A STUDY OF *SCLEROTIUM ROLFSII*, SACC.

by

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A STUDY OF SCLEROTIUM ROLFSII

Introduction

Sclerotium rolfsii Sacc. is a fungus that is prevalent throughout the southern states, and at times causes very serious losses to farm crops and ornamental plants. It causes a disease known as Sclerotium Wilt, Southern Wilt, or Southern Blight. This fungus has been reported on a large number of cultivated plants such as beans, cabbage, cantaloupes, cucumbers, clover, corn, lettuce, peanuts, peas, potatoes, soybeans, snapdragons, squashes, sugar beets, sweet potatoes, and tomatoes. It has just recently been reported on young apple trees in Virginia (13). There are not many instances of this fungus being reported on woody plants.

The Virginia Agricultural Experiment Station has received many complaints of Sclerotium Wilt on ornamental plants in the last few years, but has been unable to give any definite recommendations because no satisfactory method of control has been developed. The object of this investigation was to study the characteristics of the fungus and to discover, if possible, some method or methods of checking the disease when established.

Review of Literature

Sclerotium rolfsii was first reported on tomatoes in Florida in 1893 by P. H. Rolfs (9). At present it is classed with the Fungi Imperfecti although it is believed to be one of the higher Basidiomycetes. Rosen and Shaw (11) give four reasons to support its classification with the Basidiomycetes:

1. The main vegetative body is made of hyphae possessing clamp connections.
2. The cells of the vegetative body are binucleate.
3. The fungus readily utilizes cellulose for nutitive purposes, a feature possessed by many Basidiomycetes.
4. It produces under certain conditions an aborescent growth which is differentiated into main axes and side branches.

Recently Goto (2) (3) in Japan and Mundkur (7) in India have reported finding the perfect stage. Goto found that spore formation was correlated to a considerable extent with other growth characteristics such as mycelial characters, growth rate, color, etc. He used potato, onion, apricot, and carrot agars. The onion

agar was found to be the best medium for spore production.

Mundkur used a special medium containing onions, asparagine, and proteose peptone. The sexual stage appeared 40 to 45 days after inoculation at the optimum temperature (30° - 31° C.) for growth of the fungus. It agreed in size and morphology with the description of Corticium rolfsii by Curzi, and was tentatively placed in that species.

Rolfs (10) in 1913 reported that Sclerotium rolfsii could be controlled on tomatoes by spraying Eau celeste or an ammoniacal solution of copper carbonate on the soil about the stem of the plant.

Taubenhaus (12) in 1919 listed some of plants attacked by Sclerotium rolfsii. He described the growth of the fungus and its effect on the host. He stated that an enzyme, or enzymes, is secreted by the advancing mycelial strands, apparently to kill the host tissue.

In 1923 Edson and Shapavalov (1) studied Sclerotium rolfsii on Irish potatoes. The symptoms of the disease were described and field tests with artificially inoculated tubers were conducted. They state that the host tissue may be destroyed without hyphal penetration by digestive enzymes and that varietal strains of the fungus apparently exist.

Higgins (4) in 1927 states that the temperature and soil reaction are the most obvious factors limiting the geographical distribution of the fungus. The maximum and minimum temperatures at which the fungus will grow are 40° C. and 8° C., respectively, with 30° - 35° C. the optimum. The acid-alkali range for growth is pH 1.4 to pH 8.3 on beef-extract-peptone broth. Higgins concludes from his studies that the fungous hyphae secrete oxalic acid which kills the epidermal cells of the plant attacked and that the hyphae do not enter the host tissue until these cells have been killed.

Oserkowsky (8) in 1934 tested a number of organic compounds in the aqueous and gaseous state against Sclerotium rolfsii. Saturated vapors of trioxymethylene, benzene, toluene, xylene, nitrobemzene, and ortho, meta, and para dichlorobenzene killed the mycelium. Three days exposure at 25° - 26° C. to the saturated vapors of the following compounds killed the sclerotia: benzene, toluene, xylene, ethylbenzene, n-propylbenzene, chlorobenzene, ortho and meta dichlorobenzene, 1, 2, 4, trichlorobenzene, nitrobenzene, chloroform, carbon tetrachloride, bromopicrin, iodine, carbon bisulfide, and trioxymethylene.

Sclerotia were killed by immersion for one day in a 1:33 dilution of Merthiolate and in a 0.1 percent solution of hexylresorcinol, and by immersion for five days in a 0.1 percent solution of o-chlorophenol.

Leach and Davey (6) in 1935 reported that an aqueous solution of ammonia, 50 parts per million, killed mycelium in 24 hours in laboratory tests, and that in the same length of time 250 parts per million killed sclerotia. Equal concentrations of formaldehyde required a longer exposure to kill mycelium and sclerotia.

In field tests, anhydrous ammonia or ammonium sulphate, dissolved in irrigation water at approximately 300 parts ammonia per million increased the yield of disease-free sugar beets from 5 to 9 tons per acre over non-treated plots.

INVESTIGATION

Greenhouse Studies

Experiments were carried on in the greenhouse in an effort to find a method of checking the disease after the fungus had become established in the soil.

Procedure

The soil in the bed was sterilized with steam and then inoculated with sclerotia and mycelia. The soil was kept moist so as to give the fungus a chance to develop in the soil. When the fungus had become well established, the soil was treated, and afterwards planted to susceptible plants. The plants were carefully examined at frequent intervals for signs of the disease. Diseased plants were usually removed as soon as they were discovered. After about 70 to 80 days from the time of planting, the remaining plants were removed. The number of diseased and healthy plants was recorded.

Preparation of Cultures

The cultures used in inoculating the bed were grown on moist corn meal in 200 cc. flasks. The corn meal was moistened to a crumbly dough and then placed in the flasks and sterilized in the autoclave. In inoculating the flasks either a mycelia or sclerotia were used. The flasks were kept at room temperature until sclerotial formation had ceased. The mycelia grew luxuriantly and

sclerotia were formed profusely. The sclerotia were large, light brown in color, and often grew together in irregular masses.

Soil Inoculation

In preparation for inoculation the mycelial masses were cut up in small pieces and the sclerotial masses were broken up. A small quantity of sterile soil was then taken from the bed, sifted, and the mycelia and sclerotia mixed with it. This mixture was then spread evenly over the surface of the bed. In this way all parts of the bed were thoroughly inoculated. The soil was kept moist and after about two weeks was ready for treatment. At this time large numbers of sclerotia could be seen all over the surface of the bed (Figure 1).

Soil Treatment

The following materials were used in treating the soil: home-made formaldehyde dust, Form-O-Fume (formaldehyde dust), formaldehyde, pyroligneous acid, acetic acid, and Calo-Chlor.

Figure 1.--*Sclerotium rolfsii* in soil.

The home-made formaldehyde dust and Form-O-Fume were applied at the rate of one and a half ounces per square foot. The dust was thoroughly mixed with the soil to a depth of about four inches and the bed was then watered.

The formaldehyde dusts used contained six percent formaldehyde. The home-made dust was prepared as follows (14): A mixture of fine clay and wood charcoal was used as a carrier; 85 parts by weight of the carrier were used to 15 parts of commercial formalin (40 percent formaldehyde). Mixing was done in a small revolving barrel containing small stones.

The liquid formaldehyde was applied in a solution made up of one part commercial formalin to 50 parts water at the rate of two quarts per square foot.

The pyroligneous acid was applied in a solution made up of three parts acid to 100 parts water at the rate of two quarts per square foot.

The acetic acid was made up with water to a concentration of 1.19 percent and applied at the rate of two quarts per square foot.

The Calo-Chlor (a mixture of mercuric and mercurous chloride) was applied at two different rates; three and six ounces per 1000 square feet. The Calo-Chlor was thoroughly mixed with sterilized sifted soil, and the mix-

ture sprinkled evenly over the surface of the soil. This treatment was applied after the seed had been planted. It was the plan to have the chemicals to remain near the surface; therefore, the soil was not cultivated, and water was applied carefully so as not to wash the surface layer out of place.

Seed Treatment

Only one seed treatment was tried. The seeds were coated with Cuprocide before planting, and when the plants came up zinc oxide was applied to the soil at the rate of three-fourths ounce per square foot according to the method used by Horsfall (5) for controlling damping off.

Plants Used in the Tests

In the first test, snap beans, tomatoes, and corn were planted. A good stand was obtained with the beans and the tomatoes but rats and mice ate all but a few grains of the corn. The beans seemed sufficiently susceptible to the disease and after the first test only beans were used.

Symptoms of the Disease

In young beans the first sign of the disease is a permanent wilting. On examination the base of the stem is found to be infected and a white mycelial growth of S. rolfsii is usually quite noticeable on the surface of the lesion. Large sclerotia form later. Figure 2 shows two diseased plants.

With larger beans where the stem is quite strong the symptoms may be a little different. On some plants a small brown lesion may be noticed on the base of the stem before the plants show signs of wilting. This is followed by a white mycelial growth on the lesion and wilting of the foliage. Sometimes the plants wilt before the lesions are noticed. The infected tissue may shrink and turn dark brown or black with a rotting of the stem at the ground line similar to damping-off. In other cases the brown lesion may not be observed, the only signs of the disease being the wilting of the plant and the white mycelial growth at the base of the stem. When the stem and upper root of diseased plants is opened the white mycelium of the fungus can often be plainly seen inside.

The symptoms of the disease on young tomato plants are wilting and rotting of the stem. The small tomato

Figure 2.--Sclerotium rolfsii on young beans.

plant has a more tender stem than the bean, and at the point of infection the whole stem, with the exception of the tracheae, rots away.

First Test

In the first test the following materials were tried: home-made formaldehyde dust (1½ ounces per square foot), formaldehyde solution, 1:50 (2 quarts per square foot), pyroligneous acid solution, 3:100 (2 quarts per square foot), and acetic acid solution, 1.19 percent (2 quarts per square foot).

Results of First Test

The results of the first test are given in Table 1. The sections treated with pyroligneous acid and acetic acid had a high percentage of diseased plants and were eliminated from future tests. The formaldehyde solution showed up the best. The home-made formaldehyde dust was not effective.

Table 1.—Effect of Soil Treatments on Sclerotium rolfsii, Blacksburg, Virginia, 1934-1935.

Material	Host	Number of plants	Percent diseased
Formaldehyde dust (home-made) 1½ oz. per sq. ft.	Beans	38	50.0
	Tomatoes	50	98.0
Formaldehyde 1:50, 2 qts. per sq. ft.	Beans	25	36.0
	Tomatoes	25	0.0
Pyroligneous acid 3:100, 2 qts. per sq. ft.	Beans	66	95.5
	Tomatoes	91	35.2
Acetic acid, 1.19 percent, 2 qts. per sq. ft.	Beans	41	63.4
	Tomatoes	51	45.1
Check	Beans	36	89.4
	Tomatoes	51	13.7

Second, Third, and Fourth Tests

The following materials were tried in the second, third, and fourth tests: Form-O-Fume, 1½ ounces per square foot; formaldehyde solution, 1:50, 2 quarts per square foot; Cuprocide and zinc oxide, zinc oxide applied at the rate of ½ ounce per square foot; and Calo-Chlor, 3 and 6 ounces per 1000 square feet.

Results

The results of these tests are given in Tables 2, 3, and 4. The Form-O-Fume applied at twice the recommended strength for control of damping-off, was not effective. Planting immediately after treating the soil resulted in the germination of very few beans (Figure 3). In the third and fourth tests a good stand was obtained by planting three days after the soil had been treated with the dust. The formaldehyde solution, with the exception of the first and third tests, showed some promise as a control for the disease. The Cuprocide-zinc oxide seed treatment did not give satisfactory control.

Figure 3.—Formaldehyde and Formaldehyde Dust Treatments, showing effect of formaldehyde Dust on Germination.

Figure 4.—Cuprocide-zinc oxide Treatment and Check same experiment as Figure 3.

Figure 5.—Young Beans affected with Sclerotium rolfsii.

Table 2.--Effect of Soil and Seed Treatments on Sclerotium rolfsii, Blacksburg, Virginia, 1935.

Material	Host	Number of plants	Percent Diseased
Form-O-Fume, 1½ oz. per sq. ft.	Beans	31	32.3
Formaldehyde, 1:50, 2 qts. per sq. ft.	Beans	77	3.9
Cuprocide zinc oxide, ¾ oz. per sq. ft.	Beans	59	22.0
Calo-Chlor, 3 oz. per 1000 sq. ft.	Beans	59	1.7
Calo-Chlor, 6 oz. per 100 sq. ft.	Beans	57	24.6
Check	Beans	80	16.3

Table 3.—Effect of Soil and Seed Treatments on *Sclerotium rolfsii*, Blacksburg, Virginia, 1935.

Material	Host	Number of plants	Percent Diseased
Calo-Chlor, 3 oz. per 1000 sq. ft.	Beans	155	12.3
Calo-Chlor, 6 oz. per 1000 sq. ft.	Beans	165	1.8
Check (above)	Beans	71	76.1
Form-O-Fume, 1½ oz. per sq. ft.	Beans	148	30.4
Formaldehyde, 1:50, 2 qts. per sq. ft.	Beans	124	16.1
Cuprocide zinc oxide, ¾ oz. per sq. ft.	Beans	106	21.7
Check	Beans	125	55.2

Table 4.—Effect of Soil and Seed Treatments on *Sclerotium rolfsii*, Blacksburg, Virginia, 1935.

Material	Host	Number of plants	Percent diseased
Calo-Chlor, 3 oz. per 1000 sq. ft.	Beans	156	2.6
Calo-Chlor, 6 oz. per 1000 sq. ft.	Beans	158	3.2
Form-O-Fume, 1½ oz. per sq. ft.	Beans	145	48.3
Formaldehyde, 1:50, 2 qts. per sq. ft.	Beans	132	7.6
Cuprocide zinc oxide, ½ oz. per sq. ft.	Beans	143	20.3
Check	Beans	150	23.3

Discussion of Results

The Calo-Chlor soil treatment was the most promising method tried. It gave excellent control except in two cases. In the second test, at 6 ounces per 1000 square feet, 24.6 percent came down with the disease, and in the third test, at 3 ounces per 1000 square feet, 12.3 percent came down with the disease. In the other four cases the percentage of diseased plants was less than 3.5 percent. This material deserves thorough testing.

In the second and fourth tests the percentage of diseased plants in the checks was rather low although sclerotia were profuse throughout the entire bed each time. Several of the treated sections had a higher percentage of diseased plants than the checks.

After the tests were completed, samples of the soil from the three beds used in the tests were taken to Mr. Nelson Price who determined the pH of each. The three samples had a pH of 7.2, 7.4, and 7.8, respectively. This would indicate that the tests were not run under the most favorable conditions possible as laboratory experiments have shown that the fungus grows better in an acid medium rather than an alkaline medium (4). The writer believes that the effectiveness of various treatments could be more readily determined if an acid soil

were used. In the first test, the section treated with pyroligneous acid had a greater percentage of diseased plants than the check. A summary of the results of the four tests is given in Table 5.

LABORATORY STUDY

Characteristics of Mycelium

Potato dextrose was the standard agar used in growing the fungus in the laboratory. The cultures were incubated at room temperature and growth usually started in two or three days after inoculation with sclerotia or fresh mycelium.

After germination, if the reaction of the medium was favorable, the fungus grew rapidly, covering the bottom of the petri dish with a luxuriant, fluffy, white mat. The fungus grew outward from the point of inoculation in a circular, or radial, fashion. Figure 6 shows the characteristic radial growth. Figures 7, 8, and 9 are photographs of a Selerotium rolfsii culture and shown the sclerotial formation.

Sclerotial formation usually started about five days after germination. The immature sclerotia are first milky white, later becoming light brown. Mature sclerotia are dark brown in color, globular, and about the size of

Table 5.—Summary of Soil and Seed Treatments on *Sclerotium rolfsii* Showing Percent of Diseased Plants for Each Test, Blacksburg, Virginia, 1935.

Material	Tomatoes	Beans			
	First test	First test	Second test	Third test	Fourth test
Formaldehyde Dust* 1½ oz. per sq. ft.	98.0	50.0	32.3	30.4	48.3
Formaldehyde 1:50, 2 qts. per sq. ft.	0.0	36.0	3.9	16.1	7.6
Cuprocide zinc oxide, ¾ oz. per sq. ft.			22.0	21.7	20.3
Calo-Chlor, 3 oz. per 1000 sq. ft.			1.7	12.3	2.6
Calo-Chlor, 6 oz. per 1000 sq. ft.			24.6	1.8	3.2
Pyroligneous Acid 3:100, 2 qts. per sq. ft.	35.2	95.5			
Acetic Acid, 1.19%, 2 qts. per sq. ft.	45.1	63.4			
Check	13.7	69.4	16.3	55.2	23.3

* - Commercial formaldehyde dust (Form-O-Fume) was used after the first test. A home-made dust was used in the first test.

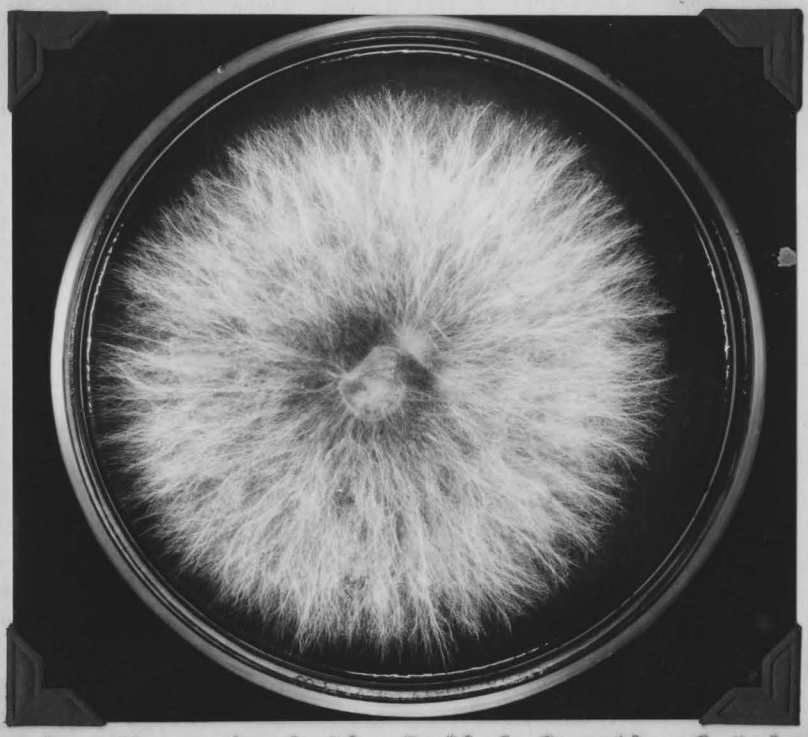

Figure 6.—Characteristic Radial Growth of Sclerotium rolfsii.

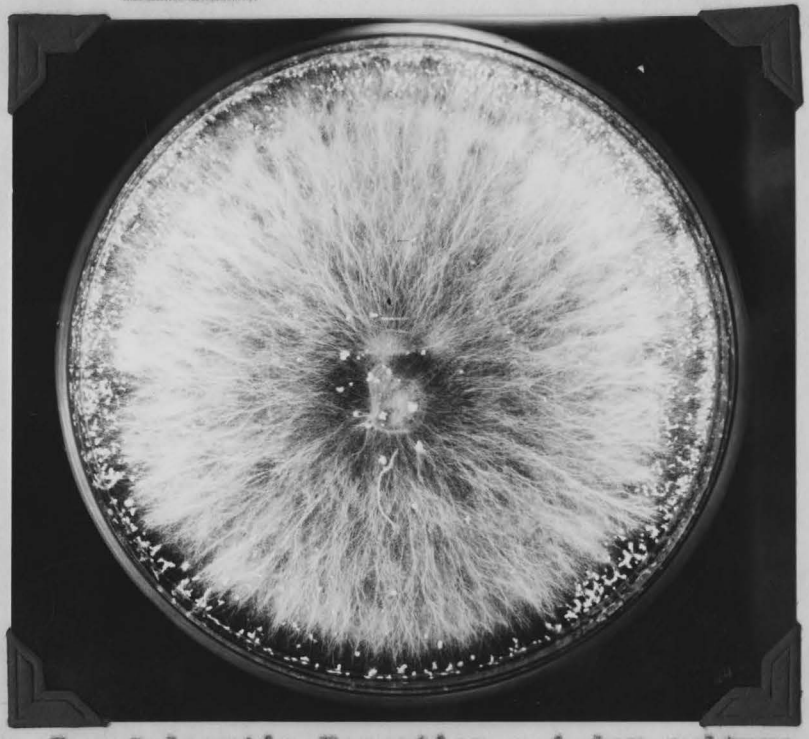

Figure 7.—Sclerotia Formation - 4-day culture.

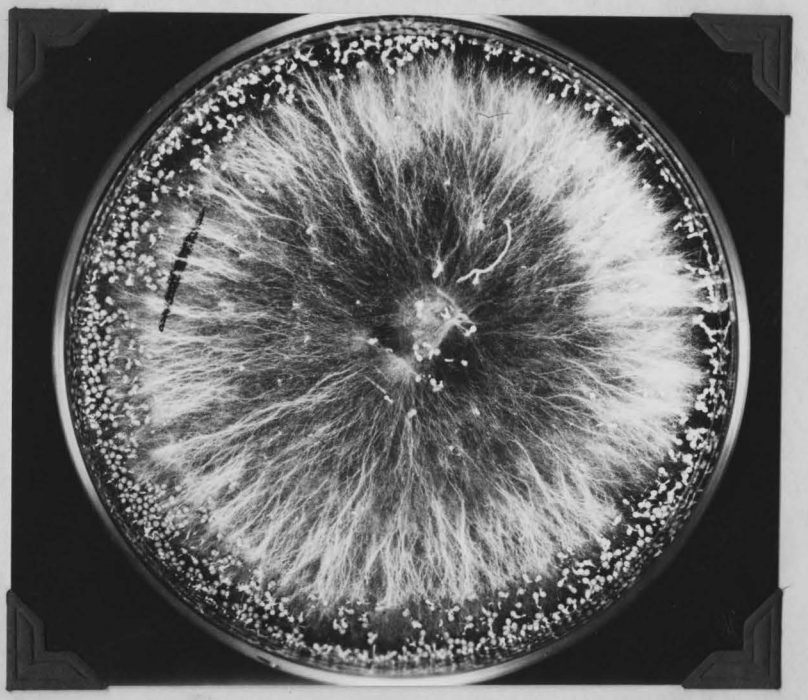

Figure 8.--Sclerotial Formation - 7-day culture.

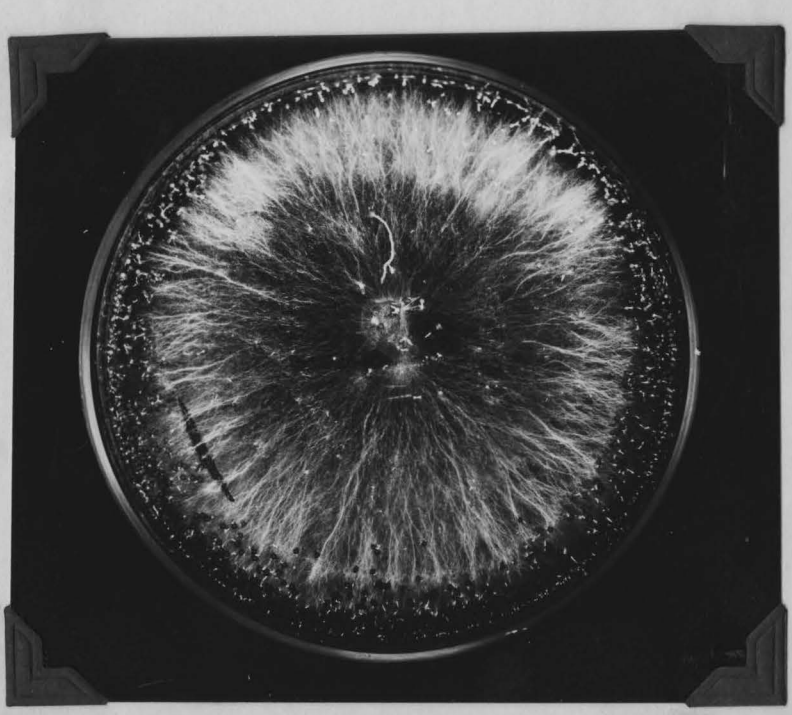

Figure 9.--Sclerotial Formation - 11-day culture.

a mustard seed. The size and shape, however, may vary somewhat according to media on which the fungus is grown. Drops of liquid exuded by the mycelium were observed in old cultures. These drops were either colorless or had a slight brownish tint.

The hyphae are septate, binocleate, and have clamp connections (11). Figure 10 is a drawing made with the aid of a camera lucida showing the clamp connections.

Growth of Fungus on Various Media

The fungus was grown on the following media: potato dextrose agar (used as the standard), potato corn meal agar, carrot juice agar, malt agar, Endo agar, and nutrient agar.

The potato dextrose, potato corn meal, carrot juice, and nutrient agars were prepared by the usual laboratory methods. The nutrient agar had a pH of about 7.2. The malt agar consisted of 1.5 percent malt and 2 percent agar. The Endo agar was prepared from commercial dehydrated Endo agar.

Three petri dishes of each media were inoculated with mycelium. The fungus grew readily on the potato dextrose, potato corn meal, carrot juice, and malt agars, and had

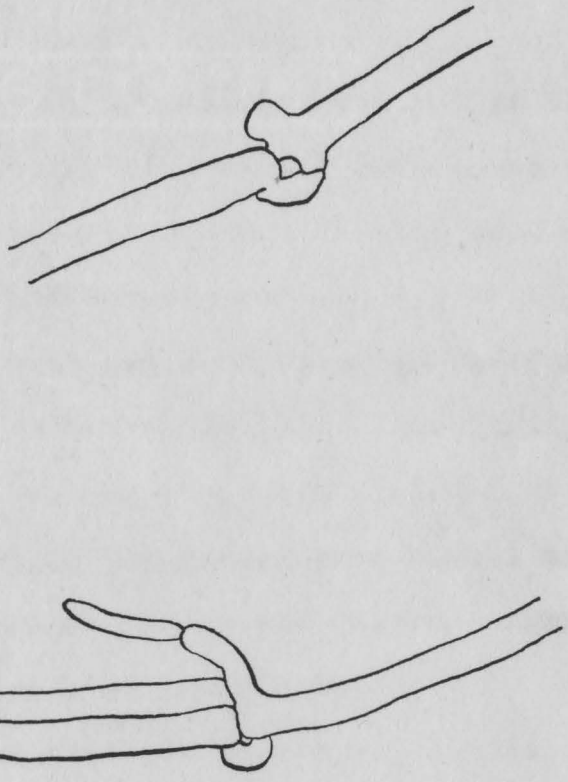

Figure 10.—Clamp connections on Mycelium of Sclerotium rolfsii (Camera Lucida).

covered the bottom of the petri dishes of all in six days. Growth was very slow on the Endo agar. The average diameter of the three cultures on Endo media 10 days after inoculation was 1 inch. There was no growth on the nutient agar.

The same type of growth was obtained on the potato dextrose, potato corn meal, carrot juice, and malt agars. A description of this growth has already been given. The potato dextrose, potato corn meal, and carrot juice agars seemed to be the best media for growing the fungus in the laboratory. The growth was a little more rapid and a little more luxuriant on the carrot juice agar than on the other two. While the fungus grew almost as rapidly on the malt agar as on the potato dextrose agar, the growth was not nearly as luxuriant.

The growth on the Endo agar was a little different from that on the other agars. It was rather luxuriant, but very slow, and was present in two concentric circles. The inner circle was raised and showed the typical radial growth. The outer circle was irregular around the margin, with the radial growth not so pronounced.

Sclerotia began to form six days after inoculation in the potato dextrose, potato corn meal, carrot juice, and malt cultures, and eight days after inoculation in the first of the Endo cultures. The sclerotia on the po-

tato dextrose, potato corn meal, and malt agars were about the same size. On the carrot juice medium they were slightly larger with a number of very large, very irregularly shaped sclerotia. The sclerotia on the Endo agar were slightly smaller than those on the potato dextrose agar. There were two large clusters of small sclerotia on the Endo agar. No clusters were formed on the other agars. The sclerotia were sometimes rugose and sometimes smooth, both kinds being found on the same medium.

Viability of Mature Sclerotia after Treatment with Various Sterilizing Agents

The procedure used in testing the viability of mature sclerotia after treatment with various sterilizing agents was as follows:

The requisite amount of distilled water was placed in 150 cubic centimeter flasks and sterilized in the autoclave, and the correct amount of chemicals to give the desired dilutions used in this test was added to the flasks aseptically after they were removed from the autoclave. The toxicity of these solutions to the sclerotia was tested by placing a number of sclerotia in the solution and then removing them at 3-hour intervals and testing

their viability on potato corn meal agar.

The materials tested were: ammonia, acetic acid, and formaldehyde. They were used at concentrations of 100, 250, and 500 parts per million.

The results of the three-hour treatments are combined and are given below:

Sterilizing Agent		Number of Sclerotia	Percentage of Sclerotia germinating
Ammonia,	100 ppm	30	0.0
	250 ppm	30	0.0
	500 ppm	30	0.0
Acetic acid,	100 ppm	30	80.0
	250 ppm	30	3.3
	500 ppm	30	0.0
Formaldehyde,	100 ppm	30	13.3
	250 ppm	30	0.0
	500 ppm	30	0.0
Check		30	96.7

Sclerotia were treated for six hours in formaldehyde, 100 ppm, and acetic acid, 100 and 250 ppm, with the following results:

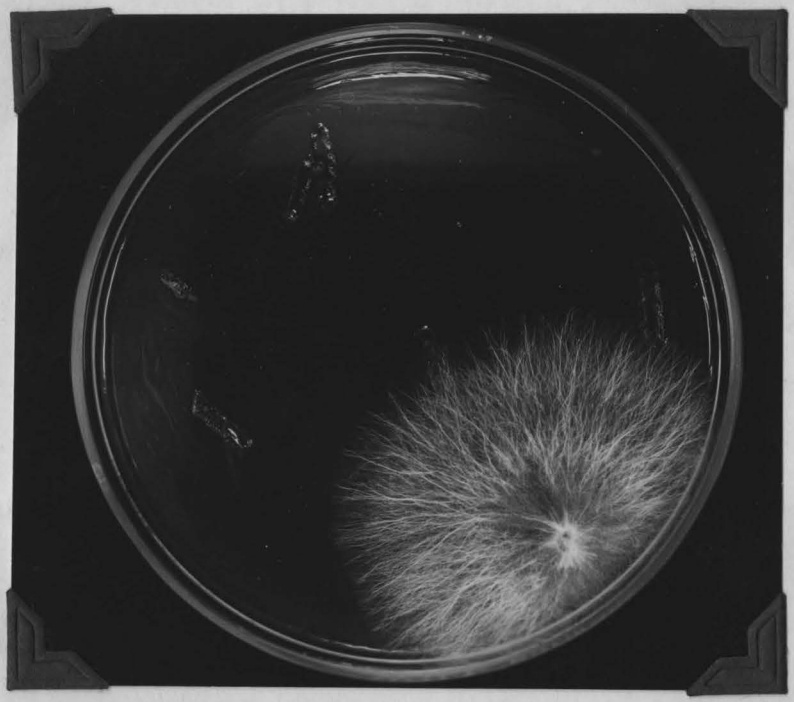

Figure 11.—Sclerotial Treatments - One sclerotia out of ten germinated after immersion for three hours in a solution containing 100 ppm formaldehyde.

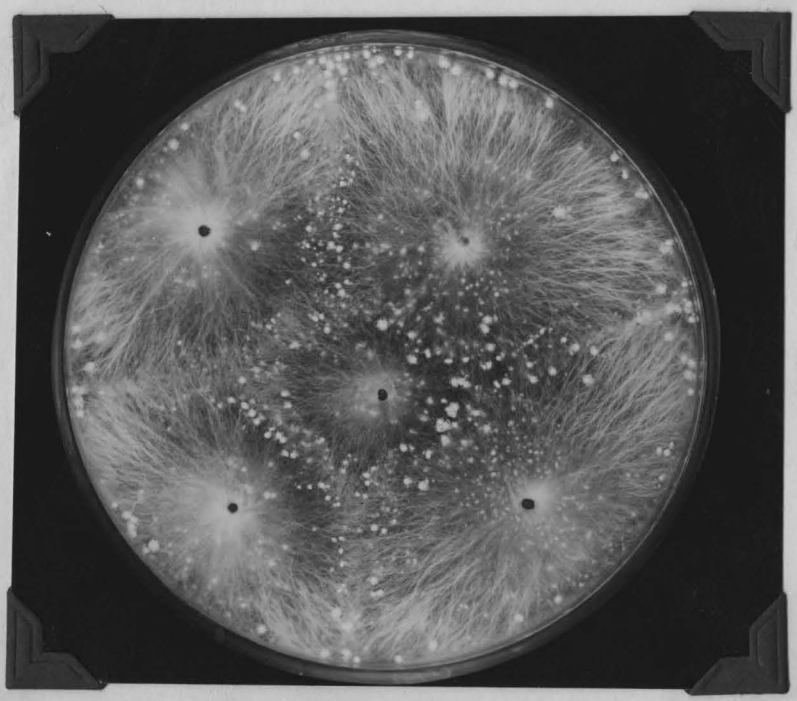

Figure 12.—Sclerotial Treatments - 100 percent germination after immersion for three hours in a solution containing 100 ppm acetic acid.

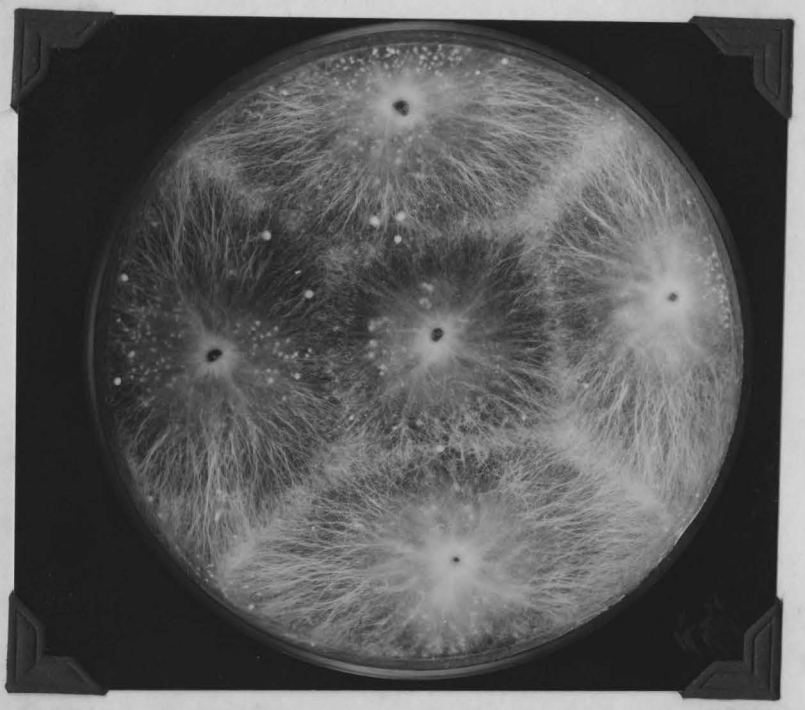

Figure 13.—Sclerotial Treatments - 100 percent germination of the untreated sclerotia.

Sterilizing Agent	Number of Sclerotia	Percentage of Sclerotia Germinating
Formaldehyde, 100 ppm	10	0.0
Acetic acid, 100 ppm	10	100.0
250 ppm	10	0.0
Check	10	100.0

Acid-Alkali Tolerance of Sclerotium Rolfsii

The fungus was grown on potato dextrose agar adjusted to different reactions. A Hellige Colorimeter with a range from pH 4.4 to pH 8.6 was used in making the adjustments. The procedure is given below:

The potato dextrose agar was placed in flasks in 100 cubic centimeter quantities and sterilized. The flasks were removed from the autoclave while hot and one flask was adjusted to each of the following reactions: pH 4.6, pH 5.0, pH 6.0, pH 6.8, and pH 8.2. The acid used in adjusting the media was .05 N. hydrochloric acid and the alkali .05 N. sodium hydroxide. After the adjustments were made, three petri dishes were poured from each flask. When the agar had hardened the dishes were inoculated with mycelium from a recent culture of S. rolfsii.

The fungus grew on media of all the reactions; however, it grew on only one of the dishes with a reaction

of pH 8.2. Growth started on all the plates about the same time, but the fungus grew more rapidly on the most acid plates and least rapidly on the alkaline plates. One day after growth had started the average diameters of the colonies in inches were: pH 4.6, 2.5; pH 5.0, 2.2; pH 6.0, 1.5; pH 6.8, 1.0; and pH 8.2, 0.4. Two days later the fungus had covered the bottom of all dishes except those having a pH of 6.8 and 8.2, and the colonies on these plates had an average diameter of 3 and 1.5 inches, respectively. The following day sclerotia had started to form in all dishes but those having a pH of 6.8 and 8.2.

The results of this test show that the fungus is markedly acid tolerant. This checks with the work of Higgins (4) and others.

SUMMARY

Soil infested with Sclerotium rolfsii was treated with pyroligneous acid, acetic acid, liquid formaldehyde, home mixed formaldehyde dust, Form-O-Fume, and Calo-Chlor. The liquid formaldehyde and the Calo-Chlor treatments gave partial control of the disease. In some instances the percentage of diseased plants in the plots treated

with pyroligneous acid and formaldehyde dust was greater than that in the untreated check plots.

Seeds treated with cuprocide were planted in soil infested with Sclerotium rolfsii. Zinc oxide was applied to the soil when the plants were about an inch high. This treatment did not control the fungus.

In the laboratory the fungus was grown on potato dextrose, potato corn meal, carrot juice, malt, Endo, and nutrient agars. The best growth was obtained on the carrot juice, potato corn meal, and potato dextrose agars. The fungus grew on potato dextrose agar adjusted to pH 4.6, pH 5.0, pH 6.0, pH 6.8, and pH 8.2. Growth was the most rapid at pH 4.6, and the slowest at pH 8.2. Only two out of three inoculations made with mycelium on agar with a reaction of pH 8.2 resulted in the growth of fungus.

Mature sclerotia were immersed for three and six hours in solutions of ammonia, formaldehyde, and acetic acid with the following results:

Sclerotia were killed by immersion for three hours in ammonia 100 p.p.m., acetic acid, 500 p. p. m., and formaldehyde 250 p.p.m. Immersion for six hours in formaldehyde, 100 p.p.m. and acetic acid 250 p.p.m. killed the sclerotia.

(1). Edson, H. A. and Shapovalov, M.

1923. Parasitism of Sclerotium Rolfsii on Irish Potatoes. Jour. Agr. Res. 23:41-46.

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