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A MAPLE LEAF DISEASE CAUSED BY CRISTULARIELLA DEPRAEDANS

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FOREWORD

In the last twenty years the writer has paid particular attention to the diseases of trees in Connecticut. These have been of various types. First there was the chestnut blight which, soon after its discovery here in 1907, developed very rapidly and killed all of the chestnut trees, though the seedlings and sprouts from the old stumps continue to exist. Then came the white pine blister rust which requires Ribes for its alternate host but which proves a far more serious enemy to the white pine, especially in its seedling stage needed for natural reproduction in the pine areas. Very recently we have had to deal with the willow scab, which suddenly developed under unusually moist spring conditions, particularly in the northwestern part of the state, and has already killed many of the large shade trees of *Salix alba* var. *vitellina,* its most susceptible host. These diseases, except possibly the last, seem to have been imported.

There has recently been called to the writer's attention another disease, on maple leaves, new to the state and possibly to the country, that developed conspicuously in the fall of 1928. It had previously been reported only in Europe. This disease, however, unlike the others, is not likely to prove a serious trouble, since it has rarely been found and then only under unusually moist conditions and at a time in late summer when the injury is not so important as it would be earlier in the season. Dr. Deuber, of Yale, while working during vacation for the Bartlett Tree Expert Company at North Stamford, became interested in this disease and brought specimens to the writer, who started on its identification. Material was given to Dr. McCormick, of the Station's Botanical Department, and she eventually obtained in pure culture a fungus that has since been identified by Mr. Bowen as the cause of the maple disease. Mr. Bowen had in the meantime been assigned the study of this fungus, as a part of his work for a master's degree at Yale, under the writer and Dr. Deuber. Since part of Mr. Bowen's investigations were carried on at the Station, where he was later employed during the summer, it was decided to publish his results as a Station Bulletin.

G. P. Clinton.

CONTENTS

A MAPLE LEAF DISEASE CAUSED BY CRISTULARIELLA DEPRAEDANS

Paul R. Bowen

INTRODUCTION

On September 3, 1928, Dr. C. G. Deuber of Yale University and Mr. Francis A. Bartlett of the Bartlett Tree Expert Company noticed that the trees and seedlings of the sugar maple, *Acer saccharum* Marsh., in the woods across the road from the High Ridge Country Club at North Stamford, Connecticut, possessed an unusual appearance in their foliage. At first glance it was thought that this phenomenon was a sun-scorch wilt, since the leaves exhibited very soft areas, as if they had been scalded, but observation revealed tiny white fruiting bodies of a parasitic fungus on the under surface of the affected leaves. The attack of the fungus had been very sudden, as evidenced by the color of the foliage, since no yellowing or gradual decomposition had occurred. The wilted leaves, though green, had a grayish tinge in their badly infected portions, which was due to external mycelium. Wilting was so severe at the base of many of the leaf blades that the blades hung limp from the petioles. A slight shaking of the trees caused the infected leaves to fall freely.

Such rapidity of attack by the fungus appeared to be intimately connected with the weather factors during the last week of August and the first few days of September. Weather conditions recorded by Dr. Deuber for this period are as follows: "On August 22, it rained all day and every day for the next week. Foliage of all trees remained in good condition. August 29, 30, and 31, and September 1 and 2, were very hot days with high humidity. On the night of September 2, a cloud-burst occurred at North Stamford, and on September 3, it rained all morning."

It was suspected that the rainy week of August 22-29, followed by extremely hot, humid weather presented excellent conditions for the fungous attack upon the sugar maples. The leaves had

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Author's Acknowledgments. The writer wishes to acknowledge his indebtedness to Dr. G. P. Clinton and Dr. C. G. Deuber, whose direction of the work, constructive suggestions, and kindly criticism have made this publication possible. Thanks are given to Dr. Florence A. McCormick for her willing assistance and suggestions in the culture work; to Dr. H. Castle for his advice in most of the histological work; and also to Dr. A. A. Dunlap and Dr. R. P. Marshall.

become water-soaked, the temperature remained high, and the atmosphere continued to be very moist. Such conditions seemed to prove favorable for fungous activity, since seedlings, surrounded by a heavy overgrowth where the most humid condition existed, were found to be the ones most severely infected. Further discussion of the diseased condition of the sugar maple trees at North Stamford is made in a later section of this report.

HISTORICAL REVIEW

REVIEW OF LITERATURE

The organism causing the disease has been studied very little, and references to it in the literature are extremely few. This scarcity of knowledge is probably due to the fact that the malady is not of widespread occurrence, being recorded abroad in only England and Germany, and apparently never being reported before in the United States. The writer understands, however, that one or two botanists have recently observed it here, although no published accounts by these have been made. In the succeeding paragraphs the writer quotes in detail from the more important publications that have been made concerning its occurrence in Europe.

The disease was first described by Cooke (1) in 1885, in a group of short articles entitled, "Some Remarkable Moulds," read before the Quekett Microscopical Club. About six years before the reading of these articles Dr. Cooke had noticed, when in Norfolk, that several young trees of *Acer pseudo-platanus* L., growing in a damp plantation, presented an unusual appearance, from the flabbiness and decoloration of the leaves. He wrote, as follows: "The green leaves had become flaccid and rotten whilst still attached to the tree; the whole surface blotched with grayish spots, which were in many cases confluent over a great portion of the leaf. The under surface, under a pocket lens, was spotted with minute white points, like the head of a small pin. These points were most numerous on, and almost confined to, the veins of the leaf. Under the microscope, these minute points were found to be the globose capituli, or heads, of a small parasitic mould, scattered over the under surface of the leaf, with its delicate mycelium penetrating into the substance. The heads were loosely scattered, and not collected in tufts, almost wholly confined to the venation. The hyphae, or threads, were short, slender, flexuous, septate and swollen at the apex, where one, or three to four larger cells formed the basis of the globular head; around these large cells were clustered a number of smaller, elliptical cells, which again were sur-

mounted by somewhat triangular, obtuse-cornered cells, and these divided at the apex in a furcate manner, each fork divided off as a globose hyaline spore. Each capitulum was, in its entirety, about one-tenth of a millimetre in diameter, and the spores 12 micromillimetres.

"Some leaves were placed under glass and kept moist for weeks, when a very peculiar phenomenon was presented, the formation of small black round sclerotia on the spots occupied previously by the mould . . . The parasite is undoubtedly an injurious one, extending speedily to every leaf on young trees, ..." To this parasite Cooke gave the name of *Polyactis depraedans* Cooke.

In 1886 Saccardo (7) made reference to the article cited above from Cooke, renamed the fungus under the genus Botrytis, and gave the following (translated) description for *Botrytis depraedans* (Cooke) Sacc.: "Grayish spots on leaves, determinate to confluent; hyphae white, ascending, septate, flexuous, simple, with oblong basidia-like cells at the apex, surrounded by an outer circle of bilobed cells; with capituli globuse, subcompact; conidia globose, hyaline, 12 micromillimetres in diameter. Discovered at Norfolk, England, infecting the foliage of *Acer pseudo-platanus*, which it destroys."

Cooke (2) in 1906, again referred to the disease as follows: "*Botrytis depraedans* (Cooke). First discovered on living leaves of *Acer pseudo-platanus* in a damp wood. Several young trees had nearly every leaf affected . . . Grayish spots were formed on the leaves, which were sometimes large and confluent. The threads were flexuous and septate, simple, crowned at the apex with elliptical basidia-like cells, ultimately two-lobed. The glomerules of conidia globose and compact. Conidia globose, 12μ diam. After the leaves had fallen to the ground, and lain for a short time, numerous minute black sclerotia were formed, the ultimate development of which was never ascertained. Certainly a most destructive pest, but it does not appear to have been recognized elsewhere, at home or abroad. This parasite has never been thoroughly investigated, and, as it has occurred so seldom, there has been no opportunity for experiment on remedies."

In 1908 Saccardo (8) described a new fungus, *Illosporium Diedickeanum* Sacc. The translation follows: "Leaf spots spreading, oftentimes confluent, grayish, more distinct on the upper surface, without a border; fruiting heads very small, somewhat flattened, compact, transparently white, more numerous on the upper side, loosely scattered, 130-160μ in diam., fastened only by one point, hence easily broken off; basidia spread out from an inner almost globular cell, 30-40μ in diam., oblong cylindrical or somewhat clavate, smooth or somewhat lobed, 30-40μ long and 14μ thick, apex obtuse, conidia occasionally somewhat roughened, globular or somewhat angular, single or clustered, 13-14μ long and 10-14μ

thick, and hyaline. On leaves of *Acer pseudo-platanus,* at Steigerwald near Erfurt, Germany, Oct. 1907 (H. Diedicke).— The small fruiting bodies are quite like insect eggs. The internal structure differs from Illosporium and needs further investigation. Basidia are oftentimes seen somewhat rough at the apex."

In 1912 the following brief (translated) account was given by Sydow (13) concerning *Illosporium Diedickeanum:* "This interesting fungus has hitherto only been known in Steigerwald near Erfurt. It was found on some trees in the park of Hohenschwangau, as well as on the Scharteweg, by Fussen, near the Tyrolean border. In both localities almost all leaves of the infected trees showed the fungus."

In 1916 von Höhnel (3) showed that *Illosporium Diedickeanum* was the same as *Botrytis depraedans.* He wrote (translated) as follows: "The author (Saccardo) in Ann. Mycol., 1908, **VI,** Bd., p. 563, Taf. XXIV, Fig. 9, described and showed this fungus, of which he had seen only the small fruiting heads (capituli), and has therefore erroneously understood and incorrectly characterized the same. It seems, therefore, that the fungus was already correctly described and classified by Cooke in 1885 as *Polyactis depraedans* [Journ. Quekett Microscop. Club, 2, Ser., II, Bd., 1885, p. 138 ff., Taf. X, Fig. 4, and Journ. Roy. Hortic. Soc., London, 1905, XXIX, Bd., p. 201 ff., Taf. XIX, Fig. 4 (n.g.)]. The fungus is, according to the original example in Sydow Mycoth. Germ. Nr. 950 and Nr. 1150 (from upper Bayern) neither an Illosporium nor a Polyactis, but a new Hyphomyceten genus which I call Cristulariella, and therefore name it *Cristulariella depraedans* (Cooke) v. H.

"This was found only on the under side of half decayed sycamore maple leaves. It appears only in such places, mostly in pallid spots, where the under leaf epidermis has been destroyed by other influences. On such places one finds in the spongy parenchyma, hyaline, thin-walled, septate, 4 to 8μ wide hyphae, mostly upright, some turned in all directions.

"Some of the hyphae ends appear on the surface and develop there straight, hyaline, 100 to 270μ long sporophores which above are 8-9, in the center 11, and on the swollen base 16μ thick The same are thin-walled and show mostly five septa, of which two are found underneath and the others more or less above. The sporophore develops above an almost round, up to 28μ wide, cell separated by a transverse wall, on which near the center, arranged in a circle, sit ten short single-celled, thin-walled, two-lobed cells, 15 to 20μ high and 12 to 15μ wide, with somewhat contracted bases.

"These bilobed cells grow in part into short and thick branched, coral-like formations, which separate further into two or three-

lobed side branches, which similarly branch. Sometimes they are rounded to about 20μ wide secondary cells, which behave in a manner quite similar to the central cell, only less plentifully. These develop also a coral-like, compressed, branch-system, which may, however, develop a pair of third lobes, which develop two to three-lobed outgrowths. From the last rounded lobes of the third and fourth orders arise finally the rounded, about 10μ large, hyaline, single-celled spores, which, as do all the branches and cells of the entire head, rest on a broad base and are separated only with difficulty.

"One understands this complicated structure of the spore head most readily when one places the same on a slide heated with caustic potash solution and subjects it to slight pressure. The branch system of the head then separates and lies in a circle around the large central cell. There is seen then mostly 10 secondary cells, each with its circle of one to two, two or three-lobed branched cell carriers with some smaller tertiary lobes surmounted by a few flaccid cells, which are cordate. The lumen of the individual cells and branches are separated from one another by transverse walls.

"The fungus cannot be considered as Cristularia (Syll. Fung. **IV,** p. 134), although it is nearly related thereto, on account of the little constricted base of the outgrowing spores, which are detached with difficulty, and the compact condition of the peculiarly constructed head."

Von Höhnel, following his description of *Illosporium Diedickeanum,* placed the fungus, as stated above, in a new genus, Cristulariella, and gave a brief description, which we translate as follows: "Cristulariella v.H. *n.g.* (Botrytidee). Parasitic Mucedineen, nutrient hyphae growing in the substratum. Fruiting hyphae upright, septate. Terminal cell large, spherical, with partially spherical, partially one to two short, two to three-lobed compact branched outgrowths, the branches of which are in part saccate. All the sac-like cells similar to the chief cell, have lobate outgrowths. Branches and cells are separated by cell walls. Spores spherical, single-celled, separated with difficulty, with a slightly narrowed base, sessile, borne singly on the tips of the ultimate branches."

SYNONYMY AND EXSICCATI

Von Höhnel in his article gave the synonymy of the fungus so completely that the writer has been unable to add others. The accepted name with synonyms and places of publication are as follows:

Cristulariella depraedans (Cooke) v. Höhn. Sitzungsber. Kais. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. I, **125**: 124. 1916.

Polyactis depraedans Cooke. Jour. Quek. Micr. Club. S. II, 2: 141-142. 1885.

Botrytis depraedans Sacc. Sacc. Syll. Fung. 4: 134. 1886.

Illosporium Diedickeanum Sacc. Ann. Mycol. 6: 563. D 1908.

Discussion of the synonymy follows later. Apparently only two exsiccati specimens have been issued, as follows: *Illosporium Diedickeanum* Sacc. Syd. Mycoth. Germ. Fasc. 19, Nr. 950. 1910. Ibid. Fasc. 23, Nr. 1150. 1912.

These few references, which were the most important of those found, were mostly of a taxonomic character, describing the fungus that caused the disease. There were even differences of opinion among the writers, concerning the description and the placing of the species in the proper genus. Very little research on the life history of the fungus has been done, so that there lies open this new field of investigation, which the writer has undertaken to unfold in the short amount of time at his disposal.

MYCOLOGICAL INVESTIGATION

LEAF STUDIES

On September 28, Dr. Clinton and the writer visited the region where the disease was first discovered, in order to observe the infected trees and seedlings, and to collect diseased leaves to be used for study. When Dr. Deuber and Mr. Bartlett discovered the fungous disease on September 3, leaves exhibiting the beginning stage showed circular, flaccid, grayish spots about one millimeter in diameter. Activity of the fungus influenced by favorable environmental conditions caused these spots to increase in size and finally merge, thus producing large, irregular, wilted portions, which covered quickly the entire area of many of the leaf blades.

By the last of September, due to much drier weather, activity of the pathogene had practically ceased. The flaccid, grayish areas of the leaves had now become grayish-brown and withered. Many of the blades cracked or were broken away from their points of attachment to the petioles. Infection had invariably followed the veins. A dropping out of the holonecrotic areas in time was common. Premature defoliation was distinctly evident, being most obvious among the tender seedlings.

On the surface of the leaves were scattered white, ball-like structures, scarcely visible to the naked eye, which resembled very much in appearance insect eggs. These structures appeared sessile, but under the microscope they proved to be fruiting heads, each borne on a short, minute stalk or sporophore. The heads were irregularly globular, somewhat flattened, and composed of rather compact cells. The sporophores were attached to very

delicate mycelial threads scantily growing on the surface. Some of the fruiting bodies appeared on the dorsal side of the leaves, but the majority were situated on the ventral side. They were sparse on some leaves and quite abundant on others, depending upon the degree of infection. Most of the fruiting bodies were located in the holonecrotic areas, but occasionally a few appeared on the adjoining green portions, which indicated that these portions were infected, but not badly enough to show plesionecrosis.

At Hurd Park, Conn., on October 10, leaves of the silver maple, *Acer saccharinum* L., were found having the same pathological condition. The blades contained grayish-brown, parched areas, but in the majority of leaves these areas were small, circular, and single, in contrast to the great number of large splotches found on the sugar maple leaves. The fruiting bodies in the necrotic regions were abundant on the top side of the leaves and rare underneath, a dissimilar condition to that of the sugar maple leaves. Adjoining green areas showed a few fruiting bodies. It seemed that the silver maple leaves were more resistant to the disease, because infection in general was neither so serious nor as widespread as it was on the leaves of sugar maples.

On October 17, at the Cathedral Pines in Cornwall, Conn., leaves of *Acer saccharum* were also found exhibiting pathogenic characteristics like those observed on the trees at North Stamford. On the same host in a ravine of the Tunxis Forest at Hartland, Conn., the disease was seen again on October 24, but the fungus was rarely in fruit on the infested leaves.

The abundance of fruiting bodies found in the grayish-brown areas and in adjoining green areas of the leaves of both species collected, and also their prominence on the dorsal and ventral surfaces for the two kinds of infected areas, are as follows:

Acer saccharum: Fruiting bodies more abundant in grayish-brown areas, few on dorsal surface, several on ventral surface; less abundant in adjoining green areas, less on dorsal surface, more on ventral surface.

Acer saccharinum: Fruiting bodies very abundant in grayish-brown areas, many on dorsal surface, very few on ventral surface; few in adjoining green areas, few on dorsal surface, very few on ventral surface.

The character of the region in which this disease was first seen at North Stamford is shown in Plate XXIX, made from a photograph by Dr. Deuber. Plate XXX illustrates the early stages of infection of the leaves of both *Acer saccharinum* and *A. saccharum,* while the later stages of injury to *A. saccharum* are seen in Plate XXXI. Through the courtesy of the officials of the Kew herbarium, Cooke's type specimen of this disease was loaned to the Experiment Station, and a photograph made of it by Dr. Marshall is shown in Plate XXXII.

CULTURE STUDIES

In order to study the organism causing the disease an attempt was made to obtain pure cultures. Sections of leaves of *Acer saccharum* approximately .5 centimeter square, each having a fruiting body on its surface, were washed in a 4 per cent formaldehyde solution and plated on oat and malt agars. Within two or three days each leaf section was covered with a copious growth of fine, downy mycelium. Transfers were made to slants of oat, potato, malt, and peptone agars, and rapid growths of the fungus resulted.

In the many transfers, vegetative growth was always rapid and abundant, consisting of fine, long, hyaline hyphae of variable but narrow widths, and exhibiting Y-shaped branching, and very few septa. All cultures were repeatedly studied in an attempt to find spores, but these rarely appeared. Later it was found that these were cultures of a Pestalozzia and, although apparently not connected with the disease, usually readily appeared in the attempts to isolate the real fungus, *Cristulariella depraedans,* which was usually crowded out by the Pestalozzia and so difficult to obtain.

At the time the disease was discovered at North Stamford, Dr. Deuber at the Bartlett Tree Research Laboratories had attempted to get cultures of the fungus. Small pieces of infected leaves were placed on agar media, and in a very few days fungal growths appeared. These cultures were sent to the Botanical Department of the Connecticut Agricultural Experiment Station for examination. There was present in all the Petri dishes the same rapidly growing fungus, exhibiting large, fluffy mycelial masses, as was obtained by the writer and previously described. Dr. Florence A. McCormick, plant pathologist of the experiment station, on examination of the cultures made by Dr. Deuber, found in one of the Petri dishes a different fungus that had previously been overlooked. This fungus was exceedingly delicate in contrast to the rapidly growing organism previously obtained, and it appeared to be very weak in mycelial production and extremely slow in growing. Dr. McCormick attempted to get this delicate, slow-growing fungus in pure culture. Failure to obtain a culture was probably due to the fact that the Petri dish cultures, made by Dr. Deuber, were rather old and the media on which they were growing had become somewhat dry. However, from diseased leaves collected at the Cathedral Pines in Cornwall, October 17, Dr. McCormick obtained cultures of the slow-growing parasite, which she kept growing during the time the writer was studying the rapidly growing fungus that he had obtained from infected leaves collected at North Stamford. The cultures of the writer and those secured by Dr. McCormick were compared, and were found to be entirely different. The mycelium of Dr. McCormick's cultures in contrast to that of the mycelium

of the other cultures was larger in width, shorter in length, and formed a more limited growth.

Since it was unknown at the time which of the two fungi had caused the infection of the sugar maple leaves, pure cultures of both were kept in active growth throughout the winter. This was done not only to study the two fungi, but with the object of using each for inoculation purposes in the spring on newly developed leaves, in order to ascertain which was the causal organism. It was thought, at this time, that perhaps one was parasitic on the other. The writer was given cultures of the slow-growing organism (Plate XXXIIIa) by Dr. McCormick,[1] and the following experiments with them were begun.

Fungous growths in Petri dishes from cultures B were produced, in which a very small number of spores was found. Thinking that perhaps differences in the degree of thickness or thinness of the media might have something to do with the formation, location, and appearance of the spores, the writer inoculated another group of Petri dishes containing media of various depths. Results indicated that the degree of thickness or thinness had no apparent effect on the cultures, only that the ones growing on the thicker media produced better vegetative growths that lasted for a longer period of time, due probably to the fact that more food was available. Eventually, an abundance of spores was produced in both groups of Petri dishes. Spore production was slow in starting in all cases.

The mycelium in the surface of the agar, in Petri dishes inoculated with cultures B, developed a very interesting but singular activity in all the media used. Very characteristic eight-sided crystals, ranging in width from 8-15 microns, were produced in the agar. Plate XXXIVb.

Other Petri dishes with media of different depths were each inoculated with cultures A and B, to see whether the presence of the two fungi had any influence on each other. The fact that neither organism produced any effect upon the other seemed to indicate that no parasitism existed between them.

Further tests were made by inoculating test tubes of the various kinds of media used with cultures B. After several days all colonies had formed spores, production being greatest on potato agar, malt agar, and green bean pods. Growth of mycelium was best on potato agar, oat agar, and green bean pods, and was always more rapid and abundant on media having a high moisture content, indicating that the natural condition for infection in nature was probably a very humid atmosphere. On agar with a low water content vegetative growth was small, but spore production was hastened. Growth appeared sooner in the tubes kept in the

[1] For convenience the first cultures studied by the writer will be called cultures A (Pestalozzia), and those procured from Dr. McCormick will be designated as cultures B (*Cristulariella depraedans*).

dark than it did in those placed in the light, and seemed to be a little better. More spores were produced in the cultures left in the dark. The presence of the fungus caused the media to harden. In the older cultures black sclerotia were formed. Plate XXXIIIb, c. Potato and peptone agars were darkened by the fungus.

Externally, the fungus, cultures B, on culture media consisted at first of small, thick, rather compact, oval bunches of short, ascending, delicate hyphae. These circular, convex bunches on converging formed a dense, cottony mass whose surface was irregularly bulbous.

Microscopically, the mycelium of cultures B was hyaline, thin walled, 4-7 microns in width, sparingly branched, abruptly sinuous, with many septa. The numerous fruiting heads appeared different from those found on the surface of diseased leaves. Plate XXXIVa. They were smaller, loosely compact, composed of a cluster of basidia-like cells, from which was borne on the upper extremity of each, a small, globular conidium, 2-3 microns in diameter. Plate XXXIVb. The entire cluster rested on a larger, somewhat globular cell-like structure which was the enlarged end of the short fruiting hypha, that had become divided off by a transverse wall. The fungus, therefore, when produced on artificial media appeared to belong to the section Cristularia of the genus Botrytis as given by Saccardo.

HISTOLOGICAL STUDIES

Besides studying the two fungi, designated as cultures A and B, the writer decided to study the internal anatomy of infected leaves to help him determine which of the two fungi caused the infection. In order to do this, it was necessary to prepare and study permanent slides of diseased leaf tissues. At the time the writer collected diseased leaves at North Stamford, Hurd Park, and the Cathedral Pines, he prepared leaf sections, and the procedure for those made from diseased leaves of *Acer saccharum* from North Stamford is given below.

Pieces of the diseased leaves about .25 centimeter square were cut in the following manner: 50 from the necrotic, grayish-brown regions, each containing a fruiting body; 50 from areas where the infected portions came in contact with green areas that in all respects seemed healthy except for the presence of a few fruiting bodies; and 50 from areas that looked perfectly normal, taken far from the visibly infected portions. Fifty sections were also made from leaves of trees of the same species having no infection.

These leaf blade sections were killed and fixed in chromo-acetic acid and imbedded in paraffin. Sections, cut 10 and 16.6 microns in thickness, were stained with Pianeze IIIb, according to Vaughan (20). A study of the prepared slides of the four different groups of sections was made. Results for the groups are as follows:

1. Necrotic, grayish-brown regions. Differential staining was not at all pronounced. This was due to the fact that infected host cells reacted differently to the dye than did the cells of healthy leaf tissues, in the fact that they did not become green, but took on a more or less pink appearance, which was very similar to the color of the stained fungous mycelium.

The delicate mycelium was found mostly on the surface, which had given the grayish appearance to the infected areas. In the majority of sections fungous activity had partially disintegrated the epidermis, whereas in others the epidermal layer was completely missing. The small number of hyphae that penetrated the leaf was, for the most part, directly under the epidermis and had caused the epidermis to split off or to become loosened from the adjoining tissues. Mycelial threads, short and slightly undulating, were thin walled, septate, 4-7 microns wide, and appeared mostly curved upright, but a few strands wandered in various directions, and had broken down some of the palisade and spongy cells.

The sporophores, each bearing a single fruiting head, were turned upright to the surface of the leaf. They were simple and varied in height, the average length being 150 microns. The fruiting heads irregularly globose, somewhat flattened, 100-150 microns in diameter, presented on longitudinal section a complex structure. Plate XXXIVa. At the end of the sporophore there was a large cell, which was the swollen end of the hypha that had become divided off by a transverse wall. Produced on this basal structure was a dense ring of somewhat smaller, irregularly elliptical to club-shaped cells, which were slightly and irregularly short branched; four to six of these rings, with more or less bilobed cells terminating the upper and outermost ring, made up the compact head.

2. Infested portions in contact with green areas. The necrotic, grayish-brown areas here appeared like those described in the slides that contained leaf blade sections cut from these only. It was thought that the few fruiting bodies appearing on the green surface that adjoined the dead areas indicated that these seemingly healthy-appearing areas had mycelium from which the fruiting bodies came. The slides showed this to be true, for a few hyphae were found. Some of the tissues were slightly broken down. The fruiting bodies were like those described above. Staining appeared a little more differentiated. Host cells which were not affected as yet took on a green color, characteristic of normal leaf cells. It would seem that probably further growth of the mycelium, along with the possible secreting of acids or enzymes by the fungus, caused the tissues in these green regions to disintegrate, thus producing eventually necrotic areas.

3. Normal appearing areas on infected leaves. The normal appearing areas taken far from visibly infected portions were healthy, the leaf tissues stained green, all parts of the leaf were intact, and no mycelium was present.

4. Areas from healthy leaves. In the leaf sections made from healthy leaves all cells stained green, and were normal in position and arrangement.

Slides made with leaf sections cut from the infected sugar maple leaves obtained from the Cathedral Pines and from infected silver maple leaves collected at Hurd Park showed the same conditions as those from North Stamford.

CONCLUSION FROM STUDY OF LEAF SECTIONS AND CULTURES

A study of the prepared slides indicated that the mycelium of the organism that had injured the leaf tissues, appeared very much like that in cultures B and not at all like that of cultures A. Mycelial growth was usually scanty in the leaf tissues, and also rather sparse on the surface, which indicated a similarity in vegetative growth to that of cultures B. The hyphae appearing short, slightly undulating, thin-walled, septate, 4-7 microns wide, were also like those found in cultures B.

The fruiting bodies on the surface of the leaves, however, were different from those found in cultures B. The sporophores bearing the fruiting heads on the leaves were longer and wider than the fruiting hyphae bearing the spore heads in cultures B; those on the leaves being 100-150 microns long and 8-10 microns wide, and the fruiting hyphae in cultures B being of much shorter, variable lengths and practically the same width as that given for the mycelium in general, 4-7 microns.

The fruiting heads in the leaf sections, when under the low power of the microscope, in general appeared about as large as did those of cultures B under the high power. Those on the leaves (Plate XXXIVa) were globular, somewhat flattened, and subcompact, while those in cultures B (XXXIVb) assumed an irregular, more or less spherical form, and were loosely compact. The subcompact heads on the leaves were much more complicated than were the loosely compact ones in culture, in the fact that they were composed of more different kinds of cells and of a greater number. The clusters of cells making up both kinds of heads were produced on swollen ends of the fruiting stalks that had been partitioned off by cross walls. The cells composing the basal cluster of both kinds of heads were elongated, rather elliptical or club-shaped, but on those in the heads produced on the leaves was another cluster of irregularly branched, elongated cells, which in turn bore other similarly shaped cells, making four to six rings

of cells to each head. The basal cells of the heads in cultures B did not bear other similar cells but produced globular conidia, 2-3 microns in diameter. The terminal cells on the heads found in the leaves were not strictly globular and not 2-3 microns in diameter, but were rather heart-shaped, measuring 10-12 microns in diameter.

A comparison of cultures B with the fungus found in the prepared slides of diseased leaf sections seemed to indicate that the organism in cultures B was the fungus that had caused the infection of *Acer saccharum* and *A. saccharinum* the previous fall. The fruiting heads of cultures B with their basidia-like, basal cells bearing conidia looked like those produced in Botrytis cultures; but because of the slow and scanty growths produced, and the kind of fruiting bodies appearing on the leaves, the writer hesitated to place the organism in the genus Botrytis.

COMPARISON WITH STATEMENTS OF OTHERS

At this time a review of the literature was made. From this it seemed that the fungus that had caused the disease of our maples was the fungus described by Cooke (1) in 1885 under the name of *Polyactis depraedans,* which was later changed to *Botrytis depraedans* by Saccardo.

Saccardo, at the time of the discovery of the disease in Germany, called the fungus *Illosporium Diedickeanum.* The exsiccati specimens of *Illosporium Diedickeanum* on *Acer pseudoplatanus* examined by the writer looked like the disease on the leaves collected in Connecticut, but according to the generic description of Illosporium and other species of Illosporium observed and studied, the writer does not see how it could be called an Illosporium. Saccardo, himself, makes a statement somewhat to the effect that it differed from a true Illosporium and needed further investigation.

Von Höhnel (3), who in 1916 was the last investigator of this fungus, according to the writer's knowledge, thought that it was neither an Illosporium nor a Polyactis (Botrytis), and placed it in a new genus Cristulariella. Description by von Höhnel of the fungus under the name of *Illosporium Diedickeanum,* which he changed in this same article to *Cristulariella depraedans,* agreed better than did the other descriptions with the fungus found in the leaf tissues by the writer. It seemed evident that the fungus in the diseased tissues of the infected leaves was the same as that described by von Höhnel.

Cooke's first description of the fungus indicated that he probably had as clear an idea as any of the early workers concerning the fruiting heads. However, von Höhnel better understood their complex structure. The conidia mentioned by Cooke were given

as 12 microns in diameter. The spores produced in cultures B were 2-3 microns in diameter. Cooke, and von Höhnel as well, evidently mistook the outermost cells of the fruiting heads for spores. Apparently the true spores are rarely seen on these spore heads on the leaves.

To see whether spores were formed from the terminal cells of the fruiting heads, fruiting bodies were removed from leaves and placed in sterilized water in Van Tieghem cells. Within a few hours these terminal cells budded small conidia that were in size and shape like the spores produced in fungous growths in cultures B. Some of the conidia became detached and moved away from the terminal cells producing them, but in a short time other buds were formed, developing into conidia that were like the first ones produced. Some remained attached, and due to successive budding, short chains of conidia were formed on the fruiting cells. Many of the spores germinated while on the fruiting heads, since within a few days mycelium was found connected to the fruiting heads and extending in all directions from them. Measurements showed that the terminal fruiting cells were 10-12 microns in diameter, and that the spherical conidia were 2-3 microns in diameter. It appears from this that previous investigators were mistaken in calling the terminal cells conidia. In deciding on a name for the fungus the writer has called it *Cristulariella depraedans*. There is some doubt, however, as to whether a new genus should be made for it.

Further verification concerning the identity of the fungus was made. Cooke's type specimen of *Acer pseudo-platanus* (Plate XXXII), bearing on its lower surface a large number of fruiting bodies, was sent upon request from the herbarium of the Royal Botanical Gardens at Kew, London. This specimen was compared with the writer's diseased leaves of *Acer saccharum* and *A. saccharinum*, also with Sydow's exsiccati specimens, and the infected tissues and fruiting bodies were found to be identical. Two of the fruiting bodies were carefully removed from the English specimen, placed in a two per cent solution of potassium hydroxide to soften them and mounted in glycerin. Fruiting bodies were also removed from our pressed leaves of *Acer saccharum* and *A. saccharinum*. They were mounted in the same way as were those from the *Acer pseudo-platanus* specimen. All the heads examined appeared the same under the microscope.

It seemed certain then that the fungus found here in the diseased leaf sections was *Cristulariella depraedans*. The next step toward positive determination of its parasitic nature was to try inoculations with cultures B on growing maple leaves.

CHEMICAL TREATMENT FOR SHORTENING THE REST PERIOD OF POTTED MAPLE TREES

FORCING TREATMENTS

Work upon cultures B during the fall and early winter had progressed to a point where it was desirable to try inoculations upon growing maple leaves. Since the writer wished to make these inoculations before the regular spring leafing, forcing methods were tried in order to shorten the rest period of potted maple trees. The results of these forcing methods have been briefly reported by Deuber and Bowen (19). More complete methods and the results are given in this paper.

On October 13, the infested area at North Stamford was visited to observe the condition of the diseased trees, and to collect small trees for inoculation purposes. At this time defoliation of the infected trees was largely complete, as can be seen in Plate XXIX. Fifty small, diseased sugar maple trees from this area were secured, and transplanted to three to six inch pots in the greenhouse of the Marsh Botanical Garden, Yale University. On October 24, 50 small, healthy sugar maple trees were collected at Hartland, and potted in the same manner. Some of the transplanted diseased and healthy trees were placed outside. Others of both groups were left in the greenhouse. Complete defoliation of all the potted trees, both in the greenhouse and outside, occurred within a few weeks. From all appearances the trees had entered their normal rest period.

In order to break this dormant period and induce leafing, so that inoculations might be tried on living leaves, a series of chemical stimulation experiments was started on December 1. Ethylene chlorhydrin treatments, as used by Denny and Stanton (17, 18) for breaking the rest period of pot-grown woody plants, were followed in these investigations.

The trees were subjected to the vapors of ethylene chlorhydrin in an air tight galvanized iron can of 121.5 liters in volume. This can was kept in the preparation room of the greenhouse at temperatures of 10-18 degrees centigrade. Two concentrations of the ethylene chlorhydrin were employed, 10 and 20 milliliters for the 121.5 liter can, or one and two parts per 12,150 parts of air. A piece of cheese-cloth placed at the top of the can was moistened with the liquid ethylene chlorhydrin to provide for the volatilization of the vapors around the trees. The length of exposure of the trees varied from one to twelve days.

Trees subjected to the vapor treatments were of both the healthy and diseased groups of those kept in the greenhouse and out of doors. Checks or control plants of each were maintained. After exposure to the gas the trees were removed to the greenhouse at a temperature of 16-22 degrees centigrade, and observa-

tions were made of the time required for the leaves to unfold. The appearance of some of the trees several weeks from the time of vapor treatments with untreated checks is shown in Plate XXXVa. Plants left out of doors and not treated were placed in the greenhouse at the time that those of the same group were removed from their vapor treatments.

RESULTS OF FORCING TREATMENTS

Data for the various groups, from December 1 to March 1, concerning the number of check plants, the number of plants exposed to the gas, the time and periods of treatment, and the date of leafing were recorded. Results were tabulated to March 1 to see whether an actual forcing had been obtained. From the middle of March untreated trees left outside and in the greenhouse began to leaf. Tables 1-3 give condensed data concerning the treatments and the results obtained.

TABLE 1.[1] RESULTS OF THE FIRST AND SECOND TREATMENTS WITH ETHYLENE CHLORHYDRIN IN FORCING HEALTHY TREES OF *Acer saccharum* INTO LEAFING. LEAFING NOTED TO MARCH 1, 1929

No. of trees	Days of treatment	Date of treatment	No. of trees leafing	Date of leafing	Trees leafing per cent
First treatment					
Trees left in greenhouse					
6	1	Dec. 8–9	0		0
6	3	Dec. 9–12	2	Dec. 25	33
6	2	Dec. 13–15	0		0
Trees placed out of doors					
8	2	Dec. 13–15	0		0
12	1	Dec. 17–18	0		0
38			2		5
Second treatment					
Trees left in greenhouse					
4	5	Jan. 12–17	1	Feb. 24	25
3	5	Jan. 12–17	1	Feb. 22	33
4	5	Jan. 12–17	1	Feb. 23	25
1	12	Jan. 19–31	0		0
Trees placed out of doors					
4	5	Jan. 12–17	4	Feb. 13–23	100
2	12	Jan. 19–31	0		0
8	5	Jan. 12–17	3	Feb. 16–19	38
2	12	Jan. 19–31	2	Feb. 14–15	100
28			12		43

[1] Since none of the checks produced leaves before March 1, they are not listed in the Tables 1-3.

TABLE 2. RESULTS OF THE FIRST AND SECOND TREATMENTS WITH ETHYLENE CHLORHYDRIN IN FORCING DISEASED TREES OF *Acer saccharum* INTO LEAFING. LEAFING NOTED TO MARCH 1, 1929

No. of trees	Days of treatment	Date of treatment	No. of trees leafing	Date of leafing	Trees leafing per cent
First treatment					
Trees left in greenhouse					
3	1	Dec. 17–18	0		0
Trees placed out of doors					
4	1	Dec. 1–2	0		0
6	1	Dec. 2–3	0		0
4	2	Dec. 3–5	1	Feb. 26	25
4	3	Dec. 5–8	0		0
14	1	Dec. 12–13	1	Feb. 26	7
15	2	Dec. 13–15	0		0
50			2		4
Second treatment					
Trees left in greenhouse					
2	5	Jan. 12–17	0		0
Trees placed out of doors					
3	5	Jan. 12–17	2	Feb. 14, 26	67
3	5	Jan. 12–17	2	Feb. 15, 23	67
1	12	Jan. 19–31	1	Feb. 12	100
3	5	Jan. 12–17	2	Feb. 16, 19	67
3	5	Jan. 12–17	3	Feb. 11–13	100
4	5	Jan. 12–17	3	Feb. 12–19	75
6	12	Jan. 19–31	3	Feb. 11–24	50
3	5	Jan. 12–17	3	Feb. 12–17	100
8	12	Jan. 19–31	4	Feb. 10–24	50
36			23		64

The results of the chemical stimulation experiments to March 1, as given in Tables 1 and 2, show that the ethylene chlorhydrin was very effective in shortening the rest period of the potted maple trees. Taking all treated trees into account, more than 25 per cent leafed before March 1. None of the check trees produced leaves by that time.

The first treatment of one, two and three days' exposure to the ethylene chlorhydrin vapors did not appear to be long enough nor of sufficient concentration for shortening the rest period of sugar maple trees. Consequently, the second treatments were of five and 12 days' duration, and the concentration of the gas was doubled. The longer periods of exposure and higher concentration of gas proved to be more effective, causing some trees to unfold leaves as early as 10 days after subjection to the gas. With the five day treatment 56 per cent of the trees so treated produced leaves, while 50 per cent of those subjected to a 12 day treatment leafed in the same period. The summarized results of the five day treatment are set forth in Table 3.

TABLE 3. RESULTS OF THE FIVE DAY TREATMENTS WITH ETHYLENE CHLORHYDRIN IN FORCING TREES OF *Acer saccharum* INTO LEAFING. LEAFING NOTED TO MARCH 1, 1929

No. of trees	Date of treatment	No. of trees leafing	Date of leafing	Trees leafing per cent
Trees left in greenhouse				
Healthy				
11	Jan. 12–17	3	Feb. 22–24	27
Diseased				
2	Jan. 12–17	0		0
13		3		23
Trees placed out of doors				
Healthy				
12	Jan. 12–17	7	Feb. 13–23	57
Diseased				
19	Jan. 12–17	15	Feb. 11–26	79
31		22		71

Two explanations for the greater effectiveness of the chemical treatments in January than in December can be suggested: first, a longer period of dormancy, and, second, the increased length of exposure and increased concentration of the ethylene chlorhydrin gas. The fact that the trees kept outside showed a higher per cent of leafing than those kept in the greenhouse, indicated that the subjection to a period of low temperatures was very helpful in breaking the rest period of sugar maple trees. The trees with diseased leaves placed out of doors showed a higher percentage of leafing than the healthy trees kept outside, due, it was thought, to the earlier potting of the diseased trees, which allowed the root systems to become better established than those of the later transplanted, healthy trees. Records of the leafing of the check trees were kept. The dates ranged from March 13 to May 4. In most cases the trees kept out of doors during the winter months leafed sooner than those kept in the greenhouse.

Most of the new leaves produced on the trees whose old leaves had been diseased, were normal in size and shape, but some were slow in growing after the buds had burst. This condition indicated that, due to the premature defoliation the previous autumn, these trees had not been able to store enough food, which must be present in the new twigs in sufficient quantity for normal development of new leaves. None of the new leaves, however, produced on these trees having infected leaves the previous fall, showed any signs of the disease. This indicated that the disease was only a leaf infection, and that it was not carried over in the trees from one growing season to the next.

By means of the forcing experiments with ethylene chlorhydrin, an abundance of new leaves on the potted maples was available for inoculation experiments 30 to 60 days before the check trees leafed out.

INOCULATION OF POTTED MAPLE TREES

MYCELIAL INOCULATION AND INFECTION

Trees were inoculated on March 7 with the mycelium of the slow-growing fungus, *Cristulariella depraedans* (cultures B), secured by Dr. McCormick. In some cases a little agar along with the mycelium was transferred. It was thought that this would prevent too much drying out of the mycelium prior to its penetration of the host tissues. Some leaves were inoculated only on the dorsal surface, some on the ventral side, and others on both surfaces. Punctures with the inoculating needle were made in some leaves which, it was thought, would help to give the mycelium a better chance for more immediate penetration of the leaves.

Before inoculations were made, an abundance of wet sphagnum moss was placed on the surface of the soil in and around the pots. After inoculations, large bell jars were placed over the pots and pushed down in the moss, care being taken to leave a few openings for air to enter. The wet moss made the atmosphere around the plants very humid. It was hoped that such a moist environment would keep the surfaces of the leaves in a damp condition, would prevent the mycelium from desiccating too much before its attack on the leaves, and would stimulate growth of the fungus afterwards, since the disease was in a very damp wood when discovered, and during a rainy period in late summer. Large strips of cheese-cloth were placed over the bell jars to diffuse the light. Paper was spread over the cheese-cloth during the brightest part of the day to assure a more or less equal degree of shading. Plants placed under the same conditions but not inoculated were used as checks.

The activity of the fungus was very rapid, for on March 11, within four days after inoculation, grayish-brown spots formed on the leaves, and on March 12, characteristic white fruiting bodies appeared, some in the necrotic areas, and some in the adjoining green portions. The infected leaves continued to show all the symptoms found on diseased leaves collected the preceding autumn. Activity of the fungus was much more rapid on the leaves, its natural host, than on the artificial media, where growth generally did not appear earlier than eight to ten days.

Since cultures B had produced the infection, no inoculations were made with cultures A. The examination of permanent slides made from the infected areas of the new leaves showed that the

fungus was *Cristulariella depraedans*. The diseased conditions of the leaves produced by artificial infection are shown in Plates XXXVb and XXXVI.

The infection of the leaves of the potted maples was found to differ with the various ways in which inoculations were made. When the mycelium was transferred along with a portion of the culture media, larger and more abundant infected areas were produced than when inoculations were made with mycelium alone. Probable reasons for this were that the presence of the media as food kept the mycelium growing before its attack on the leaf tissues, and prevented it from drying out. The ruptures made by puncturing the surface layers of the leaves also aided the hyphae to penetrate a little more readily. Leaves that were inoculated on both sides showed greater infection than leaves inoculated on only one side. On these leaves whose inoculations had been on both sides, infection in every case occurred earlier on the ventral surface than on the dorsal surface. Inoculations made only on the ventral surface produced earlier ventral infection than dorsal infection. Likewise inoculations made on the dorsal side alone produced earlier dorsal than ventral infection. Ventral infection, however, was always more serious than dorsal. This was probably due to more moisture and less light on the ventral surface.

The results of the inoculations were almost 100 per cent infection. There were, however, a few inoculated leaves on three trees that did not become infected. Also a few leaves that were not inoculated became diseased from infected leaves. Due to the very humid atmospheric condition, produced to favor the development of the disease, molds such as Botrytis and Penicillium in a few cases appeared on the leaves whose inoculations were made along with a bit of the culture media, the media chiefly being attacked by those organisms. The leaves thus attacked were early removed. Trees badly infected with the disease were defoliated.

SPORE INOCULATION AND INFECTION

Spores secured from cultures B were placed in sterilized test tubes, each containing five cubic centimeters of sterilized tap water. These solutions were kept at ordinary room temperature. Within a day the spores had germinated. Loopfuls of the spore liquid were then transferred to the leaves of potted plants, and infection took place. Symptoms were slower in appearing than they were when the inoculation with mycelium was made.

ENVIRONMENTAL FACTORS IN INFECTION

After the infection of the inoculated trees had taken place, further observations and study were made to see whether moist condi-

tions were necessary for the infection to take place, and for the continued progress of the disease. A group of the potted trees was inoculated and exposed to the ordinary atmospheric conditions of the greenhouse. No signs of infection occurred, which seemed to indicate that a moister atmosphere than that in the greenhouse had to be present for the infection to start. Another group of the potted trees, in which infection was advancing, was removed from the humid atmosphere of the bell jars and placed in the customary environment of the greenhouse. The infected areas, instead of remaining flaccid and increasing in size, became dry and brittle and stopped spreading. These conditions seemed to indicate that more moisture had to be present for the activity of the fungus.

Some of the trees not inoculated were placed on the same bench with the inoculated and infected trees, with the result that they did not become infected. Others, not inoculated, were placed under bell jars containing infected plants. The majority of their leaves became infected. The chief difference in the environment of the last two groups was the difference in humidity of the greenhouse and the bell jars.

CONCLUSION

The disease of sugar maple trees found at North Stamford, September 3, 1928, and later elsewhere in the state, is definitely identified with *Cristulariella depraedans* as the causal organism. This conclusion is arrived at from pure cultures obtained from diseased leaves, from histological examination of diseased leaves, from comparison of our fungus with European descriptions and specimens of the above fungus, and by actual inoculation experiments with this organism upon healthy leaves.

SUMMARY

1. A disease of maple leaves apparently previously unknown in the United States, was found at North Stamford, Conn., in early September, 1928.

2. Collections of diseased leaves and small trees were made at several places in Connecticut during the autumn.

3. Pure cultures of two fungi occurring on the diseased leaves were obtained and grown on a variety of culture media and under different environmental conditions.

4. A histological study of diseased maple leaves was made. This phase of the investigation, as well as examination of typical fruiting bodies on the necrotic areas, indicated that the causal organism was *Cristulariella depraedans,* as identified by European specimens.

5. Potted sugar maple trees were forced into leafing by treatments with ethylene chlorhydrin from 30 to 60 days before leafing

of check trees, and from 60 to 90 days before leafing occurred in the field.

6. Inoculation experiments with pure cultures of *Cristulariella depraedans* on the leaves of the potted sugar maple trees produced the characteristic symptoms of the disease, as found in the field the previous autumn.

7. A condition of high humidity and warm temperatures surrounding the leaves was most satisfactory for artificial infection. This simulated the field condition of warm temperatures and the high humidity noted at the time the disease was first observed in Connecticut.

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Photograph of the locality at North Stamford where the maple leaf disease caused by *Cristulariella depraedans* was first seen.

a. *Acer saccharinum,* showing leaf in the early stage of the disease.

b. *Acer saccharum,* showing leaf in the early stage of the disease.

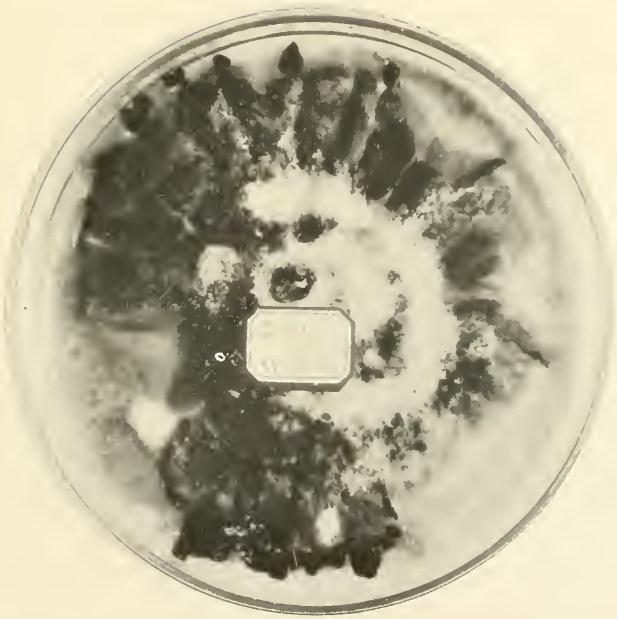
a. *Acer saccharum* leaf showing disease in fairly advanced condition.

b. *Acer saccharum* leaf in final stage of disease.

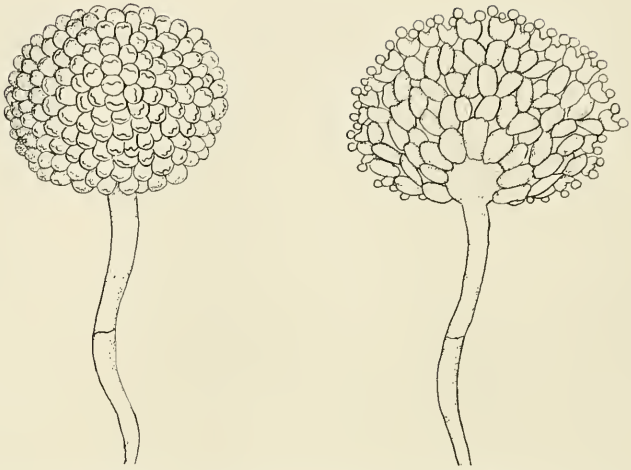
Photograph of herbarium sheet, loaned by Kew herbarium, of Cooke's type specimen of the maple leaf disease.

a. Young culture of the fungus *Cristulariella depraedans.*

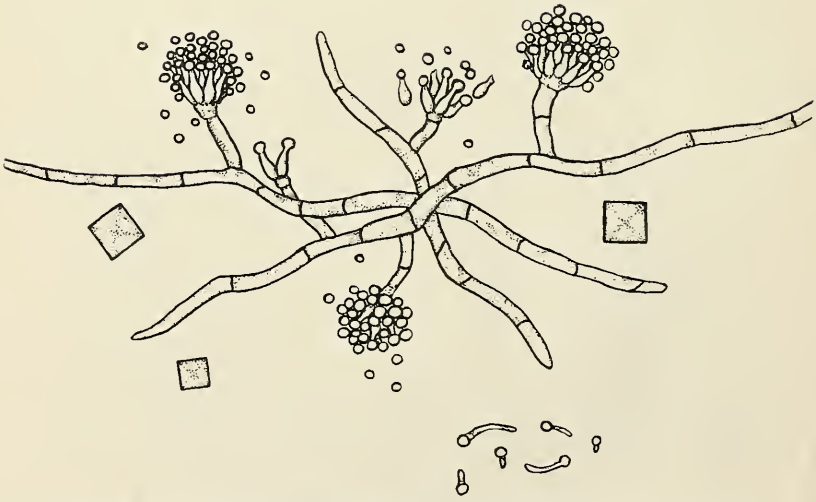
b. Older culture showing the beginning of sclerotial development.



c. Old Petri dish culture with an abundant development of black sclerotia merging together in a mat.



a. Characteristic fruiting bodies as seen on the leaves in nature.



b. Mycelium, fruiting bodies, germinating spores, and crystals as seen in the artificial cultures.

a. Effect of ethylene chlorhydrin in forcing premature foliage development. First two plants, untreated checks; second two, forced plants. *Acer saccharum.*

b. General view of artificially infected plants in pots in greenhouse experiments. *Acer saccharum.*

a. *Acer saccharum* leaf artificially infected with mycelium in agar, showing mycelium growing on infected spots.

b. *Acer saccharum* leaf artificially infected with mycelium only; no external evidence of the mycelium, but the diseased areas more extended.