PHOMA ROOT ROT OF CELERY

THESIS FOR DEGREE OF M. S.

C. W. BENNETT

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INTRODUCTION

Muck lands of Michigan equal five million acres and constitute an appreciable percentage of the total farming area of the State. Much of this is highly improved and forms some of the most valuable land to be found in any of the great agricultural sections of the country. This type of soil, on account of its high organic content and physical properties, is peculiarly adapted to the growing of truck crops. Celery, cabbage, lettuce, and onions, in particular, are crops which thrive well and which yield large returns. The growing of these crops for the markets of Chicago, Detroit, and other large cities of the north central and middle west, constitutes an important part of Michigan agriculture.

On account of the liberal returns per acre and the brisk market demand, celery has become the leading crop on much of the best muck land. The high organic content of muck soil is conducive to rapid vegetative growth which produces the very best quality in celery and also enables the grower to put his product on the market early in the season. It is often the practice to grow two or three crops on the same soil each year. In such cases the second and third crop are put in between rows just before the preceding crop goes in boards for bleaching. Thus, the land is in celery the entire growing season. Along with this intensive culture, the value of the crop has led to an almost complete absence of rotation in many of the chief celery growing districts. Not infrequently do we find fields which have

grown celery continuously for a period of more than thirty years, the fertility of the soil not only being maintained but greatly improved by liberal application of manure.

The absence of rotation, the interchange of plants, and the procuring of seeds from a great many sources has resulted in the introduction of practically every disease known to the celery plant. One of the most recent of these invaders is a Phoma disease, which, in Michigan, was first discovered at Kalamazoo in the spring of 1914 by Dr. G. H. Coons of the Michigan Agricultural College. The disease, since its introduction has not spread rapidly in an epiphylotic form, but in several cases it has been very destructive on small acreages. These destructive outbreaks have seemed serious enough to warrant a systematic study of the disease with a view to adding something to the knowledge of its relation to weather and soil conditions and to the methods of coping with the problem which this disease presents to celery growers.

NAME OF THE DISEASE.

This disease, like many others, is known by a number of common names. In Germany it is called "Scherfkrankheit" and the name "Scab" has been applied in this country. "Root Rot" is a term which is commonly used but sometime confused with rot due to Sclerotinia and other causes. In choosing a common name for a disease one should be selected which will not be confused with other troubles and one which is as descriptive as the nature of the thing permits. The diseased

area is not typically scabby, but simply composed of dead tissue. Since this is true the term "Scab" seems inappropriate for the trouble as it occurs in America; on turnip rooted celery the terms may be more descriptive. The roots constitute one of the chief points of attack and it seems that no term is more fitting than Root Rot. To avoid confusion with other Root rots of celery we suggest that the disease be called Phoma Root Rot, the causal organism being, as will be shown later, a species of Phoma.

HISTORY AND DISTRIBUTION.

There is considerable difficulty in determining the approximate time at which Phoma Root Rot first made its appearance as a disease of celery, and in tracing the history of the trouble. While the same disease has been described by certain European investigators on celeriac in Germany, Holland, and France, little has been reported regarding its occurrence in this country. It is hardly probable that this is due to a recent introduction of the pathogene, or to a limited distribution of the disease. Numerous references in literature to root rots of celery, all presenting the same pathological aspects, make it more plausible to believe that the disease has been present in certain celery districts for a number of years, and that it is generally distributed through the celery growing district or east and east central parts of the United States. Working as it does beneath the

surface of the soil, the true cause of the disease has probably escaped observers because of its obscure nature and because of confusion withother diseases.

Aside from a Phyllosticta leaf spot reported by Halsted (1891), no description of anything similar to this disease seems to have been made until Van Hook (1907) discovered and described a root rot of celery from the celery districts of Ohio. He did not identify the organism causing the trouble, but found Rhizoctonia in the diseased tissue. However, he states that he does not believe that Rhizoctonia alone was responsible for the disease. Neither can the writer believe that any species of Rhizoctonia had a part other than as a saprophyte coming in after the disease had been initiated by another organism. In our work with diseased plants, Rhizoctonia has quite frequently been associated with diseased condition, but inoculation from several isolations, and with Rhizoctonia solani and Rhizoctonia from milkweed have given no results which would indicate that any of these forms are pathogenic on celery.

Van Hook's descriptions, his photographs of diseased plants, and the environmental conditions under which the disease became destructive, furnish evidence conclusive enought to justify the assumption that he was dealing with the Phoma Root Rot which has been reported from other celery growing regions.

Van Hook also reported that he had observed the same or a similar disease on celery in New York State as early as 1903.

In 1919[1] the disease was reported from Ohio and specifically attributed to Phoma apiicola.

Klebahn (1910) described the disease on celeriac in Hamburg Lowlands. It seems to have a general distribution over Holland and southern Germany where it has caused serious loss to the growers of turnip rooted celery.

Quanjer and Slagter (1914) described the disease from Holland, and state that it isvery generally distributed in that country.

Dye and Whetzel[2] in 1918 reported the disease as present in New York State.

This disease in Michigan was first found at Kalamazoo where it appeared in a very virulent form from a seed bed infection. Since that time it has been found at Byron Center, North Muskegon, and Portage. It is extremely likely that it has been generally present throughout the celery growing districts of the State.

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1. Plant Disease Bulletin. p. 109. 1919.

2. Verbal statement to Dr. G. H. Coons to whom the author is indebted for this information.

ECONOMIC IMPORTANCE.

The sporadic occurrence of this disease makes it impossible to give even an approximate estimate of annual loss. The seriousness of the attack after infection depends entirely on environmental conditions. In wet cool seasons, the loss may reach 75% of an infested district; under other conditions, the loss may be negligible.

The disease in America has never been so serious as in Europe. Klebahn and Quanjer and Slagter report considerable damage to celeriac occurring at more frequent intervals than has been noted in America. Many plants are rotted off at the base, while the damage done by stunting and pruning away of the outer leaves, calls for serious consideration, reducing as it does the market value of stalks so greatly.

Several rather severe outbreaks of this disease have been recorded in America in certain localities. Van Hook reports that in the celery districts of Ohio in 1902 one grower suffered a loss of 75% of his crop; minor losses were reported from other sections. At Kalamazoo, Michigan, in 1914, and again in 1915[1] the first crop on an area of three acres was a complete loss. At Kalamazoo and Byron Center Coons (1918) states that the disease was very severe in the spring of 1917. In the past two seasons the

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1. Verbal report to the author from Dr. G. H. Coons.

disease has been present in a number of celery fields which have been inspected, but diseased plants have shown nothing more than black rings around the base. These seasons have been rather dry and warm in the early part of the summer; this no doubt accounts for the scarcity of the disease during these years. Where the disease producing organism is known to be present growers in cool wet seasons should expect to encounter lèss if due precaution for protections are not taken.

HOSTS.

So far as we have been able to discover by a careful survey of the literature on the subject, this disease has been reported only on celery and celeriac (Apium graveolens L.). Other Phomas have been reported on species of the Family Umbelliferae, but in many cases the available descriptions are too meager to admit of satisfactory comparison with the one on celery. It is, of course, entirely possible that this organism may have been reported on other species of plants. The genus Phoma embraces so many little-known forms, with present descriptions so inadequate, that, until a more comprehensive study of the genus is made, it will be impossible to determine the validity and host range of many of the parasitic species.

Inoculation experiments leave no doubt as to the ability of the fungus to attack plants other than celery.

Its potentialities along this lines have been tested by inoculation of a number of species of Umbellifers and several species of Crucifers.

Plants, nearly related to celery and of economic importance, were inoculated with pure cultures of the pathogene, under conditions very favorable to the production of the disease on celery. Inoculation of carrot (Daucus carota L.) produced black sunken spots on the roots and a killing of the outer leaves. Pycnidia were found scattered over the diseased parts.

Parsnip (Pastinaca sativa L.), while it is by no means immune, seems to be less susceptible than carrot. The region of invasion was largely confined to the upper part of the root and to the base of the leaf stalks, The diseased parts were typically dark brown or black. On the root the epidermis was broken and the underlying tissue invaded, which resulted in a "cankered" area. Invasion was very slow and the damage slight, except as the diseased spots disfigured the roots.

Under greenhouse conditions Moss Curled parsley (Carum petroselinum B. & H.) and celery seem to be equally susceptible to attack of the fungus. Young plants were killed, and older ones were severely "scabbed" around the base. Pycnidia of the pathogene were produced in all

diseased parts. Although it has not been reported, it would not be surprising to find this disease doing damage to parsley under field conditions, particularily in trucking districts where celery and parsley are grown extensively.

The attacks of the fungus on caraway (Carum carvi L.) were very weak. A few leaves have been killed by basal invasion but the region of attack is usually limited. Poison hemlock (Conium maculatum L.) and dill (Anethum graveolens L.) have been free from all signs of disease after inoculation with heavy doses of mycelium and spores.

SIGNS OF THE DISEASE.

General.

Cases of mild infection are often impossible to detect, unless the plants are removed from the soil and examined for dark discolorations. Above ground, the first indication of this disease is the dying of a few outer leaves. Often one or two withered leaves lying on the ground, but still attached to the plant, betray the presence of the disease, while the remainder of the plant has all the appearance of a normal, undiseased individual. As invasion progresses, more leaves may die and fall to the ground. The plant takes on a general unthrifty appearance, and, quite often, remains stunted throughout its period of growth. In the case of severe attacks, plants topple over, so completely are the

crowns and roots destroyed.

On Leaves.

Under Michigan conditions, natural infection of the leaves has not been noted, and if it does take place it is probably of rare occurrence. Wherever the disease has appeared in America, it seems to have been restricted to underground parts. In literature and references pertaining to celery diseases, there are only two citations of an occurrence of an organism of a Phoma or Phyllosticta type on leaves of celery. Halsted (1891) has described a Phyllosticta on celery leaves, which closely resembles the organism causing Phoma Root Rot, and which produced a leaf-spot such as can be produced by inoculating, under the proper environmental conditions, with the root rot organism. Recently a Phyllosticta leaf spot has been reported from Porte Rica (1918). A complete description of this fungus has not been obtained and it is not known whether or not it is a distinct organism.

In Holland, Klebahn found the disease on celeriac leaves and on the flowering parts, and also discovered pycnidia of the pathogene on the seeds.

Given the proper conditions, the fungus is capable of attacking the leaves. Spraying with spores outside and in the greenhouse has not resulted in leaf infection. Only a small amount of infection has been produced by spraying the plants with a spore suspension and placing them under

battery jars. Much more uniform infection has been obtained by first germinating the spores, then placing them on the leaves under small bits of wet cotton. On leaves inoculated in this manner, signs of the disease began to appear after two days. The results of infection may be of several kinds, depending on conditions. If there is not too great a quantity of moisture present there is first produced a light colored spot which later turns into an irregular red blotch. Given more moisture, the fungus, possibly by the aid of other organisms, produces soft, water-soaked areas, which are often studded with pycnidia. In the first type of spot, fruiting bodies begin to be formed in the mesophyll of the leaf, but seldom reach maturity unless the leaves are subjected to exceptionally moist conditions. The progress of the disease has been slow under all conditions maintained, and individual spots cease to spread when the plant is taken out of a moist atmosphere, and more or less normal relations restored. Leaves that have been killed by invasion at the base, often show pycnidia on the petioles and blades, after remaining on damp soil for a time, which indicates that the fungus is not selective as to the part of the plant attacked, but is governed chiefly by environmental factors.

Inoculation experiments, and observations on the disease in the field, establish clearly that climatic relations in Michigan are not favorable to the development

of the leaf-spot phase. In no place, either in America or Europe, has leaf infection been reported as common. The pathological significance lies in the bearing, which leaf and flower infection may have on the dissemination of the disease producing fungus.

On Crown and Roots of Plants.

The chief loss to the celery crop from this disease comes from attacks on the roots and crown of the celery plant. In the incipient stages of the disease, there is usually a bluish-green discoloration of diseased parts; which, as invasion continues, gives rise to a black scurfy surface. A bluish-green border, more marked on the leaf stalks than on any other part, is usually found around these "scabby" areas. The fungus may confine its ravages to the outer part of the crown and produces a black ring of diseased tissue. This kind of an attack, by killing the epidermal and neighboring cells, often leads to large cracks in the crown and bases of the leaf petiole, as the plant continues to grow and expand from within. The course of the disease after infection depends entirely on environmental conditions. The plant may attain normal size and reach maturity, with only a black ring around the crown to indicate the presence of the disease. In other cases, the plant may rot off at the crown or the roots may be attacked and destroyed, leaving the plant to fall over, or to maintain itself by means of new roots which may be shot out from the diseased

base. Plants of this latter class are usually stunted and are easily pulled out of the soil. They exhibit a black, ragged, con-shaped butt which is invariably studded with black fruiting bodies of the pathogene.

In some cases, when the plants are deeply set, the attack may be confined to the leaf stalks in the region where they come in contact with the first inch or two of surface soil. In such instances, a killing of the outer leaves usually occurs. In most cases, the disease spreads to the crown and roots, where the work of destruction is continued.

Root attacks are most severe in close proximity to the crown. There is rarely a general attack on the entire root system. Typically, infection takes place at some point near the base of the leaves, and spreads down and around the basal portion of the plant, involving the roots as it progresses. Roots are usually rotted from the plant before they become extensively diseased. Those roots, which are near the surface are more subject to attacks along their entire length than are those which extend down deep into the soil. The disease on the roots is characterized by a brownish discoloration. The black surface, so typical of the disease on other parts of the plant, is also noted on the larger roots; small roots usually disintegrate before this color appears. This disease as will be shown subsequently is due to the pathogene, Phoma apiicola. The following account may be given of its Morphology and life history.

ETIOLOGY.

Morphology.

Mycelium.

Within the tissue of the host plant, the mycelium is composed of septate threads, sparingly branched, and comparatively small; averaging between three and four microns in diameter. In young hyphae, cross walls are rare and the threads are very uniform throughout. With age, the hyphae sometimes increase in diameter, and the cells may bulge slightly as the cell walls thicken, and as the septa become more numerous. Very young hyphae are hyaline and the cell contents finely granular; older threads are vaculate and often densely granular within.

In pure culture, the organism is subject to great variation in size of threads,in thickness and color of cell walls, and in structure of cell contents. New hyphae vary from two to four microns in diameter. These become very closely septate, and the cells bulge and increase in size, until they are large spherical bodies, twenty or more microns in thickness. The cell walls are rather thick and have a dark-brown color. When the mycelium comes in contact with glass, it forms a black mass of thick walled cells which closely adheres to the smooth surface; in liquid cultures it forms a black ring immediately above the surface of the culture medium. Old cultures, on favorable media, form black compact masses of densely interwoven threads approach-

ing a pseudoparenchymatous tissue. On dextrose agar, sclerotium-like bodies, several millimeters in diameter, are sometimes produced in the mass and near the surface of mycelial growth. These very much resemble sclerotia of the genus Sclerotinia. Sectioning, however, reveals the same context throughout; the entire structure is composed of dark brown cells closely compacted and cemented together. Klebahn, in addition to finding these bodies in pure culture, has noted them on diseased plants.

Pycnidia.

Pycnidia are produced singly, either scattered or clustered on the surface of the host. Mycelial development around the pycnidium is sparce; nothing approaching a stroma has been observed. Young pycnidia are hyaline; older ones vary from brown to black. A few pycnidia are more or less spherical, but more commonly, they are considerably depressed; all are very symmetrical. In size they measure 80--100 x 100--250 microns. The pycnidial walls vary in thickness from 6 to 8 microns. The neck is cylindrical or expanded at the base and top. Pycnidia usually originate immediately below the epidermis; as they increase in size, they may push out-ward, until, at maturity, they are firmly seated on the surface with only the base imbedded in the host tissue; or they may remain imbedded in the host tissue with only the neck exposed.

The pycnidium has its beginning in a tangled weft of hyphae which rounds out into a globose, fruiting body. The first part to darken is the neck of the ostiole which is conspicuous as a black ring, even before any part of the pycnidium becomes superficial. The remainder of the pycnidial wall is hyaline until after the spores are formed. Then, there is a deposition of brown-coloring matter in the outer layer of cells which produces the dark colored pycnidium. The greater part of the black outer portion of the pycnidial wall consists of a layer of large, rather thick walled cells. This is rarely more than one cell in thickness. Near the base of the ostiole, the wall begins to increase in thickness, until the wall of the neck itself is two to three cells thick. To this greater number of thick walled cells, is partly due the darker color of the pycnidial neck. Within, covering the internal surface and extending well up into the neck of the pycnidium, is a layer of thin-walled cells filled with dense masses of protoplasm. These give rise to minute conidiophores which, in turn, give rise to spores by constriction and formation of cross walls near their tips.

Spores.

Spores of Phoma apiicola are small rod-shaped, thin-walled, hyaline bodies, measuring 3-3.8 x 1-1.6 microns. Staining discloses the presence of vacuoles which are surrounded by cytoplasm having a finely granular structure.

The size of spores on the host plants does not vary to any considerable degree. This uniformity apparently holds true with the organism very generally, as the following table of measurements by different investigators will show.

Spore and Pycnidium Measurement.

	Spore.	Pycnidium.
Klebahn	3-4 x 1.2--1.8 microns	90--240 microns
Quanjer & Slagter	4 x 1.5 microns	98--210 "
Author	3-3.8 x 1-1.6 microns	80--100 x 100--250 microns

The pycnidia, as shown by the above table, are subject to great variation in size. Quanjer and Slagter found that spores produced in pure culture were smaller than those on the host, a thing which has not been noted in any culture used in this work.

PROOF OF PATHOGENICITY.

The organism causing "Scab" of celeriac has been isolated by Klebahn and its pathogenicity proved,by inoculation experiments Coons (1917) isolated a Phoma from diseased celery plants. Regarding this organism he says: "This fungus was obtained in pure culture and typical lesions were obtained in inoculation experiments. The etiological relation of the Phoma thus obtained has been fully established."

The author has obtained a Phoma from celery from a

number of sources. Single spore isolations have been made from diseased plants from Kalamazoo, Portage, and North Muskegon, as well as from a number of diseased plants which were found in the greenhouse at the Michigan Agricultural College. Inoculations with these cultures have given characteristic diseased plants in a large percentage of cases. The organism has been reisolated from celery, parsnip, and parsley and the identity of the different cultures proved by comparison of cultural characteristics and by inoculation followed by typical signs of disease. Repeated inoculations and isolations leave no doubt as to the pathogenicity of the organism and its causal relationship to the disease.

NAME OF ORGANISM.

European writers have been unanimous in ascribing the cause of "Scab" of celeriac to Phoma apiicola Speg. A culture of this organism was obtained from Miss Johanna Westerdijk of the International Station for Fungous Cultures by Dr. G. H. Coons who compared this culture with the Phoma which he isolated from celery. As a result of this comparison he states that, "The fungus (the one isolated in Michigan) in culture and pathological habit greatly resembles Phoma apiicola described by Klebahn as producing the "Scab" of Celeriac."

The author has further compared this European culture with the ones which have been found in Michigan and in all cases responsible for the Phoma disease

of celery in America.

This organism is a typical Phoma and in the light of most recent taxonomic work by Diedicke on this genus, cannot be classified otherwise. The walls of the mature pycnidia are typically black and parenchymatous, the spores are small and the conidiophores are neither branched nor hooked. This organism has none of the structural characteristics of which Diedicke took advantage in his work with the species formerly embraced in the genus Phoma, and to which he attached generic value.

RELATION OF PARASITE TO HOST.

Method of Infection.

The factors involved in the physiology of parasitism have been baffling problems which investigators have attacked with varying degrees of success every since the study of plant diseases has attracted scientific research. Much of the work done has hinged around the method by which the parasite enters the host and around relations of the pathogene to the internal tissues of the host. It is to be hoped that a thorough knowledge of these phases will aid in the discovery of at least some of the fundamental factors which constitute the difference between parasites and saprophytes. Aside from the scientific value connected with this kind of investigation, there is often considerable practical significance attached to the method of host penetration and invasion.

Parasites, of course,employ a number of different kinds of methods in entering host tissue. Germ tubes may enter through stomata, as Gregory* has shown for Plasmopara viticola, and as Pool and McKay# have shown Phoma betae. Other natural openings are often very important. Wounds are recognized as weak places in any plants defensive equipment and, with many parasites, are important points of infection. Still other organisms enter by boring directly through the epidermal cell walls. In the case of the Botrytis of lily Ward## very early believed that the fungus gained entrance by dissolving the cell walls by means of a "ferment substance", an observation which has been confirmed repeatedly and which has been found to be true for a great number of other plant pathogenes.

Whether or not all organisms which penetrate cell walls employ some kind of dissolving substance as they come in contact with the host, is not definitely known. It is possible that the substances are not necessary for all parasites.

Hawkins and Harvey[1] think that in Pythium de Baryanum they have an organism capable of exerting enough mechanical pressure to enable the threads to

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*Gregory, C. T. Phytopath. 2: 233-247. 1912.

Pool, Venus W. and M. B. McKay. Jour.Agr.Res. 5:1011-1036.1916

Ward, H. M. Ann. Bot. 2: 319-382. 1888.

1 Hawkins, Lon A. and Harvey, A. B. Hour.Agr.Res. 18: 275--298. 1919.

push their way through the cell wall.

With a view to determining the relation of the Phoma Root Rot organism to its host in the early stages of infection a number of inoculations and microscopic studies have been conducted. Virulent strains of this parasite uniformly give infection regardless of whether spores or mycelium are used as the inoculum, but on account of the greater uniformity of time required for penetration sporeshave been used for studying methods of infection.

Leaf and petiole inoculations have been made to avoid the difficulty of making observations on crowns and roots. The spores were first germinated in water, then bits of filter paper were soaked in this spore suspension and placed on the plant under flecks of wet cotton. The spots inoculated were examined at different times by stripping off pieces of epidermis, or by placing bits of leaves under the microscope. In this way, it was possible to study relation of host and parasite under high power without resorting to stains.

Forty-eight hours after inoculation the germ tubes were found to have entered the host. The method of entrance was a direct penetration of the epidermal cell walls by the fungus hyphae. No cases of penetration between cells, by wedging apart of cell walls or by dissolving the cementing

substances, were discovered. A few cases of stomatal entrance were noted but these were not numerous enough to denote any special attraction on the part of the stomata for the fungus. Those tubes which entered in this manner probably did so by chance rather than as an easy method of gaining entrance to the host tissue. As normally the fungus attacks the underground parts where stomata are scarce or lacking, it is obvious that methods of entrance must be confined to other natural openings, to wounds, or to direct penetration. These observations establish the fact that the fungus is capable of making its own openings in the leaf-blade and petiole. The same is doubtless true in the case of roots and crown.

With young plants, 100% infection is quite frequently obtained; older plants, when inoculated, often fail to take the disease. This observation has been made repeatedly with greenhouse plants. Young seedlings are subject to rapid infection. As the plants increase in age, indications of extreme susceptibility become less marked, though age by no means renders them immune. Full grown plants can be made to take the disease under certain conditions. As the plants become older the tissues become tougher and no doubt offer more resistance to invasions of this fungus. Breaks in the epidermis under these conditions would offer opportunity for the pathogene to gain a foothold.

To determine the effect which wounding would have on

the percentage of infection in the case of older individuals, 50 plants were selected and wounded in the region of the crown by scratching into the tissue with sharp needles. Bits of mycelium were placed in direct contact with these breaks in the epidermis. At the same time, an equal number of checks was inoculated by placing mycelium in contact with unbroken tissue. The plants were kept under conditions favorable for the progress of the disease. At the end of two months, 48 of the 50 wounded plants had developed black rings around the crowns, and a large number of roots had been lost. Of the check plants, 32 of the total 50 showed typical signs of the disease, but the invasions were more restricted and much less serious in their affect on the normal functioning of the host plants.

Under average conditions in the field, wounds due to insect bites, fungus invasion, mechanical injuries, etc., are common. Such injuries in all probability are an aid in admitting the root rot fungus, but by far the greater percentage of infection must take place through the unbroken epidermis. Especially is this true in the case of young plants which may suffer a high percentage of infections. Older plants which offer more resistance to invasions are probably less susceptible when they present an unbroken surface.

PATHOLOGICAL HISTOLOGY.

Previous investigation by Klebahn has shown that the Phoma Root Rot organism is both an intercellular and intracellular fungus. This point was confirmed early in the course of the present work, and an attempt was made to determine something of the effect of the pathogene on the individual host cells. Liquid cultures in which the fungus had grown, extracts from mycelium, and alcoholic precipitates from both of these types of substances have produced no perceptible effect on raw sterile celery when pieces were immersed in such solutions. It is very certain that enzymes which will react on cellulose or pectin and allow the fungus to make progree between cells in the middle lamella or which would cause a falling apart of the cells of pathological tissue, if produced by the fungus, are not present in sufficient quantities to cause noticeable results in experiments of this kind. Apparently hyphae are capable of penetrating cell walls at any point at which they come in contact and of running through cell walls, protoplasm or intercellular spaces as these are encountered. Sections of diseased plants show the hyphae ramifying through all parts of the tissue, forming a perfect network of threads through the cell walls and protoplasm. Hyphae have been found in all the various tissues of the celery plant. In the later stages of disease mycelium sometimes

even produces extensive growth in the vascular system. Both stained and unstained sedtions reveal no injury preceding invasion as is noted with many rot producing organisms. Even after penetration the cell is not immediately destroyed; many invaded cells, but for the presence of mycelium, could not be distinguished from cells of normal unattacked tissue. The first indication of change in the invaded cells comes about through a disintegration of protoplasm, followed by production of brown coloring matter in the cell walls preceding a general break down and collapse of diseased tissue. Many soil organisms no doubt come in at this point and continue the work of destruction. It is to these last organisms that we attribute the true rotting found in diseased plants as the primal organism concerned, while effective in killing tissue, is incapable of producing a typical rot such as is usually encountered in diseased specimens, which are found in the field.

PHYSIOLOGICAL RELATIONS.

Cultural Characteristics.

General.

The fungus grows readily on all of the common laboratory media and on many other media which have been prepared and used in cultural studies. It is easily from spores by the dilution method. Single spore colonies

appear as thin feathery growths, so characteristic of many of the Fungi Imperfecti. The colonies are at first white; after four to eight days, on certain media, the superficial growth is bluish or bluish-green. The submerged threads are hyaline to black. Acidity, high temperature, and carbohydrates seem to favor color production in the mycelium. Proteins, low temperature, and alkalinity tend to cause little color production. A protein medium also tends to cause permanent loss of potentialities for color production, and for pycnidium production, as well as loss of virulence. Pycnidium production is sparse on all media solidified with agar and on sterile vegetables, with the exception of celery plugs. Ten to twenty days are required for pycnidia to develop. Characteristics of growth on different media are given briefly in a tabular form in the following outline.

TABLE 1.

Characteristics on Different Media.

Medium.	Vegetative growth.	Fruiting.
Standard nutrient agar	Growth good, largely superficial, slight mass colored tinge	Few pycnidia.
Dextrose agar	Growth abundant, fluffy, largely superficial; tough masses of dark mycelium formed over surface with black sclerotial-like bodies formed in the surface mycelium.	Pycnidia scarce.
Celery agar	Growth good, considerable submerged mycelium; aerial mycelium slightly tinged with blue.	Pycnidia scarce.
Corn meal agar.	Considerable growth below the surface of the substrate; aerial mycelium very faint blue.	Pycnidia sometimes numerous in old cultures.
Oat meal agar.	Mycelium abundant, sometimes filling entire slant in test-tube; black masses of mycelium formed at the edges of cultures.	Pycnidia have been found in old cultures.
Prune juice agar.	Growth moderate, largely aerial, fluffy, white.	No pycnidia were found.
Potato plugs.	Mycelium abundant, compact, white, old cultures form tough black mats.	Fruiting not abundant.
Carrot plugs.	Very much the same as with potato plugs; mycelium not so compact and slightly bluish.	Few pycnidia.
Celery plugs.	Growth moderate, fluffy and white at first, later turns blue	Pycnidia found in great numbers, seated on the surface and visible to the unaided eye
Beans	Mycelium very abundant, white or slightly tinged with blue.	No pycnidia observed.

TABLE 1 continued.

Medium	Vegetative growth	Fruiting
Rice	Color of rice changed from white to orange color; mycelium varying from light blue to black Growth abundant.	Pycnidia produced in old cultures.
Corn	Growth moderate, color mycelial mass light blue.	Pycnidia produced in old cultures.
Coons' synthetic (on filter paper)	Mycelial growth sparse; at first white, later turning dark.	Pycnidia very abundant on both side of filter paper cone.
Richard's solution	At first submerged coming to the surface; very dense masses formed; Color varying from white to black.	No pycnidia observed.

Temperature Relations.

Vegetative growth.

The seaspns of the year, spring and fall, in which this disease is prevalent, suggest a sharp susceptibility on the part of the fungus to high temperatures. This is forcefully brought to ones attention when he attempts to isolate the organism during a hot period, or attempts to grow it in pure culture at a temperature higher than 27°C. During the summer months it has invariably refused to grow in the laboratory, a fact which has previously been noted by Coons. Nearly all the cultural work at such times has been done in a basement where the temperature ranged around 21°C. Where more rapid growth was desired an ice box has been used. This gave a temperature of about 16°C, which is very favorable for vegetative growth.

In the more exact temperature studies with the fungus, a differential thermostat, modeled after that of Ganong's, has been employed. This consists, at one end, of a cubical container which is filled with ice, at the opposite end, of a compartment for water which is heated with a hot-point; a galvanized sheet iron connection is between the two. Between is the boiling water at one end, and the ice at the other, there are nine compartments, separated by cardboard partitions. A glass covering is provided so that light is admitted. This gives a range of temperatures which is fairly constant in the middle compartments, but which admits

of considerable variation near the two extremes.

Using this equipment, preliminary experiments were conducted to determine the effect of temperature on quantity of vegetative growth. Test-tube culture on agar were first used, then, later, in order to get a giant colony effect for more exact comparison, these were replaced with Ehrlenmeyer flasks. Twenty c.c. of celery agar were placed in each flask; bits of mycelium of Phoma apiicola were placed in the center of the medium and subjected to different temperatures. The experiment was run for 30 days in duplicate. The colonies progressed slowly in uniform circles and were actively growing at the time the experiment was concluded. Results in the following table are recorded in terms of average diameter of the colonies. Temperature variations are given as well as the average of twenty readings made during the experiment, values being recorded in terms of the nearest degree.

TABLE 2.

The Effect of Temperature on Growth of Mycelium.

Temp. Variation.	Average Temp.	Average diameter of colonies.
4--6°C	2°C	.5 cm.
8--12°	9°	1.6
13--15°	13°	2.5
15--16°	16°	3.1
18°	18°	3
20o	20°	2.8
21--23°	22°	2.6
25--27°	26°	1.7
27--30°	28°	.3
34--40°	36°	no growth.

These results show that minimum temperature for growth is close to the freezing point of water; maximum temperature is around 28 degrees. Optimum temperature is somewhere in the range of 16 to 20 degrees, though very good growth is produced with a few degrees variation either way.

Pycnidium Production.

Quite often pycnidia are not produced on a solid medium and when they do make their appearance it is usually in old cultures where there is great difficulty in making estimates for comparison of response to different treatments.

Cultures of the organism on filter paper, prepared as described by Coons[1] are more satisfactory for determining the value of various factors, in pycnidium production. Fruiting bodies have been found to be formed in greater numbers and results are more easily and accurately estimated. This method consists, esentially, in placing filter paper cones in a deep culture dish with the addition of a liquid medium. The nutrient solution used has been either corn-meal broth or Coons' synthetic solution. Either is very satisfactory although the latter gives a greater mycelial development and a larger number of pycnidia.

Four deep culture dishes, prepared as outlined above, were inoculated with mycelium of Phoma apiicola and placed in each compartment of the differential thermostat. They were allowed to remain thirty days. During this period, the temperature in the basement in which the test was conducted, was fortunately very constant. Therefore, the temperature in the different compartments varied little, after the first two days. The temperatures given below are averages for the period: except in the case of the last two figures the variation was not more than one degree in either direction.

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1. Coons, G. H. Jour. Agr. Res. 5: 752. 1916.

TABLE 3.

The Effect of Temperature on Pycnidium Formation.

Average Temp.	Percent Mycelial Growth.	Pycnidia.
4 C	75	-
10	80	-
13	90	+
16	100	+
18	100	+
20	80	+
23	40	+
26	10	-
35	0	-

The quantity of mycelium produced at different temperatures is in harmony with previous experiments. Color reactions on different media have been noted before, but they came in here in a very pronounced form in response to heat. At 4 degrees and 10 degrees, the mycelium was almostwhite. As the temperature increased, the color became more intense. At 26 degrees there was very little vegetative growth, but that which was produced had a very intense dark bluish cast.

Pycnidia begin to appear at the end of 14 days. Larger number of fruiting bodies were produced at 13°, 16° and 18°, though, per unit area of mycelium, there were as many produced at 25° as at any other temperature.

At 4°and 10° while there was a very good mycelial growth, there were no pycnidia; however, after these cultures were removed and placed at 21° the characteristic bluish color came in and pycnidia were produced as abundantly as in any of the other cultures.

High temperature seems to lessen pycnidium production only as it lessens mycelial growth. Low temperature, i.e. below 10°, repressespycnidium production, but a short exposure does not destroy the potentialities for fruiting.

Germination of Spores.

In moist chambers and liquid culture, the spores of Phoma apiicola have been very slow in germinating. From 36 to 48 hours have been required for the first indications of germination at room temperature. After the mycelium had shown such a marked response to relatively high temperature, it was suspected that spores were being kept at too high a temperature for the mist rapid germination.

Pycnidia from deep culture dishes were crushed and the spores placed in small test-tubes containing sterile tap water. These were incubated, four tubes being placed at each temperature recorded below. Loops from these tubes were examined every 12 hours for germination of spores. Results are given in the following table.

TABLE 4.

Effect of Temperature on Germination of Spores.

Temperatures.	Stage of Germination.			
	24 hrs.	48 hrs.	60 hrs.	84 hrs.
4°C	-	-	Swelling	budding
10°	-	-	Swelling	germ tube
13°	-	budding	germ tube	germ tube
16°	swelling	germ tube	germ tube	germ tube
18°	swelling	germ tube	germ tube	germ tube
20°	-	swelling	germtube	germ tube
23°	-	swelling	budding	germ tube
26°	-	-	-	swelling

At least 24 hours are required for germination at any temperature. The most favorable temperature for vegetative growth, 16°to 18°, is also conducive to most rapid spore germination. As indicated by weak germination at 4°, the minimum is very little below that temperature. Maximum temperature is close to 26°.

The most outstanding feature is the length of time required for germination even at the most favorable temperature. In this, we probably have on of the minor factors for making restriction of the fungus to the underground parts of the host plant under field conditions. The spores, as indicated by inoculation and germination experiments, require considerable moisture for germination.

A fungus restricted by this moisture requirement, in order to become an important leaf disease producing organism, must necessarily have spore which will germinate rapidly and produce infection before drying out takes place.

Thermal Death-Point.

The ability of the fungus mycelium to withstand low temperatures has been tested by placing flask cultures outside of a window when the temperature ranged from-18° to - 28°C. Transfers proved that the mycelium was not killed after several days exposure.

In the higher range of temperatures the mycelium is killed by subjecting it to 35° for a period of 72 hours. A ten day exposure at 32° stopped growth but was not sufficient to produce death. Shorter exposures at lower temperatures indicate that the thermal death-point of the mycelium is very close to that of the spores which is very close to 49°.

In determining the thermal death-point of spores the capillary tube method, as described by Novy, has been used. Capillary tubes, five inches in length, and about 2 mm. in cross section, were drawn out from a piece of this walled glass tubing. These were inserted in a spore suspension until there were properly filled, then sealed at each end. The tubes were immersed in water baths held at constant temperatures. At the end of the time of treatment they were cooled in water at a temperature of about 21°C.

The tip of one end was then broken off, using aseptic precautions, and the contents discharged into melted agar by applying heat to the unbroken end. The experiment was run twice in duplicate, first with an interval of 5 degrees and later with an interval of 1 degree. Results are combined in the following table:

TABLE 5.

Thermal Death-Point of Spores.

Temperature Degrees Centigrade	Germination of spores. Time of Exposure in Minutes.			
	5	10	20	60
35°	+	+	+	+
40°	+	+	+	+
45°	+	+	+	-
46°		+		
47°		+		
48°		+		
49°		-		
50°	-	-	-	-

The thermal death-point of spores, at an exposure of 10 minutes, lies between 48 and 49 degrees. Only a few spores germinated at 48 degrees, indicating that there was some slight variation in the treatment which thespores in any one tube received or that some of the spores had slightly greater potentialities for withstanding heat than did others.

Light.

Physiological investigation in this field indicated that, with many species of higher fungi, light is of little importance either as a stimulant or as a repressant of vegetative growth. With many of these same organisms, however, light is as important and often an essential factor in spore production. Levin[1], working with a number of species of Sphaeropsidales, has found that, while mycelial growth was just as abundant, fruiting was considerably lessened and, in several species, completely suppressed by absence of light. Species of Coniothecium, Phyllosticta and Cytospora showed considerable pycnidium production in the dark but at that suffered a falling off of more than 55%, when light was excluded. Species of Ascochyta, Phoma, Sphaeropsis, and Fusicoccum produced no pycnidia in the dark. Coons[2] has found that, in the case of Plenodomus fuscomaculans, darkness has little or no effect on vegetative growth, but is prohibitive to pycnidium production.

With these results in mind, experiments were planned to determine the effect of light and darkness in vegetative growth and pycnidium production in the case of Phoma apiicola. To provide for darkness and at the same time allow for the circulation of air, a method modeled after that of Coons was used. To exclude light, two battery jars were wrapped in

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1. Levin, Ezra, Report Mich. Acad. Sci. p.134-135. 1915.
2. Coons, G. H. Jour. Agr. Res. 5: 720--725. 1916.

heavy black paper. The larger was placed bottom end up over the smaller one and allowed to rest on block of wood, thus providing for a passage of air at the base and over the top of the jar within. A similar arrangement was prepared, using heavy wrapping paper, for obtaining diffused light. Two jars without any covering were used in the series exposed to light. Filter paper cones were placed in Soyka dishes containing 5 c.c. of corn meal broth as the nutrient medium. Inoculations were made directly from mycelium. Eight dishes were placed in each jar. Results are tabulated below.

TABLE 4.

Effect of Light on Growth and Pycnidium Production.

Treatment	Percentage Development, based on best culture.	
	Pycnidia	Mycelium
Light	100	90
Diffused Light	80	100
Dark	80	90

Pycnidia were produced in greater numbers in light than under any other conditions. However, it is conclusively demonstrated that light is not an essential factor in the fruiting of this organism. Light seems to stimulate fruit body production, probably due to the lessening of vegetative growth or to some other indirect action.

Greatest mycelial development was obtained in diffused light, here also the hyphae were more upright producing a looser and more fluffy mass. In darkness there was apparently as much growth as in strong diffused light. Under normal conditions on the host the organism is not subjected to strong light when it grows around the base of the plants just beneath the surface of the soil. Long continued growth under such conditions may have produced a physiological adaptation to environmental conditiens. At any rate it is a signficant fact that the light relations which are most favorable for development in culture are those to which the fungus is subjected when functioning as apathogenic organism.

Dessication.

Mycelium of this organism will live for a period of two or three months when grown on artifical medium and allowed to dry. Longevity varies with the character of the growth on the different media; rice, which produces thick tough mats of growth and hyphae with thick walled cells, is conducive to great resistance; soil and certain synthetic media produce a more delicate type of growth which succumbs more readily to dessication. The organism was grown on sterile muck until the soil was thoroughly filled with a loose growth of mycelium. Samples of this were removed, placed in sterile filter paper and subjected to drying at room temperature. Plating brought out the fact that the mycelium under these

conditions was not living at the end of 35 days but was still capable of producing growth at the end of 28 days.

With many organisms, it is unquestionably truethat longevity of spores subjected to drying on glass surfaces furnishes no reliable index as to their persistance in nature. The longevity of fungus spores as well as bacteria depends largely on the medium upon which they are subjected to drying and also on whether or not they are imbedded in a gelatinous matrix. Hansen has found that Pseudomonas radicicola dried on filter paper or cover-glasses died within 14 hours, while on seeds it lived 14 days, and, in the dried nodules of certain legumes, it was able to grow after two years of dessication. Yeast cells shew a similar but less striking response and the same is true of many fungi.

Spores of Phoma apiicola were tested in the usual manner by drying them on coverglasses, and then germinating them in broth. Under these conditions, 3 days were sufficient to eliminate all signs of life. Other methods have been employed to more nearly approximate some of the conditions to which spores might be subjected in nature. Celery seeds were treated with mercuric bichloride, thoroughly washed, inoculated with spores and subjected to drying on sterile filter paper. The seeds were placed in broth at regular intervals. Spores germinated after 38

days dessication; above this, the limit of resistance seems to be reached and no more germination is obtained. To eliminate any inhibiting or germicidal effect, which might be produced by traces of mercuric bichloride untreated seeds were inoculated with spores, divided into two lots and subjected to drying. Platings were made from the first lot while seeds from the second lot were planted in pots at the end of every seventh day. Platings gave, substantially, the same results as obtained in previous experiments. In the first three plantings of seeds, infection was produced in the seedlings; the fourth planting gave negative results, and subsequent plantings failed to produce infection in the seedlings.

These results merely indicate that free spores exposed on the surface of celery seeds, are not able to survive dessication at room temperature for a period longer than 30 days, because of the many varying factors, they cannot be taken as an accurate index as to the time which must elapse, before infected seeds may be considered safe. Spores used in the above experiments were produced in pure culture. Under such conditions, there is always the possibility of the spores being less resistant than those produced on the host plant. Under natural conditions, the spores discharged are imbedded in a gelatinous matrix which would serve to make them more resistant to adverse conditions of environment. When we consider these differences and the fact that pycnidia may be produced on the seeds, possibly existing there as immature fruiting bodies capable of later producing spores, it seems extremely likely that, while ordinary conditions of dessication may be important in killing large numbers of superficial spores, there is not enough evidence to conclude that dessication is effective in entirely freeing seeds from the living organism.

Oxygen Relations.

The seat of this disease is near the surface of the soil regardless of whether leaf stalks, crowns, or roots are located at that place. Light is evidently not an important factor in this restriction. Experiments early indicated that the organism is an aerobe. Inoculation to Giltner[1] H- tubes produced no growth and broth cultures covered with paraffin oil showed the same results. Very little growth was produced on oatmeal agar in test-tubes when the tops were sealed with paraffin though the volume of air, in such cases, was many times that of the fungus. In tubes of sterile muck, the depth to which the fungus penetrates can be regulated by changing the compactness of the soil. In very loose muck, the fungus seldom penetrates deeper than an inch and a half; in more compact or water logged soil the growth is largely superficial. The same observation has been made in flasks where, in order to get thorough penetration, it is necessary to have the proper amount of moisture and to aerate the soil by shaking the flasks at frequent intervals.

1. Giltner, W. Microbiology, p. 160--162. 1916.

To determine the effect of introducing large supplies of oxygen, an experiment was planned to be set up in the following manner: Potted plants, having a well established root system, were allowed to dry to the wilting point. They were then taken from the pots, none of the soil around the roots being disturbed, and soaked in water having a heavy charge of spores. After being placed in 6-inch battery jars and well tamped in with more soil, the entire mass was then drenched with a spore suspension. Glass tubes, bent in such a manner as to deliver air currents upward, were placed in the bottom of the jars and connected with an air line. The amount of oxygen was regulated by allowing the air to bubble through water in a filtering flask. An average of about 120 bubbles per minute was introduced for a period of two months. Checks were run where no oxygen was supplied and also where no inoculation was made. When a difference in the size of tops, between the plants supplied with oxygen and the checks, became noticeable they were removed, washed and examined for signs of disease. Plate VI shows a picture of a typical plant supplied with oxygen. The roots were attacked at many different points and many of these rotted off two or three inches from the crown; smaller roots had suffered severely and the whole root system was in an advanced stage of decay. The presence of hundreds of pycnidia scattered over the root system furnishes proof of the organism causing the destruction. On the inoculated check plants, the disease was produced only

around the crown; the roots were as thrifty and as free from disease as in the case of the uninoculated plants.

This air relation, we believe, largely explains why roots a short distance from the crown are usually free from disease. Excessively high moisture content in the soil, since it drives out the air and makes less oxygen available, would tend to still further restrict the susceptible area, though relatively wet conditions are requisite for mycelial proliferation. Lack of moisture, in nearly all of the celery growing districts where the fungus is a pest, drives it from the aerial parts. Thus, the physiological relation of the fungus to these two environmental factors, namely water and air, determines the type of disease which is produced, and the part of the host plant which is dangerously subject to attack.

Relation to the Reaction of the Medium.

Several different media have been used in determining the relation of acidity and alkalinity to the growth of Phoma apiicola. Celery agar, nutrient broth, and Coons' synthetic solutions have been employed in several experiments.

With the solid media used, a neutral or slightly acid reaction is most favorable for growth. The same is true of the liquid media used but the initial reaction for production of most growth varies with the chemical composition of the medium. This can be brought out more clearly by a description of a typical experiment. Flasks of nutrient broth were prepared

to range in reaction from -30 degrees to +30 degrees Fuller's scale. Each flask wasinoculated with a small bit of mycelium and incubated at room temperature. In the following table, results are given for different periods of time on a percentage basis, reckoning the flask showing best growth as 100%.

TABLE 5.

Effect of Acidity and Alkalinity on Mycelial Growth.

Reaction of Medium Degrees Fuller's Scale	Percentage Development Based on Flask Showing Largest Myselial Mass.		
	2 weeks	4 weeks	8 weeks.
-30	5	5	5
-20	10	5	5
-10	25	50	50
0	100	90	90
+10	90	100	95
+20	50	70	100
+30	5	5	5

At the beginning, there was a much more rapid growth at the neutral point. As development continued, those flasks having an initial reaction of +10 and +20, respectively replaced the neutral flasks in quantity of mycelium produced. This came about, both through a gradual acceleration of rate of growth in the acid and a slowing up in the alkaline media.

Flasks, neutral and alkaline had practically ceased growth at the end of four weeks. In those having an acid reaction, the fungus continued a fair rate of growth up to the end of the eighth week. The slowness of growth in the acid flasks at the beginning was no doubt due to an unfavorable reaction, the longer period of growth being made possible through the breaking down of protein molecules into alkaline compounds. This decomposition, brought about through the activities of the fungus, had the effect of making the reaction of the medium increasingly more favorable for growth up to the point at which the alkaline compounds began to exert a deleterious influence, and then, of slowing up growth until the medium became too toxic for further development. Thus, in a single flask, the organism was able to pass from the limits of acid tolerance on the one hand to an alkaline solution on the other. Various toxic substances thrown off by the fungus were no doubt important in stopping growth before the outer limits of alkaline tolerance were reached, since, titrations, at the end of the experiment, proved that the flasks having an acid reaction at the start were only slightly alkaline. Inoculated flasks of broth, acid to +20 degrees, upon being titrated every seventh day, showed a gradual change in reaction up until the end of the eighth week, when they were 6 degrees alkaline.

A series of deep culture dishes, with Coons' synthetic solution as the nutrient medium, were inoculated to determine the effect of reaction of the medium on pycnidium production.

TABLE 6.

Reaction of Medium Degrees Fuller's Scale	Comparative Development Based on Greatest Number of Pycnidia and Largest Mass of Mycelium.	
	Pycnidia	Mycelium
-20	5	5
-10	50	75
0	90	100
+10	100	85
+20	0	slight
+30	0	slight

The limits of fruiting seem to be as wide as those for mycelial development. The neutral medium again markedly favored vegetative growth, while solutions slightly acid brought forth the largest number of pycnidia. The salient features in these results is the failure of the solutions acid to +20 to produce growth in measurable quantities. At first glance, this seems contradictory to results obtained in broth cultures. However, the solution used in this experiment has more carbohydrate and less protein in its composition than has beef broth. The probability is, that, on account of the nature of the medium, the fungus was unable to adjust the reaction in its favor and that +20 is too acid for growth.

The cultures at differant reactions produced a noticable series of color changes; though there is not such a wide

variation as is produced in the case of extreme temperatures. In the alkaline solutions, the fungus produced a pink discoloration, which is never observed in neutral or acid solutions, and the color of the mycelium varied from an almost white to a light blue color. The acid solution produced a bluish or bluish-green mycelium.

DESSEMINATION.

The restriction of the pychidia of the causal organism to the roots and crown of the host plant makes air currents of little importance in the dissemination of the disease. The gelatinous matrix in which the spores are imbedded necessitates a water distribution. When pycnidia are mature and the spores pushed out in long tendril-like threads, the jelly-like matrix is dissolved away leaving the spores free to be washed through the soil.

The importance of this method of dissemination will depend largely on the frequency of heavy rains and on the amount of surface drainage. When the disease is present in the seed bed spores are disseminated by handling plants at transplanting time. It is quite a common practice to take plants from the seed bed and place the roots in water in a shallow pan until they can be placed in rows in the field. Under such conditions a single diseased plant, bearing pycnidia of the pathogene, would be capable of infecting an enormous number of sound individuals.

With an organism capable of living and growing for a period in soil, the question of lateral dissemination by

means of the growth of mycelium through the soil must be considered. To gather experimental evidence on this point plants in one side in each of five average size flats were inoculated in the greenhouse and checks placed in the same flats at a distance of 4 to 12 inches from inoculated plants. Watering was done with a sprinkler and the soil was kept moist enough for a good growth of celery. After three months the plants were pulled up and washed free from soil. Of the inoculated plants 96% were diseased; 2% of the plants 4 inches from inoculated plants were attacked, and all other uninoculated plants were free from disease. Pot experiments and observations on checks used in the greater part of greenhouse work have confirmed these results. It might be pointed out that in the above experiments conditions were very favorable for spread of mycelium as the soil was kept damp at all times. Due to its oxygen relation the fungus can penetrate soil only to a short depth, and since in the field surface soil is often dry the spread from plant to plant by means of mycelium will assume even less importance than in the greenhouse.

VARIETAL SUSCEPTIBILITY.

The whole question of varietal susceptibility is one which will require more searching investigation and critical observation to solve the problems here presented. Van Hook reports that Giant Pascal and Evan's Triumph are much freer from the disease than is Golden Self Blanching. The most destructive outbreaks of the disease in Michigan have been on Golden Self Blanching celery, but unfortunately in

these cases, not other varieties have been present in the infested districts on which to make observations, or with which to make comparisons. White Plume and Easy Bleaching are knownto be subject to attack under field conditions; other varieties have such a limited use that it is unwise to draw any conclusions regarding their predisposition to the disease, other than from results obtained from the inoculation of a limited number of plants.

A variety test was conduced using Perle La Grand, Smallage, Schumacker, Dreer's Mammoth, Perfection's Heartwell, Dwarf Golden Heart, Winter Queen, Rose Ribbed Patie, Golden Self Blanching, Boston Market, Columbia and Golden Heart. The method adapted for testing these varieties was to place 25 plants of each variety in a separate flat and thoroughly spray them with spores.

Indications of infection first became apparent on Golden Self Blanching and Golden Heart plants through a dying of the lower leaves and a general checking of growth. In time, the same symptoms were to be noted on all other varieties except White Plume and Giant Pascal. All inoculated plants of these two varieties, upon examination, proved to be diseased; but the characteristic symptoms were not to be seen on the parts of the plants above ground: At the base a thin ring of black diseased tissue showed that the fungus was active but was not

seriously interfering with growth. In the case of the other varieties there seemed to be no great difference in susceptibility.

As a result of these tests we feel justified in saying that though none of the common varieties are immune to this disease, some give indication of being more resistant than others. Golden Heart, Golden Self Blanching and Dwarf Golden Heart, seem to be the three varieties which suffer most from the disease. White Plume, Giant Pascal, and Easy Bleaching give indication of being more resistant. These last three varieties are large, rapid growing plants of a tougher texture. Herein probably lies the secret of resistance. Succulent plants of any variety are more susceptible than plants of a tougher texture. Similarly different species of Umbellifers seem to correlate resistance with woodiness.

LIFE HISTORY OF THE CAUSAL ORGANISM.

In tracing the detailed life history of the parasite in relation to disease production, it is well first to consider the periodical development of the disease in the greenhouse and under field conditions. The first plant infection quite often came in the greenhouse or in the out-door seed bed in the early spring. Clean plants of the first crop may be attacked after they are transplanted to the field. The second crop of celery growing during midsummer is free from serious attacks. As cool days of fall come on the Phoma root rot fungus again becomes active and may produce serious damage in the last crop.

There are four possible ways in which the causal organism may pass the winter: (1) in soil or trash of the greenhouse or in the cold frame; (2) the refuse of the previous year's crop in the field; (3) as a saprophyte in the soil; (4) on seeds, and (5) of course, there is always a possibility, with an organism of this type, that there is an undiscovered perfect stage produced. As to the first possibility the fungus is know to persist through one season under such conditions. In case of the most serious outbreaks of the disease at Kalamazoo, Michigan, the greenhouse was the location where infection took place in two successive years and the point from which the disease was carried to the field. The source of the organism for the initial infection is unknown, but the appearance of the

disease in a very virulent form the record year points strongly to the fungus having lived over in the soil or trash of the seed bed. In the greenhouse at Michigan Agricultural College, plants became diseased when placed in soil from around diseased plants, after this soil had been kept in flats through the winter up to February.

There has been some difference of opinion regarding the importance of trash in harboring the organism. Klebahn is inclined to the belief that seeds are very important in the distribution of this disease, while Quanjer and Slaghter minimize seed carriage and state that the chief source of infection is to be found in the trash, manure, etc. To determine something of the importance of trash and also to determine whether or not the organism lived as a saprophyte in the soil through the winter an experiment was started September 1919, on the following basis. Seven large flats were placed outside to go through the winter. Two of these contained growing plants which had been inoculated in midsummer and which were know to be diseased, in two others were diseased plants pulled from the soil and left on the surface; two contained muck soil, mixed with sterile muck on which the fungus was growing. In one flat diseased bases of plants were placed between layers of thick filter paper and covered with soil to depths varying from 1 to 6 inches. April 1, 1920, the first six flats were taken into the greenhouse and planted to celery. By May 1, the plants in the four flats containing trash were showing

signs of disease. The two flats containing soil inoculated with a pure culture of the pathogene did not produce the disease indicating that a long saprophytic existance under the above condition at least is not common. The diseased parts in the remaining flat were examined for pycnidia and for a possible perfect stage. The latter quest was wholly unsuccessful, but pycnidia containing an abundance of spores were found on nearly all diseased parts. These spores germinated readily in tap water and indeed many of them had apparently germinated between the layers of filter paper as masses of bluish mycelium were everywhere present. This mycelium after isolationcorresponded to that of Phoma apiicola and produced disease when placed in contact with celery plants.

Before the late War a great portion of the celery seeds used in the United States are grown in Holland and France, where this disease is most destructive. Since this is apt to continue to be one of our chief sources of seeds, it is a question of first importance to determine whether or not the causal organism is seed borne.

Klebahn has found pycnidia of Phoma apiicola on the seeds and suggests that seed distribution is important. Quanjer and Slagter as stated before incline to the belief that infection from the seeds is exceptionable.

This latter view seems to fit in well with the facts as observed in Michigan. The disease has, no doubt, been

introduced from Europe on the seed or on trash present with the seeds, but the occurrence of the fungus on seeds is probably rare. Examination of French, English, and Dutch grown seeds has not shown pycnidia to be present. The infrequency with which the disease is found in the seed bed indicates that in the case of seeds shipped to America from infested European countries seed distribution does not commonly occur. While not often carried on the seeds, sporadic outbreaks of the disease in one or two instances indicate that the seeds in some cases may be heavily inoculated. It is probable that seed carriage is important in introducing the disease into new territories and in occasionally being the source of bad seedling infections, but as a means of annual distribution of the disease, it is probably of minor importance.

CONTROL.

Because of the fact that plants are attacked at a point located below the surface of the soil, none of our common sprays which are used to fight other diseases of the celery plant are effective against this disease. Control measures must be based largely on certain relations of the disease to environmental conditions and on the application of sanitary principles. In applying such measures it is well to keep the following points in mind: (1) While some varieties of celery are more or less resistant, none are known to be wholly immune; (2) The disease may occasionally be seed borne; (3) low temperature and high moisture content in the soil favor the production of disease; (4) attacks are most severe in the spring and fall; (5) greatest injury comes from attacks on small plants; (6) older plants are not so seriously affected; (7) trash of the greenhouse and field are important sources of infectious material.

It is out of the question to rely on any of our common varieties to resist the attacks of the fungus. The varieties of best quality seem to be most susceptible to the disease, and some of the poorest quality seem to be

most resistant, but not enough difference has been found to justify the grower in sacrificing quality for resistance.

The possibility of the disease being seed borne leads to the question of seed treatment and sources of contaminated seeds. Phoma root rot has not been reported from any seed producing section of the United States. If it is present in any of these districts it is evidently causing little damage and chances of seed infection would be very small. European grown seeds are more apt to carry infection and treatment of such seeds may prove to be a good insurance measure against introduction of the disease into the seed-bed.

A low temperature, combined with a high moisture content in the soil, is necessary if the disease is to become of economic importance. In seed beds where conditions can be controlled as in the greenhouse, we believe that it would be possible to check the disease and in many cases prevent infection by limiting the water supply and keeping the temperature relatively high; but, since the seedling stage is the critical one for infection, it is dangerous to rely on this treatment. If seedlings of the first crop are kept free from infection until they are transplanted to the field, it is believed that in an average year the disease, caused by

infection from field sources, is not capable of producing a great amount of destruction before its progress is arrested by the warm weather of summer. It is far safer to keep watch of the seed bed and if the disease appears change or sterilize the soil. In the greenhouse, if the soil is changed, the benches should be drenched with a formaldehyde solution (1-40). This strength of solution has been found to be very effective in killing spores and mycelium of the causal organism when this fungus was grown in sterile muck. Drenching diseased soil with the same strength of solution would no doubt prove effective in destroying the fungus. Where facilities are available, steaming soil will pay. The organism is very sensitive to high temperatures and this method of freeing the soil from its presence should prove to be very satisfactory.

In the case of the fall crop, field infection may be a source of considerable loss. In dealing with this, it may be necessary to practice rotation and to remove all diseased trash from the crops of celery which are grown in the rotation. Under field conditions it is not know how long the causal organism may persist in the soil and trash, but removal of diseased parts from the field should decrease considerably the amount of infection. Crop rotation would seem to be a very effective method of control in sections where land is available and celery can be replaced by crops

equally valuable. In districts where rotation is not practiced it is believed that the removal of diseased trash and the use of disease free plants as mentioned above, will hold the disease in check and insure the grower a crop comparatively free from this type of root rot.

SUMMARY.

Phoma Root Rot is a disease of celery and celeriac known both in Europe and America and is caused by the same fungus (Phoma apiicola) in both cases.

The fungus also attacks parsley (Carum petroselinum), parsnip (Pastinaca sativa), carrot (Daucus carota), and caraway (Carum carvi).

The disease comes in on the crown of plants and causes dead leaves at the base, "stunting", and a pinching off near the surface of the soil.

The causal organism requires a relatively low temperature, (optimum about 18°C) and abundance of moisture and a large supply of oxygen for best growth.

The disease reaches its maximum of destructiveness in the spring and fall, the hot weather of midsummer checks the advance of the fungus and gives a clean crop so far as this trouble is concerned.

Overwintering is known to take place in the trash of the greenhouse and in the trash of the field.

Control measures recommended, the use of disease free plants and the destruction of trash, which harbors the pathogene or when the disease is severe the rotation of crops.

BIBLIOGRAPHY.*

Coons, G. H. Annual Report State Board Agr. p. 28, 1917.

Coons, G. H. A Phoma Disease of Celery. Report Mich. Acad. Sci. p. 444. 1918.

Halsted, B. D. Ann. Rept. N. J. Agr. Exp. Sta. p. 253, 1891.

Klebahn, Von H. Krankheiten des Selleries. Zeitschr. für Pflanzenkrankheiten. 20: 17-39. 1910.

Quanjer, H. M. and N. Slagter. De Roest of Schurftziekte van de Selderie knol en Enkele Opmerkingen over Andere Selderieziekten. Tijdschr. over Plantenzeikten. 20: 13-27. 1914.

Van Hook, J. M. Celery Root Rot. Ohio Agr. Exp. Sta. Cir. 72. 1907.

*This bibliography contains only those references which bear directly on the disease in question, others are given simply as footnotes.

Plates L-XII

Plate I.

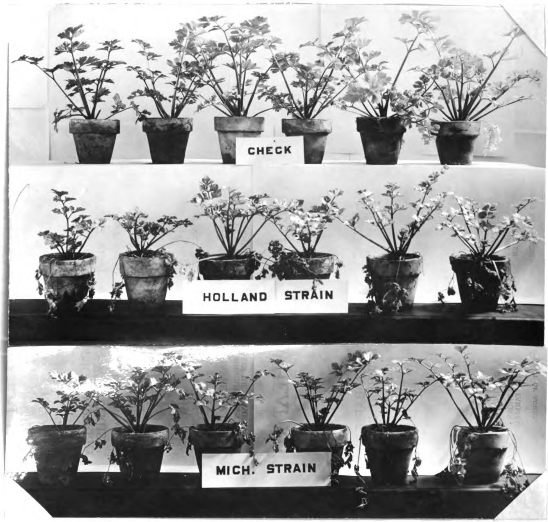

Fig. 1. Golden Heart Celery inoculated with Phoma apiicola from Holland and Michigan.

Plate II.

Fig. 2. Diseased celery plants.

Inoculated artificially with mycelium

PLATE III.

Fig. 3. Diseased (left) and undiseased (right) plants of the Same age (natural size).

Fig. 4. Diseased plant (natural size).

PLATE IV.

Fig. 5. Diseased spots on Celery leaves. These leaves were inoculated by spraying with germinated spores. Infection took place under a bell-jar.

PLATE V.

Fig. 6. Diseased Crown (Enlarged 6 times).

Pycnidia are shown on the base of one of the leaf petioles.

PLATE VI.

Fig. 7. Effect of Oxygen on root attacks.

The plant at the left was not inoculated; the one in the middle was inoculated with spores and supplied no oxygen; the plant on the right was inoculated with spores and supplied oxygen from below. Note the scarcity of small roots and the diseased condition of the larger ones.

PLATE VII.

Fig. 8. Parsley artificially inoculated.

Fig. 9. Carrots (left) and parsnips (right) artificially inocula ted

PLATE VIII.

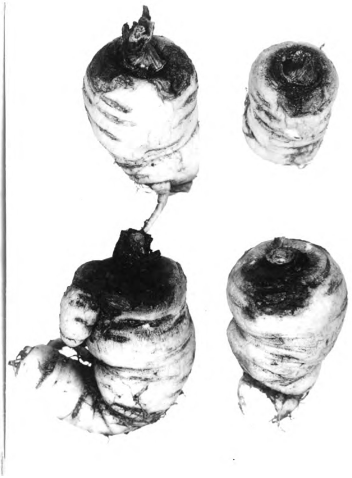

Fig 10. Showing type of lesions on parsnip caused by artifical inoculation.

PLATE IX.

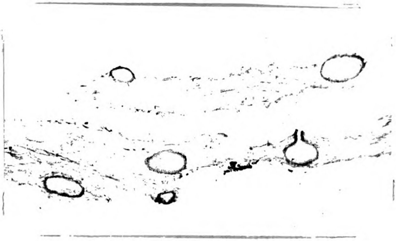

Fig. 11. Group of pycnidia from leaf petiole (magnified 150 times).

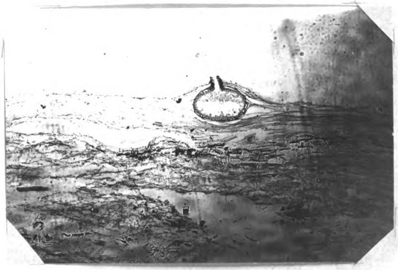

Fig 12. Pycnidiium imbedded in host tissue. (magnified 200 times).

PLATE X.

Fig. 13. Pycnidia on filter paper with corn meal broth (above) and Coons' synthetic (below).

Fig. 14. Growth on rice.

PLATE XI.

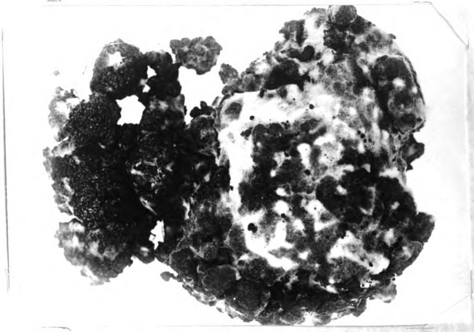

Fig. 15. Mycelium on sterile soil.

Fig. 16. Showing growth on celery agar at different temperatures. (The range of temperatures is given on page 31.)

PLATE XII.

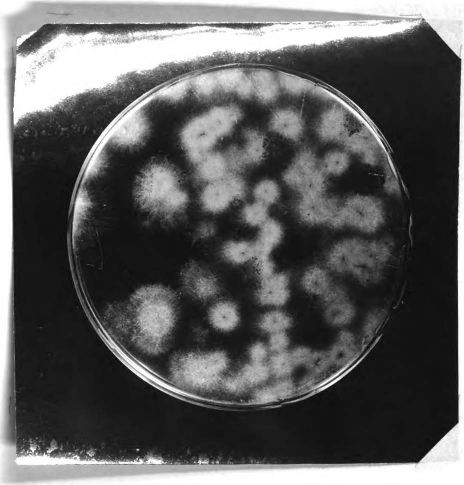

Fig. 17. Colonies on agar in Petri dish.
(Note rings of pycnidia).

PHOMA ROOT ROT OF CELERY

THESIS FOR DEGREE OF M. S.

C. W. BENNETT

1920