

STUDIES ON SCLEROTIUM-FORMING FUNGI

I. *Sclerotium cepivorum* Berk and *S. tuliparum* Klebahn

Part 1. Cultural Studies

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INTRODUCTION

A NUMBER of workers have attempted to correlate restriction of parasitic fungi to particular host plants with certain biochemical relationships, for example, with toxic properties of plant juice and with the capacity of fungi to secrete the pectinase enzyme. The primary object of the investigations in this series is to explore the position with regard to certain sclerotium-forming fungi. In the first instance the fungi selected, *Sclerotium cepivorum* and *Sclerotium tuliparum*, were chosen as being similar in habitat, in the type of plant part attacked, and to some extent at least in morphological features. The first step in the investigation was to determine how far these two fungi were restricted to their particular hosts, onion and tulip, respectively. That being established, the problem was then to try to explain the basis of the specialisation shown.

While the central idea was as outlined above, the investigation naturally followed a variety of lines, leading to a detailed cultural study of the two organisms concerned.

HISTORICAL

The literature dealing with the fungi *Sclerotium tuliparum* Klebahn and *Sclerotium cepivorum* Berk is rather extensive. In the following account each fungus will be treated separately, in the order named.

The bulb-rot of tulips was long known in Holland and Germany where it caused severe damage. It was reported from Holland at least as early as 1884 and Wakker⁴¹ appears to have been the first to describe the disease which he designated merely as the "tulpenziekte". He has given extraordinarily clear and accurate symptoms of the disease and the characters of the pathogen.

Ritzema Bos^{35,36} describes a disease which was very destructive at the time in certain parts of Holland and which from the symptoms given was obviously the gray bulb rot. However, there was a certain amount of

confusion in his account with the blight due to *Botrytis* ("fire"). He states that diseases of Iris, Hyacinth and Gladiolus due to the same *Sclerotium* also occur.

Klebahn^{20,21,22} in his earlier papers made the same confusion between two tulip diseases but later recognised them as distinct. He is responsible for the name *Sclerotium tuliparum*, and he showed that the same fungus attacks a large number of hosts, in particular *Iris hispanica*, Hyacinth, *Fritillaria imperiales*, Yellow Narcissus, *Scillia sibirica*, *Galanthus nivalis* and *Crocus vernes*.

Muller-Thurgau²⁷ and Lendner²³ reported the disease from Switzerland. The latter observed that the newly formed bulbils may also be attacked.

Whetzel and Arthur⁴⁶ have given a good historical resumé and have studied the taxonomic relationships of the fungus. They say that the first indications of the disease are the bare spots in the tulip beds in the spring. Nearly all bulbs in the soil contaminated area are usually so injured that they fail to grow. When affected bulbs do send up leaves, their growth is greatly retarded, and they soon die and wither away. Initial infection evidently occurs in the fall and early winter shortly after the bulbs are put out into the beds, or early in the spring. When diseased bulbs are dug up, they are found to be more or less rotted, the infection being usually at the tip, or nose, of the bulb. The healthy white tissue is turned to a grayish or a reddish gray colour. The soil clings to the exterior of the rotted parts and embedded in the soil or in the rotted bulbs are sclerotia. Experiments showed that the pathogen depends upon its sclerotia to tide over from one season to the next. Mycelium, which is readily produced from the sclerotia, spreads through the soil and attacks the suscept. The pathogen appears to be a low temperature parasite.

Whetzel and Arthur remark that certain distinctive features in the morphology (especially in sclerotial structure and mycelial characters) of the pathogen show taxonomic relations to *Rhizoctonia solani* and *Corticium stevensii*. They regard these as sufficient to warrant its transfer from the genus *Sclerotium* to *Rhizoctonia*.

Brooks⁵ gives an account of the disease of tulips and *Iris reticulata* caused by *Sclerotium* (*Rhizoctonia*) *tuliparum*. Tulips or other bulbous plants affected by this disease may be either completely destroyed below the soil level, or they put forth shoots which appear above the ground but are dwarfed and malformed and never flower. The sclerotia of the fungus cling to the neck of the bulb and the part of the shoot below soil level. The infection almost invariably proceeds from the soil by the formation of

strands of mycelium from sclerotia already therein. He found that Hyacinths, Daffodils, *Scilla sibirica*, *Fritillaria imperialis* and *Iris hispanica* are also attacked.

Dowson¹⁵ reports that *Sclerotium tuliparum* sometimes attack tulips and *Iris reticulata* in England causing gray bulb-rot. He gives the usual symptoms of the disease. Infection is entirely due to contaminated soil and takes place in early winter. The parasite spreads but slowly from one place to another and is probably introduced into a new locality by a few small sclerotia embedded between the scales of otherwise perfectly sound bulbs.

Van Beyma Thoe Kingma³⁸ notes the frequent association of *Sclerotium tuliparum* with *Penicillium corymbiferum* on tulip bulbs, both of these being active parasites. Weber⁴⁵ gives the symptoms, etiology and control of sclerotial disease of tulips caused by *Sclerotium tuliparum*. Kawamura¹⁹ states that tulip bulbs in Japan are liable to infection by *Sclerotium rolfsii* with which *Sclerotium tuliparum* is believed to be identical. Buddin^{11,12} reports that *S. tuliparum*, besides attacking tulips, also occurs though generally less severely on Iris, *Scilla*, Crocus, *Ixia*, *Fritillaria*, *Colchicum*, Hyacinth and Narcissus. Observations showed that bulbs planted with one-half to two-thirds of their surface protruding mostly remained healthy even in badly diseased soil. Steaming of soil completely eliminated the disease. In the control of the disease when a powder containing chloronitro-benzol was mixed with the surface soil 90 per cent. control was obtained whereas sprinkling the soil after planting the bulbs was unsatisfactory.

For the first time in 1938 *Sclerotium tuliparum* was recorded in England and Wales on Crocus.⁴⁹ Osterwalder and Camenzind³³ tested 0.5 per cent. formalin solution against *S. tuliparum* on tulips with satisfactory results.

Control measures, which rely chiefly on chemical disinfection of bulbs or soil, have been described by Caballero,¹³ Wakker,⁴¹ Ritzema Bos,³⁵ Klebahn,²² Whetzel and Arthur,⁴⁶ Dowson,¹⁵ Van Slogteren,⁴⁴ Buddin^{11,12} and Osterwalder and Camenzind.³³

White rot of *Allium* is a disease of widespread occurrence and was first recorded by Berkeley⁴ in 1841 in Great Britain who named it *Sclerotium cepivorum* Berk. Voglino³⁹ recorded severe attack of leeks in Italy by *S. cepivorum* but on the basis of his cultural study he renamed the fungus *Sphacelia allii*. Cotton and Owen¹⁴ reported that the white rot disease of onion bulbs caused considerable damage to onion crops in Great Britain. They found that shallots were markedly resistant and leeks did not appear to suffer. Caballero¹³ reports considerable damage in garlic fields in Spain

and regards *Sclerotium cepivorum* as the most destructive of the garlic parasites.

Walker in a series of papers^{42,43,44} described the white-rot of *Allium* caused by *S. cepivorum* in Europe and America and found that the disease occurred on onion, Welsh onion, leek, garlic and shallot. Leeks only suffered from the disease during cooler months. The fungus attacked the plants at any time during the growing period, provided external conditions were favourable. He observed that the disease thrives best at moderately cool temperatures and with moderate soil moisture. He found that within the temperature range favourable to growth of the plant, the fungus became less destructive as the rapidity of host-growth increased.

Dowson¹⁵ gives a brief description of *Sclerotium cepivorum* and its host. He also observed that warm and damp weather favours the disease which is spread by the planting of diseased seedlings or sets and is increased by repeatedly planting onions in the same ground. Natrass^{28,29} reports the occurrence of the disease from Egypt and Cyprus. He describes its symptoms and says that overwintering is due to sclerotia in the soil and that the fungus did not grow above 30° C. He further suggests that sets should only be planted from disease-free areas and cultivation of the different species of *Allium* should be discontinued for 8 to 10 years.

Du Plessis^{16, 17} gave a popular account of white mould on onion caused by *S. cepivorum* in South Africa and reports that disinfection of soil by formalin, heat or mercuric chloride are impracticable on a large scale though in laboratory test the sclerotia, which persist in soil and onion refuse for four or more years, succumbed to these treatments. He found that losses may increase from 20 to 30 per cent. when pink rot and bulb rot are accompanied by white mould caused by *S. cepivorum*.

Onion varieties showing marked resistance to white rot have been developed at Manchester University.⁴⁸ Matzulevitch²⁵ gives very brief description of the disease occurring in Russia. From one locality in Czechoslovakia an epidemic outbreak of *Sclerotium cepivorum* on garlic is reported.⁴⁷ Marchionatto²⁴ reports that *S. cepivorum* has been recognised since 1913 on onions and garlic in Argentina.

Bremer^{8,9} reports the presence of the disease in Germany and also recommends a well-regulated rotation in which onions are excluded from infested fields for at least 8 to 10 years. He has also given a popular note on the rots of stored onions in Germany by *S. cepivorum* and other organisms. Bremer and Nicolaisen¹⁰ have given symptoms, etiology and control of the disease. In New South Wales (Anon¹) white rot has been recorded once on garlic and thrice on onions.

Ogilvie and Hickman³⁰ found the disease widely distributed in Bristol province, mainly on white Lisbon spring onion. A soil application of a proprietary organic mercury compound in dust form, containing hydroxy-mercurichlorophenol with 20 per cent. organically combined mercury, before sowing, gave 56.8 and 17.9 per cent. infection at two localities respectively against average of 86.7 and 90.4 per cent. for the corresponding untreated control plots. Ogilvie, Croxall and Hickman³¹ report that early autumn sowing of onions were more severely affected by white rot than were late sowings. Ogilvie and Walton³² note that Up-to-Date, Rousham Park Hero, Improved Reading and White Spanish onions are moderately resistant, leeks being only occasionally attacked.

Brandão⁷ has given the symptoms and control of white rot affecting garlic in Brazil. The disease has also been recorded in Argentina by Hauman-Merck,¹⁸ in United States by Valleau,³⁷ in Holland by Van Poeteren³⁴ and in various parts of Australia.¹

Asthana² states that high potash manuring in England showed some decrease in the attack of *S. cepivorum* on onions but there was indication from plot experiments that liming reduced considerably more the incidence of the disease on onion seedlings. Moore²⁶ reports the fungus to be seed-borne and is usually transmitted by infected seedlings. Booer³ found that application of 4 per cent. mercurous chloride (calomel) dust to the seed drill at sowing time gave better results than seed treatment. One lb. of dust per 25 yd. of seed drill gave good disease control in bulb onions, and one lb. per 50 yd. may suffice for salad onions.

MATERIAL AND METHOD

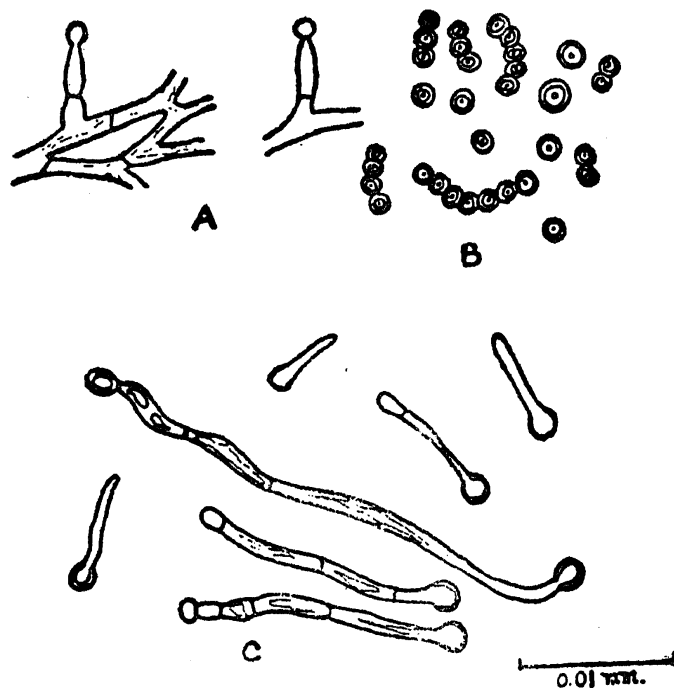
All through the experimental work pure cultures of *Sclerotium cepivorum* and *Sclerotium tuliparum* were used. Isolations were made from diseased onion and tulip bulbs and the purity of all the cultures was assured by the single hyphal tip method of Brown.⁶ All the cultures were maintained on potato-dextrose agar. Throughout the laboratory and field experiments "White Spring Lisbon" onion seeds, English onion bulbs and "Prince of Austria" tulips were used. The use of other varieties of onion and tulip will be mentioned at the appropriate places. Field and pot culture experiments were carried out at Slough and at Chelsæ Physic Garden respectively.

MORPHOLOGY

Mycelial and sclerotial growths of *Sclerotium cepivorum* and *Sclerotium tuliparum* were made on potato-dextrose agar plates. *S. cepivorum* forms a fluffy white mycelial growth, which is rather coarse with large cells. The

branches often anastomose and hold the hyphæ together in sheets or strands. The growth of the fungus is quite vigorous. After ten days of incubation at 20° C., sclerotia first appear as small white tufts of loosely intertwined branches. They become circular in form and within one to two days change into dull green from white. The outer layers darken and after a further two days the sclerotia appear as hard black bodies of 0·6 to 0·8 mm. diameter. Within two weeks of inoculation they are formed in large numbers over the whole surface of the plate (Plate III, Fig. 1). In section the sclerotia show a black cortex and inside a medulla of elongated closely packed hyphæ, in this respect resembling the sclerotium of *Sclerotinia sclerotiorum*.

The only spores produced by *S. cepivorum* were microconidia. Voglino³⁹ in the cultural study of the organism has described the production of sporodochia of hyaline conidiophores upon which were borne spherical, hyaline and catenulate conidia. Presumably the conidia of Voglino were the microconidia. It was observed that the microconidia were produced on certain media only, e.g., six week old plates of Brown's Starch agar and three to four week plates of tulip agar. These microconidia are formed on small conidiophores, 6–10 μ long, and are spherical, 2·5 μ –3·4 μ in diameter, with two walls. Very often they occur in chains, 2–8 sticking together, which arise by successive constrictions of the conidiophores. Attempts to germinate these microconidia almost uniformly failed. In a very few cases, about 14 spores altogether, short septate germ tubes appeared but these soon cut off a microconidium at the tip and ceased to grow. The germination was observed only on 20 per cent. Tulip juice (Text-Fig. 1).



TEXT-FIG. 1. Microconidia of *Sclerotium cepivorum*. A—Formation of microconidia. B—Microconidia. C—Germinating microconidia.

The hyphæ of *Sclerotium tuliparum* are long, slender and septate, the individual cells tending to be barrel-shaped when young. The mycelium grows rhizomorphically at a fairly uniform rate. It is mostly white, at first appressed and somewhat silky, later becoming more distinctly aerial towards the periphery. With age the colour of the mycelium gradually changes from white to clay. The medium soon becomes discoloured, taking on a distinctly reddish-brown tinge which deepens with age. The sclerotia first appear after 10 days as irregular, white, cottony masses on the surface of the culture, in a broad ring near the periphery of the colony (Plate III, Fig. 2). They soon turn to a pale yellow, deepening to reddish-brown, and becoming almost black when dry. They are generally globose to oblong bodies, 3–4 mm. in diameter but they vary a lot in their size and form with different media. In many cases several sclerotia are agglomerated into a large, irregular mass and in others the size varies from 1.5 to 8 mm. in diameter. In contrast to that of *S. cepivorum*, the surface of the sclerotium is dull, rough and irregular. In cross-section the medulla is seen compact and definite in form, having globose cells.

GROWTH

Nutrient Media.—Both the fungi under study were grown on 28 different natural and synthetic media. Petri-dishes of an equal depth were poured, inoculated at the centre with *S. cepivorum* and *S. tuliparum*, and incubated at 20° C. for two weeks. The results of the comparative study of the mycelial and sclerotial growths are given in Table I.

Table I shows that both fungi grow more or less freely on a large variety of media and that in general the richer the medium the greater the mycelial and sclerotial development. *S. cepivorum* growth is favoured by an acid medium whereas *S. tuliparum* prefers a neutral or alkaline one. A representative set of growth form is illustrated in Plate III, Figs. 1 to 4 and Plate IV, Figs. 5–6.

Throughout the series the mycelium of *S. cepivorum* is white and woolly while that of *S. tuliparum* is clay coloured and appressed. Characteristic features of *S. cepivorum* and *S. tuliparum* respectively are the peculiar sweet musky odour in all the cultures and the discolouring of the medium which takes a reddish-brown tinge with age.

To a certain extent the number of sclerotia produced on the same medium varies with the depth of pouring. Table II gives the number of sclerotia of *S. tuliparum* per plate as counted by the naked eye while in the case of *S. cepivorum* the numbers refer to a standard microscopic field as the sclerotia are minute.

TABLE I

Comparative study of the mycelial and sclerotial growths on a variety of nutrient media

Media	<i>S. cepivorum</i>		<i>S. tuliparum</i>	
	Mycelial Growth	Sclerotial Growth	Mycelial Growth	Sclerotial Growth
Potato extract ..	++	++	++	++
Potato mush ..	+++	+++	+++	++
Turnip agar (20%) ..	+++	+++	+++	+++
Tulip agar (20%) ..	++++	++++	++++	++++
Pea agar (20%) ..	+	+	+++	++++
Oat meal agar ..	+	+	++	+
Malt agar ..	+	+	+	+
Prune agar ..	++	++	+	+
Lettuce agar (20%) ..	++	++	++	++
Leek agar (20%) ..	++	+++	+	+
Onion agar (25%) ..	++	+++	++	++
do (20%) ..	+++	++++	++	++
do (15%) ..	++	+++	++	+
do (10%) ..	++	++	+	+
do (7.5%) ..	++	++	+	+
do (5%) ..	+	+	+	nil
do (2.5%) ..	+	+	+	nil
Brown's Starch agar ..	+++	+++	++	++
Asparagin glucose ..	+	+	++	+
Acid Asparagin glucose ..	+++	++	+	+
Glucose peptone ..	++	++	+	nil
Glucose nitrate ..	++	++	+	+
Glucose NH ₄ NO ₃ ..	++	++	++	+
Glucose NH ₄ tartrate ..	++	+	+	nil
Cane-sugar nitrate agar ..	++	+	+	+
Coon's agar ..	+	+	+	+
Chohn's nutrient agar ..	++	+	+	+
Richard's agar ..	+++	+++	+++	+++

[+ = Scanty or a few ; ++ = moderate ; +++ = good ; ++++ = abundant sclerotia and thick mycelial growth ; +++++ = luxuriant mycelial growth, large and abundant sclerotia.]

TABLE II

Amount of medium per standard plate	Number of sclerotia of <i>S. tuliparum</i> after 2 weeks		Number of sclerotia of <i>S. cepivorum</i> per microscopic field after 2 weeks	
	Mature	Immature	Mature	Immature
20 c.c. ..	136	19	2.5	1.2
40 c.c. ..	300	47	5.0	2.0

Here it will be seen that with the depth of the medium (Brown's Starch agar) the number of sclerotia is increased in both the fungi while increase

in size was only observed in case of *S. tuliparum*, no such effect being produced on the sclerotia of *S. cepivorum*.

Similarly the depth of plating influences the rate of linear growth. The figures in Table III give the increase in growth on Brown's Starch agar from the 4th to the 8th day. The effect is marked in the case of *S. tuliparum*, but negligible in the other.

TABLE III

Amount of medium per standard plate		<i>S. tuliparum</i>	<i>S. cepivorum</i>
10 c.c.	..	1.6 cm.	3.6 cm.
20 c.c.	..	2.3 cm.	3.8 cm.
40 c.c.	..	3.4 cm.	4.2 cm.

By referring to Table I it will be observed that media prepared from onion extracts were more favourable to the growth of *S. cepivorum* than to that of *S. tuliparum*. This point was investigated in greater detail.

The juice of onions and of tulips was squeezed out under a hand press, filtered through muslin and centrifuged to remove the coarse particles. Various dilutions of those extracts were then made and drops placed on cover slips in Ward-cells or on slides in moist petri-dishes. These petri-dishes contained a layer of agar to which 0.4 per cent. mercuric chloride was added and the slides were laid on this. By this method a moist atmosphere was maintained and the development of contaminating fungi and bacteria was reduced to a minimum. Each nutrient drop was inoculated with a hyphal tip of *S. cepivorum* or *S. tuliparum*. Table IV records the state of growth after 24 hours at 20° C.

TABLE IV

Mycelial growth in concentrations of crude onion juice

Fungus	Percentage of concentration of crude onion juice					
	10	20	40	60	80	100
<i>S. cepivorum</i> ..	++	+++	++++	+++	++	+
<i>S. tuliparum</i> ..	nil	nil	nil	nil	nil	nil

[+ = scanty ; ++ = moderate ; +++ = good and thick ; ++++ = luxuriant.]

The main point brought out is that *S. tuliparum* does not grow on any of the dilutions of crude onion extract (pH 6.0). The same was true after

60 hours. It is noteworthy that *S. cepivorum* is also considerably affected in its growth at the higher concentrations of the extract.

When however the onion extract was steamed for half an hour before use, the differential effect shown in Table IV was much lessened.

TABLE V
Mycelial growth in concentrations of steamed onion juice after 24 hours

Fungus	Percentage of concentration of steamed onion juice					
	10	20	40	60	80	100
<i>S. cepivorum</i> ..	++	+++	+++	+++	++++	++++
<i>S. tuliparum</i> ..	+++	++	++	++	+	+

(Notation as in Table IV.)

S. tuliparum grew quite well on this medium, especially at the lower concentrations, and the retarding effect of high concentration on the growth of *S. cepivorum* also disappeared.

That the effect of boiling was the dissipation of an inhibitory volatile substance was shown by studying the growth of the two fungi in turnip extract (20 per cent.) in the presence of crude unboiled or boiled onion extracts. These experiments were carried out in hanging drops, the turnip extract with mycelial tip being on the cover slip, and the bottom of the cell containing the onion extract. The comparative results are shown in Table VI.

TABLE VI
Mycelial growth after 24 hours on turnip extract in the presence of crude and boiled onion extracts

Fungus	Crude onion extract	Boiled onion extract
<i>S. cepivorum</i> ..	+	+++
<i>S. tuliparum</i> ..	nil	++

(Notation as in Table IV.)

The corresponding results with tulip juice, (a) unboiled (pH 5.8), (b) boiled (pH 6.2) are shown in Table VII (Notations as in Table IV). On boiling the juice a precipitate was formed which was removed and the growth of the two fungi was observed in the clear filtrate.

TABLE VII

Mycelial growth in different concentrations of tulip juice after 48 hours

Fungus	Percentage of concentration of tulip juice						
	10%	20%	40%	60%	80%	100%	
<i>S. cepivorum</i>	..	++	+	Unboiled juice nil	nil	nil	nil
<i>S. tuliparum</i>	..	++++	+++	+++	++	++	+
<i>S. cepivorum</i>	..	+	+	Boiled juice nil	nil	nil	nil
<i>S. tuliparum</i>	..	++	+++	+++	++	+	+

In contrast to the results with onion juice, boiling has no obvious effect in making tulip juice more suitable for the growth of *S. cepivorum*.

The conclusions arising from Tables IV–VII are that crude onion juice is highly inhibitory to the growth of *S. tuliparum* but that this inhibition is removed by boiling. On the other hand, tulip juice is relatively unfavourable for the growth of *S. cepivorum* and this effect is not removed by boiling.

The fact that *S. cepivorum* and *S. tuliparum* prefer the extracts of their particular host-plants is also shown, though not so markedly as in Tables IV–VII, by studying their growth rates on agar media compounded with these extracts. Comparative data after 48 hours are given in Table VIII.

TABLE VIII

Growth rates in cm. on different concentrations of onion and tulip agar media after 48 hours

Percentage of concentration of the medium	<i>S. tuliparum</i>		<i>S. cepivorum</i>	
	Onion	Tulip	Onion	Tulip
2.5	1.0	3.0	2.9	2.8
5.0	1.4	2.9	3.4	3.2
10.0	1.6	2.4	4.0	3.0
15.0	1.7	2.7	4.7	2.7
20.0	2.1	2.4	5.1	3.0
25.0	1.8	2.2	4.5	3.0
30.0	1.3	1.8	3.8	3.9
35.0	1.1	2.0	3.2	2.5

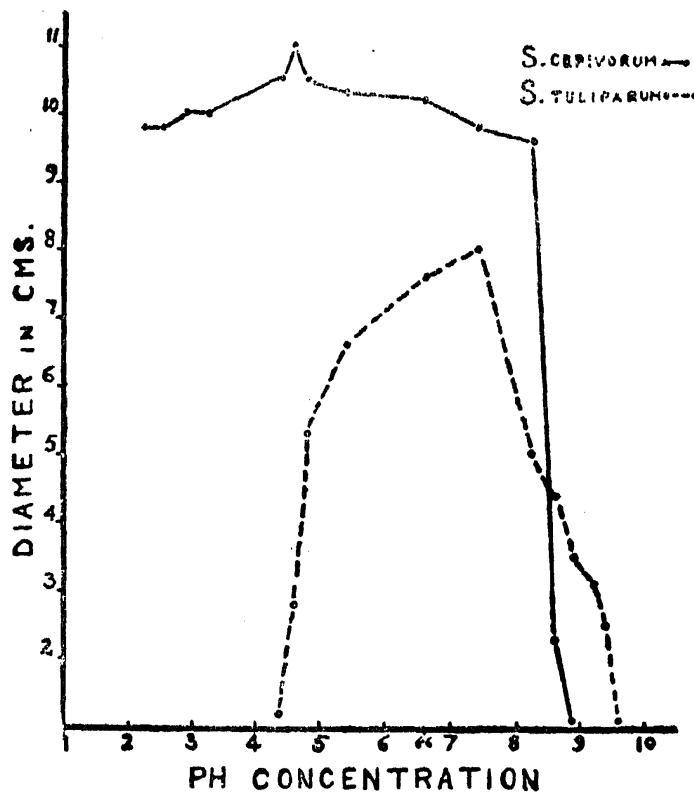
Here it is seen that each fungus grows somewhat more rapidly on an agar medium prepared from the juice of its own host plant.

Table IX, which gives the growth of *S. cepivorum* and *S. tuliparum* after 10 days at 20° C. on 20 per cent. extracts of the juice of Spanish and English onions, Spring onion leaves and Spring onion bulbs further illustrates the relatively slow growth of *S. tuliparum* on onion media.

TABLE IX
Growth of the colonies in cm. on different onion media

Fungus		Spanish onion	English onion	Spring onion leaves	Spring onion bulbs
<i>S. cepivorum</i>	..	8.0	7.8	6.5	5.4
<i>S. tuliparum</i>	..	2.8	2.7	2.3	1.9

H-ion concentration.—The two fungi were grown on plates of Brown's Starch agar (pH 6.6). The medium was adjusted to different pH values by adding malic acid or sodium bicarbonate, which were separately autoclaved and added just before pouring. Inoculations of the plates were made in the centre by a single sclerotium in the case of *S. tuliparum* and three in case

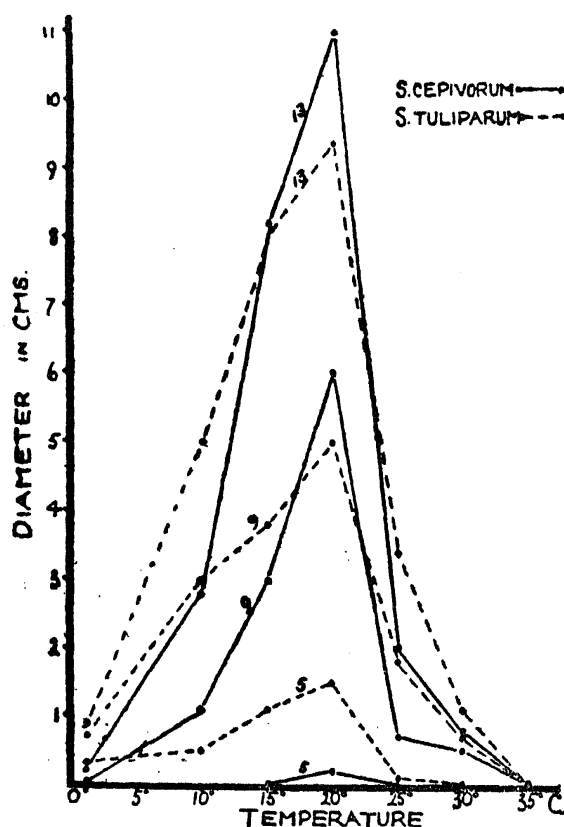


TEXT-FIG. 2. Illustrating growth of *S. cepivorum* and *S. tuliparum* at different H-ion concentrations.

of *S. cepivorum*. All the plates were incubated at 20° C. for 12 days when the colony growths were measured. The results are shown in Text-Fig. 2, in which each reading is an average of ten diameters from five different plates,

It will be seen from the figure that the growth of *S. cepivorum* is fairly constant over a wide range (2.2–8.2) of H-ion concentration. A higher pH than 8.2 causes the growth rate to fall considerably. On the other hand the curve for *S. tuliparum* shows a well-defined optimum near the neutral point. The range of *S. tuliparum* in the alkali side is greater than that of *S. cepivorum*, and conversely for the acid side.

Temperature.—The temperature response of the two fungi on potato-dextrose agar over the range 1°–35° C. is shown in Text-Fig. 3. Each point in the curves represents the average of ten measurements taken from five-fold series of plates on the 5th, 9th and 13th day.



TEXT-FIG. 3. Illustrating effect of Temperature on Growth of *S. cepivorum* and *S. tuliparum*.

The optimum temperature for both fungi is near 20° C., the minimum somewhere near zero and the maximum between 30° and 35° C. At the optimum temperature, *S. cepivorum*, though slower in beginning growth, rapidly out distances the other. At temperatures removed from the optimum, both above and below, *S. tuliparum* is the faster grower.

Light.—The effect of light factor was tested on cultures growing on 20 per cent. onion agar, Brown's Starch agar, 20 per cent. turnip agar and Richard's agar but no significant difference was observed either in growth rate or in the general appearance of the cultures.

SUMMARY

1. Brief accounts of the literature dealing with the fungi *Sclerotium cepivorum* Berk. and *Sclerotium tuliparum* Klebahn are given.

2. Morphology of the two fungi has been described. The only spores produced by *S. cepivorum* are microconidia on certain media only. Attempts to germinate these microconidia almost uniformly failed as only 14 spores altogether germinated on 20 per cent. tulip juice.

3. A comparative cultural study of *S. cepivorum* and *S. tuliparum* showed that while both grew well on a great variety of media, nevertheless there was in the case of each a certain amount of specific reaction to the juice of its own host plant. In particular, crude onion juice is markedly inhibitory to the growth of *S. tuliparum*. Boiling of the juice largely removes this effect. *S. cepivorum* does not grow well in tulip juice, boiled or unboiled.

4. *Sclerotium cepivorum* is favoured by an acid reaction of the culture medium while *Sclerotium tuliparum* by a neutral or slightly alkaline reaction.

5. The temperature range of growth for both fungi is approximately 1°–35° C., with an optimum near 20° C.

6. There is no significant difference by light factor either in growth rate or in the general appearance of the cultures of the two fungi.

REFERENCES

- | | |
|------------------------------------|---|
| 1. Anon | .. <i>Agric. Gaz. N.S.W.</i> , 1938, 49, 423–27. |
| 2. Asthana, R. P. | .. <i>Proc. Ind. Acad. Sci.</i> , 1945, 22, 168–74. |
| 3. Booer, J. R. | .. <i>Ann. appl. Biol.</i> , 1945, 32, 210–13. |
| 4. Berkeley, M. J. | .. <i>Ann. and Mag. Nat. Hist.</i> , 1841, 6, 355–65. |
| 5. Brooks, F. T. | .. <i>Gard. Chron.</i> , 1926, 79, 271–72. |
| 6. Brown, W. | .. <i>Ann. Bot.</i> , 1924, 38, 401–04. |
| 7. Brandão, J. S. | .. <i>Bol. Minist. Agric. Riode J.</i> , 1942, June 6. |
| 8. Bremer, H. | .. <i>Kranke Pflanze</i> , 1935, 12, 35–38. |
| 9. _____ | .. <i>Nachrichtenbl. Deutsch. Pflanzenschutzdienst.</i> , 1934, 14, 37–38. |
| 10. _____ & Nicolaisen, A. | .. <i>Biol. Reichsanst. fur. Land-und Forstw. Flugbl.</i> , 1934, 130, 4. |
| 11. Buddin, W. | .. <i>J. Minist. Agric.</i> , 1937, 44, 54–59. |
| 12. _____ | .. <i>Ibid.</i> , 1938, 44, 1158–59. |
| 13. Caballero, A. | .. <i>Bol. de la R. Soc. Esp. de Hist. Nat.</i> , 1922, 22, 210–12. |
| 14. Cotton, A. D. &
Owen, M. N. | .. <i>Jour. Board of Agric. of Great Britain</i> , 1919–20, 26,
1093–99. |
| 15. Dowson, W. J. | .. <i>Jour. Royal Horti. Soc.</i> , 1928, 53, 45–54. |
| 16. Du Plessis, S. J. | .. <i>Farming in South Africa</i> , 1934, 9, 70. |
| 17. _____ | .. <i>Ibid.</i> , 1932, 7, 112–14. |
| 18. Hauman-Merck, L. | .. <i>Zbl. Bakt.</i> , 1915, 43, 447. |
| 19. Kawamura, T. | .. <i>Ann. Phytopath. Soc. Japan</i> , 1936, 6, 1–14. |
| 20. Klebahn, H. | .. <i>Ztschr. Pflanzenkrankh.</i> , 1904, 14, 18–36. |
| 21. _____ | .. <i>Jahrb. Hamburg. Wiss. Anst.</i> 1905, 22, Beiheft 3. |
| 22. _____ | .. <i>Ibid.</i> , 1907, 24, Beiheft 3. |

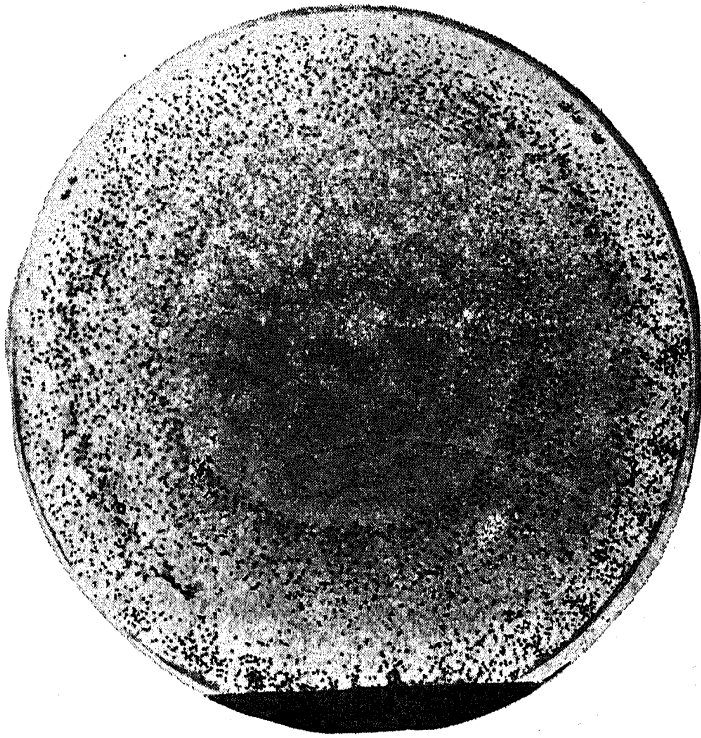


FIG. 1

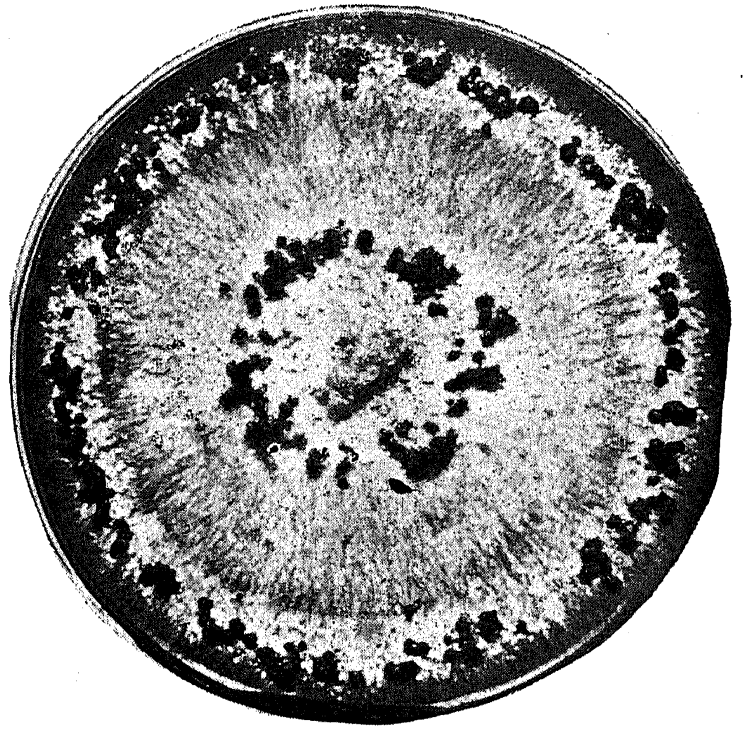


FIG. 2



FIG. 3



FIG. 4



FIG. 5

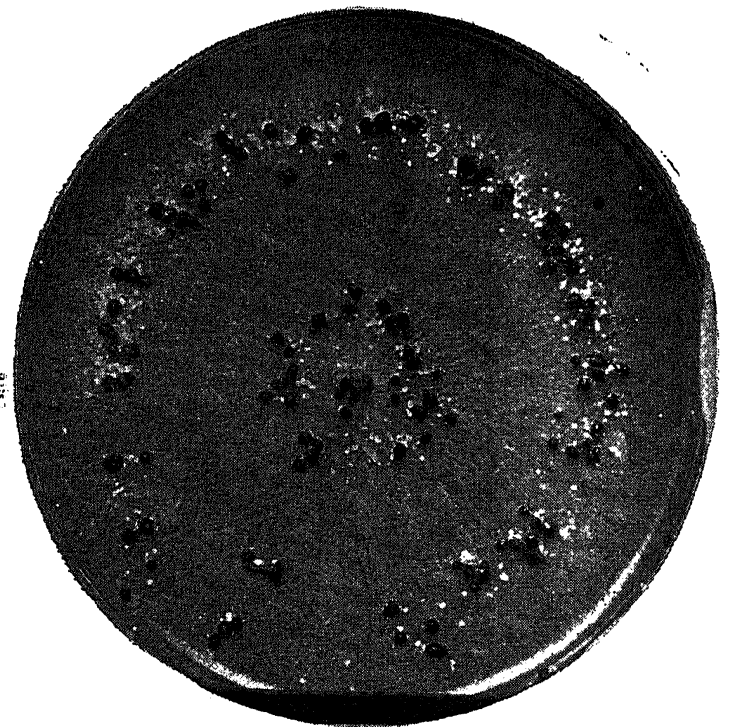


FIG. 6

23. Lendner, A. .. *Jour. hort. et. vit. Suisse.*, 1911, 7, 263-67.
24. Marchionatto, J. B. .. *Physis (Rev. Soc. Argentina Cien. Nat.)*, 1933, XI, 39, 301-05.
25. Matzulevitch, B. P. .. *Leningrad, Publ.*, 1932, 10, 24 page.
26. Moore, W. C. .. *Report on fungus, Bacterial and other diseases of crops in England and Wales for the years 1933-42*, London, H. M. Stationery Office, 1944.
27. Muller-Thurgau, H. .. *Landw. Jahrb. Schweiz.*, 1908, 22, 743-54. (Abstr. in *Ztschr. Pflanzen-Krank.*, 1910, 20, 50).
28. Nattrass, R. M. .. *Min. Agric. Egypt. Tech. and Sci. Service (Plant Protection Section)*, 1931, *Bull.* No. 107, 1-9.
29. ————— .. *Cyprus Agri. Journ.*, 1933, 28, 98-100.
30. Ogilvie, L. & .. *Rep. agric. hert. Res. Sta. Bristol (1937)*, 1938, 9,
Hickman, C. J. .. 96-109.
31. ————— Croxall, H. E. & .. *Ibid.* (1938), 1939, 10, 91-97.
Hickman, C. J.
32. ————— & Walton, C. L. .. *Worcs. Agric. Quart. Chron.*, 1941, 9, 57-65.
33. Osterwalder, A. & .. *Annu. agric. Suisse.*, 1940, 45, 389-464.
Camenzind, P.
34. Poeteren, N. van .. *Versl. Plziekt. Dienst. Wageningen*, 1928, No. 51, p. 20.
35. Ritzema Bos, J. .. *Ztschr. Pflanzenkrank.*, 1894, 4, 218-29.
36. ————— .. *Plantenzickten.*, 1903, 8, 177-202 (Abstr. in *Ztschr. Pflanzen-Krank.*, 1904, 14, 349-51).
37. Valleau, W. D. .. *Plant. Dis. Rep.*, 1925, 9, 46.
38. Van Beyma Thoe .. *Phytopath. Lab. "Willie Commelin Scholten" Baarn*
Kingma, F. H. (Holland), 1928, 12, 28-30.
39. Voglino, P. .. *Staz. Sper. Agr. Ital.*, 1903, 36, 89-106.
40. Van Slogteren, E. .. *Lab. Voor Bloembollenonderzoek te Lisse, Medded.*, 1931, 41, 3.
41. Wakker, J. H. .. *Algem. Vereening. Bloembollen-culture Haarlem. Verslag*,
1884, 1885, 22-26.
42. Walker, J. C. .. *Phytopath.*, 1924, 14, 315-22.
43. ————— .. *Ibid.*, 1926, 16, 697-710.
44. ————— .. *U. S. Dept. of Agric. Farmers' Bull.* 1060, 1931, 1-24.
45. Weber, A. .. Reprinted from *Aorbog for Gartneri* (1931), 1932.
46. Whetzel, H. H. & Arthur, J. .. *Cornell Univ. Agri. Expt. Sta. Memoir*, 89, 1924, 3-18.
47. ————— .. *Ochrane Rostlin*, 1930, 10, 1-2.
48. ————— .. *Rep. agr. Res. Inst. United Kingdom*, 1930-31, London.
H. M. Stationery Office, 1932.
49. ————— .. *Int. Bull. Pl. Prot.*, 1939, 13, 153-54.

EXPLANATION OF PLATES

FIGS. 1, 3 and 5—Plate cultures of *Sclerotium cepivorum* on potato-dextrose agar, tulip agar and onion agar respectively.

FIGS. 2, 4 and 6—Plate cultures of *Sclerotium tuliparum* on potato-dextrose agar, tulip agar and onion agar respectively.