PHYTOPHTHORA DISEASE OF GINSENG

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF CORNELL UNIVERSITY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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JOSEPH ROSENBAUM

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THE HOST

The American ginseng, *Panax quinquefolium* L., is a member of the family Araliaceæ. It was brought under cultivation about twenty years ago, but either the same or a closely related species has been cultivated in Korea for more than two centuries.

According to Jartoux (1714)[2], ginseng is a native of the North Temperate Zone. It grows in rich, damp soils, such as prevail in hardwood forests. The Chinese ginseng is found principally between the 126th and 136th meridians, east longitude. The American species has about the same range of latitude, but extends farther south. The natural environment of the plant indicates three factors as favorable to its growth, namely, shade, good drainage, and an acid soil. The failure of the grower to take these factors into consideration when removing the ginseng plant from its natural habitat to his gardens has been primarily responsible for most of his losses.

In 1821 (U. S. Secretary of the Treasury, 1822) 352,992 pounds of ginseng were exported from this country and sold for $171,786, an average of nearly 49 cents a pound. In 1913 (U. S. Department of Commerce, 1914), the exports amounted to but 221,901 pounds. This was sold for $1,665,731, an average of about $7.50 a pound. This falling off in the number of pounds exported since the first shipments must be attributed in part to the diseases that attack this crop. It is becoming a common opinion among ginseng growers that the men who would grow ginseng successfully must first know all about the diseases to which the plant is subject.

THE DISEASE

DISTRIBUTION

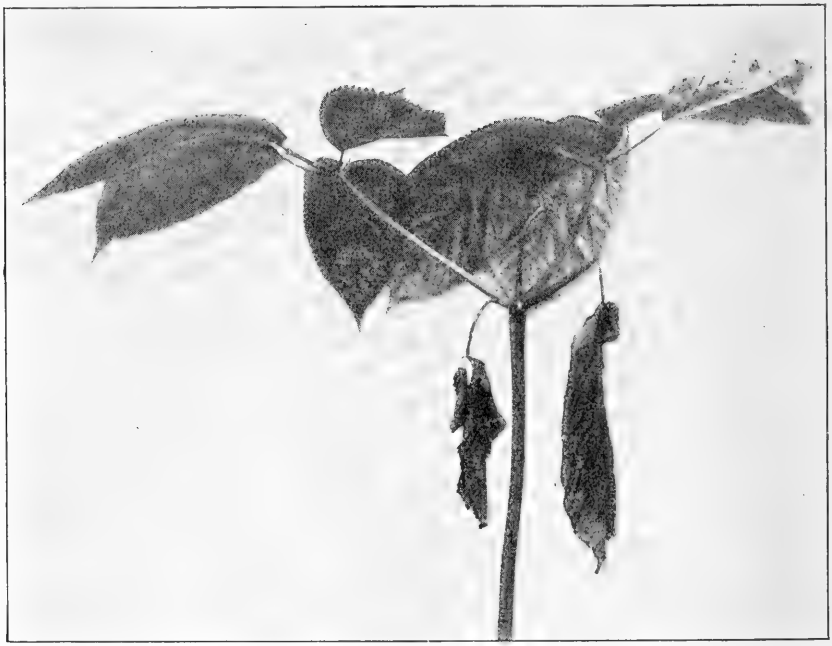
The disease known as mildew, Japanese mildew, or soft rot, attacks leaves, stems, and roots of the host. In this country it probably exists in every State in which ginseng is grown — Washington, Oregon, Nebraska, Kansas, Minnesota, Missouri, Arkansas, Wisconsin, Michigan, Indiana, Ohio, New York, Pennsylvania, New Jersey, and Maryland. It is destructive in Japan also, where it was first reported by Hanai (1900). It is known there as *Koshi-ore*, meaning bending at the loins.

[1] In making these investigations, the author had the cooperation of W. A. Orton, in charge of Cotton and Truck Disease Investigations, United States Bureau of Plant Industry. The author wishes to acknowledge also the many suggestions received during the progress of the work from Dr. Donald Reddick and Professor H. H. Whetzel, of the Department of Plant Pathology, Cornell University.

[2] Dates in parenthesis refer to bibliography, page 105.

HISTORY AND ECONOMIC IMPORTANCE

Hori (1907), who was the first to study this disease rather carefully, states that it has long been known to Japanese ginseng growers. In the United States the disease was first discovered on ginseng in the State of Ohio by J. M. van Hook (1906), and it has since been observed more or less commonly in the ginseng-growing regions of the United States. Because of its general occurrence in ginseng gardens in many States, and the fact that it attacks all parts of the plant, both above and below the ground, it forms one of the most serious disease problems that confront the grower.



PHOTOGRAPH BY WHETZEL

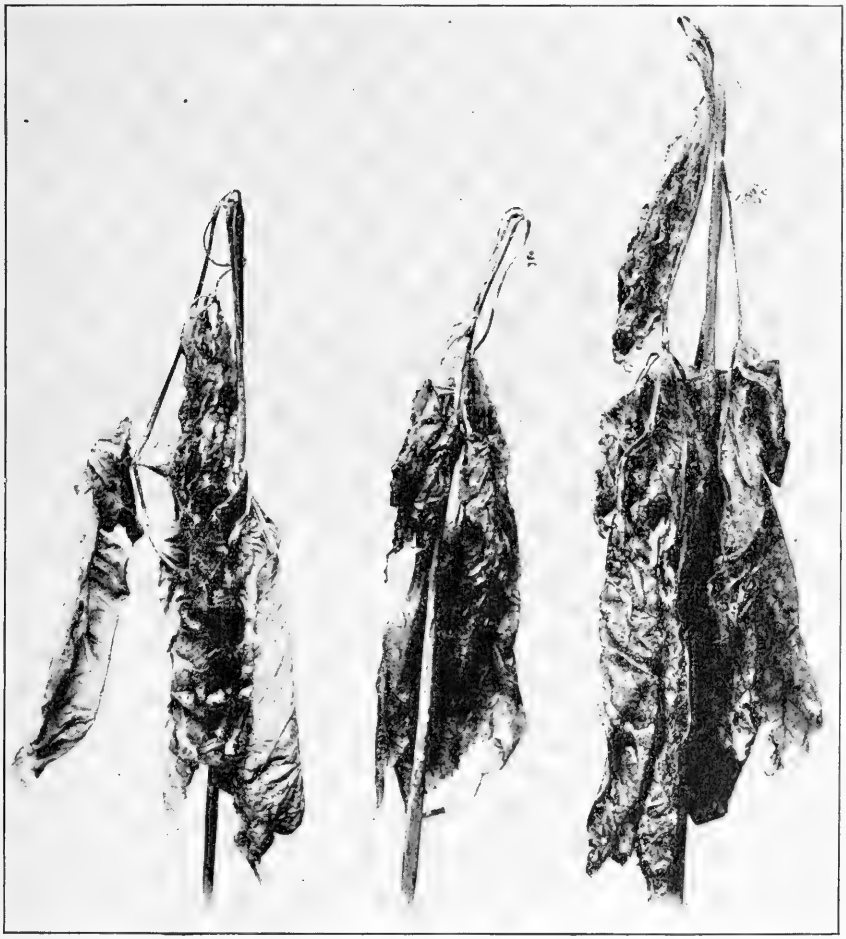
FIG. 2. CHARACTERISTIC SYMPTOM OF GINSENG MILDEW
One or more leaflets droop and hang limp and shriveled

Hori (1907) reports a loss of $25,000, due to the disease, in one province of Japan in the spring of 1904. From observations by the writer in ginseng gardens for the past four summers, it is safe to say that in the eastern United States from twenty to thirty per cent of the plants are lost through the attacks of the disease before they reach the age of five or six years.

SYMPTOMS OF THE DISEASE

The tops of the plants are affected in a characteristic manner. Usually there is a drooping of a single one or all of the leaflets at the top of the

petiole (Fig. 2). It happens in many cases that the disease attacks the main stem at the crown, or point where the leaf petioles are attached, and all the leaves droop and hang limp from the top of the stem (Fig. 3). Some other ginseng diseases exhibit similar symptoms, and microscopic



PHOTOGRAPH BY WHETZEL

FIG. 3. SYMPTOM OF GINSENG MILDEW IN LATE STAGE
The stage here shown is much later than that shown in figure 2. All the leaflets hang shriveled and dry

examination and identification of the causal organism is often necessary in order to determine definitely what disease is present. The tissues at the point of infection are rapidly injured and lose their turgidity, and the leaflets hang limp from the petiole.

The leaf blades also show characteristic lesions or spots. The spots appear dark green and water-soaked, much like those of the Alternaria blight in its early stages. A week or two after the first appearance of the spot, the center becomes white, the margins remaining a dark water-soaked green. The spots vary in size from one centimeter in diameter to lesions involving the entire leaf. The demarcation between diseased and healthy tissue is not sharply defined. The spotting shows on both sides of the leaf, but in general the differently colored regions within the spot are better shown on the upper surface (Fig. 4).

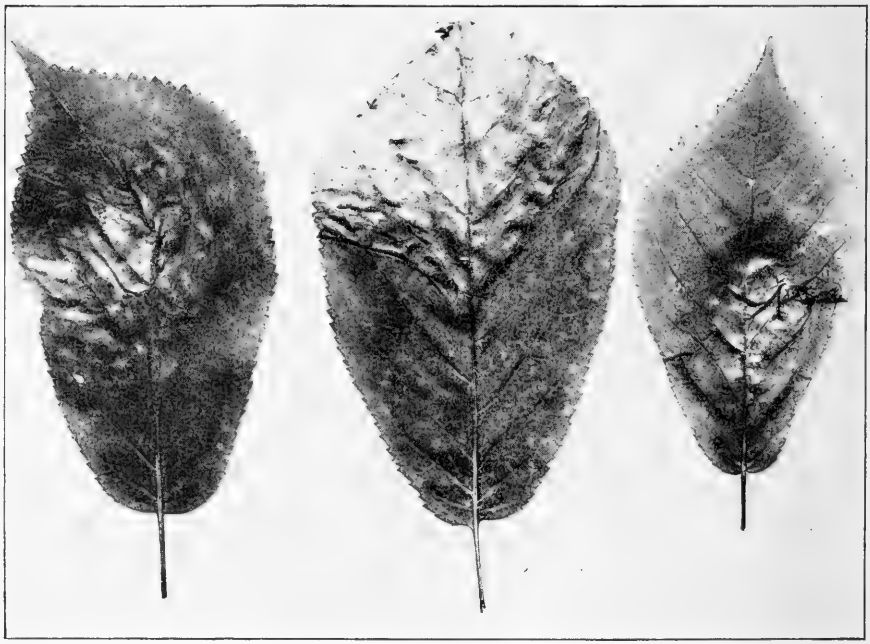


FIG. 4. LESIONS OF GINSENG MILDEW ON LEAVES
The water-soaked margin of the lesion, particularly in the leaflet at the right, should be noted

In the early stages of the disease, especially on the stalk or the petiole, the surface of the affected parts may show an almost indiscernible silvery white coating. During periods of sunshine the diseased tissues dry up quickly, leaving the dead and shriveled leaves at the top of the stem.

Under such conditions the disease does not spread down the stem with great rapidity. If atmospheric conditions remain moist and cloudy, the entire stalk is gradually involved. On pressing a diseased stalk between thumb and forefinger it is found that, in contrast with the ordinarily firm stalk, the diseased one is hollow. The hollowing of the stem is preceded by a watery discoloration of the tissues.

The roots may be attacked, showing a semi-soft rot. The lesion may start at any point on the root and in a short time involve all the tissues. When such roots are allowed to remain in the soil for any considerable

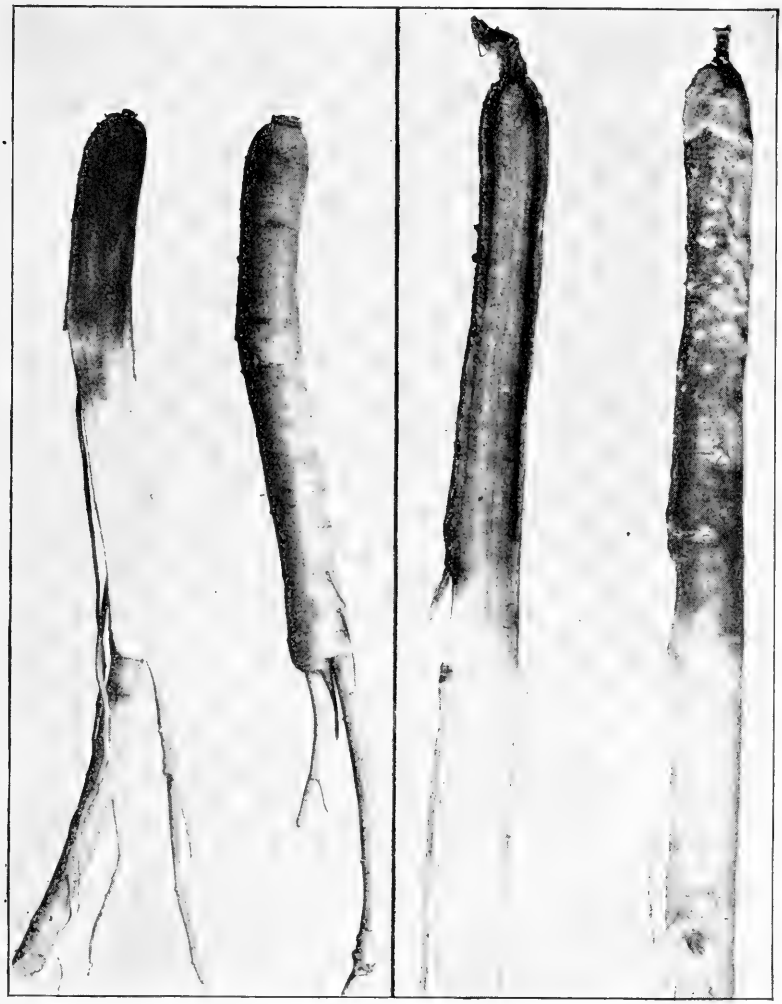


FIG. 5. SYMPTOM OF GINSENG MILDEW ON ROOT

The two roots have been cut longitudinally and show external and internal appearance. The root on the right was kept in a moist chamber for two days, and the external view shows well the growth of mycelium on the surface

time, various organisms, such as Fusaria and bacteria, invade the diseased tissues, causing them to become soft. At this stage the disease is often accompanied by a disagreeable odor characteristic of vegetable decays.

In some cases the disease starting in the root spreads up the stem. Roots cut longitudinally, showing the characteristic rotting, are illustrated in figure 5.

When the disease originates in the root and does not extend rapidly into the stem, the leaves may take on various shades of yellow and red, resembling the colors that they naturally assume toward the close of the growing period. Such discoloration of the tops in the early part of the season, however, while indicating some pathological condition, is not to be associated necessarily with the Phytophthora rot. Any disturbance in the functions of the root may cause such discoloration, and this condition may occur in the case of other rots, as, for example, the Sclerotinia white rot (*Sclerotinia libertiana* Fckl.).

SYMPTOMS OF OTHER DISEASES WHICH MAY BE CONFUSED WITH PHYTOPHTHORA

The symptoms of a number of other ginseng diseases may be confused with those of the Phytophthora disease. Such are the Alternaria blight, the Sclerotinia white rot, the Sclerotinia black rot, the Acrostalagmus wilt, and an undescribed fusarial rot.

ALTERNARIA BLIGHT

Alternaria blight is a very widespread disease caused by a fungus of the genus Alternaria, designated by Whetzel as species *panax*, although a technical description has not been published. It attacks stems, leaves, and roots of the host. The first symptoms in the spring appear as dark brown cankers on the stem near the surface of the soil. The spots on the leaves are similar in size to those caused by Phytophthora, but may be distinguished from the spotting caused by the latter in that they exhibit a broad, rusty brown border. The Alternaria lesions may also appear on the top of the plant as the point where the leaflets are attached to the petiole or where the petioles arise at the top of the stem, as in plants affected with the Phytophthora disease. The lesions are, however, readily distinguished from those of Phytophthora in that they exhibit a velvety brown coating at the point of attack. The Alternaria disease also occurs on the roots in the form of a dry rot. The tissues of the root are shrunken, and are darker in color and firmer to the touch than roots affected by Phytophthora.

SCLEROTINIA WHITE ROT

Inoculation experiments have proved that the disease known as Sclerotinia white rot is caused by *Sclerotinia libertiana* Fckl. Affected plants wilt and sometimes fall over. This is due to a rotting of the stem at its base, which usually involves also the crown of the root. The roots

become very soft and watery, but non-elastic. When placed in a moist chamber, an affected root invariably becomes covered with the white felt of the vegetative growth of the fungus. After a time numerous black sclerotia appear.

SCLEROTINIA BLACK ROT

The cause of the disease known as Sclerotinia black rot is *Sclerotinia panacis* Rankin.[3] The roots only are affected. When the disease is in an advanced stage the entire root, as the name indicates, is coal black. In the earlier stages the rot is in all external symptoms similar to that of Phytophthora; any dissimilarity before the root has turned black can be detected only by the aid of a microscope. Sections through tissue affected with the black rot disease show an abundance of dark brown mycelium.

ACROSTALAGMUS WILT

The disease known as Acrostalagmus wilt is said by van Hook (1904) to be caused by a species of Acrostalagmus. The roots are the only parts attacked. The first external symptom is a wilting of the tops. The leaves finally become dry and papery to the touch. The limp appearance of the top at once suggests the possibility of a lack of moisture in the soil. Externally the roots show no lesions, but cross sections of affected roots exhibit to the naked eye a brown ring in the region of the sap tubes.

FUSARIUM SOFT ROT

The disease called Fusarium soft rot is caused by a species of Fusarium and is the most likely of these diseases to be confused with Phytophthora rot of roots. Roots attacked by the Fusarium are softer, however, and are always accompanied in the last stages by a strong odor.

ETIOLOGY

The cause of the Phytophthora disease of ginseng is a fungous parasite, *Phytophthora cactorum* (Cohn et Leb.) Schröt. The genus Phytophthora was founded by de Bary (1876) on the potato blight fungus, *P. infestans*. The genus as it now stands includes more than a dozen species.

EARLY WORK ON ETIOLOGY

The history of the study of the organism associated with the disease can be summed up as follows: Hanai (1900:28) and Hori (1907:153) demonstrated the constant association of the Phycomycete with the lesions of the ginseng leaves. Hori, in the article cited, makes the following statement: "Since this decaying process proceeds downward to the

[3] Recent inoculations indicate that *Sclerotinia panacis* Rankin is probably identical with *Sclerotinia smilacina* Durand.

roots, the entire plant begins to wilt and drops to the ground." No experimental evidence is furnished to show that the roots really rot, or that when this does occur it is due to the attacks of Phytophthora. Van Hook (1906) reports the constant presence of oöspores of the same fungus in the stems of ginseng. Whetzel (1910) appears to be the only one who has done any work on the pathogenicity of the fungus previous to the studies herein presented. In 1909 he made a series of inoculations, employing pure cultures of the Phytophthora isolated from ginseng, and reports that "in every case there was prompt infection, with the resulting lesions characteristic of the disease." Little stress is laid, however, on the Phytophthora causing injury to the roots. In a bulletin by Whetzel and the writer (1912) the following statement is made: "Observations in the gardens show that the roots of plants, the tops of which are killed by the mildew, invariably rot unless promptly removed and dried."

For the past four seasons the writer has been investigating the diseases of ginseng, studying for the most part the root rots. During the course of the work it has developed that a large proportion of the soft rot is due to Phytophthora. This bulletin presents a study of the life history and identity of Phytophthora as it exists on ginseng.

PATHOGENICITY

Several methods have been employed for isolating the fungus and growing it in pure culture. In 1914 the organism was obtained from roots sent from a number of places — Ithaca, Scott, and Alden (New York), Kutztown (Pennsylvania), Mentor (Ohio), and Cassopolis (Michigan). Isolation from diseased roots was successful only when made from roots on which the disease had just started. Later the lesion is invaded by other soil organisms to such an extent that isolation of Phytophthora is difficult or impossible.

In most cases in which the fungus was isolated directly from the root, bits of tissue from the inside of the root at the junction of the healthy and the diseased areas were placed in tubes of oat agar. In some cases isolations were made directly from the root by placing the root in a moist chamber and carefully transferring to bean pod plugs the surface growth of mycelium which appeared in from two to three days.

Pure cultures of the fungus are obtained most readily, not from the roots, but from the stems. The method of procedure is as follows: Diseased stems are immersed in a 1-1000 solution of mercuric chloride for from two to three minutes, the stems are split longitudinally, and tissue plantings are made on poured plates of oat or bean agar. In three or four days the fungus usually will have grown out from the bits of tissue,

and subcultures can be made to test tubes of oat or potato agar. Good results may also be obtained, in case the disease is in its early stages, by washing the stems in mercuric chloride and placing them in a moist chamber. Conidia of the fungus usually appear within a period of from twenty-four to thirty-six hours. Transfers made from the surface growth usually give pure cultures.

When the fungus is obtained in pure cultures it grows readily on a number of media. The writer has grown it on oat agar as made by Clinton, on Thaxter's hard potato agar, on corn meal agar, on sterilized ginseng stems, on a ginseng decoction, on bean pods, and on lima bean agar.[4] The growth on synthetic media has been very slight.

After the ginseng fungus was isolated and grown in pure culture it was used for inoculating healthy plants. The experiments in inoculation of the tops have extended over a period of four years, but it was only in the spring of 1913 that inoculation of the roots was attempted.

Inoculation of the tops was made in the following ways: (1) The stems and leaves were sprayed with a water suspension of conidia and swarm spores, an atomizer being used for the spraying. The plants were covered with a bell jar for four or five days. Check plants were treated in a similar manner, with the exception that conidia were not added to the water. (2) By means of a platinum needle a bit of the fungus was removed from a pure culture and placed in the crotch of the plant. The inoculum was covered with moist cotton. For checks, the crotches of other plants were likewise covered with moist cotton. (3) By the use of a flamed scalpel a slight injury was made in the crotch of the plant and the inoculum was placed in the cut.

Inoculation of the roots was made in the following ways: (1) The soil was removed from one side of the root. The root was slightly cut with a flamed scalpel and a bit of a pure culture of the fungus was placed in the cut. The checks were treated in a similar manner, but no fungus was placed in the cut. All the roots were then covered with soil. (2) Roots were placed in pots and were inoculated by watering with a solution containing conidia of the fungus. Previous to inoculation certain roots were punctured with a flamed needle. As checks, similarly treated roots were kept moist with water not containing the fungus. (3) Freshly dug roots were immersed in a solution of mercuric chloride for ten minutes,

[4] The media used were composed as follows:
Hard potato agar: 200 grams of potato, 20 grams of glucose, and 30 grams of agar, for every 1000 cubic centimeters of water.
Oat agar was made according to the directions given by G. P. Clinton in the Report of the Connecticut Agricultural Experiment Station for 1909 and 1910, page 760.
Corn meal agar was made according to the formula given by C. L. Shear and Anna K. Wood in Bulletin 252 of the United States Bureau of Plant Industry, page 15.
Lima bean agar: 100 grams of ground lima beans and 15 grams of agar for every 1000 cubic centimeters of water.
Bean pods: pods of ordinary string beans placed in test tubes with a small quantity of water and sterilized.

rinsed several times with sterile water, and placed in sterile test tubes. The inoculation was made in the test tube in much the same way as a subculture is made. The roots in the test tube were cut with a flamed scalpel. By means of a platinum needle bits of the pure culture were inserted into the cuts. The checks were likewise injured with the flamed scalpel, but no fungus was placed in the cuts. The test tubes were then placed in a moist chamber for from two to three days. Later this was found to be unnecessary.

Results of inoculations on tops, on roots placed in the soil, and on roots placed in test tubes, are shown in figures 6, 7, and 8. It should

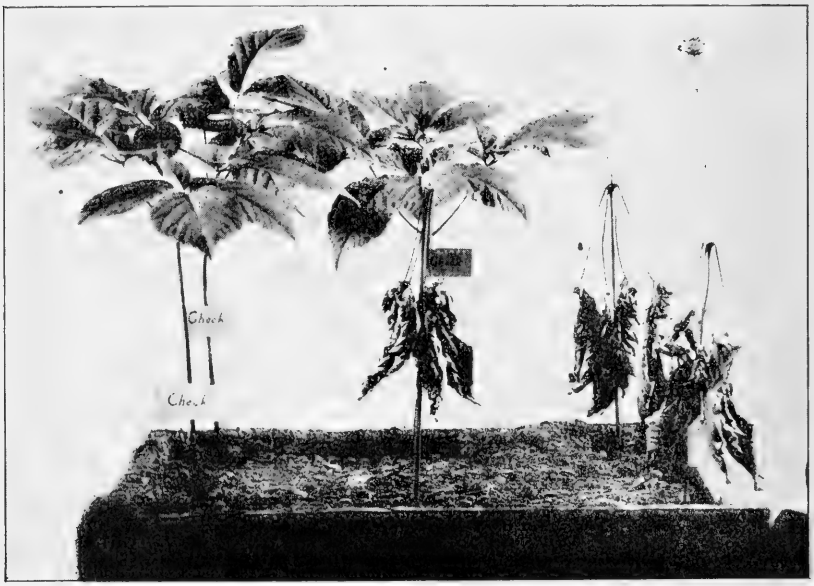


FIG. 6. GINSENG PLANTS INOCULATED WITH A PURE CULTURE OF PHYTOPHTHORA CACTORUM

be stated here that when plants were injured, either on the tops or on the roots, a higher proportion of infection was obtained than when the tissues were not injured. As far as root rot in the garden is concerned, however, this makes very little difference, for one seldom finds a root in the soil without some injury due to rodents, insects, transplanting, or other causes.

In all the inoculation work, in addition to the ginseng Phytophthora a culture marked *Phytophthora cactorum* was used. The latter was isolated by D. L. Peters, of Berlin, from Phyllocactus. This culture was found to be as pathogenic to ginseng as the Phytophthora isolated from ginseng.

An attempt was made to determine whether the use of the different organs of the fungus for inoculating — mycelium, conidia, or oöspores — made any difference in the ability to produce infection. These various organs were obtained from pure cultures of different ages and from different media. The mycelium was obtained from very young cultures on hard potato agar; the conidia were obtained from older cultures on the same medium, also from corn meal agar; the oöspores, from old cultures on bean pods. No doubt mycelium was present in every case. No difference in ability to infect was found. The period of incubation — that is, from the time when the parasite is placed on the host to the time when the first visible symptoms appear — is from three to five days on roots and from four to six days on tops.

FIG. 7. GINSENG ROOTS INOCULATED WITH A PURE CULTURE OF PHYTOPHTHORA CACTORUM

Growing roots two years old were slit with a scalpel and inoculated with a pure culture of Phytophthora cactorum. The root on the left was treated similarly but was not inoculated. The inoculations were made on August 4, 1913; the photograph was made seven days later

FIG. 8. GINSENG ROOTS INOCULATED WITH A PURE CULTURE OF PHYTOPHTHORA CACTORUM

Two-years-old roots of ginseng were disinfected, placed in sterile test tubes, and inoculated with a pure culture of Phytophthora cactorum. The root on the left was treated similarly but was not inoculated. The inoculations were made on May 15, 1913; the photograph was made ten days later

The length of time during which the fungus has been in artificial cultures does not seem to have any appreciable effect on its virulence. One culture isolated in the summer of 1911 was employed, and another isolated in the spring of 1913. They gave almost identical results in all the inoculation work. Wherever the rot was produced, it was always possible to make re-isolations by cutting out pieces of tissue at the boundary between the healthy and the diseased regions, and planting these on oat agar or bean pod

plugs. In a few instances the re-isolations were made by placing the rotted root in a moist chamber and then making plantings on oat agar from the mycelium appearing on the surface. Re-isolations from the tops were made by washing the stems for from two to three minutes in a 1-1000 solution of mercuric chloride, cutting them lengthwise, and making plantings from the interior on oat agar. The fungus obtained from such re-isolations was identical with the original culture, as regards both morphology, behavior on culture media, and ability to produce the disease. Koch's rules of proof were carried out for the Phytophthora isolated from ginseng, and also for *Phytophthora cactorum* from Phyllocactus.

In 1913 inoculations were made as shown in table 1. The percentage of infection obtained in all these inoculations leaves no doubt as to the conclusions to be reached. In 1914 a greater number of inoculations were made and the results obtained were almost identical with those of 1913.

TABLE 1. INOCULATIONS MADE IN VARIOUS WAYS WITH PHYTOPHTHORA CACTORUM FROM PHYLLOCACTUS AND FROM GINSENG. SUMMER OF 1913

Date	Source of organism used	Condition of plants at time of inoculation	Manner of inoculation	Number of plants inoculated*	Percentage of infection*	Number of plants used as checks†
April 19	Phyllocactus and ginseng	Four years old, beginning to push through soil	Tops sprayed with suspension of spores	3	66.6	1
April 19	Phyllocactus and ginseng 1911	Same as above	Plants not injured, inoculum placed in crotch	3 3	33.3 P.‡ 66.6G.‡	1
April 19	Phyllocactus and ginseng 1911	Same as above	Plants slightly injured, inoculum placed in crotch	3 3	100.0P. 66.6G.	1
April 19	Phyllocactus and ginseng 1911	Same as above	Soil removed, root injured, inoculum placed in injury	4	100.0	2
April 19	Phyllocactus and ginseng 1911	Same as above	Roots in test tubes	3	100.0	2

*The number of plants inoculated and the percentage of infection are the same for Phyllocactus and for ginseng unless otherwise stated.

† All checks remained healthy unless otherwise stated.

‡ "P." indicates percentage for Phyllocactus; "G.," percentage for ginseng.

TABLE I (*continued*)

Date	Source of organism used	Condition of plants at time of inoculation	Manner of inoculation	Number of plants inoculated*	Percentage of infection*	Number of plants used as checks†
April 19	Phyllocactus and ginseng 1911	Same as above	Roots placed in pots and watered with a suspension of inoculum	3	0	
April 19	Phyllocactus and ginseng 1911	Same as above	Same as above, but roots pricked	3 3	66.6P.‡ 33.3G.‡	1
April 20	Phyllocactus and ginseng 1911	Same as above	Roots in test tubes	3	100.0	2
April 20	Phyllocactus	Same as above	Tops sprayed with a suspension of inoculum	3	33.3	1
April 20	Phyllocactus	Same as above	Plants injured, inoculum placed in crotch	3	100.0	2
April 20	Phyllocactus	Same as above	Soil removed, root injured, inoculum placed in injury	3	100.0	2
April 20	Phyllocactus	Same as above	Roots in test tubes	3	100.0	1
April 20	Phyllocactus	Same as above	Same as above, but roots pricked	3	0	2
May 16	Phyllocactus and ginseng 1911	Five years old, full-grown in garden	Inoculum placed on leaves in drops of water	3	100.0	1
May 16§	Phyllocactus	Same as above	Same as above	3	100.0	1
May 27	Phyllocactus and ginseng 1913	Same as above	Same as above	3 3	100.0P. 66.6G.	1
May 27	Phyllocactus and ginseng 1913	Same as above	Tops sprayed with a suspension of inoculum	4 4	75.0P. 100.0G.	1

* The number of plants inoculated and the percentage of infection are the same for Phyllocactus and for ginseng unless otherwise stated.

† All checks remained healthy unless otherwise stated.

‡ "P." indicates percentage for Phyllocactus; "G.," percentage for ginseng.

§ Inoculations were made on the same day as the preceding ones, but either in a different garden or in a different part of the same garden.

TABLE 1 (*continued*)

Date	Source of organism used	Condition of plants at time of inoculation	Manner of inoculation	Number of plants inoculated*	Percentage of infection*	Number of plants used as checks†
May 27	Phyllocactus and ginseng 1913	Same as above	Plants slightly injured, inoculum placed in crotch	4	100.0	2
May 27	Phyllocactus and ginseng 1913	Same as above	Soil removed, root injured and inoculated	4	100.0	2
May 27	Phyllocactus and ginseng 1913	Same as above, but with roots removed	Roots in test tubes	4 4	75.0P.‡ 100.0G.‡	2
May 27	Phyllocactus and ginseng 1913	Same as above	Roots placed in pots and watered with a suspension of inoculum	3	0	1
May 27	Phyllocactus and ginseng 1913	Same as above, with roots pricked	Same as above	3	0	1
June 13	Phyllocactus and ginseng 1911	Four years old, roots removed from garden	Roots in test tubes	4	100.0	1
June 13	Phyllocactus and ginseng 1913	Same as above	Same as above	4	100.0	1
June 13	Phyllocactus	Same as above	Same as above	4	100.0	1
June 13	Phyllocactus and ginseng 1911	Four years old, plants standing in garden	Soil removed, root injured and inoculated	3	100.0	1
June 13	Phyllocactus and ginseng	Same as above	Same as above	3	100.0	1
June 13	Phyllocactus	Same as above	Same as above	3	100.0	1
June 14	Phyllocactus and ginseng 1911	Same as above	Plants slightly injured in crotch and inoculated	3	100.0	1

*The number of plants inoculated and the percentage of infection are the same for Phyllocactus and for ginseng unless otherwise stated.

† All checks remained healthy unless otherwise stated.

‡ "P." indicates percentage for Phyllocactus; "G.," percentage for ginseng.

TABLE I (*continued*)

Date	Source of organism used	Condition of plants at time of inoculation	Manner of inoculation	Number of plants inoculated*	Percentage of infection*	Number of plants used as checks†
June 14	Phyllocactus and ginseng 1913	Same as above	Same as above	3	100.0	1
June 14	Phyllocactus	Same as above	Same as above	3	100.0	1
June 14	Phyllocactus and ginseng 1911	Same as above	Soil removed, root uninjured, inoculum placed on surface	4 4	25.0P.‡ 0G.‡	1
June 14	Phyllocactus and ginseng 1913	Same as above	Same as above	4	0	1
June 14	Phyllocactus	Same as above	Same as above	4	0	1
July 1	Phyllocactus and ginseng 1911	Five years old, plants standing in garden	Tops sprayed with a suspension of inoculum	3 3	33.3P. 100.0G.	1
July 1	Phyllocactus and ginseng 1913	Same as above	Same as above	3	66.6	1
July 1	Phyllocactus	Same as above	Same as above	3	66.6	1
July 1	Phyllocactus and ginseng 1911	Same as above	Inoculum placed on uninjured leaves	3	100.0	1
July 1	Phyllocactus and ginseng 1913	Same as above	Same as above	3	100.0	1
July 1	Phyllocactus	Same as above	Same as above	3	100.0	1
July 1	Phyllocactus and ginseng 1911	Same as above	Soil removed, root injured and inoculated	4	100.0	1
July 1	Phyllocactus and ginseng 1913	Same as above	Same as above	4	100.0	1

* The number of plants inoculated and the percentage of infection are the same for Phyllocactus and for ginseng unless otherwise stated.

† All checks remained healthy unless otherwise stated.

‡ "P." indicates percentage for Phyllocactus; "G.," percentage for ginseng.

TABLE 1 (*continued*)

Date	Source of organism used	Condition of plants at time of inoculation	Manner of inoculation	Number of plants inoculated*	Percentage of infection*	Number of plants used as checks†
July 1	Phyllocactus	Same as above	Same as above	4	100.0	1
July 1	Phyllocactus and ginseng 1911	Five years old, roots removed from garden	Roots in test tubes	4	100.0	1
July 1	Phyllocactus and ginseng 1913	Same as above	Same as above	4	100.0	1
July 1	Phyllocactus	Same as above	Same as above	4	100.0	1
July 16	Phyllocactus and ginseng 1911	Three years old, plants standing in garden	Plants slightly injured, inoculum placed in crotch	3	100.0	1
July 16	Phyllocactus and ginseng 1913	Same as above	Same as above	3	100.0	1
July 16	Phyllocactus and ginseng 1911	Same as above	Roots not injured, inoculum placed on surface	3	0	1
July 16	Phyllocactus and ginseng 1913	Same as above	Same as above	3	0	1
July 16	Phyllocactus	Same as above	Same as above	3	0	1
August 1	Phyllocactus and ginseng 1911	Four years old, plants standing in garden	Soil removed, root injured and inoculated	4 4	75.0P.‡ 100.0G.‡	1
August 1	Phyllocactus and ginseng 1913	Same as above	Same as above	4	100.0	1
August 1	Phyllocactus	Same as above	Same as above	4	100.0	1
August 1	Phyllocactus and ginseng 1911	Four years old, roots removed from garden	Roots disinfected and placed in test tubes	3	100.0	1

* The number of plants inoculated and the percentage of infection are the same for Phyllocactus and for ginseng unless otherwise stated.

† All checks remained healthy unless otherwise stated.

‡ "P." indicates percentage for Phyllocactus; "G.," percentage for ginseng.

TABLE I (*continued*)

Date	Source of organism used	Condition of plants at time of inoculation	Manner of inoculation	Number of plants inoculated*	Percentage of infection*	Number of plants used as checks†
August 1	Phyllocactus and ginseng 1913	Same as above	Same as above	3	100.0	1
August 1	Phyllocactus	Same as above	Same as above	3	100.0	1
August 1	Phyllocactus and ginseng 1911	Four years old, plants standing in garden	Plants injured, inoculum placed in crotch	4	100.0	1
August 1	Phyllocactus and ginseng 1913	Same as above	Same as above	4	100.0	1
August 1	Phyllocactus	Same as above	Same as above	4	100.0	1
August 15	Phyllocactus and ginseng	Same as above	Plants not injured, inoculum placed in crotch	4	0	1
August 15	Phyllocactus	Same as above	Same as above	4	0	1
August 15	Phyllocactus and ginseng	Three years old, plants standing in garden	Tops sprayed with a suspension of inoculum	3	0	1
August 15	Phyllocactus	Same as above	Same as above	3	0	1
September 3	Phyllocactus and ginseng 1913	Same as above	Roots not injured, inoculum placed on surface	4	0	1
September 3	Phyllocactus	Same as above	Same as above	4	0	1
September 3	Phyllocactus and ginseng 1913	Same as above	Roots injured, inoculum placed on surface	3	100.0	1
September 3	Phyllocactus	Same as above	Same as above	3	100.0	1

* The number of plants inoculated and the percentage of infection are the same for Phyllocactus and for ginseng unless otherwise stated.

† All checks remained healthy unless otherwise stated.

TABLE I (*concluded*)

Date	Source of organism used	Condition of plants at time of inoculation	Manner of inoculation	Number of plants inoculated*	Percentage of infection*	Number of plants used as checks†
September 3	Phyllocactus and ginseng 1913	Three years old, roots removed from garden	Roots placed in pots and watered with a suspension of inoculum	3	0	1
September 3	Phyllocactus and ginseng 1913	Same as above	Same as above, but roots pricked	3	0	1
September 3	Phyllocactus and ginseng 1913	Same as above	Roots disinfected and placed in test tubes	4 4	50.0P.‡ 100.0G.‡	1
September 3	Phyllocactus	Same as above	Same as above	4	50.0	1
October 2	Phyllocactus and ginseng 1913	Four years old, roots removed from garden	Same as above	3	100.0	1
October 2	Phyllocactus	Same as above	Same as above	3	100.0	1
October 2	Phyllocactus and ginseng 1913	Same as above	Roots placed in pot, injured, inoculum placed on surface	3	100.0	1
October 2	Phyllocactus and ginseng 1913	Same as above	Same as above but not injured	3	100.0	1
October 2	Phyllocactus and ginseng 1913	Same as above	Roots placed in pot and watered with a suspension of inoculum	3	0	1
October 2	Phyllocactus and ginseng 1913	Same as above	Same as above, but roots pricked	3 3	33.3P. 0G.	1

* The number of plants inoculated and the percentage of infection are the same for Phyllocactus and for ginseng unless otherwise stated.

† All checks remained healthy unless otherwise stated.

‡ "P." indicates percentage for Phyllocactus; "G.," percentage for ginseng.

IDENTITY OF THE ORGANISM

EXAMINATION OF LITERATURE

A careful examination of the fungus from pure culture on various media shows that it resembles most closely *Phytophthora cactorum* (Cohn et Leb.) Schroeter (1889). Saccardo (1888) lists as synonyms of this species the following: *Peronospora cactorum* Cohn et Leb., *P. Fagi* Hartig, *P. Sempervivi* Schenk, *Phytophthora omnivora* de Bary. It may be well to briefly review here the history of the species.

Lebert and Cohn (1870) observed a rot on *Cereus giganteus* and *Melocactus nigrotomentosus*. From their description of the symptoms it appears that the nature of the disease was a dissolution and separation of the individual cells, resulting in a more or less soft rot. An abundance of mycelium, conidia, and oöspores was found. From the nature of the fungus Lebert and Cohn rightly determined that it was a Phycomycete, and described it as *Peronospora cactorum*. As far as they could determine, haustoria were lacking.

Hartig (1876:121) describes a fungus, *Peronospora Fagi*, as causing a disease of beech. A similar fungus was described the previous year by Schenk (1875), under the name *Peronospora Sempervivi*, found by him to be causing a disease of Sempervivum. De Bary (1881), in order to settle the identity of these forms — which he held to be identical — with little regard for priority or rules of nomenclature gave them collectively the name *Phytophthora omnivora*. Hartig (1882:42) wrote as follows in commenting on this change: " I accept this new name, since it behooves me above everything else to desire a name for the parasite that shall bear no false significance. Since I have, from the earliest time, observed that the disease attacks maple, pine, larch, and fir, I believe *omnivora* is better deserved than *Fagi*. In opposition to many systematists of later times who from their researches on priority have changed the customary name for another never accepted as valid by the science of the past one hundred years, I have been of the opinion that names existed, not for the authors, but for the scientific public." [5]

De Bary was aware of the work of Cohn and Lebert when he wrote that there was not a doubt in his mind that the two forms were identical. He proposed the specific name *omnivora* as better expressing the nature of the organism. Schroeter (1889:236), recognizing the work of Cohn and Lebert, adopted the original specific name *cactorum* and simply classed the fungus in the more recently formed genus. The name as it stands, therefore, is *Phytophthora cactorum* (Cohn et Leb.) Schroeter.

[5] Translation from the German.

Syn. *Peronospora cactorum* (Cohn et. Leb.). Bietr. biol. pflanz. **1**:51–57. 1870.
Peronospora Sempervivi Schenk. Bot. ztg. **33**:690–693. 1875.
Peronospora Fagi Hartig. Zeitsch. forst.- u. jagdw. **8**:117–123. 1876.
Phytophthora omnivora De Bary. Bot. ztg. **39**:585–595, 601–609, 617–626. 1881.
Phytophthora cactorum (Cohn et Leb.) Schroeter. Krpyt-fl. Schlesien **3**:235–236. 1889.

The following is the original description as given by Lebert and Cohn (1870):

"Mycelii tubi graciles nonnunquam torulosi ramosi, ramis angulo recto patentibus, haustoriis destituti. Stipites conidiophori tenues, in modum cincinni unilateraliter pauce-ramosi, sub apicibus ramorum conidiferis non raro vesiculoso-inflati. Conidia in stipitibus pauca hyalina, ellipsoidea vel ovata, apice papilla prominente munita majuscula = 0,048 mm. (1/28–1/15 mm.).

"Oogonia conglomerata membrana tenui marcescente munita, singula oosporam singulam exacte globosam episporio valido luteo-fusco pellucido laevi praeditam foventia, diametro = 0,024 mm. (1/40 mm.).

"Habitat in meatibus intercellularibus parenchymatis variorum Cactorum quorum morbum putredine quadam finitum efficit. Observ. hieme 1868–1869 in viridario excellentissimi ducis a Jacobi Vratislaviae."

Unfortunately it has thus far been impossible for the writer to examine the type material or to make isolations from the original host, and he is compelled to rely entirely on the above description and the descriptions of the later workers for the identity of this fungus. For comparison, a culture marked *Phytophthora cactorum* was obtained from the Bureau pour le Distribution de Cultures de Moisissures, of the International Association of Botanists in Amsterdam, with the information that it was isolated from Phyllocactus by D. L. Peters, of Berlin.

COMPARISON OF CULTURES

Since the Phytophthora of ginseng resembles the culture marked *Phytophthora cactorum*, a detailed comparison is here given.[6]

MACROSCOPIC GROWTH ON VARIOUS MEDIA

Phytophthora from ginseng and *Phytophthora cactorum* from Phyllocactus were grown on hard potato agar, oat agar, corn meal agar, bean pod plugs, and sterilized ginseng stems. Various synthetic media were also employed, but the growth on these was so small as to make them unsuitable for this work. On all the media no difference in the two forms was noticeable, either in rapidity of growth or in luxuriance of growth.

Hard potato agar.—A white, fluffy, aërial growth was produced in five or six days on hard potato agar. The growth was profuse.

[6] A comparison of the Phytophthora of ginseng was also made with nine other species of Phytophthora. The results of this study will be published in a subsequent paper.

Oat agar.—The growth on oat agar was profuse and was beneath the surface as well as at the surface, making a slightly yellowish, mealy growth.

Corn meal agar.—On corn meal agar there was a slight growth, mostly subsurface.

Bean pod plugs.—The growth on bean pod plugs was aërial as well as embedded in the tissues of the pod. The growth was white, but was less fluffy than on hard potato agar.

Ginseng stems.—On ginseng stems there was a very scanty surface growth.

KINDS OF SPORES PRODUCED ON VARIOUS MEDIA

The kinds of spores produced and the time of appearance of these are shown in table 2:

TABLE 2. KINDS OF SPORES PRODUCED ON VARIOUS MEDIA

At the end of two weeks

Organism	On potato	On oat	On corn meal	On bean pods	On ginseng stems
Phytophthora from ginseng	Numerous conidia	Oögonia, oöspores, conidia	Few conidia	Few oögonia	Numerous conidia, few oögonia, few oöspores
P. cactorum from Phyllocactus	Numerous conidia	Oögonia, oöspores, conidia	Few conidia	Few oögonia	Numerous conidia, few oögonia, few oöspores

At the end of six weeks

Organism	On potato	On oat	On corn meal	On bean pods	On ginseng stems
Phytophthora from ginseng	Numerous conidia	Conidia, few oögonia, numerous oöspores	Few conidia, few oögonia, few oöspores	Numerous oöspores and conidia	Numerous conidia, few oögonia, few oöspores
P. cactorum from Phyllocactus	Numerous conidia	Numerous conidia, few oögonia, numerous oöspores	Few conidia, few oögonia, few oöspores	Numerous oöspores and conidia	Numerous conidia, few oögonia, few oöspores

It was found in several series of the above that, while the time of appearance of the different spore forms may vary for the different strains of Phytophthora, eventually the same spore forms appear on a given

medium. Oat agar and bean pods are especially favorable for the production of the sexual bodies. In the case of bean pods, the conidia appear mostly on the aërial growth, while the oöspores are imbedded in the tissues of the pod.

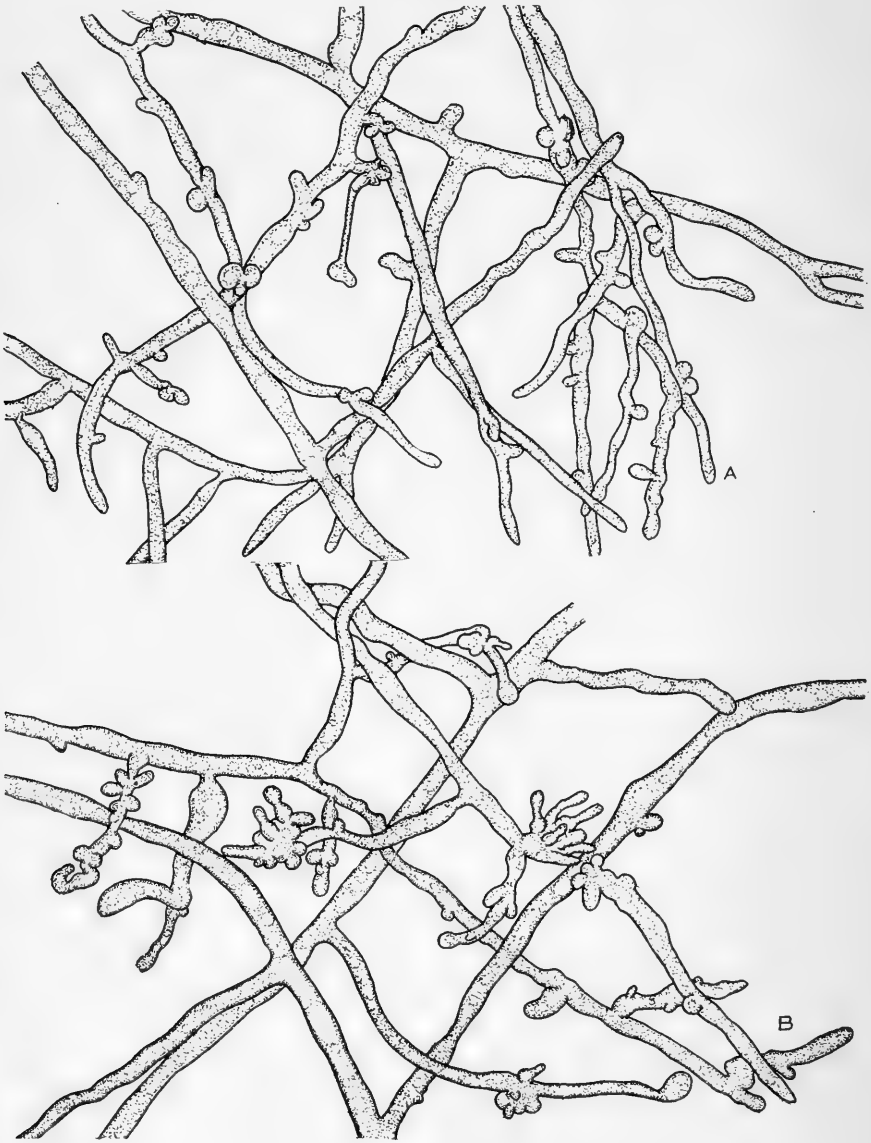


FIG. 9. MYCELIUM OF PHYTOPHTHORA, (A) FROM GINSENG, (B) FROM PHYLLOCACTUS
The drawings were made from mounts of mycelium of the two organisms growing on oat agar. No constant difference can be noted

COMPARATIVE MORPHOLOGY

The morphological studies were made from cultures of the same age, grown on the same medium. No differences were noted in the mycelium of the two cultures (Fig. 9). In very young cultures there is a slight tendency to branch at right angles, but there is such great irregularity in the mycelium that such can hardly be taken to be a constant character.

The conidia vary greatly in shape and size, but no difference between strains could be detected either when grown on the same or when grown on different media (Fig. 10). Over four hundred measurements were made for each form from cultures of different ages and grown on different

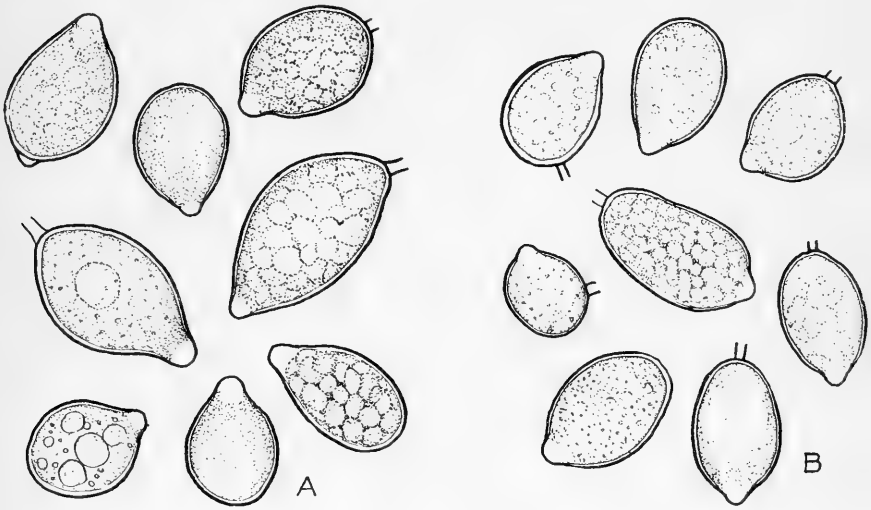


FIG. 10. CONIDIA OF PHYTOPHTHORA, (A) FROM GINSENG, (B) FROM PHYLLOCACTUS
The drawings were made from mounts of cultures of the two organisms growing on oat agar. Both drawings are made to the same scale

media. The measurements most commonly obtained are the same for the Phytophthora from ginseng and for *P. cactorum* from Phyllocactus, 34.5 x 27 μ.

Inoculations were made on freshly disinfected ginseng roots in test tubes and the two forms were re-isolated from the inoculated roots. Pedigreed single-spore cultures were then made, and conidia and oöspores were again measured.

The same differences appeared as were shown previously. Different workers have given different measurements, though working presumably with the same species. This is brought out in table 3, in which the measurements given by individual workers for the same species are presented.

TABLE 3. SHOWING VARIATION GIVEN BY DIFFERENT WORKERS FOR PHYTOPHTHORA CACTORUM

Author	Measurements of conidia (micromillimeters)	Diameter of oöspores (micromillimeters)
Cohn and Lebert	48 x 35–68	20–70
Hartig	25–40	
Schenk	*	20 (oögonium)
De Bary	35–40 x 50–60–90	16–24†
Schroeter	35–40 x 50–60	24–30
Osterwalder	14.64–24.4 x 119.56	24 (oögonium)
Himmelbaur	Not given	30–45
Zimmerman	17–30 x 25–60	None found
Hori	30–50 x 50–60 Abnormal 29 x 85.5	26–28
Bubàk	15–25 x 15–120	Not given
Van Hook	30–42 x 40–58	Not given
Author	34.5 x 27	27

* Measurements of conidia given by Schenk are as follows: "Die kleinsten derselben sind 5, die grössten 36 Theilstriche meines Zeiss'schen Mikrometers lang und 4 bis 25 Theilstriche breit."

† De Bary states that the oöspores are in general from three-fourths to four-fifths the diameter of the oögonium. He gives the measurements of the latter as from 24 to 30 microns in diameter. The measurements given above for the diameter of the oöspores were derived accordingly.

It is thus shown that great variations may occur, apparently in the same species. De Bary, knowing of these variations as given by Lebert

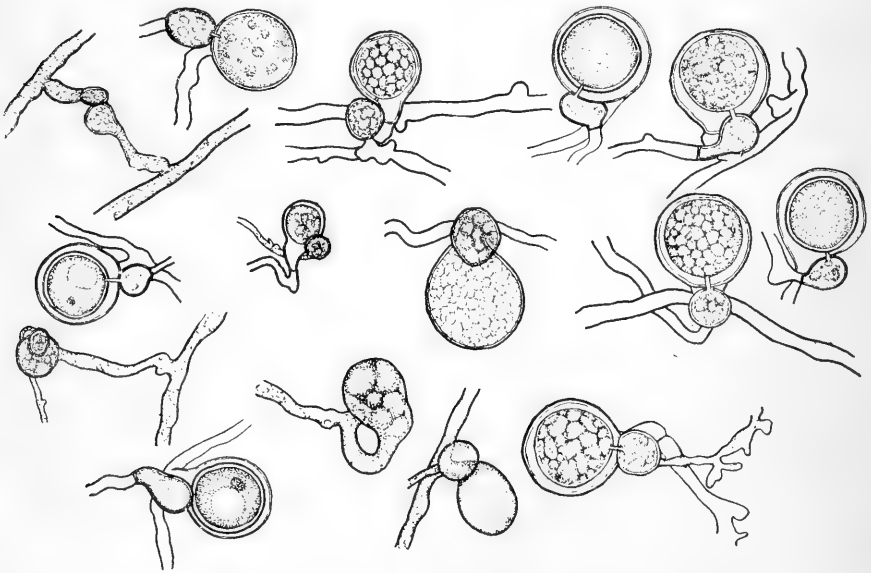


FIG. 11. THE SEXUAL PROCESS IN PHYTOPHTHORA FROM GINSENG

Various stages are shown in the development of antheridium, oögonium, and oöspores. Fertilization tubes are very evident in a number of cases

and Cohn, by Hartig, and by Schenk, did not hesitate to place all forms in a single species. Likewise, Osterwalder (1906), notwithstanding the fact that his measurements do not agree with the others, does not consider this of sufficient importance to establish a new species.

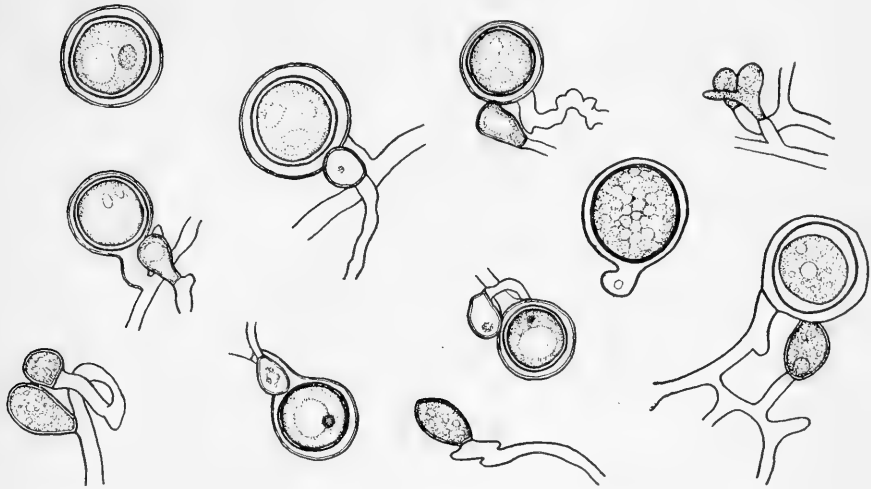


FIG. 12. THE SEXUAL PROCESS IN PHYTOPHTHORA FROM PHYLLOCACTUS

More than four hundred measurements of oögonia were made for each form. Measurements of oöspores taken from various media, and the cultures of different ages, show this spore form to be very constant. The measurements of the two forms are identical, being 27μ in diameter. The method of fertilization likewise does not differ in the two forms (Figs. 11 and 12).

A summary of the above comparisons, taken together with the results of the inoculations as shown previously, proves that the Phytophthora isolated from ginseng is identical with the culture marked *Phytophthora cactorum* (*Cohn et Leb.*) *Schroeter* isolated from Phyllocactus.

LIFE HISTORY

Careful studies of the fungus have been made in order to determine its various relations to its host and the morphological characters of the parasite itself. These studies have been made with fresh material from the garden and with pure cultures on various media. Plants which early in the spring show characteristic drooping usually exhibit no external evidence of the fungus. They show a slight shrinkage and browning of the tissues of the stem, and often the shrunken tissue appears water-soaked. If the stems are placed in a moist chamber for a day, a silvery

white coating of conidia appears, characteristic of Phytophthora. Microscopical examination shows an abundance of the large ovate conidia of the pathogen. An excess of moisture in the chamber will force the fungus to the production of a large amount of mycelium with little conidial formation. In a few cases, by taking a microscope directly into the field the writer was able to find conidia on the surface of the stems. This was the case during a continuance of from two to three days of warm, muggy weather, accompanied by dews in the mornings.

The conidia are spread by the wind or by other usual means of dissemination; very often, no doubt, by the bodies of the workers while weeding. One who knows the conditions in a ginseng garden can readily understand how such is the case, since the weeders get down on their hands and knees. The rows, and the individual plants in a row, are so close together that it is almost impossible not to disseminate the spores when these are present. A spore falling on a healthy plant, under the right conditions of temperature and moisture, germinates, and within from four to six days infection becomes apparent.

When a plant is infected in the tops, the fungus travels from the petioles into the main stem, through that, and into the root, where it rots the root. That the fungus travels downward is shown by the following experiment: On July 11, 1913, twelve plants were inoculated in the tops with a pure culture of the fungus. Six other plants were used as checks. Half of the plants inoculated were injured by means of a flamed scalpel and the inoculum was placed inside the cuts; for the uninjured plants the inoculum was placed at the base of the petioles and covered with moist cotton. The check plants were treated in a similar manner, both as regards injuring half of them and covering the others with moist cotton, but the inoculum, of course, was not placed on them. Four days later (on July 15) the moist cotton was removed. On the same day all the plants injured and inoculated showed the characteristic drooping of the leaflets from the crotch of the plant. Within three days more four of the uninjured inoculated plants likewise showed the characteristic wilting. All the checks, whether injured or not, remained healthy. On July 20 it was apparent, by the hollowing out and discoloration of the stems, that the fungus was traveling downward. On pressing a stalk between thumb and forefinger, it was found that the ordinarily firm stalk felt hollow in the region of the discoloration. By marking the point of discoloration on the stem with india ink, it was ascertained that the fungus traveled about one-half to two-thirds centimeter each day, as indicated by the new limit of discoloration. On August 1 the stems were hollow for the entire distance to the ground. On that day one of the hollow stems was examined microscopically, mounts of tissue taken

from the inside being used, and oöspores were found in great abundance. The oöspores were most abundant near the origin of infection, and gradually diminished in numbers as the distance from this point increased. Thus all developmental stages of the fungus were found on the inside of the stem — oöspores, oögonia, and mycelium. On August 7 the roots of some of the infected plants were completely rotted, and the crowns of others deeper in the soil were just beginning to rot. All the checks remained perfectly healthy during the entire time.

Similar sets of inoculations were made on August 1 and August 15, with identical results. On inoculated plants that were slightly injured, 100 per cent of infection was obtained in every case; on plants that were not injured, the percentage of infection was only from 25 to 50 per cent.

In the early stages of the rot in the root, mycelium may be found in the tissues. In the summer of 1913 an examination of a rotted root showed that one of the smaller rootlets contained numerous oöspores. In the fall of the same year, an inoculated four-years-old root was placed in a test tube filled with sterile distilled water. An examination of the root ten days later showed numerous oöspores. This experiment has since been repeated many times, but in all cases it has given negative results. In general, in the later stages of the rot the roots become soft due to the presence of soil organisms. When the roots are in this condition, nothing definite can be learned of the relation of the Phytophthora to the host.

The preceding data show how the fungus starts in the tops by infections from conidia and travels down through the stem to rot the root. It was found, however, that in many cases of artificial infection of the top, if the root was some distance below the surface of the soil the fungus did not attack it. In order to determine this point more definitely eighteen plants were carefully transplanted at varying depths in the soil. The depths used were $\frac{1}{2}$, 1, 2, 3, 4, and 5 inches below the surface, three roots being planted at each depth. The depth at which roots are commonly found varies from $\frac{1}{4}$ to 2 inches below the surface. The plants used as checks were likewise transplanted. After transplanting, sufficient time was allowed for the plants to establish themselves before inoculations were made. The plants were inoculated by slightly injuring the tops and placing the inoculum in the injured parts. As before, the fungus traveled down the stems, this being made evident by a discoloration and hollowing of the stems. In the case of roots $\frac{1}{2}$, 1, and 2 inches below the surface, all the roots rotted; of those planted 3 inches below the surface, one root rotted and two remained healthy; while of those planted 4 or 5 inches below the surface, none rotted. The experiment was repeated, but instead of transplanting plants at various depths the stems

of a number of plants were covered to various heights with soil. In these experiments, while the number of plants rotting at each depth did not correspond with the former, the experiments did agree in that they showed a correlation between the number of roots rotting and the depth at which they were planted. They also showed that in case of infections of plants of the same age and with stems of approximately equal length, the fungus took much more time to reach the root in the case of those stems that were deepest in the soil. These facts are suggestive in considering the control of the disease in the garden.

It was supposed that the fungus might pass from the tops to the roots by conidia being washed down into the soil by rain water, and that those coming into contact with the tissues of the roots would produce infection. Numerous experiments were performed in order to determine this point, by planting roots at various depths in both heavy and light soils and drenching the plants with water containing conidia of the fungus. Conflicting results only were obtained, and in no case were there any signs of infection unless the roots had previously been injured. Infection by this method, if it occurs at all in nature, is of relatively slight importance.

The primary infection may start in the tops or in the roots. When the roots are first attacked, the spread of the pathogen may be just the opposite from that already described. Instead of traveling down the stem and rotting the root, the fungus rots the root and continues its course upward into the stem. Root infection may start from mycelium in the roots or from oöspores that have wintered in the soil. Hartig (1875) has shown that oöspores of the Phytophthora on beech, left in the ground for four years, are still capable of germination and infection of healthy plants. Repeated inoculations of ginseng roots placed in soil have shown that the fungus, after rotting the root, can travel upward (Fig 13). The following details of one series of these inoculations are offered: On June 15, 1913, six roots of four-years-old plants were inoculated with a pure culture of the fungus by removing the soil from one side of each root, making a slight incision near the top, or crown, of the root with a flamed scalpel, and placing a bit of inoculum at the point of injury. Four check plants were injured in a similar manner, but were not inoculated. On July 17 all the tops of plants with inoculated roots showed a characteristic drooping and evidence that the roots were rotting. An examination proved this to be the case. The tops were carefully removed, and mounts were made from the inside of the stem at different distances from the crown of the root, which was the original point of infection. The following conditions were found: Two centimeters above the crown the stem was hollow, and mounts showed an abundance of oöspores, oögonia, and mycelium. The tissues of the stem were brownish and water-soaked.

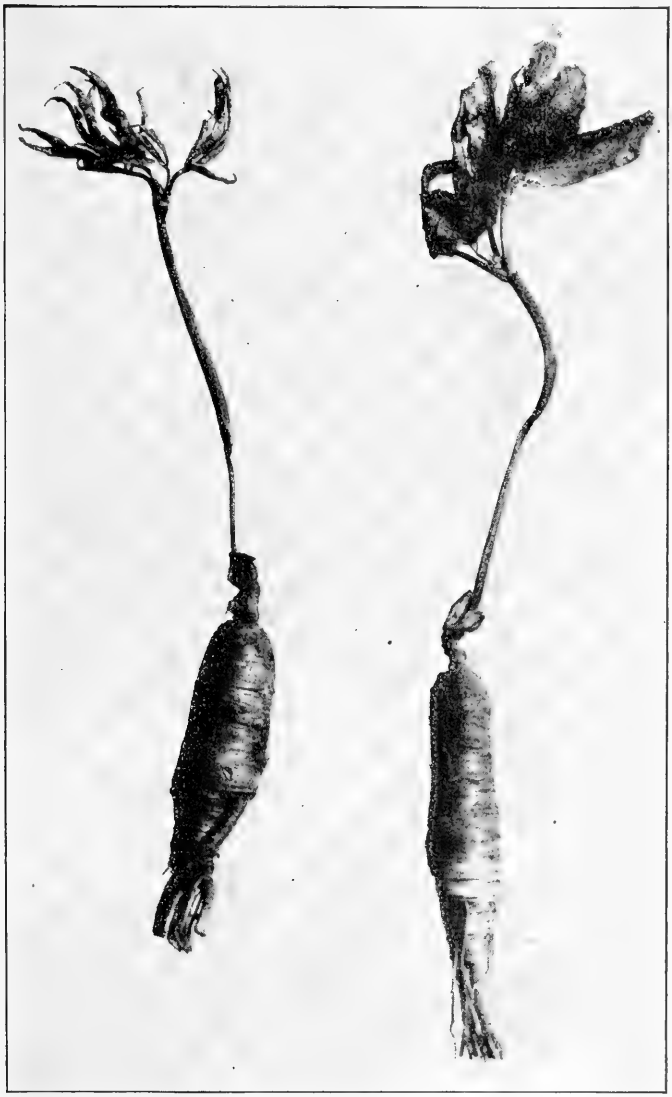


FIG. 13. CHARACTERISTIC SYMPTOM OF GINSENG MILDEW
The drying and shriveling of the stem near the crown of the root is an indication that the Phytophthora is spreading from the root upward

Five centimeters above the crown the stem was firmer to the touch, though still showing an abundance of oöspores, oögonia, and mycelium. The stem still presented a water-soaked appearance. Seven centimeters above the crown the stem was not hollow, but was still slightly discolored and mounts showed an abundance of mycelium. Evidently the mycelium at this point was not old enough for a differentiation into spore forms. Ten centimeters above the crown the stem was perfectly healthy and the microscopic examination showed no evidence of the fungus. In order to make sure that the mycelium and the spore forms seen belonged to Phytophthora, plantings from the interior of the stem from these various points were made on poured oat agar plates. The fungus obtained from these plantings was Phytophthora, identical with the fungus with which the roots had been inoculated.

MORPHOLOGY OF THE FUNGUS

MYCELIUM

The mycelium of the fungus in culture is very characteristic and can be distinguished readily as a Phycomycete if examined carefully. Septa, as a rule, are absent, except for an occasional one in very old cultures. The branching is irregular, and consists of threads of varying diameter, as well as knoblike and button-shaped protuberances. The protoplasmic contents are granular, intermingled with oil globules and other larger bodies. The larger bodies are found for the most part in mycelium of an advanced stage.

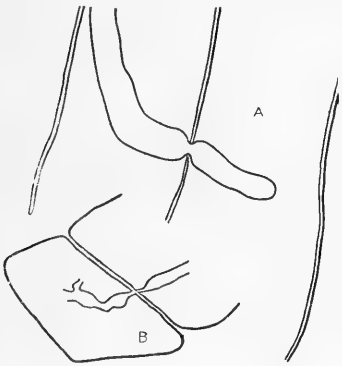


FIG. 14. PASSAGE OF MYCELIUM FROM CELL TO CELL

The mycelium is very much constricted at the point where it passes through the cell wall of the host. In B is shown a fragment of mycelium branching within the host cell. The drawing A was made with a 2-millimeter objective, B with a 4-millimeter objective

In plant tissue, especially in the root, the mycelium is scanty. The main branches are intercellular. Small branches are often seen to penetrate the cell walls. There is a constriction at the point of passage and a broadening out on each side of the cell wall (Fig. 14). In other words, the hyphæ are reduced to mere threads in passing through the wall. Hartig (1882) describes and illustrates the haustoria of *P. omnivora* as spherical. Klebahn (1909:75) and Coleman (1910:61), however, figure them as rather elongate and finger-like, in which case they agree closely with those of the Phytophthora of ginseng.

In making mounts of affected stems, it was found in many cases that where the conidiophores arose in great abundance the mycelial branches

had also penetrated within the cells. Morphologically the haustoria-like branches are not particularly specialized, since the conidiophores arise directly from them or from short inner branches extending from these swollen threads.

CONIDIOPHORES

The conidiophores arise from the mycelium within or between the epidermal cells. When about to produce conidiophores, the branches of the mycelium within these cells become slightly swollen at their tips. On emergence from the tissues, the swelling is immediately followed by a reduction in the diameter of the thread. The reduced thread pushes or dissolves its way directly through the cuticle and the cell wall. In many cases the conidiophores arise at the point of junction of two cell walls, but this is by no means always the case. The conidiophores, after passing through the small opening in the cell wall, may swell slightly and then grow into a long, almost straight stalk, which is usually not more than one-half or one-third the diameter of the hyphæ of the mycelium from which it arose. The conidiophores never exhibit the peculiar swelling just back of the point where a conidium is borne which is so characteristic of *Phytophthora infestans*. (Fig. 15.)

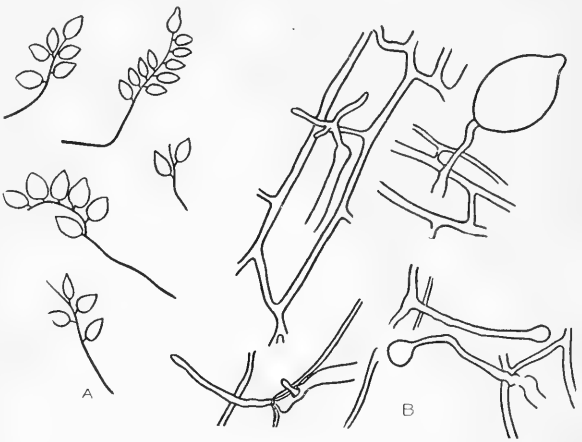


FIG. 15. CONIDIOPHORES OF PHYTOPHTHORA FROM GINSENG
(A) Manner in which the conidia are borne on the conidiophores (16-millimeter objective); (B) manner in which the conidiophores emerge from the tissue of the host

CONIDIA

The conidia are ovate, with a prominent apical swelling, or papilla (Fig. 16). The papilla is lighter in color than the remainder of the cell, and apparently thinner also. At the larger rounded end of the spore the short broken attachment of the conidiophore is seen in some cases. The walls are smooth and hyaline. The double walls of the conidium, which indicate its sporangial nature, are quite evident, the inner one being much thinner than the outer. The contents are alveolate and granular. Often the center of the spore shows a large, clear spot, re-

sembling a large vacuole. Conidial measurements, as pointed out previously, average 34.5 by 27μ.

The conidium arises as a swelling. At first, and often until near maturity, it is almost globose in form. It gradually becomes ovate, the papilla being the last part of the spore to take its form. This appears about the time of maturity. In many instances, especially in cultures of considerable age, a tube is sent out from a little below the papilla,

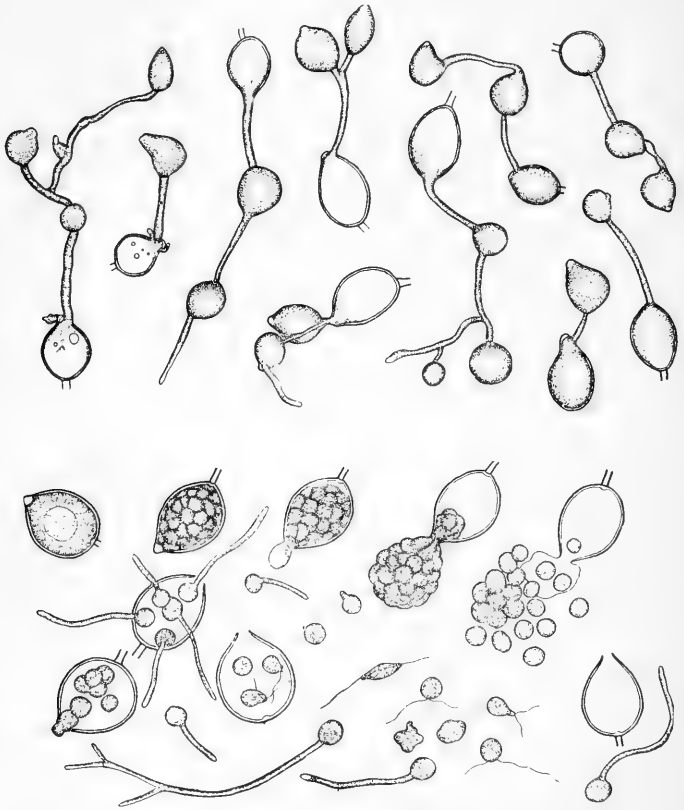


FIG. 16. GERMINATION OF CONIDIA OF PHYTOPHTHORA CACTORUM

Various stages in the germination of conidia by tubes, with the production of secondary and tertiary conidia, are shown above. Below are shown the germination of conidia by means of swarm spores, and the germination of swarm spores

and at the apex of this another conidium is formed. The contents of the first conidium pass through the tube into the second conidium. The process may be repeated two or three times, giving the appearance of a chain of conidia. As a rule, each succeeding conidium is slightly smaller in size than the preceding one.

GERMINATION OF CONIDIA

The conidium is potentially a sporangium. It germinates normally in one of two ways, either directly by the production of numerous germ tubes, or by the production of swarm spores.

GERMINATION BY GERM TUBES

The most usual and most abundant type of germination observed is by means of germ tubes produced directly from the sporangium itself (Fig. 17). The number of germ tubes varies. The tubes are large and

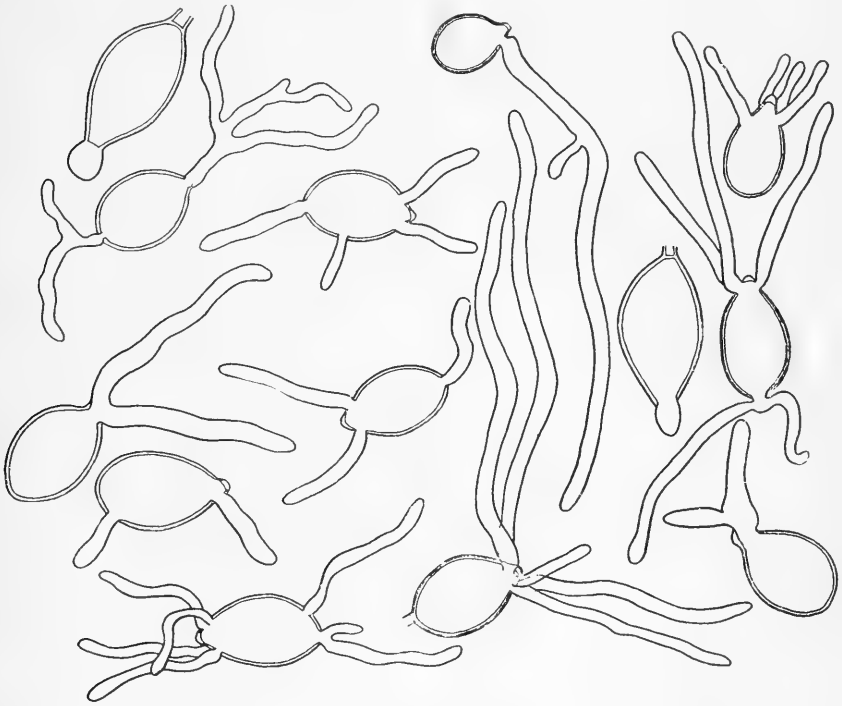


FIG. 17. GERMINATION OF CONIDIA OF PHYTOPHTHORA CACTORUM
Various stages in the germination of conidia of Phytophthora cactorum from ginseng by means of germ tubes

rather straight, with relatively few branches. They arise most commonly from the ends of the sporangium. Those arising from the proximal end of the sporangium ordinarily are fewer in number than those arising from about the apex. Moreover, the germ tubes from the proximal end commonly emerge directly from the point where the spore was attached to the conidiophore. At the apex the germ tube very seldom arises from the papilla, but usually from the side of the spore a short distance below the papilla. It is very characteristic of many of these germinations that the

germ tubes at the distal end arise in a whorl, or cluster, around the papilla. These germ tubes are densely granular, the contents of the sporangium passing out into them. The first evidence of loss of protoplasmic contents of the sporangium is the appearance of rather large vacuoles here and there through the spore.

GERMINATION BY SWARM SPORES

Germination by means of swarm spores (Fig. 16) was observed a number of times by making mounts from fresh cultures in drops of water in van Tieghem cells or on object slides. Swarming has also been seen on an object slide in a drop of water under a cover glass. The time for such germination varies. The first evidence of the presence of swarm spores in a sporangium is the movement of the protoplasmic granules in certain centers, or areas, in the sporangium. In some cases swarming is seen fifteen minutes after the conidia have been placed in drops of water. In other cases no signs of swarming are evident for from two to three hours. In examining a mount made for this purpose, there is often noticed a sudden and tremendous swarming in practically all of the sporangia. The swarm spores appear in the mount by hundreds, and a rapid review of the sporangia under low magnification shows swarm spores rolling out of nearly every one. It is a most exciting and extraordinary sight.

The manner of emergence varies. Usually the swarm spores emerge singly and very quickly from the thin, narrow, papilla-like opening. The time usually required for the emergence of the mass of spores is from four to ten seconds. After emergence the spores are usually held together at the opening for from two to three seconds, after which they all swim apart in different directions. Often they do not congregate near the opening, but swim away as fast as they emerge. It often happens that for some reason two or three swarm spores do not emerge when the majority escape. In one case those left were noticed apparently trying hard for fifty-five minutes to get out, without success. At times, in their movements, they were at the very opening. It gave the impression that the opening where the majority of swarm spores escaped became closed again, and thus prevented the emergence of those left behind. In one particular case the spore came to rest and germinated inside the sporangium, the germ tubes extending through the walls of the sporangium. Very often two swarm spores, after emerging together, seem to stick together for two or three seconds, held by a fine protoplasmic thread or connection, after which each darts off by itself.

SWARM SPORES

The number of swarm spores in a sporangium varies with the size of the sporangium — the larger the sporangium, the greater the number

of swarm spores produced. The greatest number ever observed was thirty-six. Each swarm spore is provided with light-colored spots, or vacuoles, which are located nearer one end of the concave side. The concavity is near the smaller end of the pyriform swarm spores. It appears that here also are attached the flagella. These are two in number and of unequal size, varying in size from one and one-half to two times the length of the body of the swarm spores.

After swimming for a time, the swarm spores come to rest, withdraw their flagella, become round, and germinate by sending out germ tubes. Germ tubes of considerable length are formed within a short time after the swarm spores have come to rest. Ordinarily one single germ tube is formed by each spore.

It has always been a matter of interest to discover what determines whether germination is to be by swarm spores or by germ tubes. Different investigators have attributed the phenomenon to different causes. Klebahn (1909) cites a case showing that oxygen apparently is necessary for the emission of zoöspores, while Coleman (1910) states that the formation and emission of zoöspores in a sporangium is clearly influenced by external factors, chief of which is a certain strength of light. Coleman states further that zoöspores can be obtained in the Areca Phytophthora by suspending sporangia in water, placing them on the stage of the microscope, and illuminating them by means of the mirror and the condenser. This has been tried by the writer, not only for the ginseng Phytophthora but for the Areca Phytophthora as well, but for some unknown reason without success. In the same paper Coleman cites an experiment in which a number of cultures were kept in the dark and an equal number in the light. He draws no conclusions from the experiment, but from the results obtained one is led to believe that light is necessary for the formation of sporangia. In the writer's experience with *Phytophthora cactorum* from ginseng, this has not been the case.

After numerous attempts to germinate conidia both from plants in the field and from growths on various media, the writer is inclined to the opinion that the age of the conidium has more to do with its manner of germination than external conditions. Conidia taken from young cultures — that is, cultures just beginning to form conidia — are more likely to germinate by swarm spores than are older conidia. In the case of conidia formed on the host, those having favorable conditions for germination soon after formation will germinate by swarm spores, while the others will germinate by germ tubes.

SEXUAL ORGANS

The antheridia and the oögonia form from the mycelium of the fungus when the fungus reaches a certain age. This varies with the medium

on which it is grown. The antheridium and the oögonium may arise as branches of the same thread or from different threads (Figs. 11 and 12), but always the threads are close together. The antheridium and the oögonium are terminal swellings of their branches. The formation of the two structures takes place simultaneously. In some cases the stalks bearing the antheridium and the oögonium are on the same side, and the antheridium then falls on the oögonial stalk. Under the microscope, such a condition may present the appearance that the oögonium has grown through the antheridium (Fig. 11).

The antheridium at maturity is from elliptical to reniform in shape, and is cut off by a cross wall from the main part of the thread. The oögonium is globose and much larger than the antheridium, and is also cut off by a septum. The protoplasm in the early stages, in both the antheridium and the oögonium, is finely granular, with a number of oil globules varying in size.

FERTILIZATION

Preceding the formation of the oösphere a change takes place in the oögonial contents. The protoplasm becomes denser, and, together with the oil drops, collects in the center. After the oösphere has been formed in the oögonium, one may detect in some cases a fine, light-colored passageway extending from the antheridium to the wall of the oösphere (Fig. 11). This is the fertilization tube. The passage of any contents into the oösphere from the antheridium has never been observed, though it undoubtedly takes place as an examination of later stages shows that the contents of the antheridium are less dense. But at this time the fertilization tube has disappeared. In one case on five-days-old oat agar culture numerous fertilization tubes were seen. The tubes were present also on the following day, but on the seventh day all signs of them had disappeared. In no case do all the contents of the antheridium pass into the oösphere in the act of fertilization, as is the case in many of the Phycomycetes.

OÖSPORE

After fertilization has taken place the oöspore gradually changes color, from hyaline to yellow or brown. During this change in color there is a gradual thickening of the wall of the oöspore. The oögonial wall persists, but without any change. In most cases, however, in this species, careful focusing will show that the antheridium is superimposed on the oögonium.

OÖSPORE GERMINATION

All attempts by the writer to germinate oöspores during the year 1912 and a part of 1913 met with failure, even though the oöspores were taken

from cultures almost a year old. Accordingly, in the fall of 1913 it was decided to place the oöspores under as nearly natural conditions as possible. Transfers of pure cultures of the Phytophthora isolated from ginseng and of *Phytophthora cactorum* from Phyllocactus were made on sterilized bean pod plugs in test tubes. At the end of two weeks these were examined and were found to contain numerous oöspores. Some of the test tubes containing the oöspore material were sealed with paraffin and covered

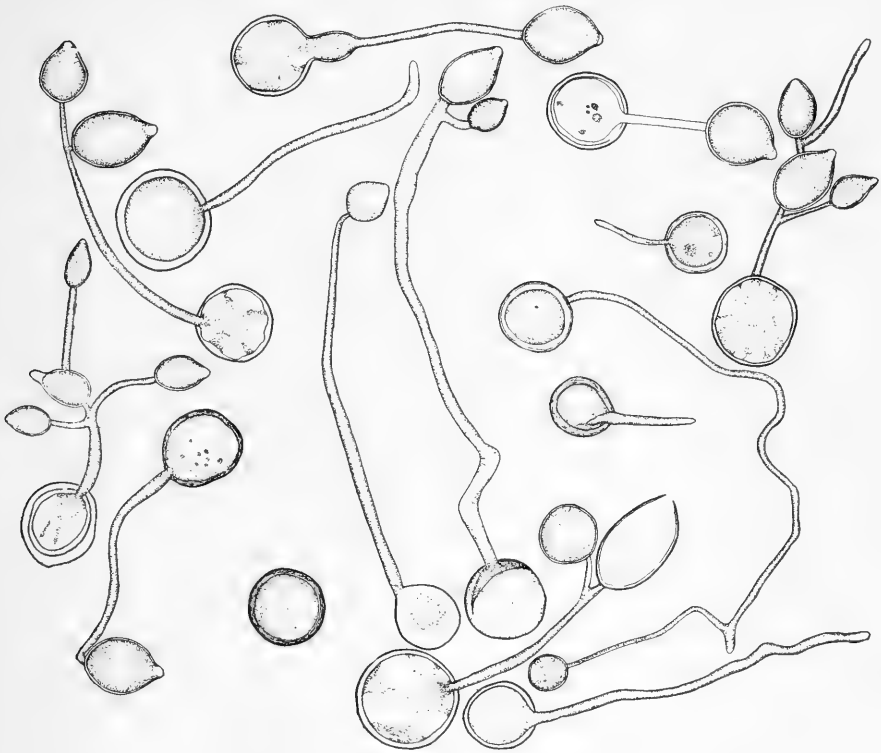


FIG. 18. GERMINATION OF OÖSPORES OF PHYTOPHTHORA CACTORUM

with small rubber caps. From the remainder of the test tubes the bean pod plugs were removed and placed in small five-inch flowerpots containing sand. On December 2, 1913, the covered test tubes and the pots were taken out of doors and buried an inch below the surface of the soil. On January 8 following, one of the pots was dug up. Small bits of the bean pod material containing oöspores were placed in hanging drops of water to germinate. They were examined on several successive days, but no signs of germination were visible. On January 29 one of the covered test tubes was brought in and bits of material were placed

in drops of water, but no signs of germination could be seen. Instead of discarding the remaining material, the test tube was partly filled with sterile distilled water. Mounts in van Tieghem cells and in drops of water were again made from this test tube on February 20. An examination on the following day showed that many of the oöspores had germinated. On the next day the remaining material was brought in and placed under water. At the end of two weeks no difficulty was encountered in germinating oöspores from any of the material. The germination of oöspores of the Phytophthora from both ginseng and Phyllocactus was identical.

The oöspores of the wintered material are granular, some being denser than others. In some cases the oögonial wall is broken or entirely gone and only the thick wall of the oöspore is seen, in other cases the oögonial wall still persists. Just before germination the granular substance becomes denser near the periphery and the wall of the oöspore takes on a striated appearance. Through a break in the wall a germ tube is sent out. The contents of the oöspore gradually pass into this tube, which, when it has attained a sufficient length, bears a conidium. More than one conidium may be borne, as is the case with ordinary conidiophores. As germination proceeds, the striations on the oöspore wall disappear and the wall itself diminishes in thickness. Various stages in the germination of the oöspores are represented in figure 18.

The conidia thus produced have been seen to send out ordinary germ tubes, as well as to break up into swarm spores.

CONTROL

The various methods of control of Phytophthora of ginseng fall under the following heads: (1) spraying with fungicides; (2) removal of diseased plants or parts of plants; (3) deep planting; (4) crop rotation; (5) sterilization of the soil; (6) drainage.

SPRAYING

As has been pointed out, the most favorable time for infection is very early in the spring, just as the plants are pushing through the soil. At this time the plant tissues are succulent and tender, and the temperature and weather conditions, as a rule, are most favorable for spore germination and infection. In order to prevent this early infection of the tops, a fungicide should be applied as the plants are pushing through the soil, and the application should be continued at intervals until all the plants have made their appearance. Spraying after this period will depend on weather conditions and on the amount of growth that the plants have made since the last application. Spraying should be done before rainy periods, and all the new growth should be covered with the fungicide.

Various fungicides, such as lime-sulfur solution, bordeaux mixture, and bordeaux mixture with arsenate of lead, have been tried for two seasons. Lime-sulfur solution in some cases causes injury to the foliage. As between bordeaux mixture with and without the addition of arsenate of lead, the former has been found to be the more satisfactory. Arsenate of lead seems to improve the adhesive quality of the mixture, which remains on the foliage longer when this is used. The fungicide employed should therefore be bordeaux mixture 3-3-50, to which has been added two pounds of arsenate of lead for every fifty gallons of mixture.

REMOVAL OF DISEASED PARTS

It has been found that if the diseased tops — that is, those that show a wilting and drooping — are removed just as soon as they are noticed, the fungus will be prevented from traveling down the stem. When the root is believed to be affected, it should be carefully removed from the bed. It is a good practice to disinfect the soil with a fungicide at the place from which the root has been removed. Formaldehyde, one part to twenty-five parts of water, or copper sulfate, one pound to ten gallons of water, is a good solution for this purpose.

DEEP PLANTING

During the summer of 1911, in a garden three-quarters of an acre in size, almost every plant was lost through attacks of Phytophthora. The disease seemed to start in the tops, and in a short time nearly every root in the garden was affected. There were, however, about a dozen plants scattered throughout the garden which did not seem to be affected. These came up again the following spring, and on examination it was found that without exception the roots of the plants not attacked were planted at least four inches below the surface of the soil. From artificial infections recorded in the preceding pages, it is seen that the fungus can travel down the stem and rot the root. When the crown of the root is several inches below the surface of the soil, however, there is less likelihood that the root will rot. In the case of the potato Phytophthora, hilling up of the rows has been suggested as a means of reducing the rot of the tubers; but the method appears to be impracticable, since it causes a considerable reduction in yield. In the case of ginseng, the roots are left in the ground for five years or longer.

ROTATION OF CROPS

A garden once affected with Phytophthora cannot be again used for ginseng for some years. A number of growers whose land had become

infected allowed the land to lie fallow for two years. They then planted seed, and the seedlings were attacked to a considerable extent by the disease. Hartig (1882) showed that the oöspores can live for a number of years in the soil. Financially the grower cannot afford to let his land lie fallow for a period of years, since it is almost impossible to move the shade. The writer therefore suggests that a rotation with some other crop, requiring the same conditions of shade, be practiced. Golden seal (*Hydrastis canadensis*) is a good plant for this purpose. A number of inoculation experiments have been made with the ginseng Phytophthora on golden seal, and in no case has there appeared any evidence of infection.

STERILIZATION OF SOIL

Where it is not desirable to practice rotation of crops, the sterilization of soil by means of steam will prove of value. The steam pipe method and the inverted pan method have been tried. The inverted pan method is by far the more satisfactory, because of the greater ease with which the pan is handled and because less of the steam is lost than in the pipe method.

The pan should be of galvanized iron, of a width equal to that of the beds in the garden, a length of ten or twelve feet, and a depth of from five to seven inches. The sides should be provided with sharp edges, which are forced down into the soil. The soil is prepared as for planting. It is necessary to have a pressure of from seventy-five to one hundred pounds for forcing the steam into the soil. The length of time required for each pan will vary with the kind of soil, owing to the fact that steam penetrates a sandy soil with greater ease than one of clay. Depending on these conditions, the time will vary from twenty to forty minutes.

DRAINAGE

When plants are growing naturally in the forest, the excess water in the soil is removed by the roots of trees and shrubs. Under cultivation some artificial means of removing the excess of water from the beds must be employed. In one experiment it was found that a much greater abundance of oöspores was produced when the root was placed in an abundant supply of water. Vegetable rots in general are favored by an abundance of moisture. It is therefore suggested that some type of underground drainage be employed to carry off the excess of water in the soil. Ordinary hard-burned clay tiles have proved most effective and permanent for this purpose. The depth, interval, and size of the drains must vary with the character of the soil and of the subsoil and with the amount of rainfall. In general the drains should be placed at a depth of from two to three feet in sand and gravel, and from one and one-half

to two feet in clay. Where possible a tile drain should be placed under the center of each bed, or the drains may be placed at intervals of from six to eight feet. The size of the tile depends on the volume of water to be carried. In the ginseng-growing sections of New York State a three-inch tile has been found satisfactory.

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