7. Growth of five isolates of S. sclerotiorum (27B, 27C, 27S, 34, 35) on potato dextrose agar at pH 9.24.

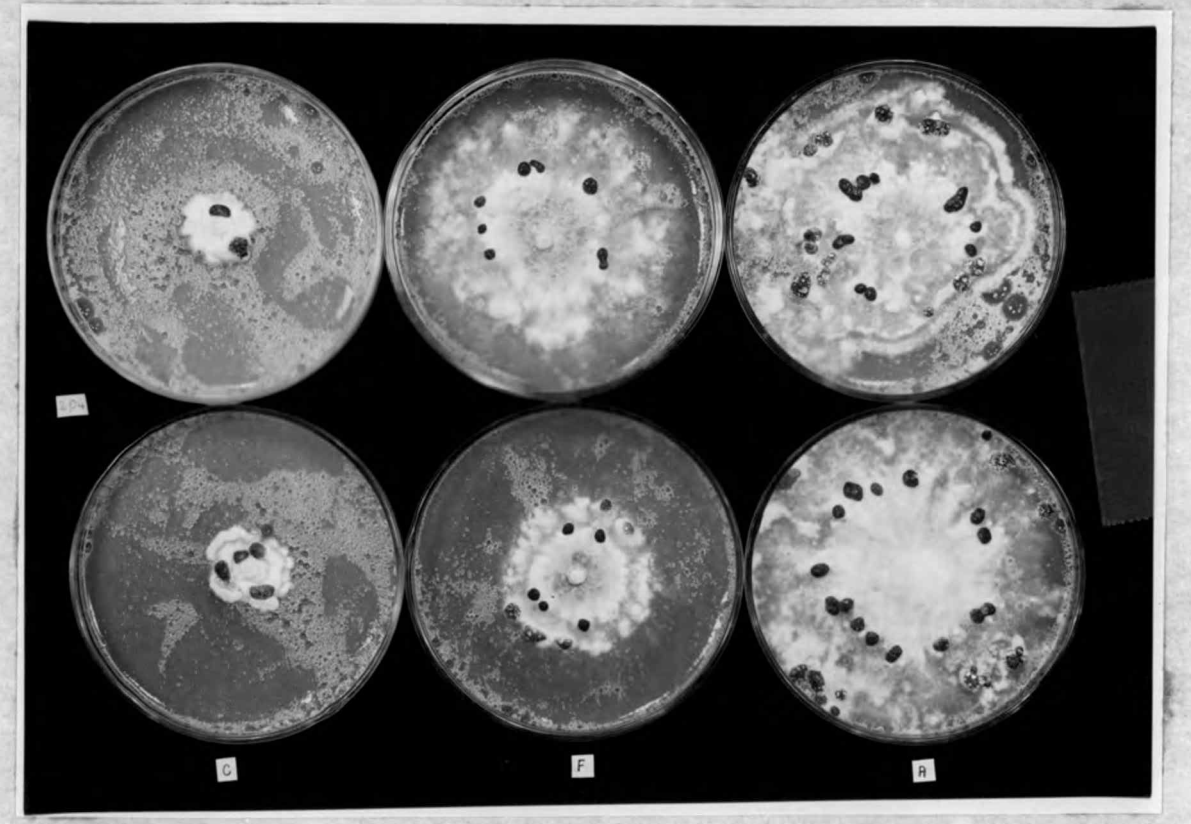

Fig. 8. Growth of S. sclerotiorum on Richards' Solution at pH 2.04 (Isolates C, F and A).

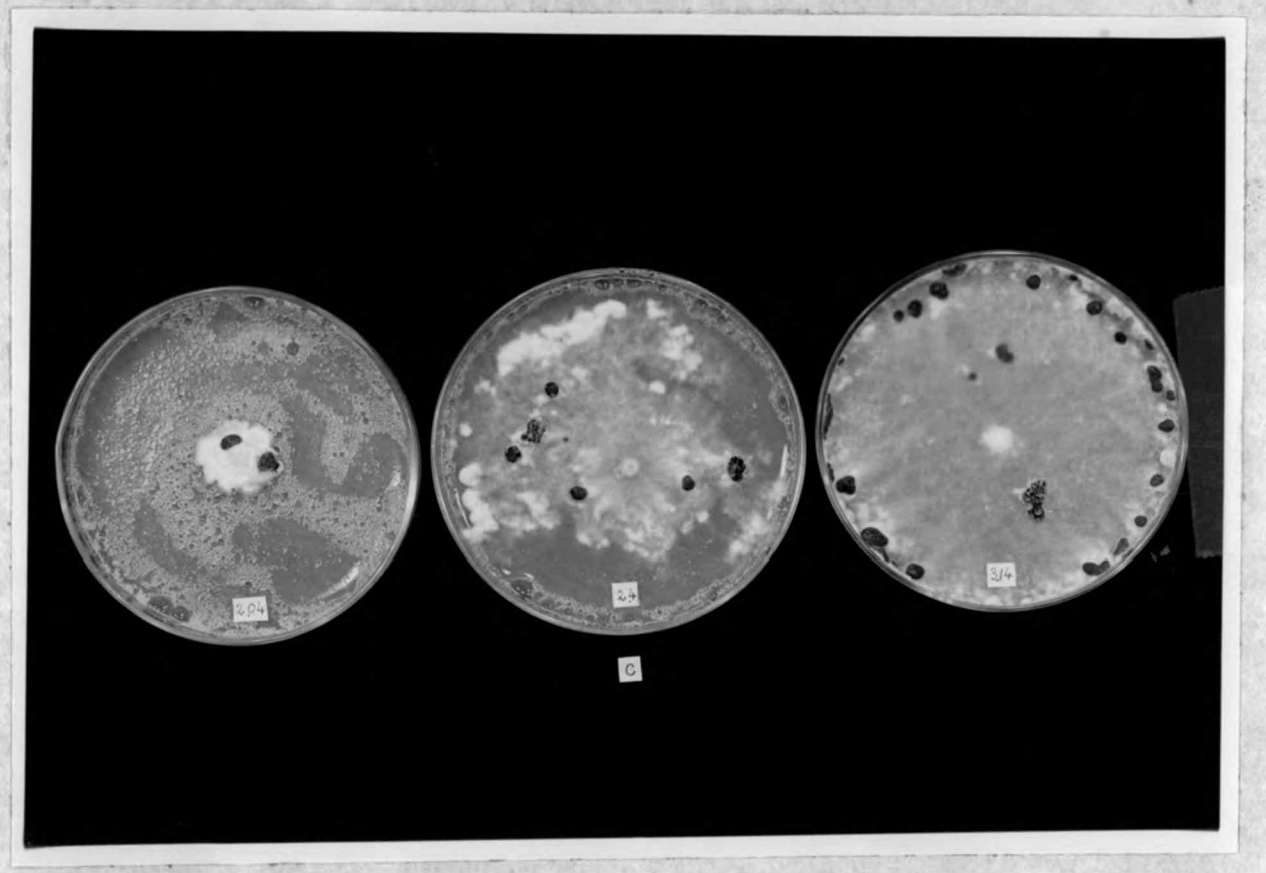

Fig. 9. Growth of S. sclerotiorum on Richards' Solution agar at pH 2.04, 2.4 and 3.14 (Isolate 27C).

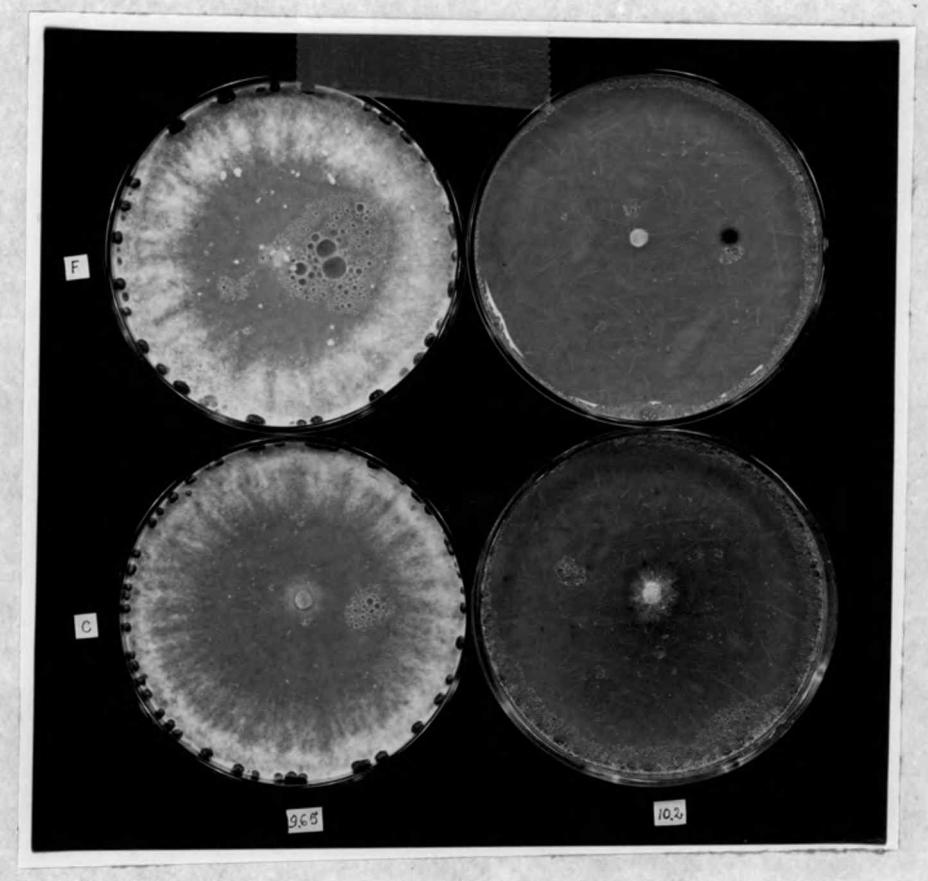

Fig. 10. Growth of S. sclerotiorum on Richards' Solution at pH 9.65 and 10.2 (Isolates F and C).

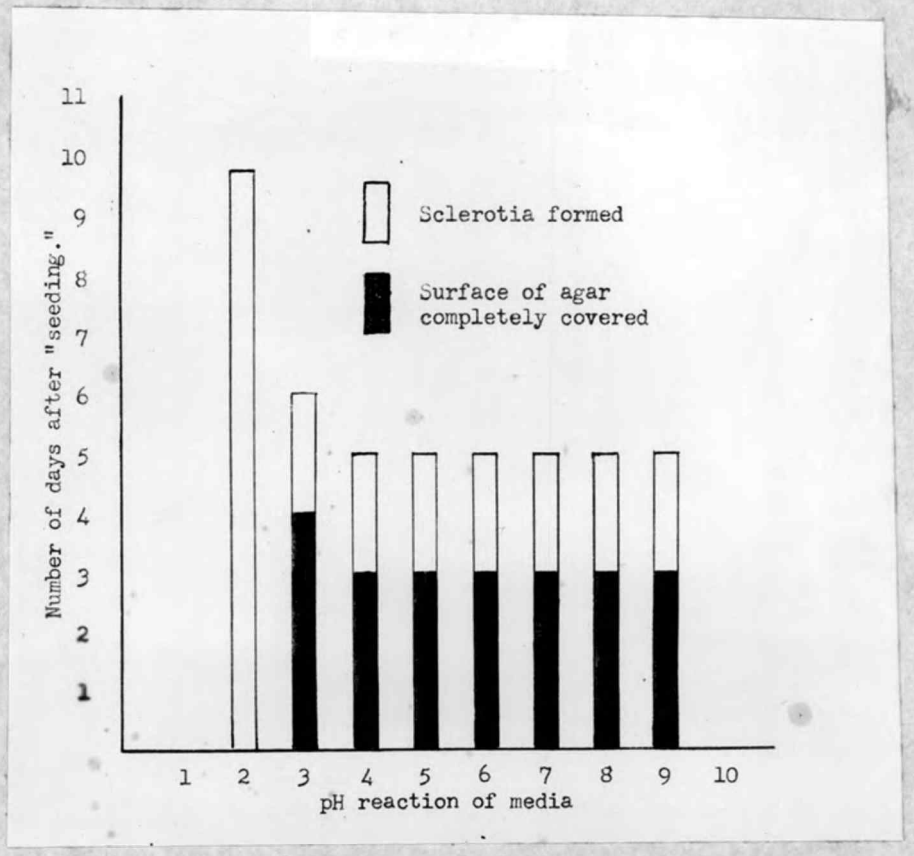

Fig. 11. Effect of pH on average rate of growth of three isolates of S. sclerotiorum (Isolates A, C and F) and on production of sclerotia.

(Note: At pH 1 and 10 there was slight growth, but fungus never completely covered the surface of the agar and did not form sclerotia.)

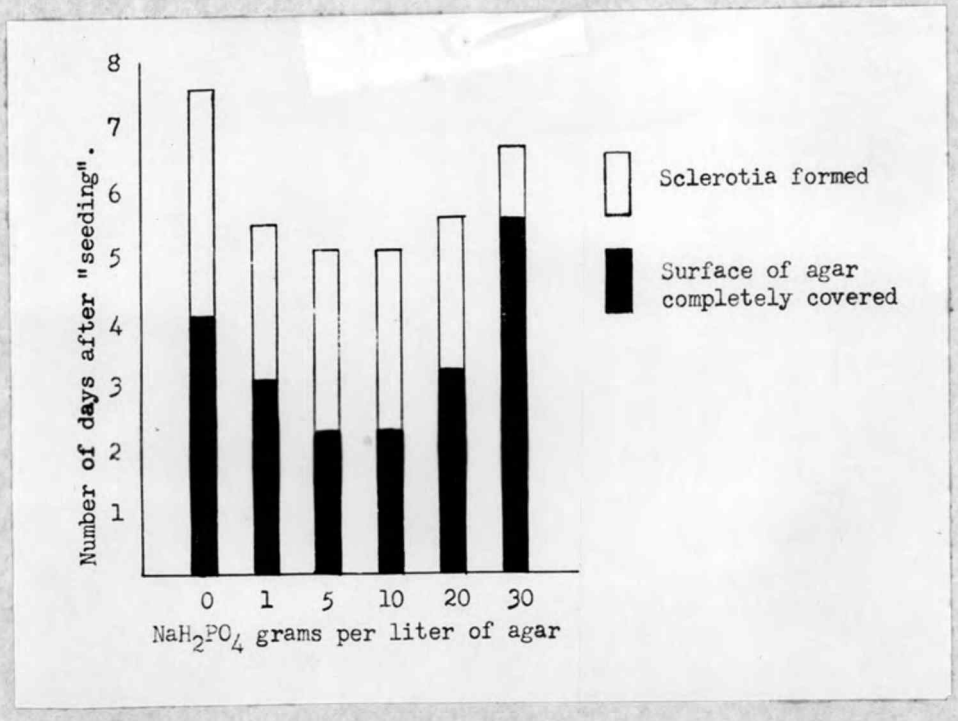

Fig. 12. Effect of phosphorous on the rate of growth and formation of sclerotia by S. sclerotiorum.

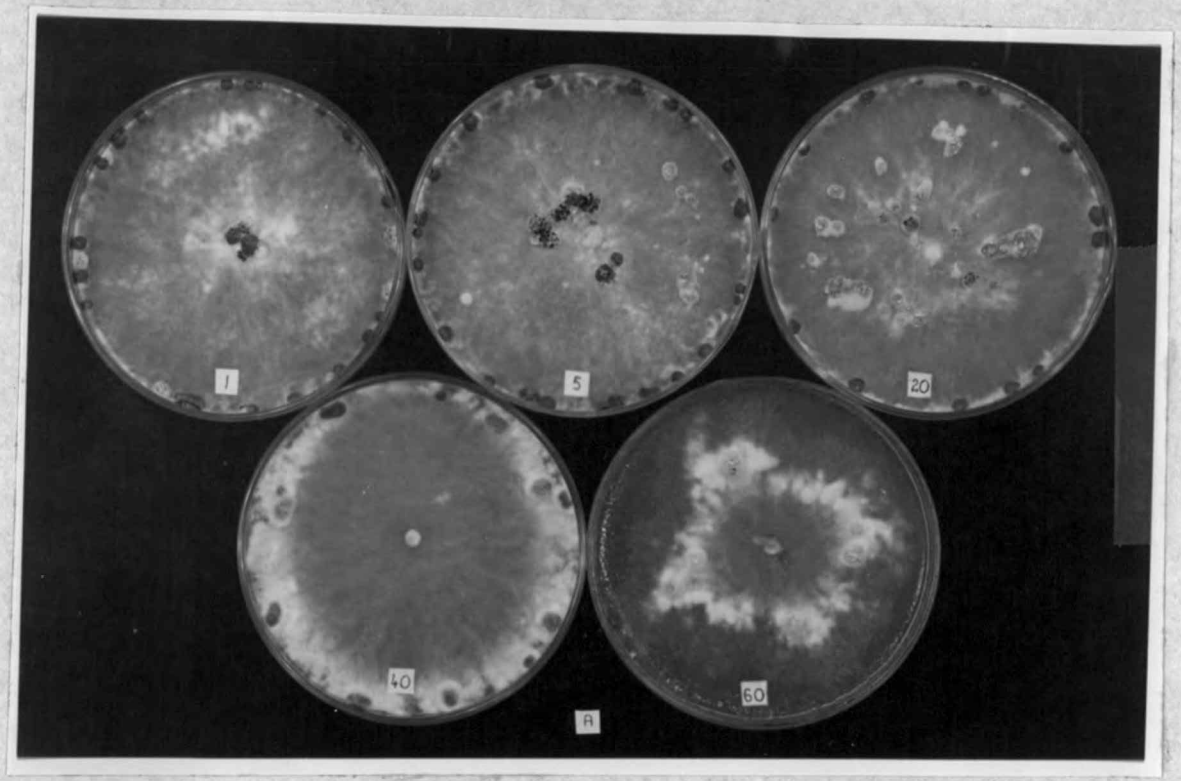

Fig. 13. Growth of S. sclerotiorum (Isolate A) on Richards' Solution agar containing 1, 5, 20, 40 and 60 grams of $NaNO_3$ per liter.

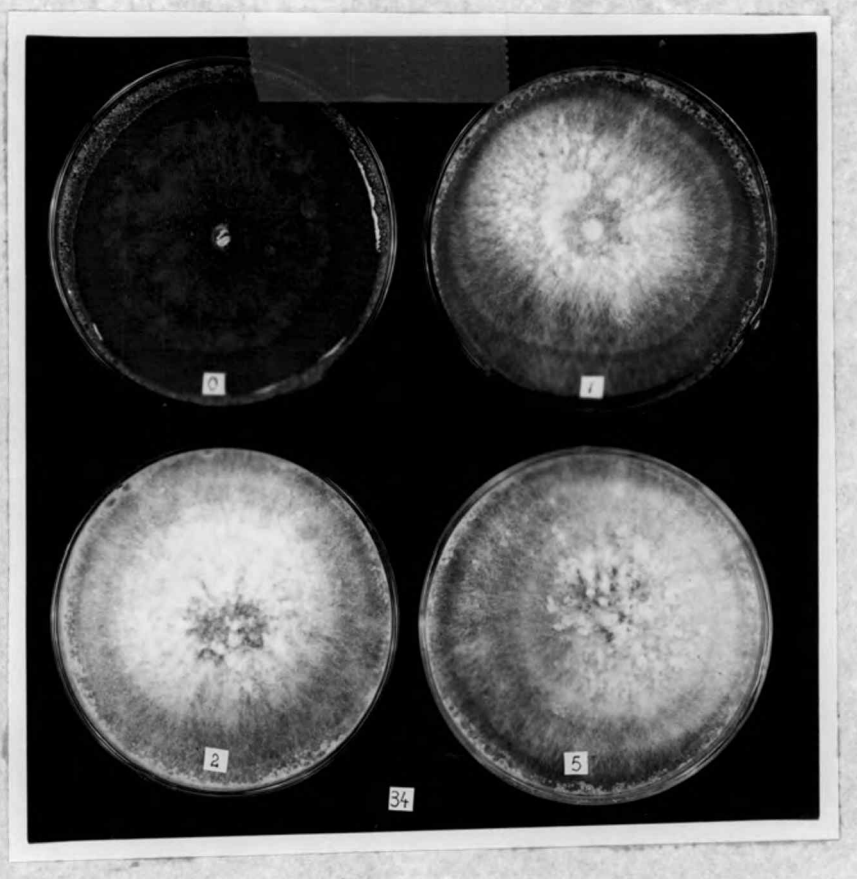

Fig. 14. Growth of S. sclerotiorum on Richards' Solution agar containing 0, 1, 2, and 5 grams of NH_4NO_3 per liter (Isolate 34).

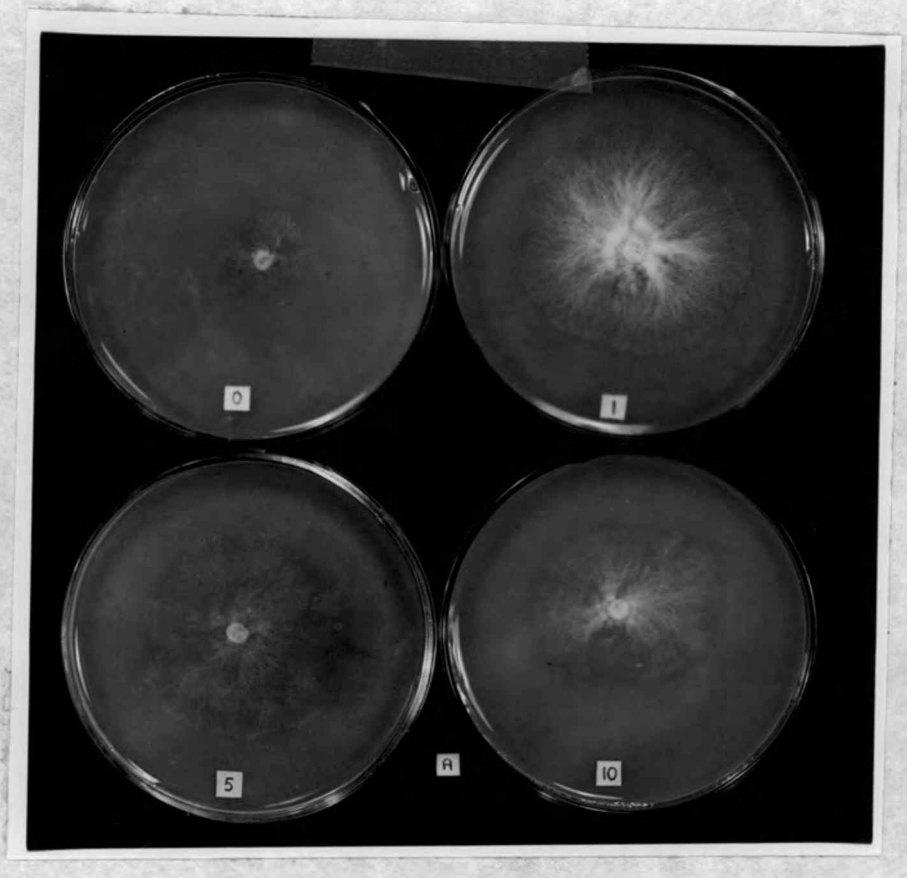

Fig. 15. Growth of S. sclerotiorum on Richards' Solution agar containing 0, 1, 5 and 10 grams of asparagine per liter (Isolate A).

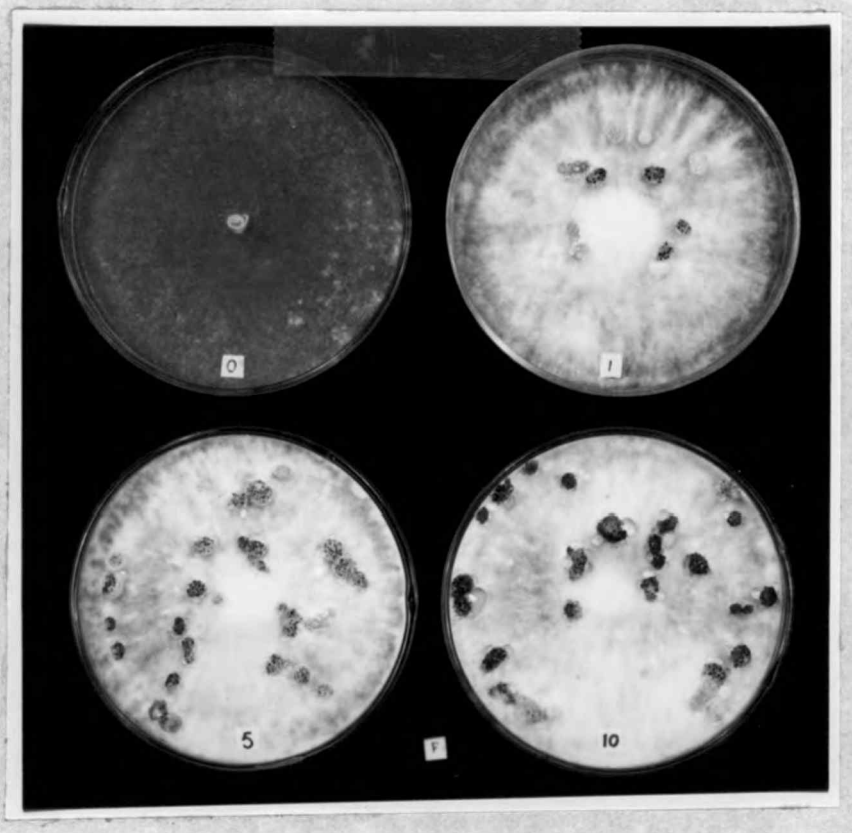

Fig. 16. Growth of S. sclerotiorum on Richards' Solution agar containing 0, 1, 5 and 10 grams of asparagine per liter (Isolate F).

Fig. 17. Growth of S. sclerotiorum on Richards' Solution agar containing 0, 1, 5 and 10 grams of asparagine per liter (Isolate 52).

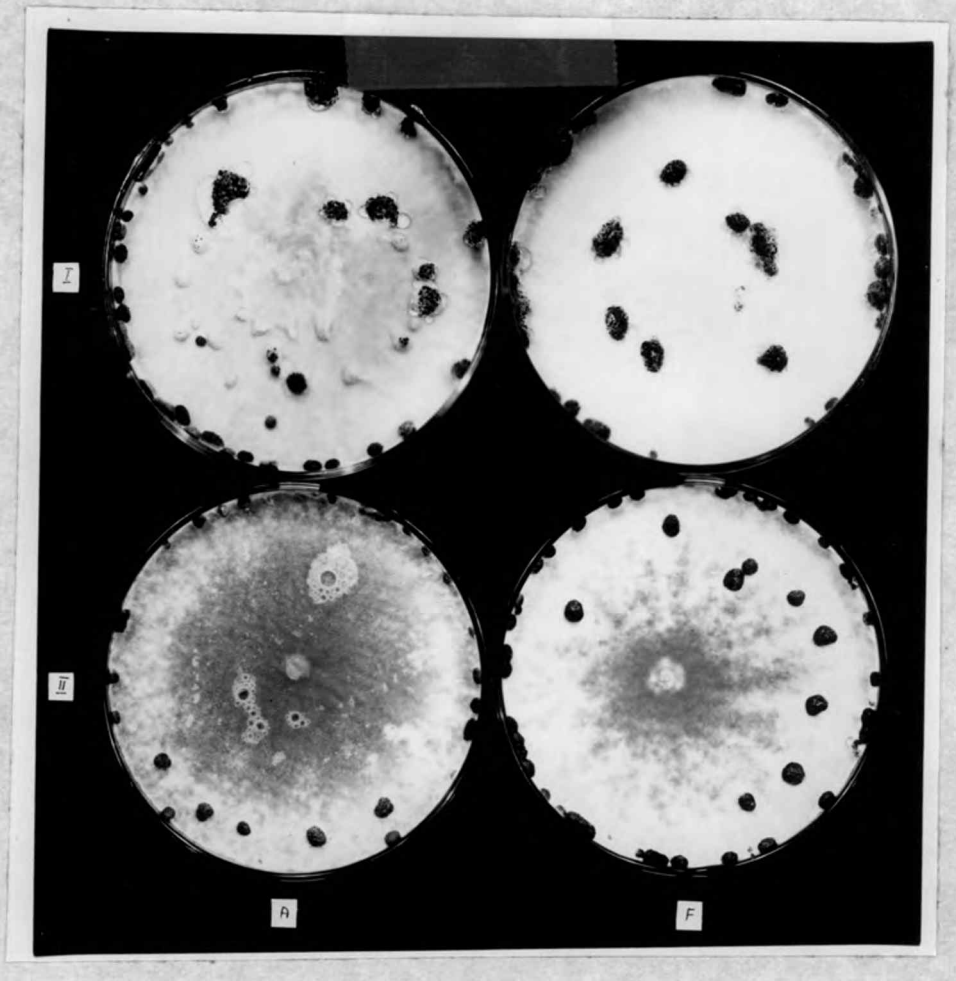

Fig. 18. The effect of asparagine upon sclerotia formation of S. sclerotiorum.

I Growth on Richards' Solution containing 5 grams of asparagine per liter.
II Growth on potato-dextrose agar.

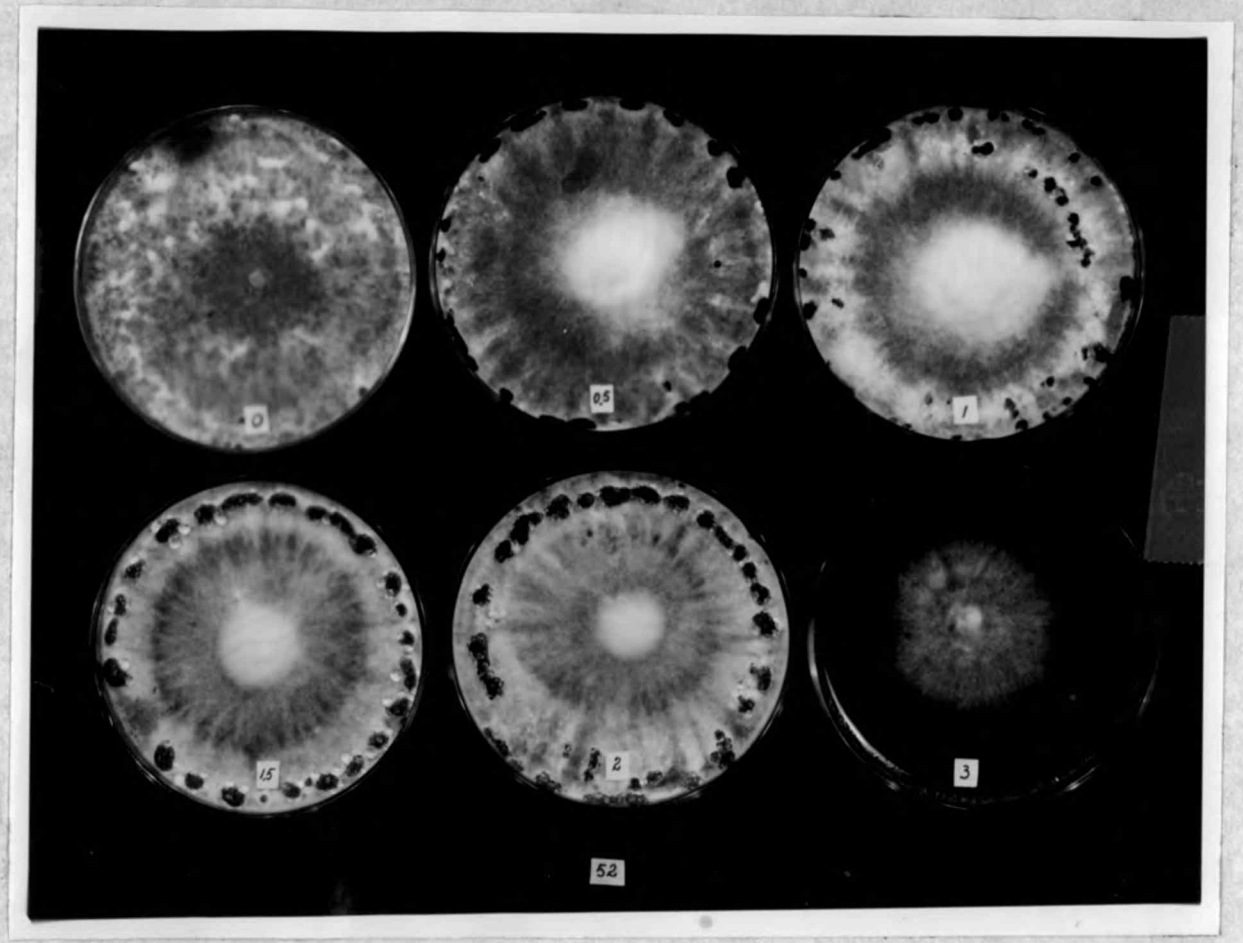

Fig. 19. Growth of S. sclerotiorum on Richards' Solution agar containing 0, 0.5, 1, 1.5, 2 and 3 grams urea per liter. (Isolate 52)

Fig. 20. Growth of S. sclerotiorum on Richards' Solution containing 0, 0.5, 1 and 1.5 grams of urea per liter (Isolate A).

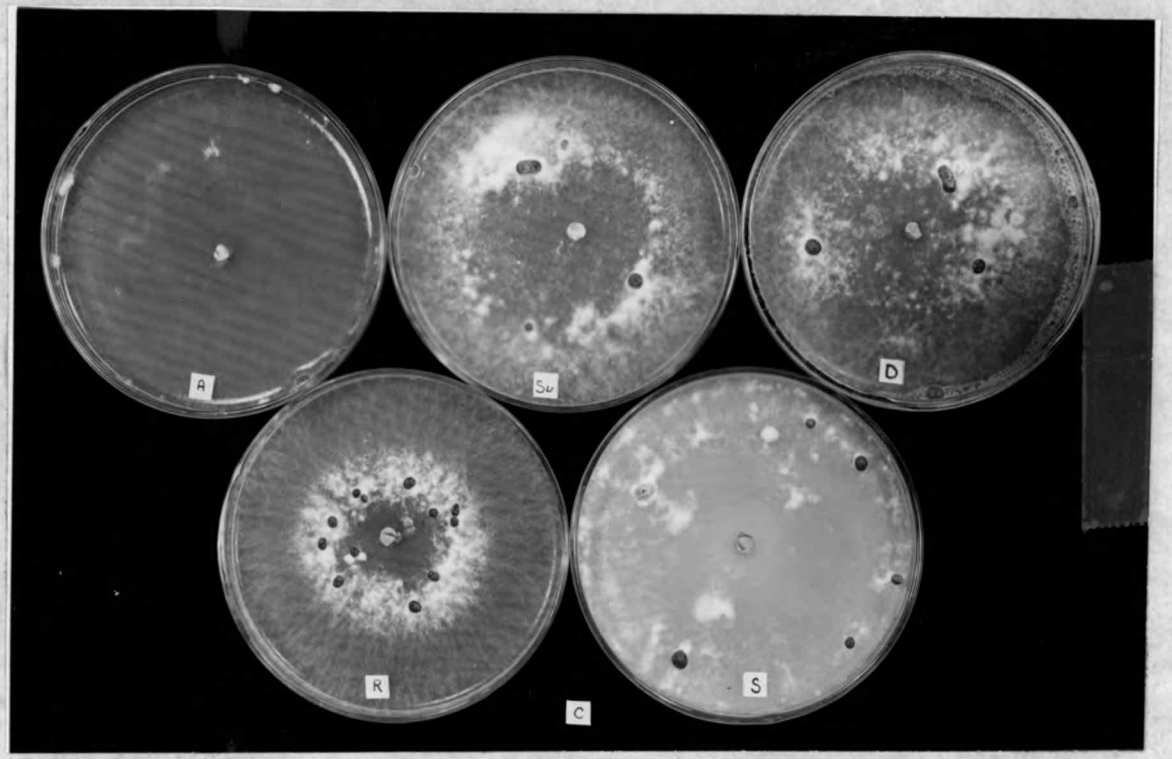

Fig. 21. Effect of carbohydrates on growth of S. sclerotiorum (Isolate C). Water agar was used as a media. A received no nutritional value, Su, D, R and S received 50 grams of sucrose, dextrose, raffinose and potato starch per liter respectively.

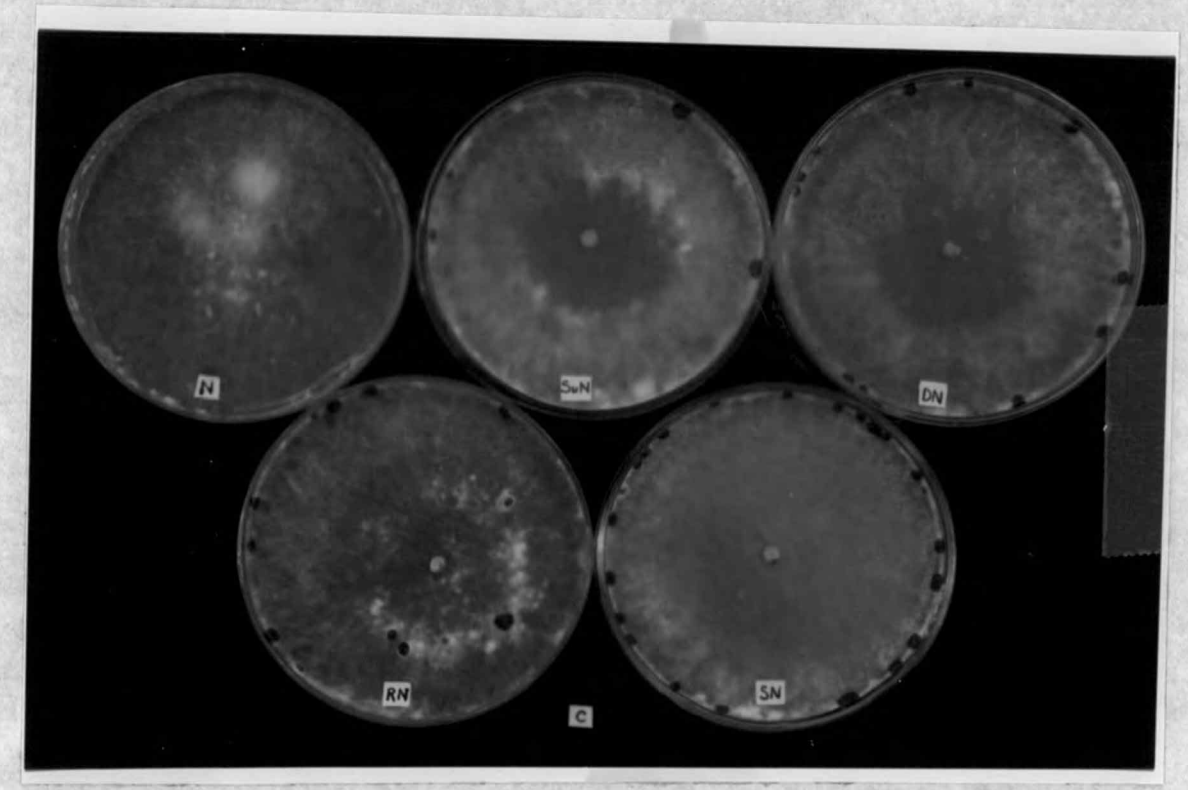

Fig. 22. Effect of nitrogen and carbohydrates on growth of S. sclerotiorum (Isolate 27C). N received only 5 grams $NaNO_3$ per liter. SuN, DN, RN and SN received 5 grams $NaNO_3$ per liter plus 50 grams of sucrose, dextrose, raffinose and potato starch per liter respectively. (Water agar was used as a media.)

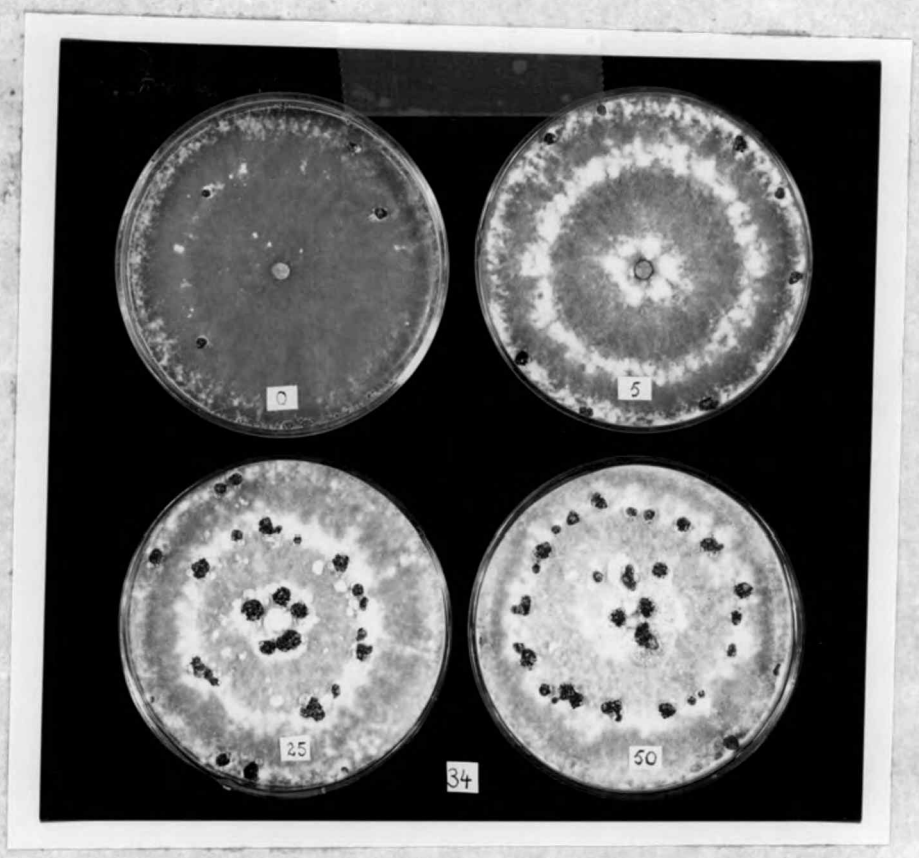

Fig. 23. Growth of S. sclerotiorum on potato agar containing 0, 5, 25 and 50 grams of sucrose per liter. (Isolate 34)

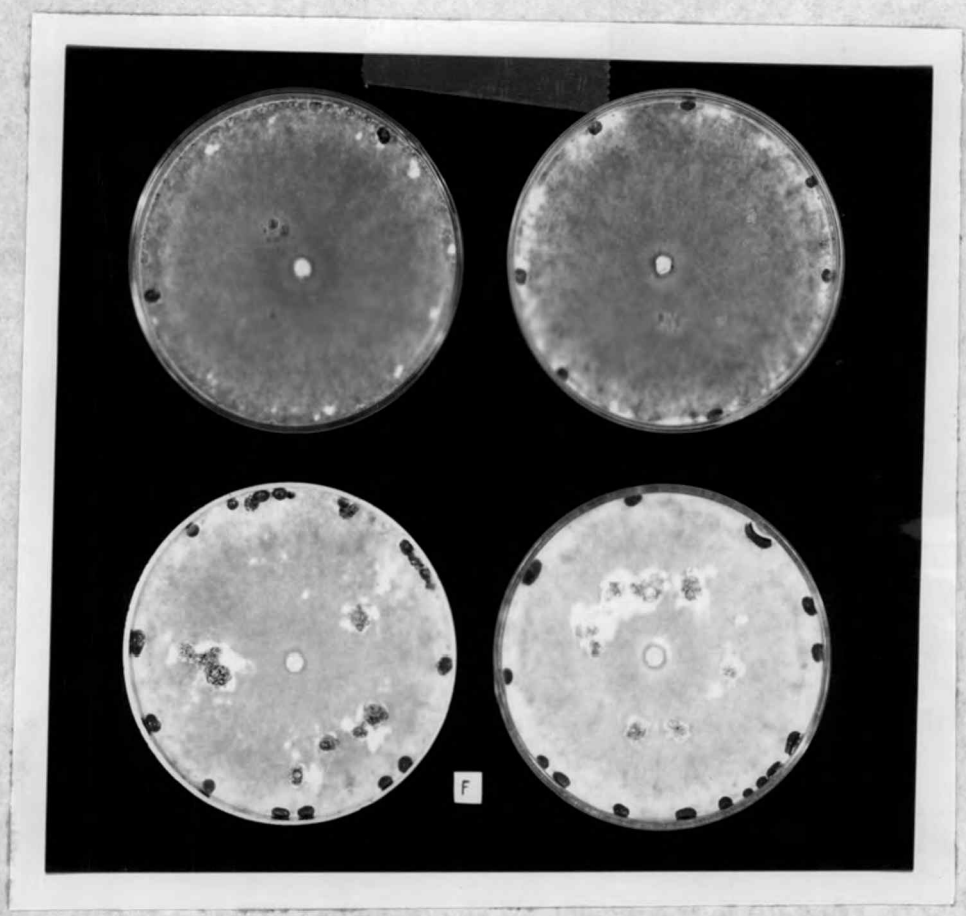

Fig. 24. Growth of S. sclerotiorum on potato agar containing 0, 5, 25 and 50 grams of sucrose per liter. (Isolate F)

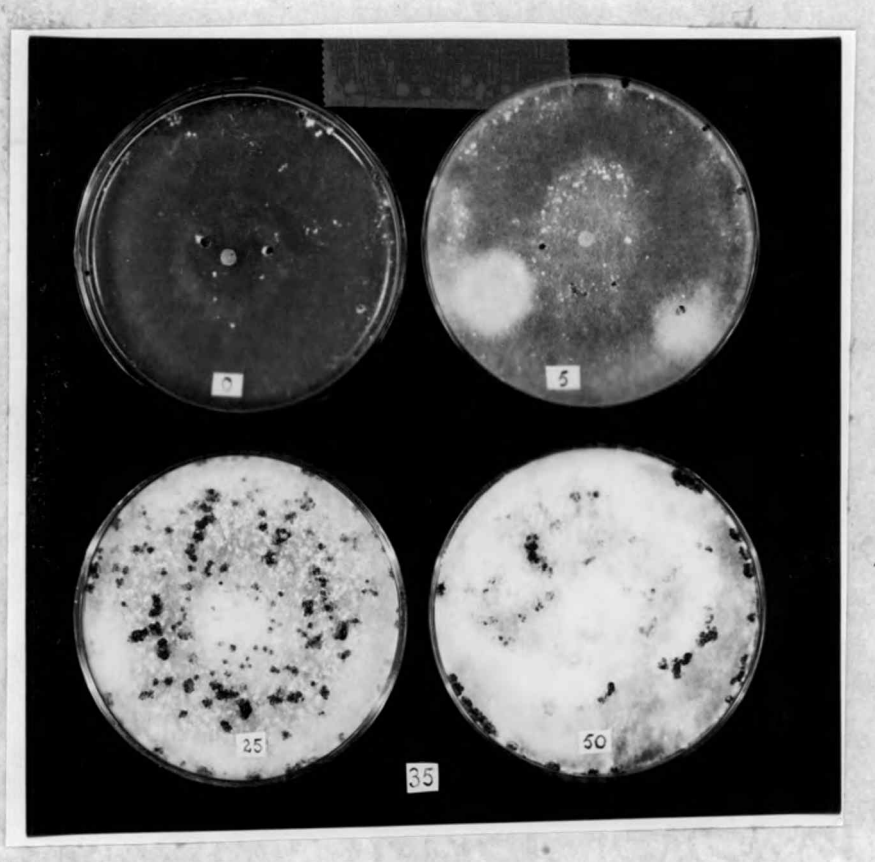

Fig. 25. Growth of S. sclerotiorum on potato agar media containing 0, 5, 25, and 50 grams sucrose per liter. (Isolate 35)

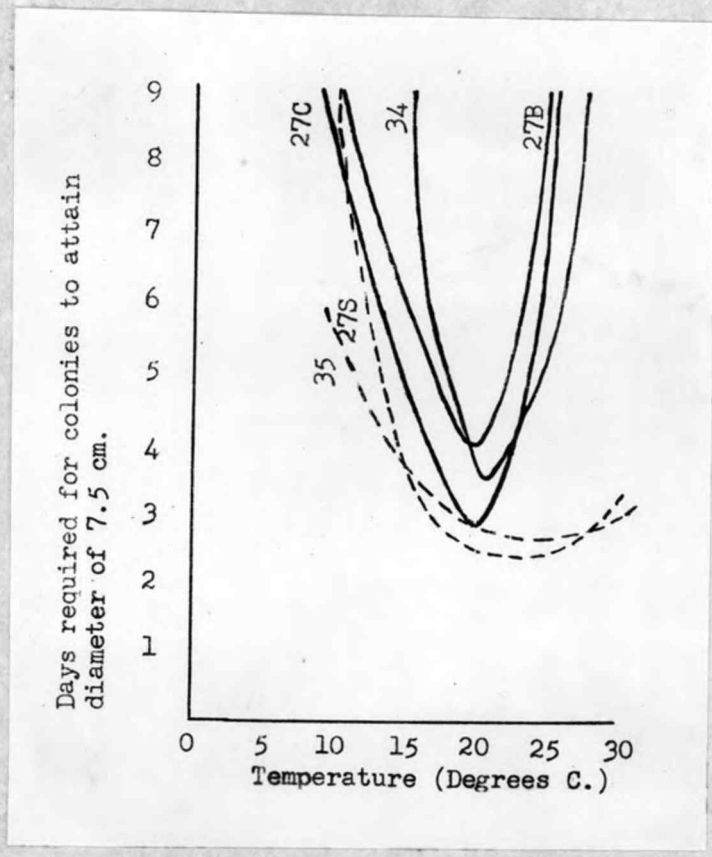

Fig. 26. Influence of temperature on growth of five isolates of S. sclerotiorum.