Life History Study

of

Sclerotinia fructigena (Persoon) Schroeter

A thesis submitted to the faculty of the Graduate School of the University of Minnesota by Robert Andrew Jehle in partial fulfillment of the requirements for the degree of Master of Science in Agriculture, May 17, 1910.

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Economic Importance

The fungus Sclerotinia fructigena (Persoon) Schroeter (Monilia fructigena Persoon) attacks many of the fruits of the Rose Family. Its most common hosts are the peach, plum, cherry and apricot but occasionally it attacks apples, quinces and pears.

The disease seems to be distributed in all countries where drupaceous fruits are grown. In Sylloge Fungorum[1] it is reported from Germany, France, Italy, Great Britain, Austria, Belgium, and the United States.[2]

In the United States it causes great losses to fruit growers in almost every state.

It was reported prevalent on Kelsey and other varieties of plums in Texas in 1894.[3] In western New York it is very common on twigs and fruits of peaches.[4] Chester reports it on blossoms, twigs and fruit of the peach in

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1) Saccardo - Sylloge Fungorum IV : 34 - 1882
2) Tubeuf-Smith - Diseases of Plants induced by Cryptogamic Parasites-p. 497 - 1897
3) Price, R.H. - Texas Bul. No. 32: 494 - Sept. 1894
4) Bailey, L.H. - New York Cornell Bul. No. 74:379-381 Oct. 1894

Delaware.[5] He also reports that in 1896 this fungus was responsible for the loss of one half of the Delaware peach crop.[6] According to Earle[7] it was very destructive to the flowers of the peach thruout the south in the spring of 1897. It is common on twigs and fruits of plums in Michigan, causing severe losses some years on peaches and attacking apricots and cherries.[8] McCarthy[9] reports the disease on peaches, plums and apples in North Carolina, but most serious on peaches and according to Massey[10] practically all of the unsprayed peaches were destroyed by brown rot. In Oregon in 1897 this disease caused a serious rotting of prunes.[11] Quaintance[12] reports the disease as causing heavy losses of the plum and peach crop in Georgia in 1900 and Kinney[13] states that in 1896 the disease almost destroyed the crop of plums in Rhode Island, just as they were ripening and that it was severe on sprayed as well as on unsprayed trees. Card and Sprague[14] report that in 1901 and 1902 the

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5) Chester,F.D. - Delaware Bul. No 19 - 1892
6) Chester,F.D. - Delaware Bul. No 34 : 3-13 - Jan. 1897
7) Earle,F. S. - Alabama College Sta. Bul.No 85: 439-44 Aug. 1897
8) Taft, L.R. - Michigan Bul. No 103 : 55,56 - Feb. 1894
9) McCarthy,G. - North Carolina Bul. No 76:14 - Mar. 1891
10)Massey,F.W. - North Carolina Bul. No 94:17 - Jan. 1894
11)Cordley,A.B.- Oregon Bul. No 57 : 15 - Apr. 1898
12)Quaintance,A.L.-Georgia Bul. No 50:237-269 - Oct. 1900
13)Kinney,L.F.-Rhode Island Report : 191,192 - 1896
14)Card,F.W. & Sprague,L.P.-Rhode Island Report : 246,247 - 1902

disease was very prevalent on the sand cherry in Rhode Island. The flowers, fruit and twigs were all said to have been infected. In Kentucky it caused a loss of 25% and in some cases 50% of the apples and plums which set in 1889.[15] In 1898, 25% of the peach and plum crop was destroyed by brown rot in Maryland.[16] Goff[17] reports the disease as attacking the fruit, flowers, leaves and even fruit spurs of the native plums in Wisconsin. In Connecticut it attacked the peach and double flowering almond.[18] Lodeman[19] states that in New York brown rot was less prevalent on unsprayed than on sprayed plums. Whittier[20] reported similar results on the Missouri Experiment Station orchard. It was prevalent in Ohio in 1896,[21] in California,[22] and caused heavy losses in the Delaware and Chesapeake peninsula, where about one sixth of the crop was destroyed, an estimated loss in peaches (at 50¢ per basket) of $400,000 for this dis-

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15) Garman, H. - Kentucky Report : 31-36 - 1889
16) Townsend, C.O.- Maryland Bul. No 71:115-127 - Feb.1901
17) Goff, E.S. - Wisconsin Bul. No 63 : 18,19 - Oct. 1897
18) Sturgis, W.C.- Connecticut State Report:261,262- 1898
19) Lodeman, E.G.-New York Cornell Bul.No 86: 70 - 76 - Mar. 1895
20) Whittier, J.C.- Missouri Bul.No 31 :4,8,17 - July 1895
21) Selby, A. D. - Ohio Bul. No 79 : 113,114 - Apr. 1897
22) Bioletti, F.T.-California Report :pt. 2,:330-333 - 1899-1901

trict alone.[23]

Brown rot has been very common in Minnesota during the years 1907, 1908 and 1909 on plums and cherries. Following heavy rain fall during the spring of these years the plums began to rot as soon as the petals had fallen. The trouble continued during the entire season and many fruits began to rot even after they had been picked and were ready to ship to the market. The writer estimates that in his orchard of one hundred and fifty plum and cherry trees about 1/3 of the crop was destroyed by brown rot in the years 1907, 1908 and 1909.

Historical Study.

Owing to the serious losses due to brown rot, the fungus which causes the disease has been made the subject of a large number of scientific studies. Thümen[24] and Hallier[25] were probably the first to regard the fungus as being of economic importance and as causing disease in fruits. Thümen considered it the most harmful and most widely dis-

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23) Smith, Erwin F. - Journ. of Myc. V - No 3 - 1889
24) Thumen-Oesterr-landw. Wochenbl. 1875, N. 41 : 484 - Fungi Pomicola, 1879 : 22
25) Hallier-Wiener Obst. u. Gart. Zeit : 117 - 1876

tributed of fruit diseases. The later European investigators did not consider the disease as serious as did these earlier writers. Thus Sorauer[26] gives the fungus only a very brief discussion and although Prillieux[27] discusses it at some length he does not consider it serious in Europe. Frank[28] also fails to mention its economic importance.

Peck[29] first described the disease in the United States in 1881. Arthur followed in 1884.[30] Since that time the disease has received the attention of a large number of investigators. Only the more important papers will be considered here.

Erwin F. Smith[28] found that in the spring time new tufts of conidia (chlamydospores) are produced on mummified fruits which cling to the trees thru the winter and that these conidia again produce the disease. In a later paper Smith[31] observed the twigs to be infected thru the blossoms at times of flowering and that spore infection of the twig, if it took place at all, was exceptional.

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26) Sorauer - Pflanzen-Krankheiten - 1886
27) Prillieux - Maladies des Plantes, 1897, Tom.II : 449
28) Frank - Krankheiten der Pflanzen, Bd. :360 - 1896
29) Peck, Dr. C.H. - New York State Museum,34th Report-1881
30) Arthur,J.C.-New York Exp.Sta.,4th Report : 254 - 1884
31) Smith,Erwin F.-Journ. of Myc. VII : 36

On examining the twigs in cross section it was found that the cambium and soft bast had almost disappeared and in their place were gum pockets filled with active mycelium.

Humphry[32] noticed that certain mummified fruits placed in the moist chamber especially in late autumn after vegetative activity had ceased, failed to produce the typical chains of conidia (chlamydospores). Upon examination they were found to produce large numbers of closely set flask shaped sterigmata, at the outer end of which were formed small globular spores about 3μ in diameter and each one containing a conspicuous oil globule. Seldom more than one spore was found attached to the sterigma but occasionally they were found united, which showed that they were produced in chains like Aspergillus spores. When these spores were sown in nutrient media they swelled to double their original size and produced a many-septate mycelium, which after a few days formed the typical chain chlamydospores of the fungus. A culture made in the fall of 1890 on prune gelatin continued to grow until most of the nourishment was exhausted. At the end of a month it was examined and a number of spores, undoubtedly of Sclerotinia fructigena were found germinating.

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32) Humphry, J. E. - Bot. Gaz. XVIII : 85 - 1893

Each spore gave rise to a single germ tube, or rarely two, which became divided by a few transverse septa. Usually one or more of the basal cells remained sterile while some of the others produced one or two flask shaped outgrowths. At the tip of each of these outgrowths a globose spore was produced 2.5 to 3 μ in diameter. Not more than one spore was seen attached to each sterigma.

"On another occasion a stout hypha in a culture of prune gelatin was observed to produce, at the ends of short branches and on slight outgrowths from its sides, long chains of globose spores. In this case the spore-chains, having been quite undisturbed, could be plainly recognized, though they readily fell into short sections or into their component spheres. In spite of the absence of sterigmata, it seems probable that this is essentially the same form as the previously described one, since the spores are produced in the same way, are of about the same size, and contain the characteristic oil globules."

In another culture when the spores were sown directly on nutrient gelatin, it was noticed in some cases that when found a short distance from the gelatin drop the hyphae grew slowly towards the drop. When they reached the drop they grew more rapidly but instead of remaining naked threads they produced on their sides oblong bodies so abundantly

that they completely filled the gelatin in some places. Their capacity for germination was not determined.

The conclusions drawn from these phenomoma were that the so-called spores of the ordinary characteristic mycelial chain were not true conidia in the sense in which Brefeld uses that term; that is they are not spores produced in fructificative fashion on special spore bearing threads. They must therefore be considered as slightly individualized portions of the mycelium with the physiological characteristics of spores. Though differing in detail of formation from the chain spores of Erisyphe they are morphologically similar to them and may be considered as chlamydospores of the most primitive type. The spores produced on the sterigmata, which are formed on distinct sporophores, may be regarded as conidia. Tulasne[33] found similar spores on Peziza (Sclerotinia) tuberosa and on Peziza balans. Brefeld[34] found them on his cultures of Sclerotinia tuberosa and Sclerotinia libertiana. DeBary[35] saw them in Sclerotinia fuckeliana. Zopf[36] saw similar struc-

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33) Tulasne - Selecta Fungorum Carpologia III t.221,865
34) Brefeld - Bot.Unters, uber Schimmelpilze
35) De Bary - Comp. Morph.&Biol. Fungi 1884,Eng.transl.243
36) Zopf - Nova acta - A. C. L. - C. U. C. XLII, No. 5

tures in Chaetomium, and Norman[37] in Sordaria. In all of these cases the general structure and mode of formation of the conidia is the same. Tulasne[33] found them sparingly produced on young germ tubes in Peziza (Sclerotinia) tuberosa, developed without sufficient nourishment. Brefeld[34] found them abundantly produced in nutrient media on Sclerotinia tuberosa and Sclerotinia libertiana, while De Bary[35] had similar results with Sclerotinia fuckeliana. The same writers found that on these species above mentioned, they were produced almost as abundantly on a resting mycelium as in a nutrient medium. Neither of these writers observed their germination and they were classed by De Bary[35] as "doubtful spermatia".

Woronin[38] found in his study of several species of Sclerotinia which attack leaves and fruits of the European Vaccinium, a chlamydosporic stage very closely resembling the one in Brown Rot. His form, however, developed cellulose plugs between the spores, which assist in their separation. Woronin states that although this is in no way represented in Brown Rot, yet similarity of growth and method of spore formation suggest relationship between the two.

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37) Norman - Beitr. z. Morph. u. Phys.
38) Woronin - Ber. d. D. Bot. Gesell III : lix, 1885
- Mem. Acad.Sci. St. Petersburg, No 6, 1888

The next important contribution was that of J.B.S. Norton.[39] He first noticed the apothecia developing on the ground of an old peach orchard in Charles County, Md., April 10th, 1902. Upon digging them up he found them to be attached to buried peaches. They were also found two days later in the young peach orchard of the Maryland Experiment Station. Here they were found on plums and peaches. They were also found in other orchards at College Park. On May 8th the dried up remains of many apothecia and a few fresh specimens were found in Washington County, and on May 9th a single one was found in Garrett County. Norton found apothecia where brown rot had been prevalent two years before, but failed to find it where it was prevalent only one year before. From this he concluded that like many well known fungi, including some species of Sclerotinia mentioned by Woronin, they do not produce certain stages of their life history for years, while in certain other years they are produced in abundance.

Norton found the earliest apothecia on April 1st and the last fresh one was seen April 27th. They last, accord-

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39) Trans. Acad. of Science of St. Louis - Vol XII, No 8
1902 - (J. B. S. Norton)

ing to Norton[40], only about two weeks and arise from the sclerotia on the surface of the mummified fruits beneath the soil, or occasionally on the surface in moist places. From one to twenty were observed growing from one fruit. They came from the lower side and appeared in a ring at the surface.

They are described by him as follows:- "The sinuous stipe is .5 - 3 cm. long, depending on the length it must grow to bring the spore-bearing surface above the ground. It is from.3-1.5 mm. thick. The lower part is covered with closely adherent particles of soil entangled in a mass of slender dark-colored septate rhizoids 1 mm. or less in length. These gradually disappear upward, the upper part of the stipe being smooth. The color is dark brown below running into the lighter brown of the disk above. The body of the stipe is made up of somewhat elongated cells in the center with shorter dark-colored cells on the outside, composing the cortex which continues around the outside of the disk and projects at the edges somewhat beyond the hymenium. The subhymenium is composed of elongated intertwined cells much like those in the center of the stipe."

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40) loc. cit. p. 93

"The stipe enlarges into the at first campanulate disk, slightly broader below the top. The disk widens out until cup-shaped and finally flat. Older ones often have the edges torn and recurved. The disc becomes again campanulate in drying up and is then darker colored. The expanded disk is 2-15 mm. wide, usually about 5-8 mm. In its later stages it is often whitish from a deposit of spores."

"The line of demarcation seen in sections between the hymenium and subhymenium is composed of a dense mass of small hyphae from which the asci and paraphyses arise. These are of the usual form of the Pezizaceae and of the genus. The paraphyses are very slender and slightly enlarged at the apex. The asci are 45 - 69μ long and 3 - 4μ wide, with 8 spores in the apical half."

"The spores are thrown off as the disk dries up. If fresh apothecia are kept for a time in a moist atmosphere and then exposed to a dryer or blown upon by currents of air or the breath, a distinct cloud of spores can be seen discharged which ascends several centimeters high and is wafted away by the slightest air current. When held in the sunlight the individual spores can apparently be seen with the naked eye. The discharge of spores can be several times repeated by additional drying, and after a rest

still others may be given off. Not nearly all the spores are ejaculated, as old dry individuals contain many asci intact and others with a few spores."

Of the germination of the ascospores he says:- "The spores germinate in water in 6 - 10 hours, sending out a small germ tube or promycelium after swelling to twice their diameter. Usually this tube does not attain in water more than 30 - 40μ in length. I have not observed the formation of sporidia such as those described by Woronin and Humphrey. The outer coat of the spore is probably thrown off in germination".

"In bouillon prune juice a much more vigorous growth takes place. A small branched mycelium is formed, which in drop cultures, which I have made, has rarely developed conidia. A few cells in some of the hyphae after long standing and slow growth took a form approximating that of Monilia spores and in some cases a few small sporidia (?) were developed."

Agar plate cultures were made by holding a sterile cover glass over the discharging asci and dropping it into tubes containing melted prune agar, and plating out in the usual manner. So many colonies were formed which were identical with typical brown rot cultures, that it seemed almost certain that they came from the discharged spores,

as it would have been almost impossible for a sufficient number of brown rot spores to be floating in the air to produce so many colonies.

Norton[41] made test inoculations of fruit and flowers and describes these tests as follows:- "Some of the fresh apothecia were placed in contact with peach flowers moistened and enclosed in paper sacks and some on mutilated buds. Probably owing to dry weather no results were obtained. With twigs of blooming peach and plum at the same time placed under sterile jars in-doors and similarly treated, in two or three days the peculiar browning of the petals seen in the Monilia blossom-blight appeared, followed by tufts of conidia. Although a great deal of blight appeared in the check cultures from Monilia spores already on the peach buds, several spots started in such places as to indicate undoubted infection from the ascospores. Finally, after a few weeks all the flowers blighted and became covered with dense masses of white hyphae often hanging down 2 - 3 cm. Although this may be some other mould, it appears to be connected with Sclerotinia."

"On May 12th, peach petals collected the day before in Garrett County, Md., were placed in a sterile petri

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41) - loc. cit. p. 95

dish. On some were placed sterilized drops of water, others were touched by wet, fresh apothecia, and others with Monilia conidia from peach flowers mentioned above. In three days those inoculated were blighted, turned brown, and later developed clusters ofconidia. Those simply wet remained perfectly fresh and white three weeks later."

"On May 19th through the kindness of Mr. A. M. Ferguson of the University of Texas, I received some well developed peaches and partially ripe plums from Texas. I inoculated some of these by piercing them with a needle which had just been touched to conidia developed in plate cultures from ascospores or which held a section containing germinating ascospores. These were placed under a bell jar with check cultures of fruits pierced with a sterile needle. Most of those inoculated developed Monilia conidia in three to five days, preceded by the characteristic 'brown rot'. The checks remained fresh for two weeks. In the damp atmosphere of the bell jar the hyphae on some of the inoculated fruits developed a dense white mass over the surface of the fruit 3 - 8 mm. thick, much like that found on the flowers kept under jars mentioned previously. This does not have the usual appearance of Monilia on rotted fruits, but since it is preceded

by the usual form of conidiophores in hemispherical clusters and the long white hyphae bear similar chains of spores, I do not believe that it is anything else."

Norton summarizes and concludes his paper as follows: "The apothecia of a Sclerotinia were found abundantly in April, 1902, developing from sclerotia in buried mummified peaches and plums in Maryland orchards. The ascospores developed coincident with the peach flowers. The ascospores were readily germinated in water, bouillon and prune juice and cultures made in agar and on sterilized dried apple and prune, from which conidia were developed not distinguishable from the Monilia associated with brown rot of fruits. Inoculations of peach and plum flowers and fruits from ascospores or from those conidia developed in two to four days brown rot and clusters of Monilia conidia. Monilia fructigena, Persoon is then properly referred to Sclerotinia fructigena (Persoon) Schroeter."

Longyear[42] of the Michigan Agricultural College succeeded in getting the perfect stage on an old mummy which he laid on the ground over winter, the cup containing the ascospores being produced the following spring.

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42) Longyear, B. O.- Mich. Exp. Sta., Spec. Bul. No 25, March 1904

Experiments in 1909-10 at St. Anthony Park.

Beerwort-gelatin plate cultures were made from mummified plums which were picked from the trees in Sept. 1909. Within three days the colonies had reached a diameter of about 2 cm. and the typical ash gray chlamydospores were being produced at the circumference of the colony. As the colonies grew many ash gray concentric rings radiating from the centre could be seen, and upon examination these rings were found to consist of chains of chlamydospores. From these colonies beerwort-gelatin test tube cultures were made and the fungus grew in the same way as it did in the plate cultures. Some of the chlamydospores were taken from these test tube cultures and mounted on sterilized cover glasses in sterilized 5% sugar solution. These cover glasses were placed on sterilized "Ward Cells". One of these was placed under a microscope equipped with a micrometer, and a single chlamydospore was observed at intervals of fifteen minutes for six hours and forty-five minutes. At the end of each fifteen minutes the growth of the germ tube during the preceding fifteen minutes was measured.

The results of this test were as follows:-
For four hours no germ tube was observed. In four hours, fifteen minutes the germ tube was 2 μ long; four hours,

thirty minutes, 4 μ ; four hours, forty-five minutes, 7.8 μ; five hours, 13.5 μ ; five hours, fifteen minutes, 19.9 μ ; five hours, thirty minutes, 28.6 μ ; five hours, forty-five minutes, 36.9 μ ; six hours, 40.8 μ ; six hours, fifteen minutes, 51.7 μ ; six hours, thirty minutes, 57.8 μ ; and in six hours, forty-five minutes, it was 61.5 μ long (Plate I).

This test shows the extremely rapid growth which may take place when chlamydospores are placed in proper conditions for germination and explains the rapidity of infection in some inoculation tests described below. It was found that in similar cultures in distilled water chlamydospores were produced in 64 hours (Plate VIII) and in beerwort in 71½ hours (Plate VII). It was found that the chlamydospores germinate and produce chlamydospores very easily in the following media: distilled water, sugar solution 2½%, 5% and 10%, manure decoction, soil infusion and beerwort (Plate II), showing that if the chlamydospores alight in nutrient solutions they may germinate and produce more chlamydospores without necessarily infecting the fruit.

It is apparent that the disease can be easily and rapidly spread, under favorable conditions by means of the chlamydospores, which are produced in concentric rings in

the cultures and in ash gray tufts on the surface of diseased fruits. These chlamydospores are produced in chains which vary in length, some of them growing to be several hundred μ long. These chains are usually branched in an indefinite manner (Plates V, VI, VII, VIII, X and XIV). The chlamydospores vary from lemon shape, to oval and cylindrical. The lemon shaped chlamydospores are the most common and the little prot uberance which always appears at the end or sometimes at both ends attached to the adjoining chlamydospore probably assists in separating them. The chlamydospores vary somewhat in size, the average being about 8 x 12μ. Under the microscope they appear colorless and filled with grandular protoplasm.

Humphrey[12] interpreted these chlamydospores as specialized resting cells of the mycelium, and this interpretation seems to be correct. The chlamydospores are not born on any special spore-bearing cells but are formed on any part of the mycelium. They are not necessarily produced at the end of a hypha but sometimes a few cells in the middle of a branch round up into chlamydospores and the cells on either side of them remain normal (Plate VIII). The chlamydospores are irregularly formed and those that are produced on the same chain vary in shape and size . Some normal cells are usually found between them in the chains.

The chlamydospores germinate easily and very frequently were found germinating without breaking away from the chain. Another common occurrence is the germination of several chlamydospores which are in the process of formation and have not yet had their cross walls formed (Plate V).

Cells formed anywhere on the mycelium may separate and each individual cell is then capable of germinating like a chlamydospore, the germ tube usually arising from one of the ends where the cell was formerly attached to the adjoining cells (Plate IX).

Chlamydospores, taken from a mummified plum which had been hanging on the tree all winter, were placed in distilled water in "Ward Cell" cultures and were found to germinate readily. Ash gray tufts of chlamydospores can be seen on mummified fruits after a few damp warm days in spring and these must have come from the resting mycelium in the fruit, or from conidia formed in fall which had germinated in the following spring. It is evident that the yearly cycle can be completed without the aid of any other kind of spore, but there are other kinds of spores produced and their function will be described below.

The vegetative hyphae that grow from these chlamydospores are many septate and very much branched. The

cells vary in length but they are usually from 20 to 30μ long, about 5μ in diameter, but are smaller when grown with little or no nutrition. Hyphal fusion is very common and it seems that it is possible for two hyphae to unite whenever they meet (Plates VI and XII). In this respect the chlamydospores act like any of the other cells and a germ tube may meet a chlamydospore and fuse with it.

Without taking into consideration any of the other kind of spores produced by this Sclerotinia, its cycle may be as follows: Ash gray tufts of chlamydospores are produced in chains on the diseased portion of the plant. These chlamydospores are carried to other parts of the same plant or to other plants by means of the wind or by rain drops. Here they infect the plant upon which they alight and within three days again produce chlamydospores and the same cycle is repeated.

In October 1909 some mummified plums from the plum trees were picked and from these a few which were not producing the typical chlamydospores were selected and placed under a bell jar, some were buried in moist earth and placed under another bell jar and others were put into a beaker of water under a bell jar. When examined about a month later the surface of all of these plums was found to

be covered with flask-shaped sterigmata 4 to 5μ long. At the end of these, a spherical conidium was found. Many of these spherical conidia were found detached and some were found still attached to one another in chains. They were 2 to 3μ in size and each contained an oil globule and were similar to those found by Humphry[32] on mummified fruits under similar conditions.

On Feb. 22, 1910 a "Ward Cell" culture was made from chlamydospores in distilled water. Some of the chlamydospores germinated and produced a mycelium upon which chlamydospores were formed in three days. On March 20th the culture was again examined and one chlamydospore was found which gave rise to several sterile cells. One of the branches of the mycelium gave rise to 5 flask-shaped cells and these bore at their ends chains of spherical conidia, each containing an oil globule, (Plate XI, fig.1). They were identical in shape, size, and method of formation with those found on the mummified plums in November. It was also observed where the chlamydospores had produced a large mycelium, that the protoplasm had withdrawn into a few cells and when the culture was examined two weeks later, these cells were also found to be producing the flask-shaped cells and chains of conidia in large numbers. The flask-shaped sterigmata were produced in

large groups at one place on the hypha. At different times in March and April similar cultures were made and five of these cultures were kept for one month. In every case similar results were obtained (Plate XI, figs. 2,3,4,5,and 6). In one of the cultures made March 4th, a chlamydospore was found on April 1st germinating directly into a flask-shaped cell which produced a chain of conidia (Plate XI, fig.3). In a culture made March 20th flask-shaped cells bearing chains of seventeen conidia were found on April 20th (Plate XI, fig. 5). These flask-shaped cells and conidia were exactly identical with those obtained by Humphrey[32] in his culture in 1892, except that in Humphrey's cultures only one spore was found at the end of each flask-shaped outgrowth while in these cultures they were invariably produced in chains. It is probable that either the chlamydospores which he observed were weak and unable to produce more than one conidium or that the chains were washed off of the sterigmata in handling. They are undoubtedly typically produced in chains.

On April 13th plums that had been hanging on the trees all winter were brought into the laboratory and examined. Their surface was found to contain only chlamydospores. They were placed under a bell jar on moist cotton

and on April 23rd both the chlamydospores and the groups of flask-shaped cells and chains of conidia were found growing on their surface in large numbers. These flask-shaped cells and conidia were identical in every respect with those produced in November and with those obtained in cultures during the winter. On April 25th other mummified fruits were brought in from the orchard and examined and found to contain only the common chlamydospores. They were placed under a bell jar on moist cotton and when examined on April 30th were found to be producing similar results.

When the sclerotinia stage was found on April 30th, cultures were made of the ascospores in beerwort-gelatin and when these were examined on May 2nd, they were found to be producing these same flask-shaped cells and conidia. There can be no doubt as to their origin as the hyphae upon which they were growing could be traced back to the ascospore, which had germinated while still in the ascus (Plate XII). Several attempts were made to germinate these conidia in beerwort, sugar solutions and distilled water but none of them were successful. Humphrey[33] had succeeded in germinating them in prune agar. None of them were found germinating in the distilled water in which they were produced and it is probable that they do not

germinate as easily as do the common chlamydospores. These conidia are probably normally produced in the spring and their function is probably that of aerial distribution.

On April 30th search was made for the ascus stage of brown rot. Two apothecia were found under some leaves directly under a plum tree and were dug up. One was found to be attached to a mummified plum. The other one was not attached but was probably broken off in digging. The stipe of the one which was attached was 2 cm. long and 1.5 mm. thick and the expanded disc was 1 cm. in diameter. In appearance it was essentially the same as the apothecia found and described by Norton[39](Plate XIII, fig. 1). The asci were found to be about 10 x 150 μ and to contain eight ascospores in the apical end, each about 8 x 14 μ in size. The paraphyses are about 6 x 150 μ and are a little broader at the apex than at the base (Plate XIII, fig. 2). Some of these asci were removed with a sterilized needle, into hanging drop cultures of beerwort and it was found that they germinate within four hours. Their rate of growth was as rapid as that of the common chain chlamydospores. On May 2nd when these cultures were again examined they were found to be producing both chlamydospores and the flask-shaped cells and chain conidia. One of them pro-

ducing the latter kind of spore could be traced back directly to the ascus in which the other seven spores could be seen (Plate XIII). These conidia were identical in every respect with those obtained in former cultures and with those obtained from mummified fruits. On May 6th similar cultures were made and when they were examined on May 9th an ascus was found which had been broken but still contained four ascospores, all of which had germinated. Two of them had formed a mycelium which produced, at the ends of several of its branches, chlamydospores (Plate XIV). These chlamydospores were identical in every respect with those obtained in former cultures and with those found on mummified plums. This evidence together with the evidence produced by Norton proves conclusively that Monilia fructigena (Persoon) is really Sclerotinia fructigena (Persoon) Schroeter.

The role of the ascus stage exclusive of its probable sexual nature is very apparent. When mummified fruits become buried, the disease could no longer be disseminated from them if it were not for this stage. But by means of the apothecia the fungus is again carried above the ground by the stipe and the ascospores are placed in a position where they can be easily disseminated by the wind. They germinate easily and produce both kinds of spores so that

the disease may be rapidly and easily spread by means of the chlamydospores or conidia.

Inoculation Experiments.

Two small plum trees were inoculated in the greenhouse with brown rot by taking chlamydospores from pure cultures and carefully placing them on the plum foliage without injuring it. Nine leaves on two different plants were inoculated in the same manner on March 21st. The plants were placed under a bell jar in the greenhouse and two check plants which had not been inoculated were placed on the bench outside of the bell jar. When the plants were examined on March 25th it was found that all of the inoculated leaves had taken on the brown color characteristic of brown rot and pieces had fallen out of the centre giving it much the appearance of shot-hole disease. On March 28th when the plants were again examined, tufts of ash gray chlamydospores could be seen. Upon examination these were found to be the typical chlamydospores of brown rot (Plant X). The notable difference between this and shot-hole lies in the fact that in shot-hole disease the destruction of the tissue stops at the larger veins. Brown rot spreads over the entire leaf and does not stop even at the largest veins. It causes a complete disin-

tegration of the tissue and the infected part withers and droops and finally falls. The disease does not stop at the leaves but runs down the petiole into the twigs.

It is probable that much of disease on plum leaves in Minnesota which is attributed to the shot-hole fungi (Cylindrosporium padi Karst, Septoria cerasina Peck and others) is really due to brown rot. On material collected at Excelsior, Minn., in 1909 for shot-hole fungus, chlamydospores were found on the leaves showing that both diseases were present. A superficial examination easily leads to a confusion of the two diseases.

On March 25th six plum leaves were inoculated the same as in the first experiment except that in one case the epidermis of the leaf was broken open. The check plums were not inoculated. The plants were examined 20 hours after the inoculation and a small circular brown spot could be seen on one of the leaves which had not been injured by the inoculating needle, thus showing the rapidity of infection under favorable conditions. On March 28th when the plants were again examined it was found that all of the leaves which had been inoculated, excepting one, were infected with the disease and the characteristic tufts of ash gray chlamydospores were being produced. These plants were examined again on April 29th and the

inoculated plants had then been almost destroyed by the disease while the check plants were still perfectly healthy (Plate XV).

On April 12th some apple twigs containing young leaves were brought into the laboratory and inoculated with brown rot in the same manner as described for the plum leaves. Five leaves were inoculated and the twigs were placed under a bell jar. On April 15th one of the inoculated leaves was found to be infected appearing the same as the diseased plum leaves and on April 18th typical ash gray chlamydospores were produced.

Inoculations on apple and plum leaves and blossoms were also made in the field but unfortunately two heavy frosts followed shortly after the inoculation and the leaves and blossoms were so seriously damaged that no results could be obtained from them. The disease has been reported on the leaves and twigs in several different states. (See above.)

Spore germination and Spraying solutions.

Hanging drop cultures were made of the chlamydospores in different strengths of bordeaux mixture, copper sulphate and of copper acetate. In each of these tests check cultures of the chlamydospores in distilled water were made.

Results of these tests were as follows:-

Bordeaux Mixture

First test, Feb. 18, 1910

Dist. water - Most of the chlamydospores germinated and in three days were producing chains of chlamydospores.

1 - 1 - 50 - One chlamydospore was found germinating. The germ tube grew to a length of about 70 μ and then ceased to grow.

2 - 2 - 50 - Two chlamydospores germinated and grew very short germ tubes and then ceased to grow.

3 - 3 - 50 - None of the chlamydospores germinated.

4 - 4 - 50 - None of the chlamydospores germinated.

5 - 5 - 50 - None of the chlamydospores germinated.

Second test - Feb. 22, 1910.

Dist. water - About 50% of the chlamydospores germinated and in four days the hyphae were producing chlamydospores.

1 - 1 - 50 - No chlamydospores germinated.

2 - 2 - 50 - No chlamydospores germinated.

3 - 3 - 50 - No chlamydospores germinated.

4 - 4 - 50 - No chlamydospores germinated.

5 - 5 - 50 - No chlamydospores germinated.

Third test - Feb. 25, 1910.

Dist. water - Most of the chlamydospores germinated and produced chlamydospores in three days.

1 - 1 - 50 - No chlamydospores germinated.

2 - 2 - 50 - No chlamydospores germinated.

3 - 3 - 50 - No chlamydospores germinated.

4 - 4 - 50 - No chlamydospores germinated.

5 - 5 - 50 - No chlamydospores germinated.

Copper sulphate solution

First test - March 2, 1910.

Dist. water - Most of the chlamydospores germinated and in three days produced chlamydospores.

1% - One chlamydospore germinated. Its germination tube grew to be about 50 μ long and then ceased growing.

2% - Four chlamydospores germinated but none of their germ tubes grew to be over 40 μ long.

3% - One chlamydospore germinated and grew a very short germ tube and then ceased growing.

4% - Results were the same as in 3%.

5% - No germinating chlamydospores were found.

Second test - March 4, 1910.

Dist. water - Most of the chlamydospores germinated and in four days chlamydospores were produced.

1% - Two chlamydospores germinated, grew a very short germ tube and ceased growing.

2% - One chlamydospore germinated, grew a very short germ tube and ceased growing.

3% - No germinating chlamydospores observed.

4% - No germinating chlamydospores observed.

5% - No germinating chlamydospores observed.

Third test - March 8, 1910.

Dist. water - Most of the chlamydospores germinated and

in four days chlamydospores were produced.

1% - No germinating chlamydospores observed.

2% - No germinating chlamydospores observed.

3% - No germinating chlamydospores observed.

4% - No germinating chlamydospores observed.

5% - No germinating chlamydospores observed.

Copper acetate solution

First test - March 21, 1910.

Dist. water - Most of the chlamydospores germinated and in five days chlamydospores were being produced.

2 oz. to 50 gal. water - One chlamydospore germinated and gave rise to a small germ tube and then ceased growing.

3 oz. to 50 gal. water - Similar results were obtained with two germinating chlamydospores.

4 oz. to 50 gal. water - No germinating chlamydospores observed.

5 oz. to 50 gal. water - No germinating chlamydospores observed.

6 oz. to 50 gal. water - No germinating chlamydospores observed.

Second test - March 22, 1910.

Dist. water - Most of the chlamydospores germinated and in three days produced chlamydospores.

2 oz. to 50 gal. water - No germinating chlamydospores observed.

3 oz. to 50 gal. water - No germinating chlamydospores observed.

4 oz. to 50 gal. water - No germinating chlamydospores observed.

5 oz. to 50 gal. water - No germinating chlamydospores observed.

6 oz. to 50 gal. water - No germinating chlamydospores observed.

Third test - March 23, 1910.

Dist. water - Most of the chlamydospores germinated, and in four days chlamydospores were produced.

2 oz. to 50 gal. water - One chlamydospore germinated and produced a short germ tube and then ceased growing.

3 oz. to 50 gal. water - Seven chlamydospores germinated and gave rise to a good growth of mycelium which became branched. The culture was kept until May 1st. No spores were produced on the hyphae.

4 oz. to 50 gal. water - No germinating chlamydospores observed.

5 oz. to 50 gal. water - One chlamydospore germinated producing a germ tube 15μ long and then ceased growing.

6 oz. to 50 gal. water - No germinating chlamydospores observed.

It may thus be seen that the common chlamydospores are easily killed by bordeaux mixture, copper sulphate and copper acetate solutions. The ascospores and conidia were not tested but it is probable that they too would be easily killed by those solutions. At the various Experiment Stations where experiments for the prevention of brown rot

have been carried on, the results have in many instances been quite contradictory. In some tests spraying with bordeaux gave very good results while in others no beneficial results could be observed. In Maryland,[43] bordeaux mixture (3-6-50) is recommended for first sprayings of peaches and ammoniacal copper carbonate for later sprayings. Five sprayings are recommended. Intervals of two or three weeks should be allowed between sprayings.

In Connecticut where peaches and almonds had twigs, flowers and fruit seriously injured by brown rot, bordeaux mixture (6-4-50) caused defoliation of peaches, Japanese plums and apricots.[44]

In Delaware, bordeaux (6-6-50) did not damage peach foliage much and checked brown rot. Copper acetate solution (8 oz. to 45 gal. of water) did not discolor fruit.[45]

In Ohio experiments were tried on plums by using early spraying with copper sulphate and later sprayings with bordeaux. Results were only partially successful.[46]

In Georgia experiments showed that spraying of the trees with bordeaux helped the keeping qualities of peaches.

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43) Maryland bul. No 71
44) Connecticut Report for 1898, pp.261-262
45) Delaware Exp. Sta. Report No 29, Oct. 1895
46) Ohio bul. Vol. II, No 7, p. 188

It was also noticed that different varieties of peaches and plums vary in resistance to the disease. It was found that bordeaux (3-6-50) did not injure peach foliage.[47]

Alwood of Virginia recommends a thorough washing with concentrated lye (8 cans to 50 gals. water) or copper sulphate (2 pounds in 50 gals. water) just before the buds swell, followed by the usual bordeaux sprayings.[48]

Experiments with bordeaux mixture (different strengths) sulphuric acid (1 part in 1000 parts water), lime-sulphur wash (1½ pounds sulphur, 3 pounds lime, to 50 gals. water, boiled 45 minutes), and self-boiled lime sulphur, were tried by the U. S. Department of Agriculture on peaches for brown rot and other diseases. These experiments were conducted in the orchard of Hitt Brothers, at Koshkonong, Mo., in cooperation with the Missouri Fruit Experiment Station. The bordeaux mixture, sulphuric acid and lime-sulphur seriously injured the peach foliage while the self-boiled lime-sulphur did not injure either the foliage or fruit and proved to be an efficient fungicide for brown rot and other diseases.[49]

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47) Georgia bul. No 50
48) Virginia bul. No 67
49) Galloway,B.T. - U.S.Dept. of Agr., Bureau Plant Industry,Circular No 1,Apr.18,1908.

The following sprayings would seem to be advisable in the treatment of Minnesota plums and cherries: All mummified and diseased fruits should be removed from the orchard and burned. If the mummified plums are plowed under, the land should be plowed very deep; because when the mummies are near the surface the apothecia may be produced and the ascospores will give an early start to the disease. While the trees are still dormant they should be sprayed with a 2% copper sulphate solution, or with lime-sulphur wash, to kill any chlamydospores that may be clinging to the bark. The trees should be sprayed with 4-5-50 bordeaux containing 3 pounds of arsenate of lead just as the buds swell, just after the petals fall, when the plums are the size of peas, and ten days later. Where the disease continues throughout the summer, sprayings with bordeaux should continue at 10 day intervals until the fruit commences to color. If spraying is still necessary after that, copper acetate solution (6 oz. to 50 gal. water) or ammoniacal copper carbonate (copper carbonate 5 oz., ammonia 3 pints, water 45 gals.) should be substituted for bordeaux to avoid discoloration of the fruit. Varieties which are much subject to the disease should not be planted. Self-boiled lime-sulphur may prove to be as

beneficial as bordeaux mixture and there does not seem to be as much danger of burning the foliage.

Summary and Important Results.

Sclerotinia fructigena (Persoon) Schroeter is distributed in almost every country where drupaceous fruits are grown and causes serious losses to fruit growers in almost every state in the United States.

Chlamydospores germinate very easily and rapidly in many nutrient solutions and in distilled water. In three to five days the mycelium which grows from them gives rise to chains of chlamydospores.

THE TRUE CONIDIA ARE PRODUCED IN DISTILLED WATER CULTURES FROM RESTING MYCELIUM OR DIRECTLY FROM GERMINATING CHLAMYDOSPORES AFTER ONE MONTH AND THEY ARE PRODUCED ON MUMMIFIED FRUITS IN FALL AND SPRING. THEY ARE TYPICALLY FORMED IN CHAINS AT THE END OF FLASK-SHAPED STERIGMATA.

Fresh apothecia were found in the Experiment Station orchard on April 30th and asci were taken from them and placed in "Ward Cell" cultures.

THE ASCOSPORES GERMINATE READILY AND IN THREE DAYS THE HYPHAE WHICH GROW FROM THEM PRODUCE CONIDIA AND CHLAMYDOSPORES.

THE SEQUENCE OF ALL THREE SPORE FORMS AS SHOWN HERE

FOR THE FIRST TIME IN CULTURES PROVE CONCLUSIVELY THAT ALL ARE FORMS OF THE ONE FUNGUS, SCLEROTINIA FRUCTIGENA (PERS) SCHROETER.

This substantiates the conclusion arrived at by Norton [50] that "Monilia fructigena, Persoon, is properly referred to Sclerotinia fructigena (Persoon) Schroeter."

INOCULATION TESTS SHOW THAT THE DISEASE ATTACKS PLUM AND APPLE FOLIAGE CAUSING THEM TO APPEAR MUCH LIKE LEAVES ATTACKED BY SHOT HOLE FUNGI AND SINCE CHLAMYDOSPORES OF BROWN ROT WERE FOUND ON MATERIAL COLLECTED FOR SHOT HOLE AND WHERE SHOT HOLE WAS PRESENT, IT IS PROBABLE THAT SOME OF THE TROUBLE WHICH IS COMMONLY ATTRIBUTED TO SHOT HOLE FUNGI IS CAUSED BY SCLEROTINIA FRUCTIGENA (PERSOON) SCHROETER.

Chlamydospores are easily killed in weak solutions of bordeaux mixture, copper acetate, and copper sulphate.

The life history of brown rot in the light of the results obtained in these experiments in Minnesota may be described as follows:-

Throughout the spring and summer the mycelium lives in the leaves, twigs, blossoms and fruits and chlamydos-

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50) Trans. Acad. Sci. of St. Louis, Vol XII, No 8 : 97

pores are produced in abundance. The chlamydospores may spread the disease. The mummified fruits cling to the trees or fall to the ground.

Mummies that hage rested a month or more or a mycelium in a starved medium, e.g. in distilled water may produce in addition to chlamydospores the true conidia.

The disease lives over the winter in the mycelial (sclerotial) stage or in the chlamydospore and probably in the conidial stages.

Mummies collected in spring produce the conidia abundantly. Mummies buried near the surface of the ground may in April and May produce from the sclerotia the apothecial stage. The ascospores germinate readily and produce a mycelium on which conidia as well as chlamydospores are abundantly formed. From these (and possibly also from wintering mycelium in the twigs) and from the hibernating chlamydospores and conidia infection is started in the spring.

Explanation of Plates.

Drawings outlined with Abbe Camera lucida.

Plate I - Rate of Germination of a chlamydospore in 5% sugar solution

Fig. 1 -	Chlamydospore						
Fig. 2 -	"	"	after	4	hours,	15	minutes
Fig. 3 -	"	"	"	4	"	30	"
Fig. 4 -	"	"	"	4	"	45	"
Fig. 5 -	"	"	"	5	"		
Fig. 6 -	"	"	"	5	"	15	"
Fig. 7 -	"	"	"	5	"	30	"
Fig. 8 -	"	"	"	5	"	45	"
Fig. 9 -	"	"	"	6	"		
Fig.10 -	"	"	"	6	"	15	"
Fig.11 -	"	"	"	6	"	30	"
Fig.12 -	"	"	"	6	"	45	"

All figures magnified x 500.

Plate II - Germinating chlamydospores, in different media

Fig.1 - 5 hours, 30 minutes in 5% sugar solution
Fig.2 - 5 hours, 45 minutes in 2½% sugar solution
Fig.3 - 6 hours in 10% sugar solution
Fig.4 - 6 hours, 15 minutes in beerwort
Fig.5 - 6 hours, 10 minutes in distilled water
Fig.6 - 7 hours in manure decoction

All figures magnified x 500.

Plate III - Germinating chlamydospores in beerwort and sugar solution

Fig.1 - 24 hours in 2½% sugar solution
Fig.2 - 27 hours in beerwort

All figures magnified x 500.

Plate IV - Chlamydospore and mycelium produced from it (in beerwort)

Magnification x 500.

Plate V - Production of chlamydospores in $2\frac{1}{2}\%$ sugar solution in 200 hours

Magnification x250.

Plate VI - Production of chlamydospores in 5% sugar solution in 78 hours

Magnification x250.

Plate VII - Production of chlamydospores in beerwort in 71 hours, 30 minutes

Magnification x500.

Plate VIII - Production of chlamydospores in distilled water in 64 hours

Magnification x250.

Plate IX - Germination of individual cells of the mycelium

Magnification x500.

Plate X - Production of chlamydospores on the surface of inoculated plum leaf

Magnification x250.

Plate XI - Production of flask-shaped sterigmata and true conidia on mycelium produced from chlamydospore

Magnification x500.

Plate XII - Ascospores germinating in the ascus and producing flask-shaped sterigmata and true conidia

Magnification x500.

Plate XIII - Fig.1 - Apothecium found under a plum tree, April 30, 1910, attached to a buried mummified plum.
Fig.2 - Ascus and paraphysis

Magnification.
Fig.1 x 1/2.
Fig.2 x 250.

Plate XIV - Ascospores germinating in the ascus and producing chlamydospores

Magnification X250.

Plate XV - Fig. 1 - Young plum trees 35 days after inoculation with brown rot chlamydospores. Almost all destroyed.

Fig. 2 - Young plum trees not inoculated. Check plants.

Magnification X 5/13.

PLATE I

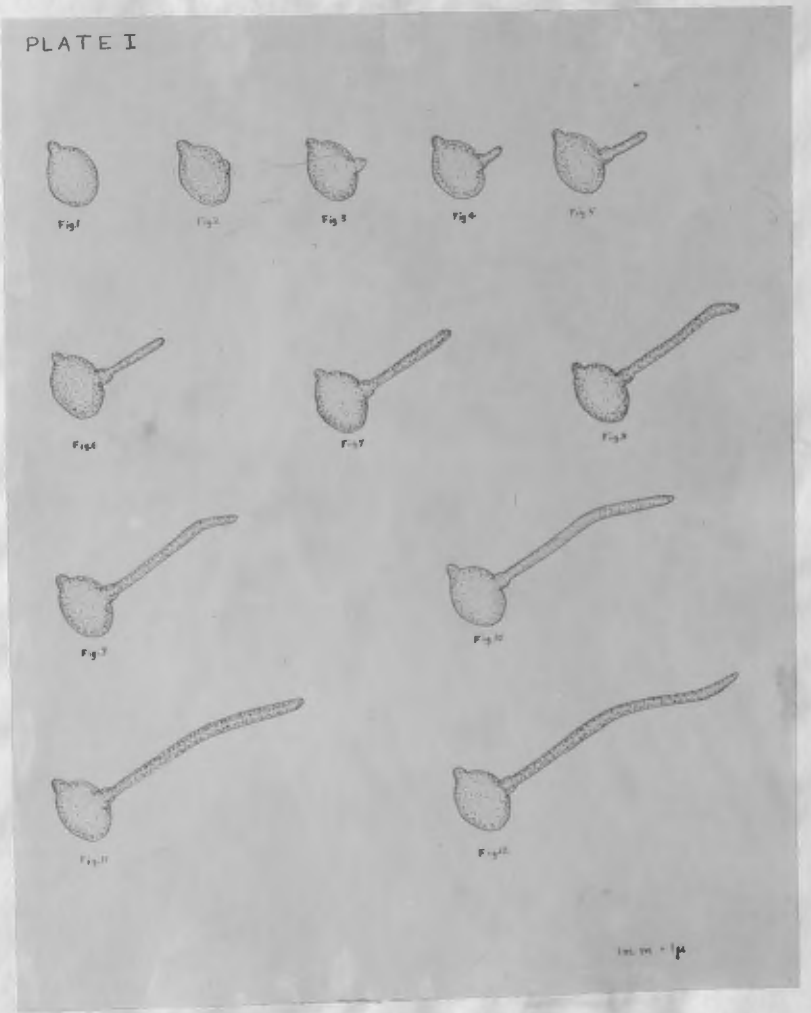


PLATE II

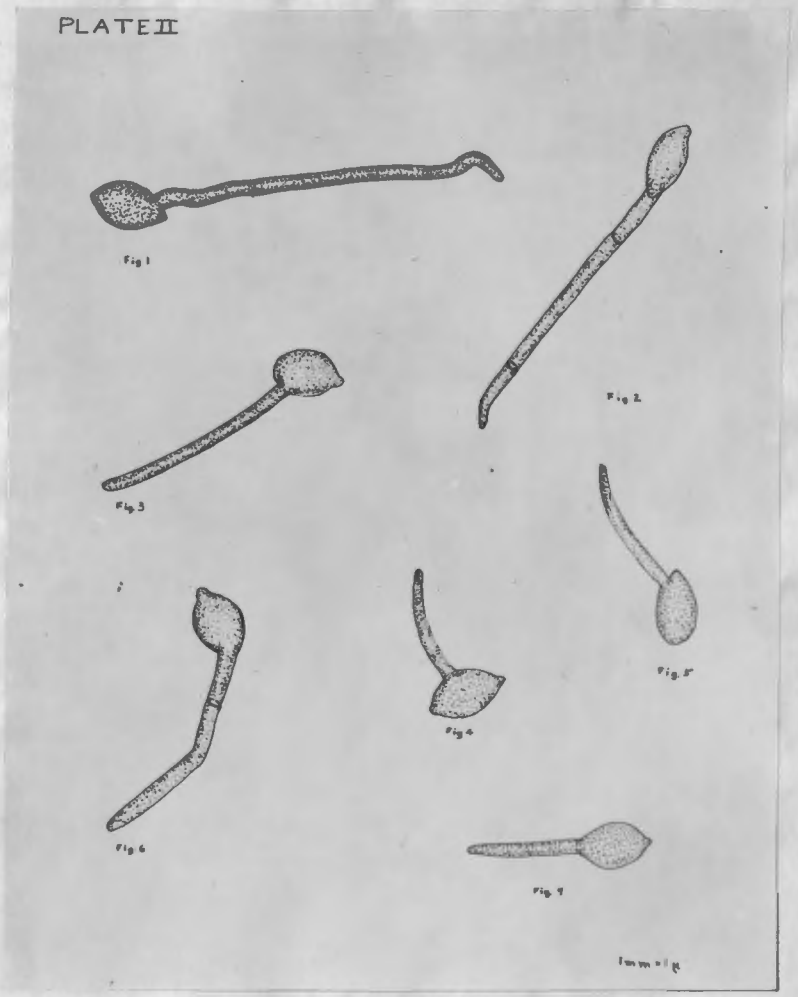


PLATE III

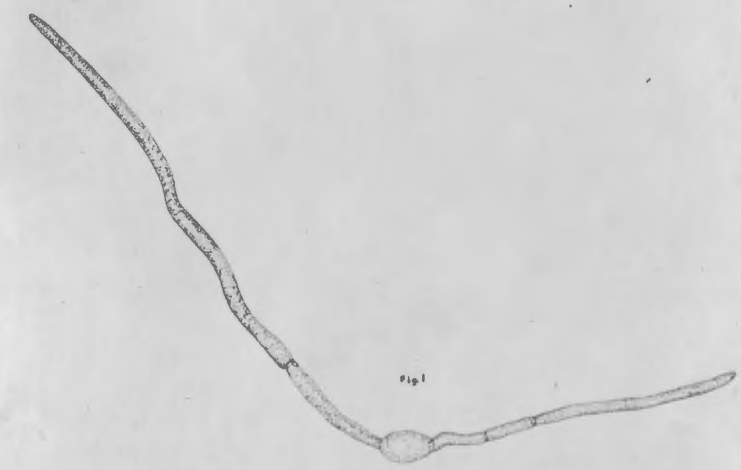


Fig 1

Fig 2

PLATE IV

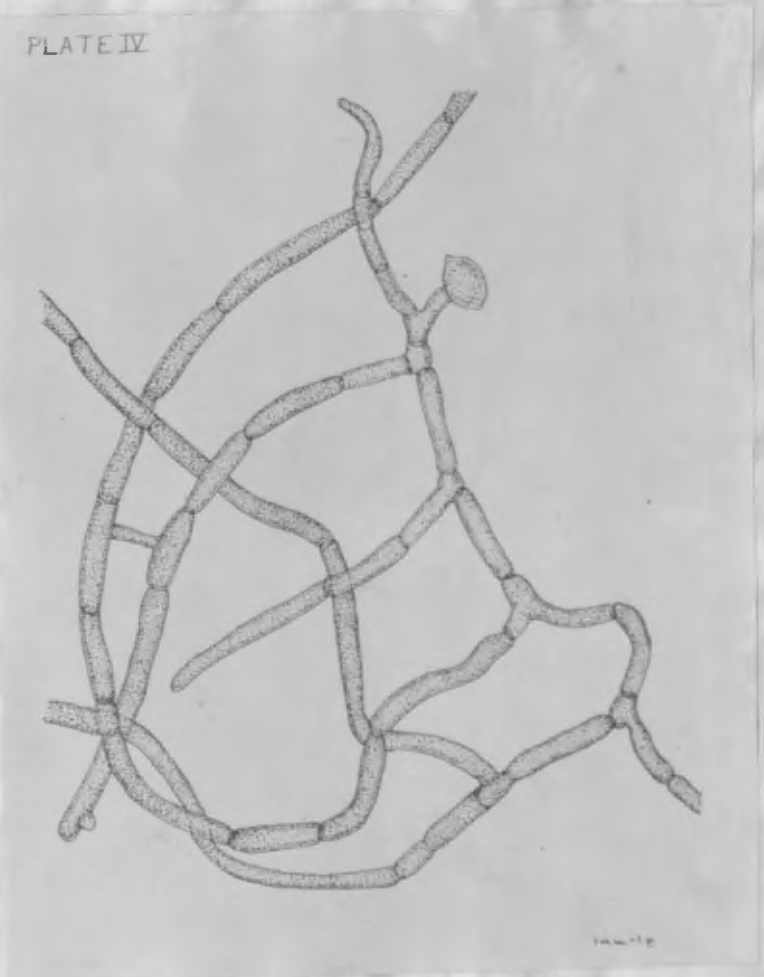


PLATE V

1mm = 2μ

PLATE VI.

PLATE VII

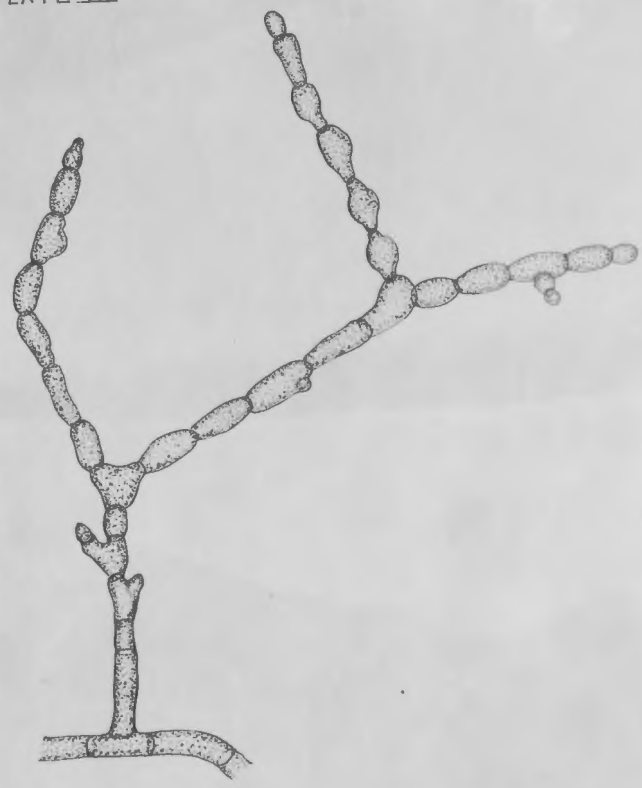


PLATE VIII

1mm = 2k

PLATE IX

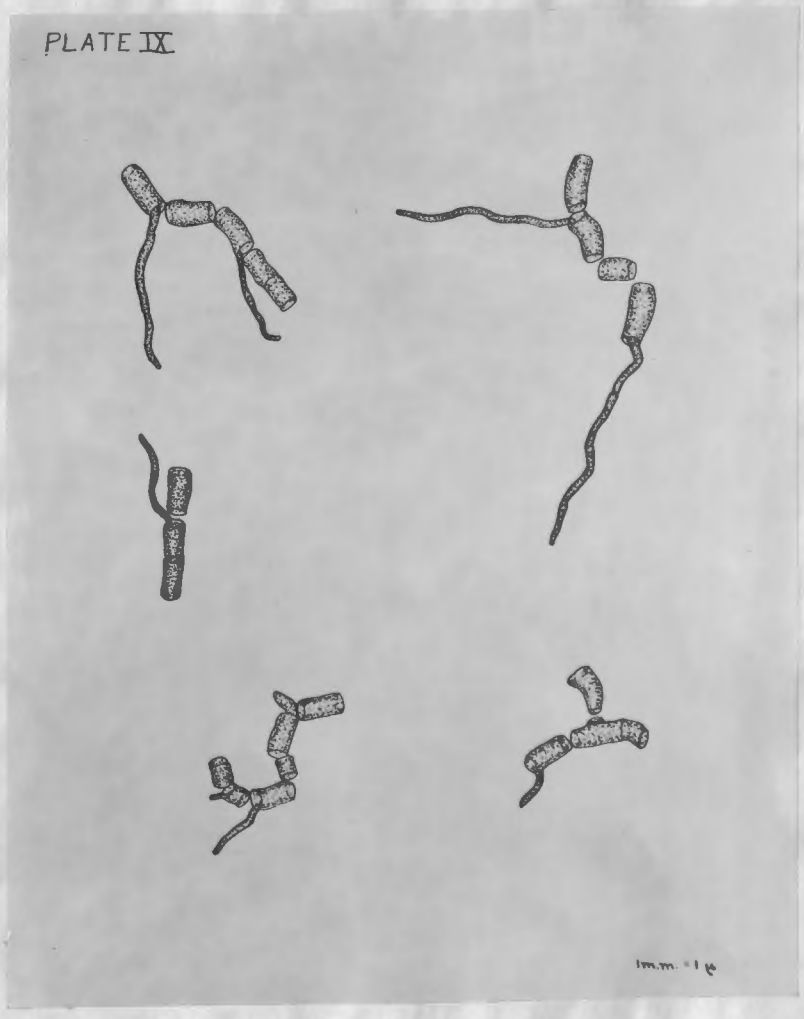
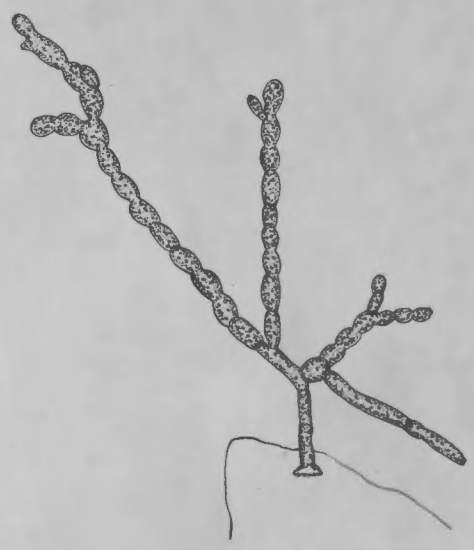


PLATE X



1mm = 2μ

PLATE XI

Fig 1

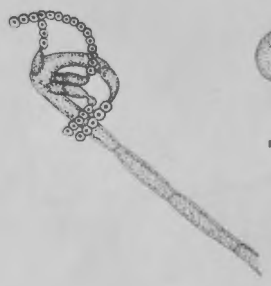


Fig 2

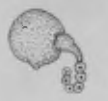


Fig 3

Fig 4

Fig 5

Fig 6

1m m = 1 μ

PLATE XII

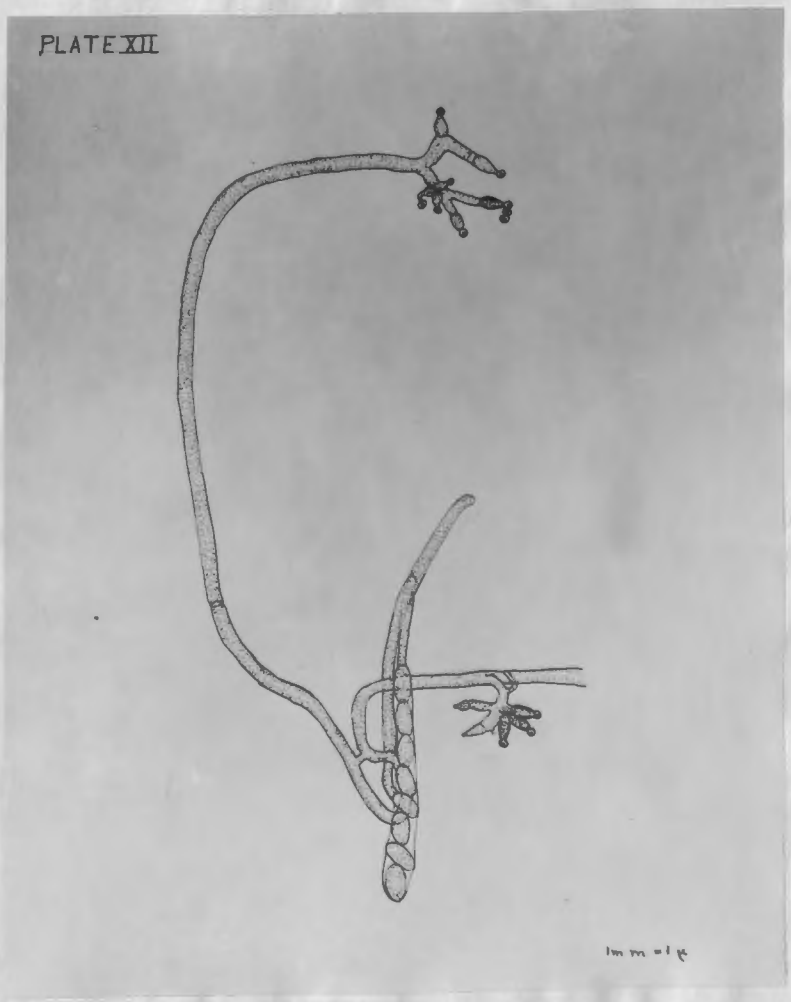


PLATE XIII

Fig 1

Fig 2

Fig 1 Natural
Fig 2 1mm = 2μ

PLATE XIV

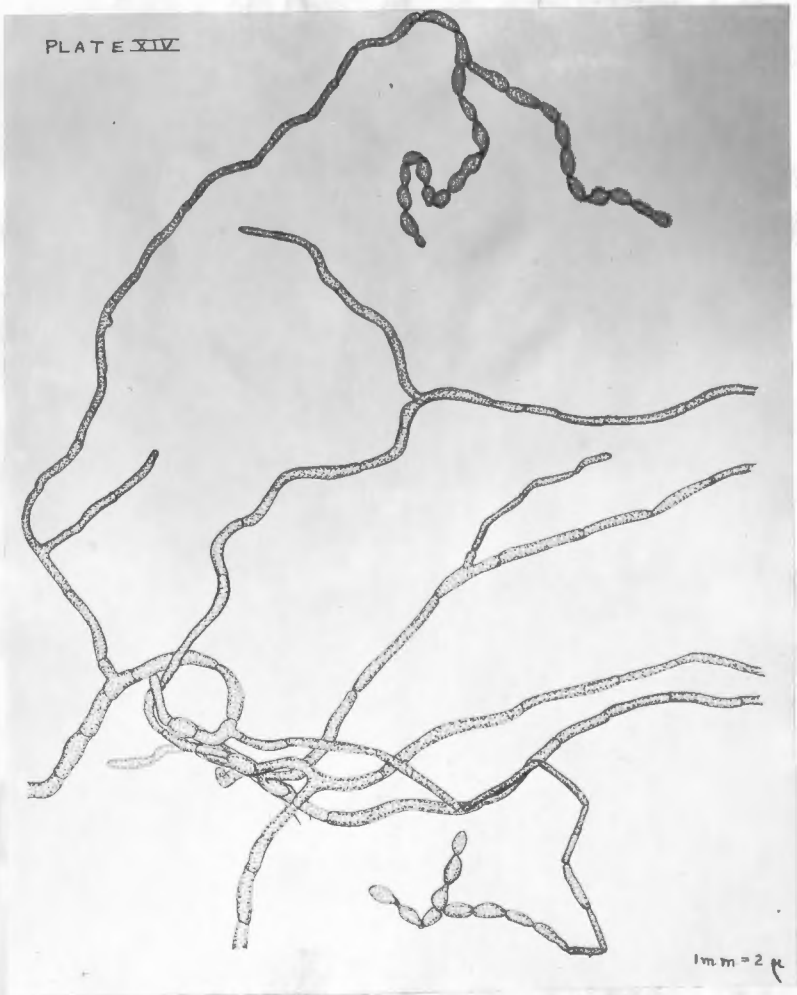


PLATE XV

Fig. 1.

Fig. 2.