THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Philip Brierley final oral examination for the degree of

Master of Science.

We recommend that the degree of

Master of Science

be conferred upon the candidate.

E. C. Stakman
Chairman

R. B. Harvey.

C. O. Rosendahl

Date May 5, 1922

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Philip Brierley for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

E. C. Stakman
Chairman

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Date May 5, 1922

Some Factors Affecting the Development of the Blackleg Disease of the Irish Potato

A thesis presented to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the Degree of Master of Science

by

Philip Brierley

June 1922

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Introduction

The potato is an important crop in Minnesota. The state produced over 28 million bushels in 1920. In some regions, especially the northern cut over lands, it is the principal cash crop. In the Red River Valley in particular the production of seed potatoes is also an important industry. In 1920 over 300,000 bushels of seed tubers were certified by the Minnesota Board for Potato Certification.

Blackleg is one of the most important diseases of the potato. It has been known for many years and even its etiology presumably has been well known. However deviations from the accepted course of development observed by pathologists and growers show that the occurrence of the disease is governed by the conditions of the environment and the host. The present investigation was undertaken to determine more exactly some of the more important factors involved in the development of the disease.

History of the Disease

The early descriptions of wet rots of potato tubers are not sufficiently clear to allow their certain identification as blackleg tuber rot. According to Appel (3), Hallier in 1878 was the first to recognize bacteria as invaders of sound potato tubers. Hallier did not use pure cultures, but was able to produce complete decay of healthy potatoes in 48 hours after inoculating with a mixed culture. This rapid progress of the rot and the fact that dry air reduced the rate of development would indicate that the active parasite involved was a bacterium.

In the following year Reinke and Berthold (3) suggested the connection of potato tuber rots with certain "potato diseases" but were not able to establish the relation of bacterial rot to any specific disease of the vines. They found bacteria constantly associated with the wet rot and succeeded in producing the disease in sound tubers by transfer of diseased juice. Infection was possible only in a saturated atmosphere; the inoculated wound was otherwise corked over.

Prillieux and Delacroix (33) described a basal stem-rot of potato and Pelargonium in France in 1890, which they ascribed to *Bacillus caulivorus* n. sp. The disease symptoms on potato according to these authors consist of a brown, more or less depressed lesion extending from the ground level upward, followed by rapid death of the plant. Pelargonium was affected similarly with a soft black stem rot. Cross inoculations from potato to Pelargonium and vice versa were successful. A bacillus was found in great numbers constantly associated with the disease. The descriptions of the organism in this publication (33) and in Prillieux's "Maladies des Plantes Agricoles" (34) are not sufficient for identification, but the disease symptoms are suggestive of blackleg. Infection of tubers is not mentioned, but a statement is made in the latter publication (34) that the disease attacks especially plants grown from tubers which are cut before planting.

In 1896 E. F. Smith (39) described a bacterial wilt of Solanaceous plants in the southern United States due to Bacterium solanacearum. The symptoms of this disease differ from those of blackleg. The wilt disease causes the potato vines to wilt from the tip downward, while blackleg lesions appear first near the base of the plant and then spread up the stem. B. solanacearum is a typical vascular parasite, while the blackleg organism causes parenchyma necrosis. Also the cultural characteristics of the organisms are distinctly different. Smith (39) first definitely showed the causal relation of a bacterial organism to a stem and tuber disease of the potato.

Frank (11) was the first worker to prove the connection of the "Schwarzbeinigkeit" or "Stengelfäule" of potato vines with a wet rot of the tubers. He found the same organism in both stem and tubers for four successive years, and was able to produce both the blackleg and the tuber rot on inoculation with pure cultures. He also showed that healthy tubers rotted when a small cube of the blackened stem tissue from a blackleg vine was placed in a wound. Frank ascribed the disease to a coccus which he named Micrococcus phytophthorus, but he did not describe it fully. Appel (3), on examining some of Frank's preserved material was convinced that the disease was identical with that caused by Bacillus phytophthorus.

He suggested that Frank might have been confused by a commonly associated non-parasitic coccus form, or that he possibly mistook short plump rods for cocci. Frank indeed made the statement that straight chains of cocci were easily mistaken for bacilli, especially when they were not stained.

In 1901 another bacterial stem disease of potatoes in France was described by Delacroix (8,9) and attributed to Bacillus solanicola. Bacteria were abundant in the living stem, especially in the vessels, and produced a wilting of the foliage from the base up. Brown spots and gum appeared in the vessels and woody elements were formed in the parenchyma. The disease was considered distinct from the "gangrene de la tige" caused by Bacillus caulivorus, but it was said to be very similar to Smith's bacterial wilt of Solanaceae. Smith (40) found that Bacillus solanicola was non-pathogenic and was undecided as to whether Delacroix's disease was due to Bacterium solanacearum, to Bacillus phytophthorus, or to a third parasitic form.

The name Bacillus phytophthorus was first published by Appel (2) on March 27, 1902. In this and an earlier preliminary note he reported that he had isolated this organism from diseased tubers and blackleg vines. He characterized the pathogene as a fairly plump rod. Some of the rods were nearly coccoid in form and others were several times as long as wide. It was readily cultivated on neutral or alkaline but not on acid media. Potato sap gelatine was liquefied with the formation of a distinctive liquefaction cavity, and on slices of sterile raw potato a characteristic brown rot appeared in from 12 to 18 hours after inoculation. The rot spread continuously with the production of a watery exudate abounding in bacteria. The characteristic black wet-rot of tubers and the basal stem-rot of vines were produced when pure cultures were introduced into wounds. Natural infection of sprouting tubers was obvserved when they were placed in soil in which diseased tubers had lain, or when the soil in which they were planted was watered with an infusion of the bacteria.

The priority of the name Bacillus phytophthorus rests upon the publications already briefly summarized. The description of the organism in full, which was promised in the second of these, appeared the following year in Appel's most complete work upon the subject.(3) This paper, one of a series of several that appeared from 1902 to 1906, still contains the most extensive treatment of the subject. It included a review of much of the older literature. Appel dealt fully with the conditions affecting the development of the disease in storage pits and in the field and made some preliminary experiments on control measures. He recommended selection and cool dry storage of seed stock. In spite of the fact that he repeatedly isolated Bacillus phytophthorus from diseased tubers and vines during a period of several years, Appel was convinced that a whole group of organisms similar in character and chemical action were capable of causing blackleg and tuber rot. Accordingly he designated "Schwarzbeinigkeit" as a disease phenomenon rather than a specific disease.

Van Hall published a dissertation in Holland, which has not been seen by the writer, in which he named a new species Bacillus atrosepticus, and stated that it was the cause of tuber rot and blackleg. The date of publication of this paper as stated by Smith (42) was May 21, 1902, nearly two months later than the appearance of Appel's paper in which he named Bacillus phytophthorus. Appel (3) compared his organism with a culture of Bacillus atrosepticus furnished by Van Hall and found the two closely similar but not identical.

The first observation of the occurrence of blackleg in the United States was that made by Jones (19) in Vermont in 1906. Jones (18) had recently examined specimens of the "Schwarzbeinigkeit" in Appel's laboratory and had also seen the disease in the field in Germany and England. He found that the disease which he observed in Vermont apparently was identical with the one which he had seen in Germany.

In 1906 F. C. Harrison (13) described an organism differing slightly in cultural characters from Bacillus phytophthorus as described by Appel. This "bacterial rot of the potato" which resembled closely the blackleg disease was found widely distributed and destructive in Canada. Harrison named the pathogene Bacillus solanisaprus and described it in great detail. His paper includes a chart, compiled from the published descriptions, showing the differences in morphology, cultural characters and host range of the several organisms B. solanacearum, B. phytophthorus, B. solanicola, B. atrosepticus, and B. solanisaprus, which had been associated with blackleg or similar diseases up to 1906.

E. F. Smith (41) published in 1910 a complete description of Bacillus phytophthorus, in which he confirmed and extended Appel's cultural studies. He worked with a culture "received from Berlin in 1906", but he also isolated this organism from potatoes grown in Maine and Virginia. Smith stated that

"Bacillus solanisaprus Harrison is a very closely related but not identical organism causing a similar disease in potatoes. The same may be said of Bacillus atrosepticus Van Hall, cultures of which are not now available".

Pethybridge in 1910 (30) mentioned a disease appearing in Ireland which resembled blackleg but which he preferred to term "black stalk-rot". In a paper read before the Royal Irish Academy and published in 1911 (31) Pethybridge and Murphy described the pathogene as Bacillus melanogenes. In comparing this organism with Bacillus phytophthorus as described by Appel differences were found in three cultural characters. But these differences were constant and were considered sufficient basis for establishing a new species. Slightly greater differences were found to exist between Bacillus melanogenes and the published descriptions of Bacillus atrosepticus and Bacillus solanisaprus.

Morse (21) found blackleg appearing in Maine when he took up work in that state in 1907. In the following year (22) he isolated the organism from blackleg stems and began a general study of the disease. His final paper (25) in 1917 was devoted chiefly to a comparison of the several organisms which had been described as causing blackleg or similar diseases. This article included a review of the literature up to 1916. Morse studied in parallel B. atrosepticus, B. solanisaprus, B. melanogenes, two cultures furnished by Appel under the name B. phytophthorus, and three organisms which he had isolated in different sections of the state of Maine. All these with the exception of the reputed B. phytophthorus agreed so closely in their reaction to the various differential tests that Morse regarded them "as one species or at the most strains of the same species". Since the organisms supplied by Appel as _B_. _phytophthorus_ were non-pathogenic and culturally distinct, Morse had no basis for using this name. Smith's statement already quoted indicated that even the true _B_. _phytophthorus_ differed from _B_. _solanisaprus_ and _B_. _atrosepticus_. Accordingly, excluding Appel's name from consideration, Morse on priority grounds designated _Bacillus_ _atrosepticus_ Van Hall as the name of the blackleg organism.

Smith (42) in his "Introduction to Bacterial Diseases of Plants", published in 1920, included a summary of the "black rot of potato" ascribed to _B_. _phytophthorus_. In this account was a parenthetical statement that _Bacillus_ _melanogenes_ as received by him was a mixture of _Bacillus_ _phytophthorus_ and _Bacillus_ _solanisaprus_. He did not mention upon what characters he distinguished the two latter organisms and in choosing the binomial _B_. _phytophthorus_ he seems to have ignored such differences and decided entirely upon priority grounds.

Jennison (16) in 1921 published a short note in Phytopathology which has considerable significance. Cultures of _B_. _atrosepticus_, _B_. _phytophthorus_, _B_. _solanisaprus_, and _B_. _melanogenes_, a strain isolated in Maine, and several strains isolated in Montana were compared and found "specifically identical". Jennison decided that "because of priority Van Hall's name (_Bacillus_ _atrosepticus_) should stand". This conclusion was based upon the assumption that Appel did not describe _B_. _phytophthorus_ until 1903, when his monograph (3) appeared.

In summary it may be stated that the nomenclature of the blackleg organism is still a contested point. Morse, Jennison, and

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Smith have made comparative studies of the causal organisms, Morse found that three of the described species were very similar but B. phytophthorus was different. Jennison concluded that these several strains, including B. phytophthorus, were specifically identical. This evidence, therefore, indicates that blackleg is caused by a single species of Bacillus. If we accept this as demonstrated, Bacillus phytophthorus Appel is the correct binomial inasmuch as the name antedates by nearly two months Bacillus atrosepticus Van Hall. The objection which might be raised that Appel did not describe his organism fully until 1903 is met by the fact that Van Hall's description in 1902 was by no means complete, and has never been extended by anyone working with a culture of authentic origin. Morse (25) obtained his culture of B. atrosepticus from Austria with no statement as to origin or authenticity. Jennison (16) made no statement as to the source of his culture, but according to Smith (42) "no one now has transfers from Van Hall's original culture".

Smith apparently still recognizes both B. phytophthorus and B. solanisaprus. In this respect his viewpoint is different from that of Jennison. He has made no detailed statement of his position in this respect to date but evidently (40, Vol. III, p.207) intends to deal with the blackleg disease at length in a later volume of his "Bacteria in Relation to Plant Diseases". In the meantime the name Bacillus phytophthorus Appel is in common use in this country for the blackleg organism. (35,37,38,42)

Distribution and Economic Importance

The blackleg disease is known to occur in England (45,29), Ireland (31), France (10,18), Germany (3), Belgium (3), Holland

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(3), Denmark (3), Canada (13,17,28,47), and the United States (46, etc.). An insufficiently characterized disease reported near St. Petersburg, Russia, by Iwanoff (15) may also have been blackleg. (3,40) It may also occur in Italy (40) and in Australia (13,40) but it is impossible to draw definite conclusions from the literature. Smith (42) considers the distribution of the disease co-extensive with potato culture, and Murphy (28) regards it as world wide wherever conditions are favorable for its development.

The severity of the disease in Germany according to Appel (3) was sufficient to prohibit the growing of two susceptible varieties "Frühe Rose" and "Welkersdorfer". Local losses of from 40 to 60 percent and even up to 80 percent were mentioned. The losses in England in the average season are considerable, and in wet seasons they are really severe. Tuber rot due to blackleg is often very destructive in storage in England when storage pits are not well ventilated. (29) In the maritime provinces of Canada local losses of 10 to 50 percent occur almost every year. (28). In 1915 the disease destroyed 10 percent of the crop in these provinces and, in some fields, up to 80 percent of the hills were missing. (27) The Canadian Plant Disease Survey for 1920 (45) records small losses in every potato region except British Columbia. According to verbal statements of Canadian pathologists the blackleg disease is one of two really serious potato diseases in Saskatchewan and Alberta.

Blackleg has been reported from every state in the United States except Oklahoma, Mississippi, and Alabama, and it is quite likely that it occurs in these states also. In most of the southern states the disease has been reported to the Plant Disease

Survey for only one year. Such sporadic outbreaks are attributable to importation of the disease on northern seed tubers. (44) In the northern potato regions and at the higher altitudes the disease is commonly severe.

The most widespread losses in this country occurred in 1919 when over two million bushels were destroyed. (44) In that year losses in both Minnesota and Nebraska reached 305,000 bushels. In Kansas 738,000 bushels or 10 percent of the crop was destroyed. Blachly (44) reported 5 to 25 percent losses locally in the latter state and mentioned the occurrence of excessive local rainfall in that season. Melchers (44) named blackleg as the most serious tuber-borne disease in Kansas.

In 1920 the damage due to blackleg was more localized, but it was serious in some of the important potato growing states. The loss was greatest in Minnesota where 486,000 bushels were destroyed; but the yield was reduced by 3 percent in Montana, Utah, Nevada and Idaho. In the Norfolk and East Shore trucking districts of Virginia the losses were severe and in the upper peninsula of Michigan the loss was 5 percent.

In 1920 in Minnesota application was made for certification of 610 fields of potatoes. At the time of the first inspection there was blackleg in 288 (47 percent) of these fields; one percent or more of the vines in 109 fields were diseased, and in one field 15 percent of the plants were destroyed. Blackleg was a factor in the rejection of 74 applications for certification. In the Red River Valley, by far the most important potato district in Minnesota, the disease was much more prevalent than elsewhere. Of 213 fields offered for certification in Clay, Norman, Polk, Marshall, and Kittson Counties 157 or 73 percent had some blackleg at the first inspection, and in Polk County the disease was present in 68 out of 73 such fields. The general prevalence of the disease in these selected fields where every effort is made to eliminate disease is an eloquent suggestion of its importance in fields receiving less care.

The above figures and all available estimates of the losses caused by blackleg are based on the percentage of infected vines in the field. This is a fair index of the tuber rot that takes place in the ground before digging time, but the whole question of storage rots is ignored.

Losses from soft rots during storage are often very heavy, and necessitate a complete resorting of tubers before they are shipped to market. Growers have learned to expect this"shrinkage", and pathologists have given much less attention to the problem than it deserves. Just how large a part the blackleg organism plays in storage losses is uncertain. The symptoms of the blackleg rot are often masked beyond recognition by a close succession of secondary organisms which follow the parasite. It must be remembered that these secondary forms are dependent upon pathogenic bacteria or fungi to initiate the rot, and are able to make no advance into sound potatoes.

Morse (25) regards this phase of the disease as of slight importance in Maine. Harrison (13) has laid great stress upon blackleg rot in storage and has estimated that in 1905 the loss in Ontario alone was 1,800,000 bushels. D. H. Jones (17) reported that a bacterial soft rot caused heavy losses in the same province in 1915. Coons (7) states that blackleg is the cause of serious losses in storage in northern Michigan, a potato district continuous with the Ontario region in which Jones (17) worked. Smith (41) holds Bacillus phytophthorus responsible for a large proportion of the storage rots of potatoes in the United States.

Symptoms

The most characteristic symptom of blackleg is the inky black discoloration of the base of the stem; hence the common names "blackleg", "basal stem-rot" and "basal stalk-rot". Before this blackening is visible above ground, affected plants have a stiff, erect appearance with an incurving of the leaves along the midrib. The lower leaves turn yellow and droop, the plant is dwarfed and unthrifty, and finally colapses and dies. Vines in any of the later stages are easily pulled up. The part of the stem between the seed piece and the groundline is blackened for a part or all of its length, and the seed piece is rotted. The black discoloration may be limited to that part of the stem below the groundline or may extend a few inches above the ground when the soil is moist and the humidity high. The rot advances from unsound seed pieces into the growing sprouts and under very favorable conditions for the pathogene the sprout is destroyed before reaching the surface of the ground. If the development of the disease is retarded by dry weather, the affected vine may put out roots above the lesion and maintain a healthy appearance. Such plants are not firmly anchored, and form small tubers, if any. Aerial tubers have been occasionally observed on such plants. When plants are artifically inoculated just above the first node the black lesion advances in both directions. The downward progress is usually limited at the base by the hard, woody tissue below the node. In its upward progress the rot encounters no such obstacle; and under moist conditions frequently advances into the lower leaf petioles and even into the lamina, converting them into a moist, black pulp. If the tip of a half matured vine is inoculated the lesion will destroy the end of the shoot and advance a considerable distance downward. See Plate I. Fig. 1 and Plates II and XI.

The pathogene may invade the stolons under diseased stalks and then may finally progress into the young tubers. If only some of the stalks in a hill are affected, the new tubers under them may be rotted while those under sound stalks remain healthy. Diseased tubers may rot completely before harvest, or may be so slightly decayed that the invasion of the pathogene is not apparent unless the potato is cut open. All intermediate stages of decay may occur. The less severely affected potatoes are put into storage where they may rot further and contaminate sound tubers with which they come in contact.

The symptoms upon tubers are less constant. Typically there is a zone of dark soft decay extending inward from the point of attachment of the stolon or from a wound. The rot is not limited to any special region of the tuber but advances evenly in all directions from the point of origin. The dark brown or distinctly black discoloration when present is most intense at the boundary between sound and diseased tissue, thus forming a sharp line of demarkation. When the decay is colorless the extent of its progress is still clearly marked by the contrast between firm,healthy tissue and a loose mass of disconnected cells. The soft, colorless type of rot is characterized in its later stages by viscous, ropy strands, and a distinctive odor. Under moist conditions tubers are converted into a watery pulp without any collapse of the skin, but when dried out in its early stages the rotted tissue shrinks and the extent of the lesion is marked by an abrupt depression.

Etiology

It has been shown beyond question that Bacillus phytophthorus, and possibly other similar organisms, cause the blackleg of potato. Primary infection in the spring comes from diseased seed tubers. When tubers are planted in wet soil they rot before sprouting or the sprouts are blackened and destroyed before appearing above the ground. Under slightly different environmental conditions the rot attacks the base of the stalks after they have developed. Stalk necrosis and consequent wilting and collapse of the plant may take place at any stage in the development of the potato vine. In Minnesota outbreaks have been observed in late July or early August.

As the rot advances from the seed piece into the base of the stem, it also invades the stolons and enters the newly formed tubers from the stem end. These new tubers may be destroyed completely in the ground. More frequently the decay merely gains entrance at this stage but continues the destruction of the tuber in storage.

The development of the blackleg rot in storage is not fully understood. Infected tubers may rot completely and spread the disease throughout the bin. Again the rot may be corked out during storage. Correlated with this erratic behavior of the disease in storage are some appearances in the field which do not conform to expectations. Apparently sound seed tubers may give rise to diseased plants, or severe outbreaks may develop from seed presumed to be nearly free from disease. On the other hand attacks do not always develop from seed tubers known to be infected.

Secondary infections in the field are not known to occur commonly. If the disease appears in one stalk, it usually spreads to involve the other stalks in the same hill, but does not spread to adjacent hills. Morse (25) records one instance of the spread of the disease by a pool of water standing in a field after heavy rains.

There is no evidence that the pathogene can overwinter in this country in the soil or in diseased tubers that are left in the field. Morse (24) and Ramsey (35) in Maine, and the latter author in Virginia also, have only negative results from a large number of controlled experiments. No blackleg appeared in potato crops raised on soil heavily loaded with inoculum in the preceeding autumn provided no disease was introduced on the seed tubers. Ramsey (35) found no viable blackleg organisms in infested soil kept overwinter out of doors or in the greenhouse. In experiments carried out at the Minnesota Experiment Station soil mixed with rotten tubers and remnants of blackleg plants in the fall produced a healthy crop of potatoes the next year.

Appel observed the occurrence of blackleg in potato crops following an outbreak of the disease in a preceeding year. So far as the author has been able to find, he did not exclude the possibility that the inoculum was seed borne in these instances. In addition, Appel succeeded in producing severe blackleg infections by mixing quantities of diseased tubers with the soil immediately before planting. From these two lines of evidence, he concluded that infection from the soil frequently occurred in Germany. Appel's evidence,published in 1903, has never been substantiated. Recent experiments have in every case strengthened the view that blackleg is carried only on seed tubers.

Recommendations for control are based on the fact that blackleg overwinters in diseased seed tubers. Rogueing in the field, selection of seed tubers from disease free stock, storage under cool dry conditions, elimination of diseased tubers in the spring at the time of cutting, seed treatment, disinfection of cutting knives in case rots are encountered, and the cultivation of the host on well drained land, are the usual protective measures.

Objects of the Present Investigation

The present investigation was undertaken with the object of determining more exactly the relation of some of the important factors of the environment to the development of the pathogene. The organism was studied under controlled conditions and in its normal relation to the host. The influences of temperature, humidity and host varieties seemed to warrant detailed studied.

Experimental Methods

A large number of specimens of blackleg tuber rot were examined, and three strains of the pathogene were isolated and identified. A fourth strain was supplied from the herbarium of the Division of Plant Pathology of the University of Minnesota. This culture was isolated and identified by M. Shapovalov in Maine in 1919. The other three cultures were isolated and identified by the author after the work was begun and for the sake of uniformity subcultures of the herbarium culture were used in all experimental work.

Stock cultures of the organisms were kept on potato dextrose agar and in beef bouillon.. Transfers were made at intervals of six weeks or less, and young cultures were used in tests of pathogenicity, growth relations and viability. Some use was made of other culture media. With one exception these special media were made according to the directions given in Smith's "Bacteria in Relation to Plant Diseases".(40) The potato broth medium used was made according to Morse's directions. (25)

The methods used in the following studies were for the most part those in general use in Bacteriology and Plant Pathology. Where special methods or apparatus were used, they are briefly described under the appropriate experiment.

Experimental Results

Characterization of the causal organism

Bacillus phytophthorus Appel is an actively motile rod,readily stained by the ordinary methods. When stained with carbol fuchsin its dimensions are .5x1.0 to 1.5 u. It grows well on neutral beef bouillon, producing a faint ring pellicle. strong persistent clouding, and abundant sediment, viscid on agitation. Potato broth is clouded in 18 to 24 hours on continued growth a flocculent precipitate separates out and the supernatant liquid becomes clear. On potato dextrose agar surface colonies are slightly raised with a smooth border, and white or faintly bluish white at the margin. On steamed potato growth is scanty, on the surface only, flat, smooth, in color honey-yellow. Fermi's solution is clouded slightly. Sterile skimmed milk is coagulated and the supernatant liquid is clear. Litmus milk is reddened.

For fermentation studies a 2 percent solution of each of the various sugars and a 5 percent solution of glycerine was made in a standard Witte's peptone medium which had been adjusted colorimetrically to pH 7. The d-glucose, d-fructose, maltose, and mannitol used were Pfanstiel products of highest purity. The glycerine was marked "C.P.", the saccharose "H.P.Merck" and there was no statement regarding the purity of the lactose. As an indicator of acid production .8 cubic centimeters of a saturated water solution of Brom-Thymol-Blue was added to each 100 cubic centimeters of solution. The sugars were steamed in the Arnold sterilizer for 30 minutes on three successive days. Three tubes of each sugar were tested and a fourth tube prepared in the same way was reserved as a color standard.

Eight days after inoculation growth in glycerine was slight in the open arm only. No acid or gas were formed in glycerine. Abundant growth appeared in dextrose, fructose, saccharose, maltose, lactose, and mannite, both in the open and closed arms. Acid was produced on all these. A small amount of gas was formed in saccharose, maltose, lactose and mannite, but none appeared in dextrose or fructose in eight days.

The organism is pathogenic to tubers and young growing vines of the potato. On tubers it produces a characteristic soft black rot. See Plate VI. On young vines it forms a soft wet rot with distinct blackening of the tissues attacked. See Plate I.

Temperature relations of the causal organism.

The organism was grown on 1 percent potato dextrose agar in petri dishes at various temperatures. The two lower ranges of temperature, -5.2 to -2° and -0.5 to 4.0°C, were available at the cold storage plant at University Farm, St. Paul. The other ranges were maintained in constant temperature incubators in the Division of Plant Pathology. Three cultures of the organism were kept at each temperature and the measurements in Table I are averages for the three colonies.

Table I.

Effect of Temperature on growth of B. phytophthorus on Potato Dextrose Agar

Temperature range	Average diameter of colony in millimeters		
	9 days	14 days	23 days
-5.2 to -2°C	0.0	0.0	0.0
-0.5 to +4°C	0.0	0.0	0.0
5.5 to 6.5°C	2.7	5.0	7.0
11.5 to 13.0°C	6.5	10.5	14.0
17.0 to 20.5°C	12.0	18.5	21.0
19.0 to 23.0°C	13.0	16.5	23.0
27.3 to 28.3°C	13.0	16.5	18.0(1)
30.0 to 32.8°C	0.0	0.0	0.0
34.2 to 35.8°C	0.0	0.0	0.0
38.5 to 39.5°C	0.0	0.0	0.0

(1) Cultures at 28°C dried out most rapidly.

Bacillus phytophthorus has a limited range for growth on potato dextrose agar. The minimum is near 5 degrees and the maximum near 30 degrees centigrade. The optimum is not clearly shown in this experiment because rapid drying at 28 degrees inhibited growth. The organism grows well at temperatures between 20 and 28°C.

B. phytophthorus was cultivated on beef bouillon at several temperatures. The medium was made neutral to phenolphthalein and 10 cubic centimeters were put in each tube. Five tubes were placed at each temperature and the first appearance of clouding was taken as the index of growth. The results are shown in Table II.

Table II.

The Effect of Temperature on Rate of Growth of B. phytophthorus in Beef Bouillon

Temperature range	First Clouding Observed
-5.2 to -2°C	No growth in 56 days. Viable.
-0.5 to 4°C	No growth in 35 days.
5.5 to 6.5°C	165 hours; 1 tube clouded 138 hours.
12.0 to 12.5°C	50 hours
19 to 21°C	27 hours
21.8 to 22.5°C	27 hours
28.5°C	27 hours
30.5 to 32.0°C (1)	52 hours, slight (1)
33.6 to 35.8°C	No growth in 42 days. Not viable.
38.0 to 39.5°C	No growth in 42 days. Not viable.

(1) Temperature dropped to 19.5°C for a few hours. Clouding appeared in 4 tubes immediately after the drop. No further growth occurred in these tubes in 40 days and the fifth tube remained clear. Viable after 42 days at 31°C.

A second trial was made in the same manner as in Table II. to determine the optimum temperature of the organism and to explain the peculiar behavior of the cultures at 31 degrees. The

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results of this trial appear in Table III.

Table III.

The Effect of Temperature on Rate of Growth of *B.phytophthorus* in Beef Bouillon

Temperature range	First Clouding Observed
11.0 to 12.3°C	121 hours
17.0 to 18.5°C	65 hours
20. to 24.5°C	39 hours
27.3 to 28°C	28 hours
30.8 to 32°C	No growth in 27 days. Not viable.

Tables II and III show that *B. phytophthorus* has a limited temperature range for growth on beef bouillon. The organism is able to live on this medium for two months or more at temperatures below freezing but is not able to multiply much below 5 degrees centigrade. Growth is slow at 5 to 6 degrees and the rate increases steadily with increased temperatures. The pathogene grows rapidly at 20 to 28 degrees, and has an optimum close to the latter temperature. It not only does not grow at 31 degrees but dilute cultures are killed on long exposure to this temperature.

Vitality of the causal organism

The vitality of the causal organism on cover slips under laboratory conditions was determined as follows: dry test tubes containing a glass cover slip and fitted with the usual cotton plug were sterilized by exposure to dry heat at from 160 - 180°C for

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2 hours. A one millimeter loop of a culture of B. phytophthorus on neutral beef bouillon was placed on this sterile cover slip by the usual technique for transfers. After a definite time, which was varied within the experiments, the cover slip was dropped into a sterile broth by inverting the dry tube over a tube containing the medium. Check tests were made by dropping one of the sterile cover slips into a tube of broth by the same process. The results of these tests are summarized in Table IV.

It is shown beyond doubt that the organism is very sensitive to drying. This fact no doubt accounts for the fact that the tuber rot and stem necrosis are often checked abruptly in dry air. In no case were the organisms in smears viable after an exposure of 30 minutes; some survived after exposure for 25 minutes, and some lived 20 minutes, but, in general, the vitality of the organism under these conditions does not exceed 20 minutes.

Observations were made during the course of the work on the length of time the organism lives on potato dextrose agar and on beef bouillon. Most of these trials were made by making transfers from cultures kept at ordinary laboratory conditions. When the usual subcultures from old agar slant cultures failed to produce colonies, a part of the dried slant was chipped off and dropped into sterile broth. Old cultures were not viable.

Since the organism failed to develop at temperatures above 30 degrees, several cultures were removed from these temperatures to the 28 degree incubator. One test was made in the same manner to find whether cultures kept at temperatures below freezing remained viable. The results of these experiments are tabulate in Table V.

Table IV.

Viability of Smears of Bacillus phytophthorus from a 1 millimeter loop of a two to six day beef bouillon culture exposed to drying on cover slips

Number of experiment	Number of cover slips used	Length of exposure	Growth in broth after exposure
1 (4 day culture)	3	None (Check)	3 viable
1	3	2 days	No growth
1	3	4 days	Do.
1	3	9 days	Do.
2 (6 day culture)	2	None (Check)	1 viable
2	1	30 minutes	No growth
2	3	1 hour	Do.
2	3	2 hours	Do.
2	2	3 hours	Do.
2	4	4 hours	Do.
2	2	5 1/4 hours	Do.
2	2	9 hours	Do.
2	2	12 hours	Do.
2	3	24 hours	Do.
3 (4 day culture)	2	None (Check)	2 viable
3	2	2 minutes	Do.
3	2	4 minutes	Do.
3	2	6 minutes	Do.
3	2	8 minutes	Do.
3	2	12 minutes	Do.
3	2	15 minutes	No growth
3	2	20 minutes	2 viable

Table IV.
(Cont'd.)

Number of experiment	Number of cover slips used	Length of exposure	Growth in broth after exposure
3	2	30 minutes	No growth
4 (2 day culture)	5	10 minutes	Do.
4	5	13 minutes	2/5 viable
4	5	15 minutes	2/5 viable
4	5	18 minutes	No growth
4	5	20 minutes	Do.
4	5	25 minutes	1/5 viable

Table V.

Effect of Culture Media and Temperature on the Vitality of *Bacillus phytophthorus*

Culture Medium	Temperature	Length of time on Medium	Viability after test
Potato dextrose agar	Laboratory temperature	61 days	Viable
Do.	Do.	70 days	Do.
Do.	Do.	90 days	Do.
Do.	Do.	95 days	Do.
Do.	Do.	96 days	Do.
Do.	Do.	97 days	Do.
Do.	Do.	121 days	Not viable
Do.	Do.	141 days	Do.
Do.	Do.	148 days	Do.
Do.	31°C	12 days	Do.
Beef bouillon	Laboratory temperature	130 days	Viable
Do.	38°C	14 days	Not viable
Do.	35°C	14 days	Do.
Do.	31°C	27 days	Do.
Do.	31°C	16 days	Do.
Do.	-5.2 to -2°C	56 days	Viable

The organism lives on potato agar for over three months, but is no longer viable after four months. The agar slant medium has dried to a hard, brittle mass after this time. On beef bouillon the pathogene is viable after more than four months. On either medium the organism dies after a short exposure to temperatures above 30 degrees, but its vitality is not injured by long exposures to temperatures below freezing.

Agents of transmission and inoculation

Seed pieces

On October 11, 1921 three sprouting seed pieces of Early Ohio potatoes from the 1920 crop were inoculated. A tuber two-thirds destroyed by artificially produced blackleg rot was placed beside each seed piece with as little injury to the roots as possible. The soil was thoroughly soaked with water and the pots were kept moist throughout the duration of the experiment.

The plants from all three seed pieces grew normally for about 7 weeks. At the end of this time some of the vines began to turn yellow and wilt without a trace of blackleg, and after 9 weeks all were dead. Several of the checks wilted in the same manner. No pathogenic organism could be isolated from the check stalks or from any part of the inoculated plants. The wilting seemed to be the result of low seed vitality, unfavorable light conditions, and insect injury.

On January 26, 1922 the three plants were dug up. In every case the rotted tubers used as inoculum, and also the seed piece had been completely destroyed. Water extracts of the soil including remnants of these tubers were not pathogenic to sound potatoes when several cubic centimeters were poured in a freshly made wound. Tubers inoculated in this manner with beef broth, or with water in which rotting tubers had lain, rotted promptly. The young tubers produced during this experiment were all sound.

In an experiment carried out at the same time tubers artificially infected with blackleg rot were placed in contact with the seed

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pieces of healthy growing vines. The seed pieces and some of the roots were purposely injured to afford easy entrance to the pathogene. The soil was wet thoroughly and kept moist during the experiment. One of the plants inoculated in this manner began to turn yellow in three days, and both plants were completely wilted in three weeks. No signs of blackleg appeared. When the plants were dug up the seed pieces were both rotted. One plant had produced several small tubers and an extensive root system. The other had no tubers and very few roots. The remains of the inoculum and of the rotted seed pieces were still evident in the soil. Attempts to isolate a pathogenic bacterium from these remnants failed and a water infusion of this refuse was not pathogenic to sound tubers.

These experiments indicate that seed pieces may rot completely in the soil without producing any signs of blackleg on the vines. No serious injury to the vine was caused by the destruction of the tuber in this manner under the conditions of the experiment. The pathogene is not readily demonstrated in the soil or in remnants of seed pieces completely rotted in the soil, and there is presumptive evidence that it does not live long under such conditions.

Soil

The rotted seed pieces, remnants of tops, and other plant refuse, were mixed in the soil after the experiments on transmission by seed pieces. Sound Early Ohio seed tubers of the 1921 crop were soaked in mercuric chloride, and on January 26, 1922, one tuber was planted in each of three pots prepared in this manner. Early Ohio tubers were planted in sterilized soil at the same time as controls. After three months the plants growing in the soil containing the inoculum were slightly more vigorous than the check plants. At

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this time they were dug up, and all new tubers that had been formed were sound.

Under the conditions of this experiment the blackleg pathogene did not persist in the soil to an extent sufficient to produce disease after three months.

Insects

Insect transfers of the pathogene from one stalk to another apparently took place in one instance. Inoculations were made near the top of an Irish Cobbler vine on March 25, 1923, and the plant was then placed in a moist chamber for 54 hours. After this incubation period the whole top above the inoculation point had collapsed and the rot was progressing down the stem. The plant was then placed at 80 percent relative humidity in the control chamber. On April 4 the rot had advanced down this single stem a distance of 16 centimeters and was still black, soft, and moist. Great numbers of the white fly Aleurodes sp. and a few of the small black Phora sp., which feeds on decaying vegetables and plant parts in general, were present in the humid chamber. On April 14 three black lesions were noticed on an uninoculated stem in the same pot. These lesions were triangular in shape with the broad base of the triangle uppermost. In each case the point of entry of the pathogene was evidently the scar left by a leaf petiole when the leaf had dropped off. The reduced light, high humidity, and white fly injury frequently caused such dropping of the lower leaves in this cage.

On April 17 the plant was removed from the cage to be photographed. See Plate II. The lesion dried out during this interval and no further advance has occurred. Three young Irish Cobbler plants were kept in the cage for two weeks after the unexplained

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lesions appeared, and leaves were broken off at intervals to offer points of entrance for the inoculum. No lesions appeared on these vines. The old infection was much less active and moist than at the time the secondary infection occurred.

The fact appears that an aerial transfer of blackleg has occurred and a secondary infection has resulted. Transfer of inoculum by actual contact of the diseased tissue with the wound is unlikely, especially since three secondary infections appeared at the same time on three sides and at three heights of the uninoculated stem. Spattering of the organisms in watering the plant was avoided. This possibility also fails to explain the appearance of the infections on opposite sides of the stem. In the opinion of the author the evidence points most strongly to a transfer of the pathogene by one of the two types of insects present.

Further experiments should be made on the possible dissemination of the organism by insects. It is almost impossible to explain some of the facts observed on the spread of the disease in the field except by the hypothesis that insects are carriers of the organism.

Points of Infection

Roots

A potted plant of the King variety was inoculated by pouring over the uninjured plant and over the soil 5 cubic centimeters of a virulent two-day old beef broth culture. The plant was kept at 80 percent relative humidity, and frequently examined for signs of disease. The inoculated plant remained as healthy as the check plant and showed no evidence of blackleg in 62 days. At this time it was pulled up and found to have developed four sound tubers.

Tubers

Tubers were readily infected when young cultures were placed in wounds. The potato was surface sterilized in 1:1000 mercuric chloride and a four sided section about one centimeter on a side was cut out with a flamed knife. The inoculum from broth or agar cultures was inserted in the resulting cavity with a platinum needle, and the pyramidal section was replaced. The inoculated tubers were placed in contact with one to two centimeters of sterile water at the bottom of stoneware jars. A ring of wet cotton was kept between the top of the jar and the lid to prevent too rapid evaporation. Tuber infections are illustrated in Plates VI to IX inclusive.

Stems

Stems were inoculated in needle pricks made with sterile steel needles. A small amount of inoculum was placed in these wounds with a sterile platinum needle. The inoculated plants were usually kept in moist chambers for two days and then put in a special cage in which the relative humidity was about 80 percent.

Inoculations of virulent cultures into stems at all points from the base to the growing tip caused disease. Infections near the tip were the more active. Stem infections are mentioned in many of the experiments below and are illustrated in Plate I. Fig. 2., Plate II, Plate III and Plates X to XIII inclusive.

Leaf petioles

On January 10, one stem and one leaf petiole of a Burbank Russet vine were inoculated with each of two stock cultures of B. phytophthorus of different ages. The inoculum was placed in needle punctures as usual. The plant was put in a moist chamber for two days and kept in a chamber in which the relative humidity was 80 percent.

Two days after inoculation one well marked lesion had appeared on the stem and one leaf petiole had rotted off at the inoculation point. In four days the whole leaf was converted into a soft black pulp, and the black zone had invaded the lamina, involving all but the margin of the leaf. Fig. 1 in Plate I. is a close view of this stalk on the fourth day showing the blackening resulting from inoculation at the base of the stem and the collapse of the petiole and invasion of the leaf lamina resulting from petiole inoculation. After five days this whole stalk collapsed from the progress of the lesion at the base. See Plate I. Fig. 2.

The stalk and petiole inoculated with the older culture developed weak lesions in four days. Seven days after inoculation the lesion at the base of the stem was two centimeters in length and one centimeter in width. The lesion on the leaf had made no progress and produced no visible injury to the leaf. This stalk is shown in Plate I. Fig. 1.

Leaf lamina

A single attempt to produce disease by inoculation into a leaf blade failed. The inoculation was made from an agar culture into a needle prick and the plant kept in a moist chamber for three days. At the end of this period a whitish slime was present at the point of puncture, but this dried out after three minutes exposure to the greenhouse air, and no further development took place.

Flower

On February 14 the flowers of a Burbank Russet vine were inoculated. A sterile cotton plug moistened with a two day beef bouillon culture was brought into contact with the petals, stamens, and stigmas of six fully expanded flowers. Some injury to the floral parts was caused in this process.

After 72 hours in a moist chamber the inoculated blossoms were entirely blackened and separated from the stem. A black spot appeared in the pith of the stem below the point of separation of the pedicel. The plant was held under observation at 80 percent relative humidity for eight days more but no further evidence of progress of the lesion into the stem below the pedicel could be detected. The six flowers were surface sterilized and plated out on agar. Two of the six developed pure bacterial colonies and the other four remained sterile.

Factors affecting infection and development of the disease

Temperature

The effect of temperature on infection and development of tuber rot was determined as follows: Three thicknesses of filter paper were placed in a deep petri dish and moistened with 1:5000 mercuric chloride. The petri dishes were then sterilized in the autoclave at 15 pounds pressure for 20 minutes. Early Ohio tubers were soaked for an hour in 1:1000 mercuric chloride, then rinsed and sliced in sterile tap water and one slice placed in each sterile petri dish. The circular slices were then cut in half, one half in each petri dish serving as a check. Forceps and knives were flamed each time before touching the potato tissue. Inoculation was made by scratching the cut surface of the tuber slice with a needle transfer from an agar culture. The petri dishes were then placed at various temperatures and the progress of the rot was recorded at intervals. Two dishes were placed at each temperature, and the results in the following table are averages of the two. The checks remained sound in every case.

Table VI.

The effect of temperature on the rate of progress of the blackleg rot in potato tubers

Temperature range	Diameter of rot in millimeters	
	2 days	6 days
11.5 - 14°C	9.0	18.0
15 - 17.5°C	9.5	15.0
20 - 22.5°C	15.0	27.5
26.5 - 28.5°C	16.0	16.0

In a similar experiment the potato slices were all incubated at 28 degrees for 24 hours. At the end of this time the rot had begun to develop in every slice. Two slices were placed at each of several temperatures, and the increase in diameter of the rot was recorded at intervals. The average progress of the rot is summarized in Table VII.

Tables VI and VII show that the blackleg rot develops most rapidly at first at a temperature near the optimum for the organism in culture, 25 to 28 degrees C. Under the conditions of the experiment, however, the rate of progress of the rot at the higher temperatures is soon checked by the drying out of the potato slice. The two lower temperatures 6 - 8°C and 11.5 - 14°C were maintained in an ice box and in an incubator cooled with

Table VII.

The effect of temperature on the rate of progress of blackleg rot in potato tubers

Temperature range	Average diameter of rot after 24 hrs. at 28°C.	Average increase in diameter in millimeters		
		1 day	2 days	6 days
6-8°C	8.2 mm.	1.2	2.2	6.8
11.5 - 14°C	12.0 mm.	2.0	4.0	15.5
20.5 - 22.5°C	9.0 mm.	1.5	2.0	5.2
26.5 - 28.5°C	7.2 mm.	2.0	3.0	3.0

running water respectively. The rate of evaporation from the potato slices and from the filter paper pads was considerably slower at these temperatures. Within the temperature limits for its growth, the blackleg organism is clearly influenced more by humidity and the water content of the host tissues than it is influenced by temperature.

<u>Humidity</u>

The effect of humidity on tuber infection was tested in air-tight chambers over sulfuric acid. Large glass chambers of the type of the ordinary dessicator, with a raised platform to support the tubers above the solution and a lid which could be sealed with vaseline were used. The percentages of sulfuric acid used to produce the various relative humidities were those recommended by Wilson.(43)

They were as follows:

Percent H_2SO_4	Percent distilled water	Relative humidity (percent)
58.5	41.5	20
48.7	51.3	40

Percent H_2SO_4	Percent distilled water	Relative Humidity (percent)
38.5	61.5	60
27.2	72.8	80
0	100	100

On November 25 one tuber of each of the varieties Burbank Russet, Early Ohio, and Green Mountain were inoculated and placed at 100 percent relative humidity. A like set of tubers were inoculated and placed at 80 percent relative humidity. No rot developed in any of the eight tubers. On December 21 each of the eight tubers was again inoculated at the opposite end, and all were returned to the humidity control jars as before. On January 4 all the tubers were cut open and examined. The wounds were corked out in every case, and no rot had developed.

On January 25 one King and seven Early Ohio tubers were inoculated and placed at 100 percent relative humidity. At the same time one King and three Early Ohio tubers were inoculated and placed at 80 percent humidity. The jars were kept in darkness at a temperature of 22 degrees C. On February 13 all tubers were cut open. All the wounds were corked out and no rot had developed. At this time two of the tubers at 80 percent and four at 100 percent relative humidity were inoculated by pouring one cubic centimeter of a 6 day broth culture of B. phytophthorus into a freshly made wound. A like number of half tubers were kept as checks. All of the inoculated tubers developed small lesions, but all but one dried out as soon as the broth had evaporated. Plates III and IV show them as they appeared on February 21, eight days after inoculation. At this time the rot was active in only one tuber and this rot was due to a mixed infection. The other tubers had formed a distinct layer of cork under the lesions.

On March 7 an experiment was carried out to determine the effect of relative humidity on the progress of rot in previously infected tubers. Twenty-six tubers were inoculated and kept in stoneware jars in contact with sterile water. Two days later all of the tubers were rotting. The four-cornered sections of tissue removed in making the inoculation were softened at the inner apex, and a white ooze was exuded when the tubers were squeezed. Four of these tubers were placed at each of five relative humidities and six were left in contact with water as controls. Sixteen days later all tubers were cut open and the condition of each was recorded. The results are summarized in Table VIII.

Table VIII

Effect of Relative Humidity in Checking Tuber Rot

Relative humidity percent	Progress of the rot after 16 days
20	3 lesions checked; 1 tuber half rotted.
40	3 lesions checked; 1 tuber two-thirds rotted.
60	2 lesions checked; 2 tubers two-thirds rotted.
80	3 lesions checked; 1 tuber half rotted.
100	1 lesion checked; 2 tubers one-third rotted; 1 tuber half rotted.
Check in contact with water	4 tubers one-third rotted; 1 tuber two-thirds rotted.

It is shown in Table VIII that the blackleg tuber rot may continue to develop in the interior of tubers even if the relative humidity of the surrounding atmosphere is as low as 20 percent. The rot is checked in some cases at much higher humidities. The effect of humidity is the limiting factor in the infection of potato tubers but has a less profound influence on the rate of progress of the rot when once initiated.

For the control of humidity in the greenhouse two special cages were used. The plan of these was a chamber 120 x 120 x 85 centimeters with the four sides and top of glass sash and the bottom of tight boards. A door was cut in the front of such size as to include the whole sash. A row of five ventilation holes 2.5 centimeters in diameter were cut in one side near the floor of the chamber and an equal number were cut at the top on the opposite side. The humidity was maintained fairly constant at 80-90 percent in these chambers by means of two pans of water, one supported above the other. A piece of coarse burlap served to maintain a slow, steady flow of water from the upper to the lower pan and offered a large surface for evaporation. The humidity in these cages could be controlled almost perfectly when the direct rays of the sun were excluded from the chamber by means of a white muslin screen. The temperature and humidity in these cages and also in the open greenhouse in certain experiments were recorded by means of combination thermo-hygrograph instruments.

In an experiment designed to show the effect of relative humidity on infection of vines by _Bacillus phytophthorus_, four Early Ohio vines were used. Two of these plants, A and B, had 2 shoots each 12.5 centimeters tall, and the other two, C and D, had a single shoot 15 centimeters tall. Plants A and B were inoculated at the fourth node from the base. Plants C and D were inoculated between the second and third nodes from the top and also one centimeter from the base. A and C were then placed at 80 percent, and B and D at 60 percent average relative humidity. The temperature range was from 15.5 to 21°C in both places and all the plants were screened from direct sunlight. The higher humidity fluctuated between 75 and 85 percent, with a few momentary drops to 60 percent. The lower humidity varied from 50 to 70 percent with brief rises to 80 percent.

After six days a lesion 15 millimeters long and half circling the stem had developed on each stem of plant A, and on plant C there was one 3 millimeters in length. The two plants B and D, at 60 percent relative humidity, had developed no signs of disease but the points of inoculation were slightly browned.

Nineteen days after inoculation there were lesions 17 and 26 millimeters long on the stems of plant A and in one the top above the lesion was drooping. Plant C had a 6 millimeter lesion at the upper point of inoculation, but no sign of disease at the base. Plants B and D, at 60 percent humidity, were free from disease. Plate V. shows the lesions on the four plants. When plant C was cut open longitudinally the pith was seen to be discolored for 10 millimeters beyond the external boundary of the lesion, meaning that the infection was more severe than it appeared.

Humidity is a limiting factor in infection of potato vines above the ground. The critical relative humidity for infection with the strain of B. phytophthorus used by the author lies between 60 and 80 percent. The dependence of the blackleg organism on high humidities explains some common field observations. The basal stem-rot of the vines seldom extends above the ground level and the appearance of the lesions on the green stems of the host is correlated with wet weather. The results also correlate well with the observed facts regarding the sensitiveness of the pathogene to drying.

Age of tissue

Some of the seed tubers of the 1920 crop were planted in December 1921 and produced young tubers without tops. Wound inoculations were made into two such tubers and the young tubers developed from them. One of the old tubers and one of the new rotted completely while the remaining two were partly sound. No effect of age of tissue appeared.

On March 25, 1922 two tubers of the 1921 crop and four tubers just grown in the greenhouse of each of the varieties Early Ohio, Burbank Russet, Triumph, Green Mountain, and Rural New Yorker were inoculated under the same conditions. Ten days later all the tubers were cut open and the progress of the rot measured. In the Early Ohio and Green Mountain varieties the new potatoes rotted slightly more rapidly, but in the Burbank Russet, Triumph, and Rural New Yorkers the older tubers rotted faster. No general effect of age of tissue on the tuber rot could be detected in this experiment. Plate VI. shows the extent of the rot in old and young tubers of two varieties.

The influence of age of tissue in vines was also tested. Three Green Mountain plants three months old and three plants one month old were inoculated at the base and near the tops. After 23 days small lesions 5 millimeters or less in length were formed in the old vines. In the younger vines the lesions at the base were from 15 to 50 millimeters long but had ceased to advance. Actual lesions appeared on the tips of each of the young vines which had been inoculated. One was blackened externally for 3 centimeters and for 18 centimeters in the pith. The top rotted off completely in another. The third of the younger vines had an external lesion 2 centimeters in length and the rot extended 10 centimeters farther down the pith.

Young shoots are much more susceptible to infection by _B. phytophthorus_ than older plant parts. Especially virulent strains of the organism, very susceptible varieties, and excessive humidity must be present to make late infections possible. These conditions are evidently fulfilled in some seasons when late attacks occur in the field. The results show clearly that _B. phytophthorus_ is a parenchyma parasite which can develop rapidly only in thin-walled parenchymatous tissues. This explains why the disease is most prevalent in the tubers, on young plants early in the season and in moist seasons. When old plants are infected, usually only the younger parts or the pith is destroyed.

Varietal Susceptibility

Tubers

A preliminary experiment with whole tubers of Early Ohio, King, Bliss Triumph, Green Mountain, Rural New Yorker and Burbank Russet varieties was first carried out. Five tubers of each of the above varieties were sterilized in mercuric chloride and inoculated with material from the same culture. They were then placed on cotton saturated with 1:5000 mercuric chloride, four tubers in each of six stoneware jars and three in each of two bell jars. To insure high humidity moist filter paper was placed over the tubers. The results of this experiment are summarized in Table IX.

An experiment already mentioned in connection with the effect of age of tubers on infection also furnished some data on the susceptibility of the several varieties. The results of this experiment are summarized in Table X.

The differences in susceptibility are not sharp. The apparent resistance of Bliss Triumph and King is due in part to the fact that the size of the tubers of these two varieties kept the inoculum raised high above the moisture at the bottom of the jar. Plate VII shows the effect of the rot on the eight varieties in this experiment.

In later experiments sections of tubers of uniform size, 4x4x2 centimeters, were cut from tubers which had been surface sterilized. Sections were placed on filter paper saturated with sterile water in two 25 centimeter stoneware jars, the lids of which were made tight with a cap of cotton which could be moistened when necessary. The eight standard potato varieties grown in Minnesota, namely Early Ohio, Irish Cobbler, Bliss Triumph, Green Mountain, Rural New Yorker, Burbank Russet, Burbank, and Kind (Spaulding Rose No. 4), were tested in duplicate. One set of eight sections, one of each variety, was placed in each of two jars. The eight varieties were thus tested under identical conditions and each of the duplicate sets was a perfect check on the other. One tenth cubic centimeter of a beef bouillon culture was dropped into a small conical hole made in the upper surface of the tuber section with a sterile scalpel.

Table IX.

Varietal Susceptibility of tubers to blackleg rot

Variety (5 tubers of each variety	Progress of the rot after inoculation 6 days	10 days	27 days	34 days	41 days (tubers cut open)	Class[1]
Early Ohio	1 incipient	2 well started 3 sound	2 well started 3 incipient	2 well started 3 incipient	5 local lesions only, corked out	S-
King	2 incipient	2 well started 1 incipient 2 sound	2 completely rotted 2 well started 1 sound	3 completely rotted 2 well started	3 completely rotted 2 corked out	S+
Bliss Triumph	2 incipient	3 well started 1 incipient 1 sound	5 well started	1 completely rotted 4 well started	1 completely rotted 4 corked out	S
Green Mountain	2 incipient	3 well started 1 incipient 1 sound	5 well started	1 completely rotted 4 well started	1 completely rotted 4 corked out	S
Rural New Yorker	2 incipient	4 well started 1 sound	2 far advanced 3 well started	1 rotted 2/3 1 far advanced 3 well started	1 rotted 3/4 1 rotted 1/3 3 corked out	S+
Burbank Russet	1 incipient	3 well started 1 incipient 1 sound	5 well started	5 well started	5 corked out	S-

(1) S = Susceptible
S- = Moderately susceptible
S+ - Very susceptible

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Table X.

Varietal Susceptibility of tubers

Variety	Diameter of lesions in cms. 4 days after inoculation: Old tubers (2 half tubers of each)	4 days after inoculation: Young tubers (Average of 4 half tubers)	10 days after inoculation: Old tubers (2 half tubers of each)	10 days after inoculation: Young tubers (Average of 4 half tubers)	Class
Burbank	1.1 1.0	______	4.0 1.5	______	S+
Burbank Russet	0.0 1.3	1.2	2.8 3.5	1.2	S+
Rural New Yorker	0.0 2.2	1.6	1.3 4.3	1.6	S+
King	0.0 0.0	______	0.0 0.8	______	S-
Irish Cobbler	1.1 2.5	______	1.8 3.0	______	S
Early Ohio	0.0 1.6	1.0	0.7 1.8	1.8	S
Bliss Triumph	0.0 0.0	0.5	0.0 3.5	0.5	S
Green Mountain	0.0 0.0	0.9	1.8 2.5	2.0	S

Two experiments were carried out in this manner. The results are summarized in Tables XI and XII. The classes of susceptibility are arbitrarily assigned from the data in the tables.

These experiments show that none of the standard varieties of potatoes grown in Minnesota are resistant to blackleg tuber rot. Slight differences in degree of susceptibility do exist. The Burbank, Burbank Russet, and King were in general most susceptible while Green Mountain, Early Ohio, and Irish Cobbler were slightly resistant. Too much significance must not be attached to these results, however, as it seems highly probable that temperature and especially moisture relations exert a greater influence than varietal differences on the development of blackleg tuber rot.

Vines

Whole tubers of each of the eight standard varieties of potatoes were planted in sterilized soil in wooden boxes 40 x 40 x 40 centimeters in size. Four varieties were grown in each of two such boxes. Three stems of each variety were allowed to develop. Provision was made for humid conditions during the incubation period by supporting over each box a water reservoir from which the water could be drained slowly by cheese cloth curtains. These curtains passed thru the water of the reservoir and down to the top of the box where they were fastened in place to form a continuous cloth-walled chamber. The water of the reservoir was replenished and the cloth walls were soaked from the outside with a spray of water once or twice daily. The reservoir and curtains were kept in place throughout the experiment except during frequent periods when the cloth was removed from one side to admit light.

In the first test of varietal susceptibility by this method

Table XI.

Varietal Susceptibility in Tubers

Variety (2 tuber sections of each variety)	Progress of rot after inoculation 3 days	12 days	16 days	Class
Burbank	Incipient	2 completely rotted	2 completely rotted	S+
Burbank Russet	Incipient	2 completely rotted	2 completely rotted	S+
Rural New Yorker	Incipient	2 rotted 1/3	2 rotted 1/3	S
King	Incipient	2 rotted 2/3	2 completely rotted	S+
Irish Cobbler	Sound	1 rotted 1/3 1 sound	1 rotted 1/3 1 sound	S-
Early Ohio	Incipient	1 completely rotted 1 rotted 1/3	1 completely rotted 1 rotted 3/4	S
Bliss Triumph	Incipient	2 completely rotted	2 completely rotted	S+
Green Mountain	Incipient	2 rotted 1/2	2 rotted 2/3	S

Table XII.

Varietal Susceptibility in tubers

Variety (2 tuber sections of each variety)	Extent of lesions after 14 days: Average Diameter in centimeters	Extent of lesions after 14 days: Depth of Penetration in centimeters	Class
Burbank	3.3 2.0	2.0 2.0	S+
Burbank Russet	3.0 3.0	2.0 1.5	S+
Rural New Yorker	1.0 0.5	0.5 0.5	S
King	4.0 4.0	2.0 2.0	S+
Irish Cobbler	1.5 2.3	0.5 0.5	S
Early Ohio	1.0 3.0	2.0 2.0	S
Bliss Triumph	0.7 0.7	1.0 1.0	S-
Green Mountain	0.0 0.0	0.0 0.0	S-

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the eight varieties were inoculated six weeks after planting. The inoculum was inserted in a needle prick at the second node from the top and the inoculation point was kept moist by wrapping it with moist cotton. In the second test plants were inoculated three weeks after planting and the inoculum was inserted at the base just above the lowest node. The results of the two experiments are summarized in Tables XIII and XIV, and the appearance of the inoculated vines in the first of these is shown in Plates X to XIII inclusive.

The classes of susceptibility of the eight varieties as determined in the several experiments are summarized in Table XV.

Differences in susceptibility were more sharp in vines than in tubers. Irish Cobbler and Early Ohio were most susceptible; the first of these two was injured most severely. Bliss Triumph, Burbank Russet, King, and Green Mountain were slightly resistant. These differences seem sufficiently well marked to exert an important influence on the severity of the blackleg disease in the field. It must be emphasized, however, that none of the standard varieties were immune, and the degree of resistance in no case approached immunity.

A number of other experiments shed light upon the question of varietal susceptibility. The severely affected plant shown in Plate I. is a Burbank Russet. Green Mountain was heavily infected in the experiments on effect of age of tissue on infection. These varieties, then, are distinctly susceptible while the tissues are young; blackleg may prove destructive on them if the early part of the growing season is wet. In two experiments in which Burbank, King, and Irish Cobbler were inoculated under similar conditions, Irish Cobbler only developed blackleg lesions. Plate II shows

Table XIII.

Varietal Susceptibility of Vines to Infection near the top

Variety	Wilting of stem above infection court (1)		Degree of stem blackening	Downward progress of lesion in 16 days	Injury to plant	Class
	In 3 days	In 16 days				
Burbank	+ +	+ +	Distinct Distinct	4 cm. 5 cm.	Slight	S
Burbank Russet	- -	- -	Very slight Very slight	None None	None	R
Rural New York-er	+ -	+ +	Moderate	10 cm. 10 cm.	Slight	S+
King	+ -	+ -	Moderate	7 cm. 2 cm.	Slight	S
Irish Cobbler	+ +	+ +	Moderate	30 cm. 24 cm.	Very Severe	S++
Early Ohio	+ +	+ +	Moderate	5 cm. 15 cm.	Slight Severe	S+
Bliss Triumph	- -	+ -	Moderate None	1.5 cm. None	Slight None	R-
Green Mountain	- -	+ +	Slight Slight	5.0 cm. 0.0 cm.	Slight	R-

(1) + indicates the stem wilted.
- indicates that it did not.

S - Susceptible
S+ - Very susceptible
S++ = Extremely susceptible
R- = Slightly resistant
R - Moderately resistant

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Table XIV.

Varietal Susceptibility of vines to infection at the base

Variety	Wilting of leaves above infection court after 13 days	Blackening of stem	Length of lesion after 13 days	Class (1)
Burbank	Slight	+ -	5.0 cm. 0.0 cm.	S
Burbank Russet	-	+ +	3.0 cm. 2.5 cm.	S-
King	-	- -	2.0 cm. 1.0 cm.	R
Irish Cobbler	Slight	+ -	6.0 cm. 1.0 cm.	S
Early Ohio	Decided	+ +	2.5 cm. 7.0 cm.	S+
Bliss Triumph	-	- -	2.0 cm. 1.5 cm.	R
Green Mountain	-	Brown Brown	2.0 cm. 2.0 cm.	S

(1) See Table XIII for explanation of class designations.

Table XV.

Summary of Varietal Susceptibility

Variety	Tuber Susceptibility: Table IX Whole tubers	Tuber Susceptibility: Table X Half tubers	Tuber Susceptibility: Table XI Tuber sections	Tuber Susceptibility: Table XII Tuber sections	Vine Susceptibility: Table XIII Inoculated at top	Vine Susceptibility: Table XIV Inoculated at base
Burbank	-	S+	S+	S+	S	S
Burbank Russet	S-	S+	S+	S+	R	S-
Rural New Yorker	S+	S+	S	S	S+	-
King	S+	S-	S+	S+	S	R
Irish Cobbler	-	S	S-	S	S++	S
Early Ohio	S-	S	S	S	S+	S+
Bliss Triumph	S	S	S+	S-	R-	R
Green Mountain	S	S	S	S-	R-	S

For classes see Table XIII.

one of these infected plants which rotted very rapidly. The extreme susceptibility of this variety was apparent in every test in which it was used.

The records of the Potato Certification Board of Minnesota show that in 1920 the maximum percentages of blackleg in fields of different varieties were: Irish Cobber 15 percent infected, Early Ohio 8 percent, Green Mountain 12 percent, and Rural New Yorker 3 percent. In 1921 the corresponding maximum infections recorded were: Irish Cobbler 3 percent, Early Ohio 3 percent, Rural New Yorker 8 percent, and Green Mountain 1 percent.

Discussion and Conclusion

Blackleg is essentially a cool climate disease. Prolonged exposure to temperatures above 30°C or short exposures to dry air are fatal to the causal organism. Morse (25) has found the organism sensitive to direct sunlight also. It must accordingly lead a precarious existence in southern potato regions where soil temperatures may easily exceed 30°C (86°F) and where the sunlight is direct and intense. The low humidity which commonly accompanies high temperatures is perhaps the most important factor in limiting infection and development of the disease.

Although the organism is rapidly killed by exposure to any of these external influences it is somewhat protected in tubers. The water content of potato tubers is sufficiently high to permit the organism to live and even develop at low humidity, and the bulk of the tuber doubtless serves as a protection against sudden rises of temperature. The exact influence on tuber rots of temperature, atmospheric humidity, and water content of potato tubers is a problem well worth further investigation.

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The blackleg organism may lie dormant in stored potatoes. If humidity is low and temperature moderate, or if temperature is below 5°C and relative humidity is high, the rot does not develop but the organism retains its virulence. A slight rise in either temperature or humidity is followed by renewed activity of the pathogene, and a great increase in the amount of inoculum. Continued spread of the rot destroys the infected tuber and spreads the highly virulent inoculum over the tubers below it. Wounds, lenticels, or sprouts in these furnish suitable infection courts for the bacillus.

Varietal susceptibility and age of tubers appear to be of little significance in limiting infection. Temperature affects the rate of development, but relative humidity appears to be the factor of critical importance. If the liquid inoculum exuded from rotted tubers remains moist for 24 hours or more the organisms in contact with the wound or other infection court have the requisite conditions for multiplication and penetration. The pathogene fails to infect if the atmosphere is very dry and the inoculum quickly dries out. Spread of the disease in storage, the most important repeating stage in the life history of the pathogene, is thus governed by atmospheric moisture.

The importance of blackleg in initiating storage rots of tubers and the role of the secondary bacteria and fungi involved are problems of great interest and importance. In the author's experience the blackleg organism has been hard to isolate from the mixed bacterial infections known as wet rots or soft rots. The predominance of the saprophytes encroaching upon the pathogene may obscure its presence, or possibly inhibit its development in later stages of the rots. E. F. Smith (42) has suggested the very sketchy nature of our present knowledge of tuber rots, and the economic importance of these is sufficient to justify exhaustive study.

Tubers infected before storage time from diseased vines, or during storage by contact with diseased potatoes in the following spring frequently are cut into seed pieces and planted. Careless workmen make cuts thru blackleg lesions and then thru sound tubers and not even the most careful worker can detect every lesion at this time. Much emphasis has been placed on the spread of blackleg by cutting knives. (25,28 etc.) In a preliminary trial by the author a large quantity of broth cultures was poured over tuber sections heaped in a jar containing some water at the bottom. Only those pieces in contact with the water rotted. The humidity in the laboratory was between 20 and 30 percent. There is evidence that humidity is a limiting factor in spread of the disease at this stage also.

Moist soils in the early potato season furnish optimum conditions for the development of the blackleg organism. Morse (25) has correlated heavy infections of blackleg with wet soils and high precipitation; and Rosenbaum and Ramsey have correlated them with low temperatures and high precipitation. The same limiting factors are operating here as in development of tuber rot.

Early infections are most active and general because the pathogene attacks chiefly parenchyma tissues and flourishes in these when they are young. The limitation of the lesions to the underground portions of the plant under field conditions is a humidity relation. No such limitation is evident when the pathogene is inoculated into various green parts of the plant under moist conditions. In late infections the lesions are largely confined to the parenchyma tissue of the pith.

Where lesions appear on the plants above ground and moist weather conditions prevail, the transfer of the organism by the white fly (*Aleurodes* sp.), or similar insects, is possible. Field observations by pathologists and growers have pointed to this possibility and three secondary infections in the greenhouse strengthen the evidence. The fact that many bacterial diseases (*Bact. solanacearum*, *Bact. campestre*, etc.) are known to be carried by insects has suggested this method of transmission for the blackleg organism. Transfer by soil inhabiting insects is also possible. Hegyi (14) found wire worm injury so commonly associated with blackleg lesions in German and Swedish fields in 1910 that he was convinced that the pathogene was secondary to these wounds.

Differences in varietal susceptibility of vines are probably of some importance in the field. Morse (25) stated that Maine growers were little troubled with blackleg while Green Mountain potatoes were grown. When Irish Cobblers were grown for the southern seed trade the disease increased in importance. Appel (3) fround differences in susceptibility in varieties grown in Germany, but no variety was immune. It is unfortunately true that the varieties of chief importance in Minnesota are most susceptible. Irish Cobblers and Early Ohio are very severely attacked, and Green Mountain is also infected moderately in the field.

Fluctuation in virulence of the blackleg organism has been recorded by Appel (3) and VanHall.(3) Jennison also mentioned it in correspondence and Morse (25) described a reviving process to bring his various strains to uniform vigor. Smith (42) observed no loss of vigor in *Bacillus phytophthorus* after 14 years growth on artificial media. The writer is convinced that the organism with which this work was done has been more virulent at some times than at others. The evidence presented on page 32 indicates that the age of a colony on artificial media also has an effect on its pathogenicity. Plate I. Fig. 2 shows two stalks from the same seed tuber, the one at the left inoculated with a culture of _B. phytophthorus_ 44 days after transfer, and the one in the center inoculated with the same strain 2 days after transfer. The older culture was much less virulent than the younger on both stem and leaf petiole. Too little data in regard to these questions are available to warrant definite statements at this time. However, the factors governing the virulence, and in turn influencing the pathogenicity of the blackleg organism, are worthy of detailed investigation.

Summary

1. The blackleg disease of the Irish potato, caused by <u>Bacillus phytophthorus</u> Appel, is an important factor in potato production in Minnesota and the northern potato districts in general.

2. The cardinal temperatures for growth of the causal organism are approximately: minimum 5°C, optimum 28°C and maximum 30°C. The organism is killed in broth cultures after 16 days exposure at 32°C.

3. The organism is killed by exposures of 30 minutes or less on dry cover slips.

4. <u>B. phytophthorus</u> retains its viability on agar cultures after 97 days, but not after 121 days. It remains alive in broth cultures after 130 days.

5. The disease was not shown to be transmitted by seed pieces or soil.

6. There is some evidence of insect transmission.

7. Tubers, stems, leaf petioles, and flowers were artificially infected. Inoculations of the soil, roots, and leaf lamina failed to develop infections.

8. Tubers rot more rapidly at 25°-28°C than at lower temperatures if the moisture supply is adequate.

9. Actual contact with water was necessary for infection in inoculated tubers. No infection resulted at 100 percent or at 80 percent relative humidity.

10. Tuber rot once initiated continued at 20 percent relative humidity, but its development is favored by higher humidities.

11. Vines were infected at 80 percent but not at 60 percent relative humidity.

12. The age of tubers had little effect on infection. Old vines were much less readily infected than young vines.

13. The eight standard varieties of potatoes grown in Minnesota showed no significant difference in susceptibility to blackleg tuber rot.

14. The varieties Irish Cobbler and Early Ohio were very susceptible to blackleg infection in the growing vines. Burbank and Rural New Yorker were moderately susceptible. Bliss Triumph, King, Burbank Russet, and Green Mountain were slightly resistant.

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Explanation of Plates

Plate I.

Figure 1. Burbank Russet potato vine inoculated in three places with *Bacillus phytophthorus*. Photographed 4 days after inoculation. The base of the stem nearly rotted away, a black lesion near the top of the stem, and a leaf infection which has blackened the petiole and leaf lamina.

Figure 2. At the left, at the arrow, a petiole inoculation that failed. In the same vine a local infection at the base of the stem showing a drooping leaf. In the center the plant shown in Figure 1, five days after inoculation. The stalk rotted off completely, and supported against the card background to show the extent of the lesion. At the right an uninoculated stalk.

Plate II.

Irish Cobbler vine 23 days after inoculation with *Bacillus phytophthorus* near the top of the stem. On the uninoculated stalk at the left 3 black lesions developed from possible insect transfer. Inset shows the lesions more plainly. See text for detailed explanation.

Plate III.

Four Early Ohio tubers inoculated thru the wedge shaped incisions at the top with agar cultures of *Bacillus phytophthorus* and placed at 80 percent relative humidity. No infections resulted. The two tubers at the left inoculated again with broth cultures of *Bacillus phytophthorus*. Photo 8 days after second inoculation.

Plate IV.

Early Ohio tubers inoculated as explained under Plate III, but placed at 100 percent relative humidity. The upper row inoculated twice. Photo 8 days after second inoculation.

Plate V.

Early Ohio stems inoculated with _Bacillus phytophthorus_. A and C kept at 80 percent relative humidity; B and D at 60 percent.

Plate VI.

Effect of age of tubers on the progress of blackleg rot. At the left 2 old and 3 young tubers of Early Ohio; at the right 2 old and 4 young tubers of Rural New Yorker. One young tuber of Early Ohio was completely destroyed and is not included in the photo.

Plate VII.

Tubers of the eight standard varieties of potatoes inoculated with _Bacillus phytophthorus_. 1. Burbank, 2. Burbank Russet, 3. Rural New Yorker, 4. King, 5. Irish Cobbler, 6. Early Ohio, 7. Bliss Triumph, 8. Green Mountain. Photo 10 days after inoculation.

Plates VIII and IX.

Susceptibility of potato tubers to blackleg rot. From left to right the varieties are as shown in the photo. Sections in the top row incubated under identical conditions. Sections in the bottom row a similar but independent series.

Plates X to XIII

Eight varieties of potatoes inoculated with _Bacillus phytophthorus_ near the top and kept under parallel conditions. Photo after 16 days.

Plate X Bliss Triumph (Tr) Burbank (Bbk).
Plate XI King, Irish Cobbler (Cobb).
Plate XII Early Ohio, Green Mountain (G.M.)
Plate XIII Burbank Russet (Russ), Rural New Yorker (R. N. Y.)

The infection in Burbank Russet (Plate XIII) is slight; the drooping of leaves is due to lack of light.

Plate I.

Figure 1.

Figure 2.

Plate II.

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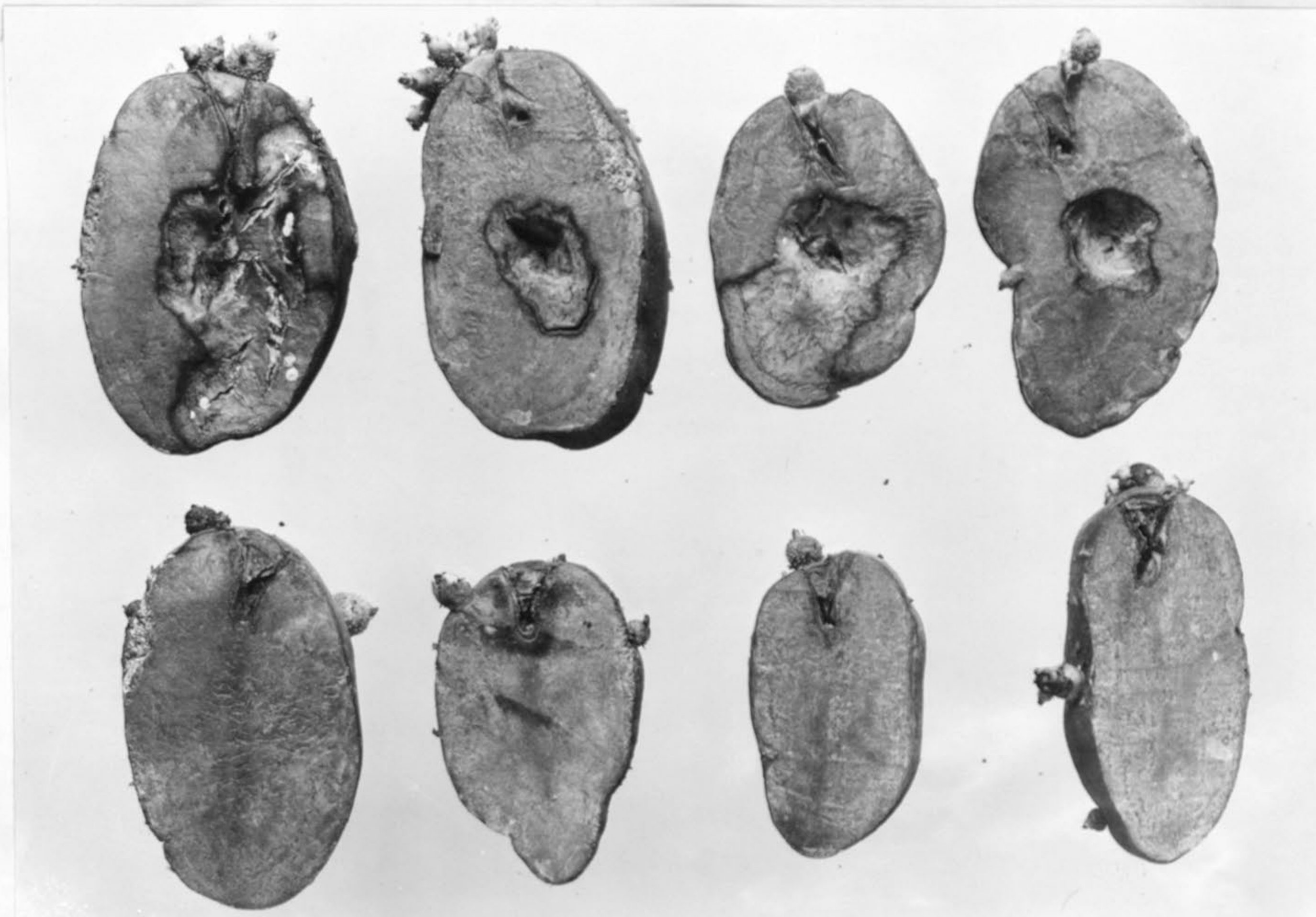
Plate III.

Plate IV.



100% Relative Humidity

Plate V.

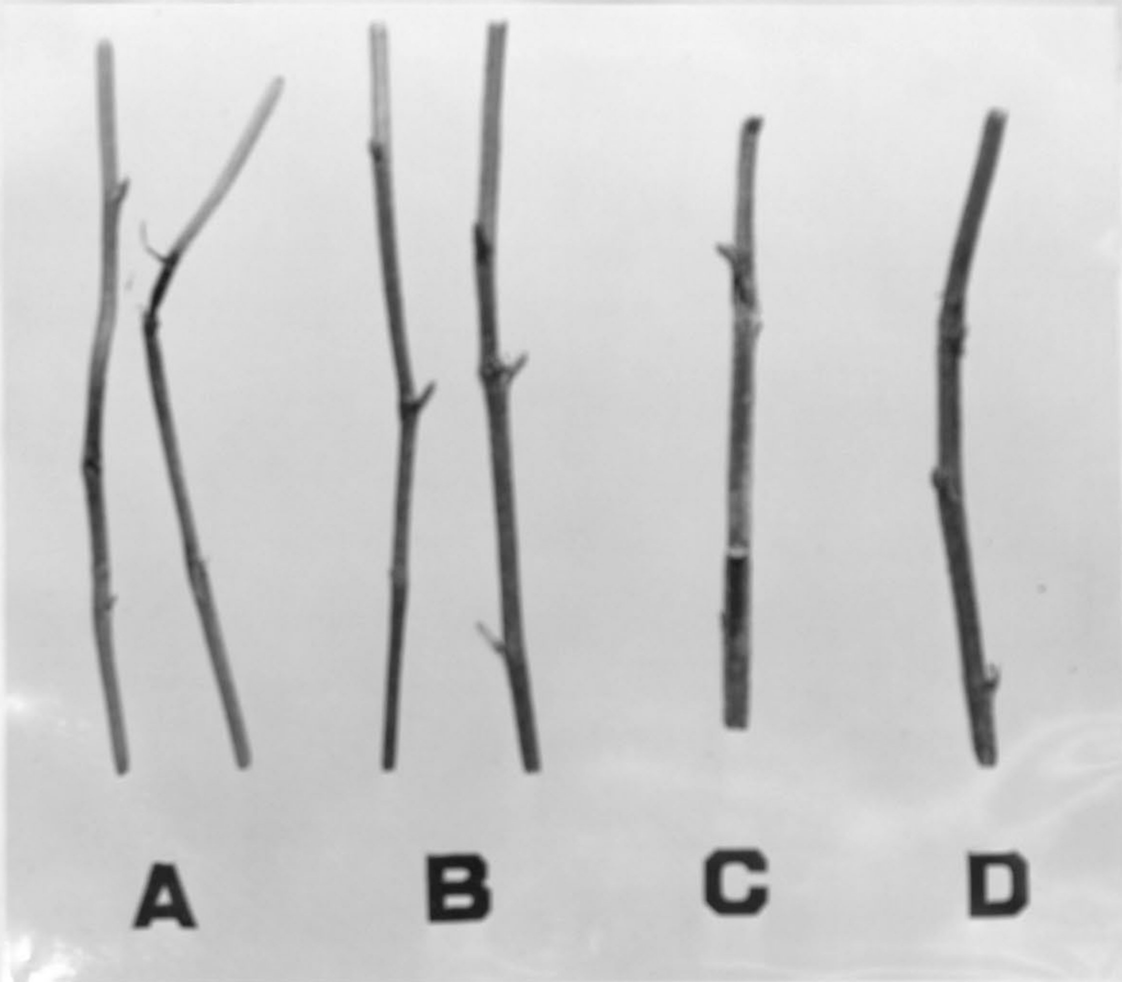


Plate VI.

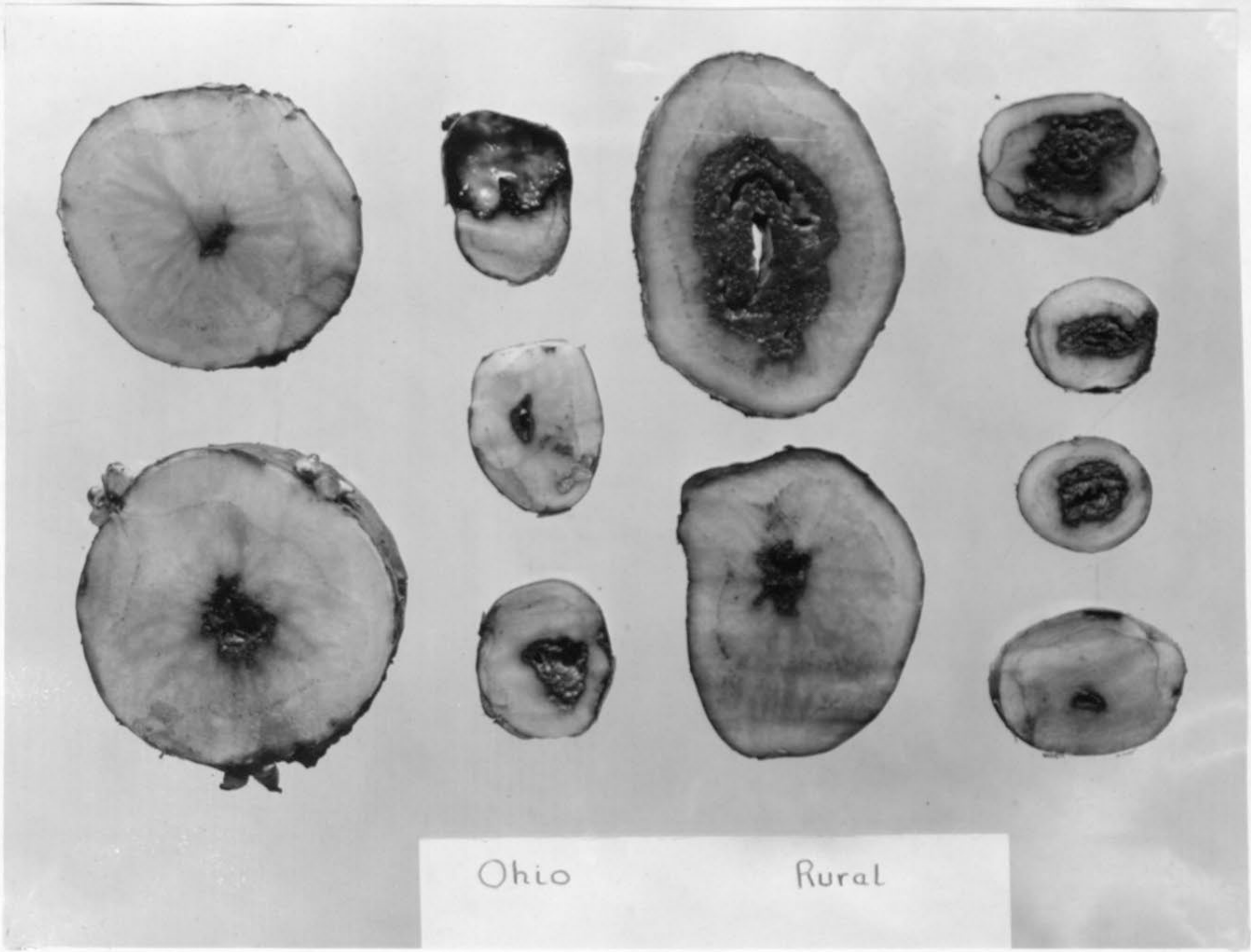


Plate VII.

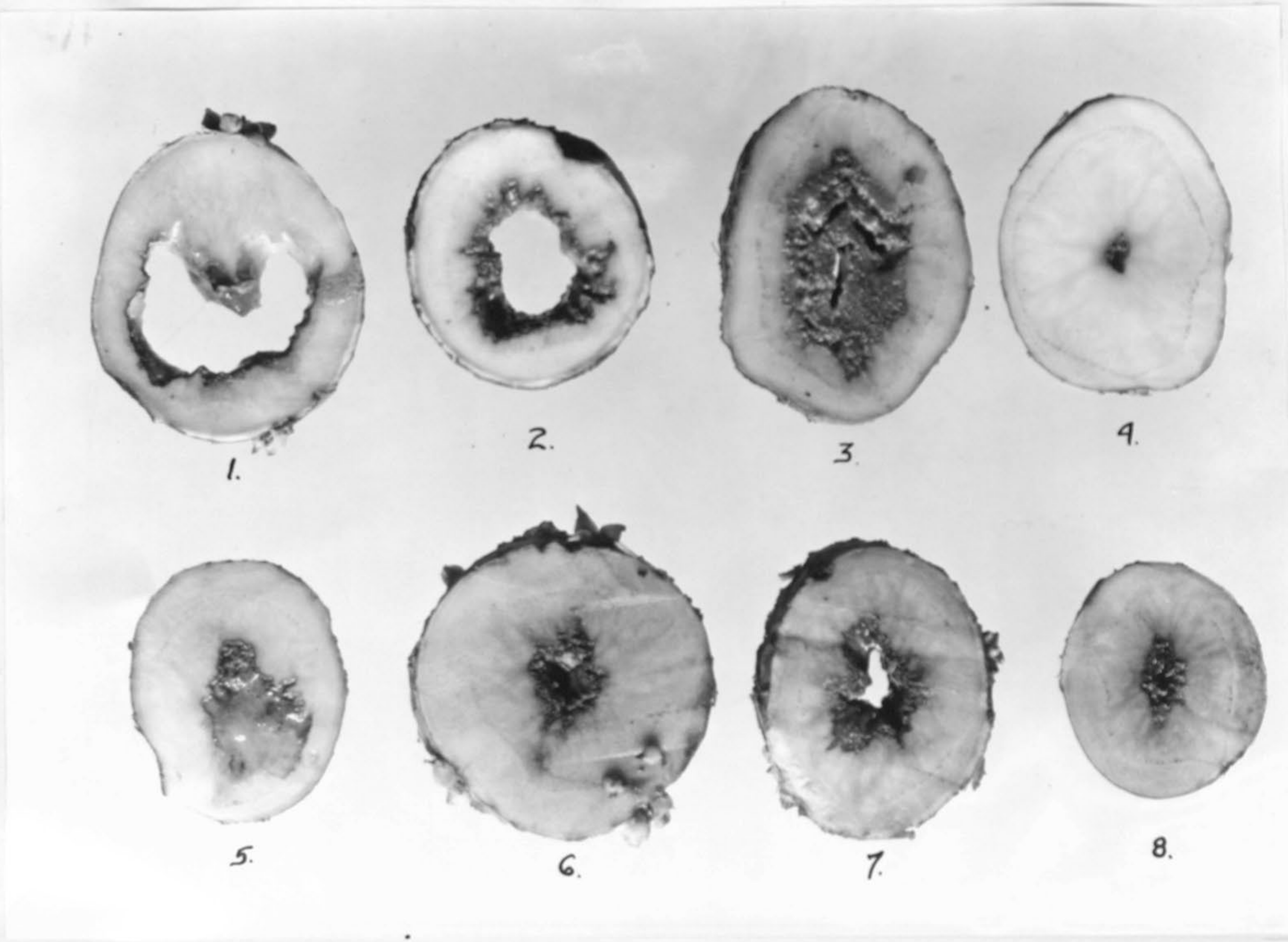
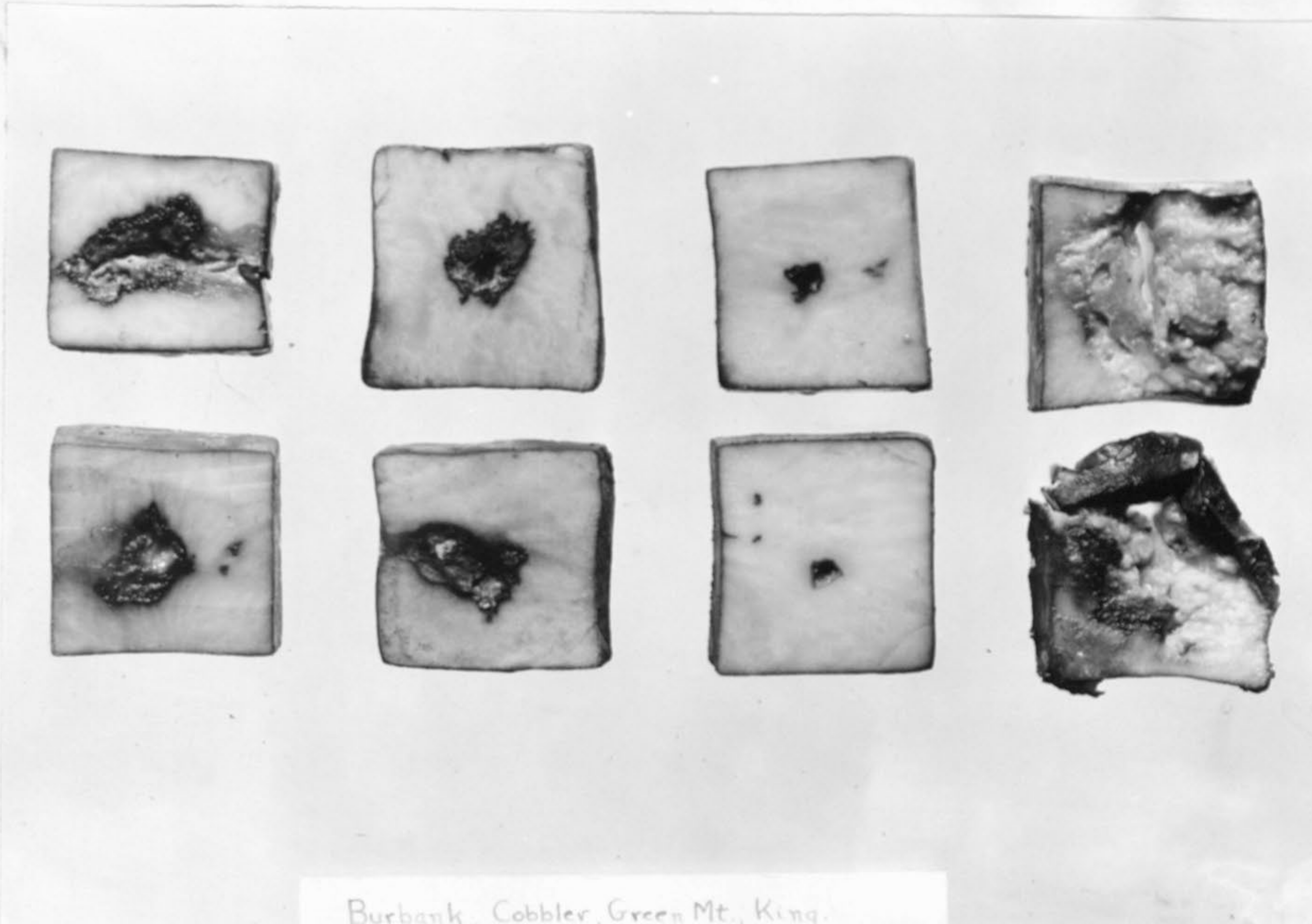
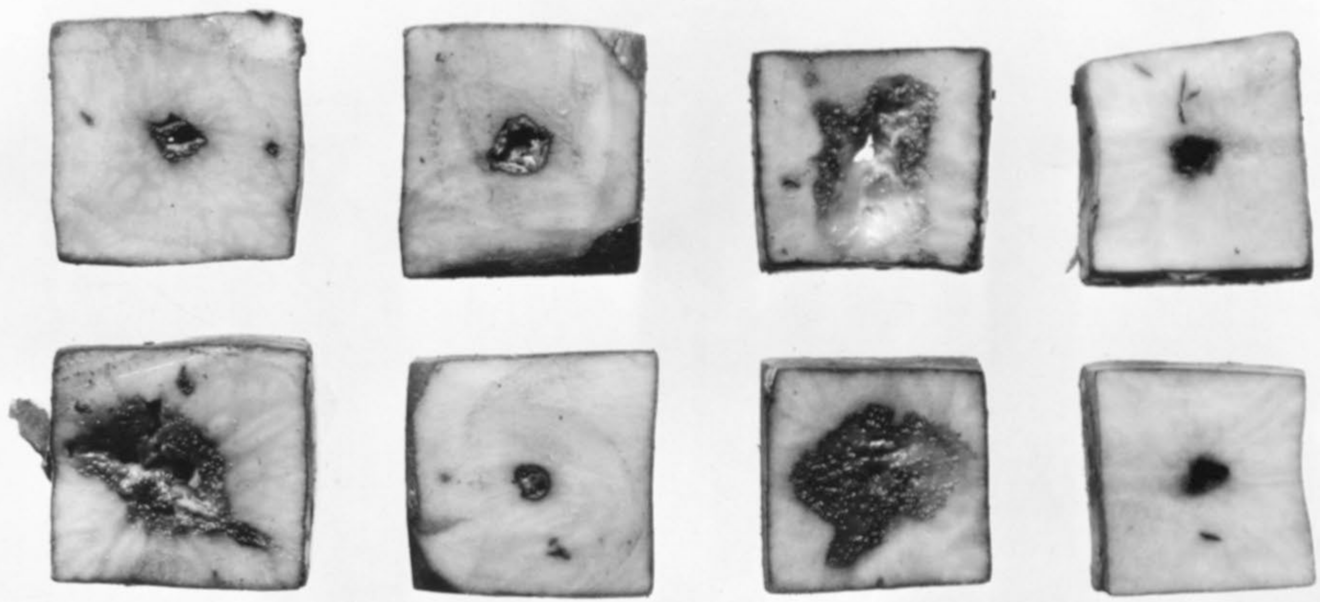


Plate VIII.



Burbank, Cobbler, Green Mt., King.

Plate IX.



Ohio, Rural, Russet, Triumph.

Plate X.

Plate XI.

Plate XII.

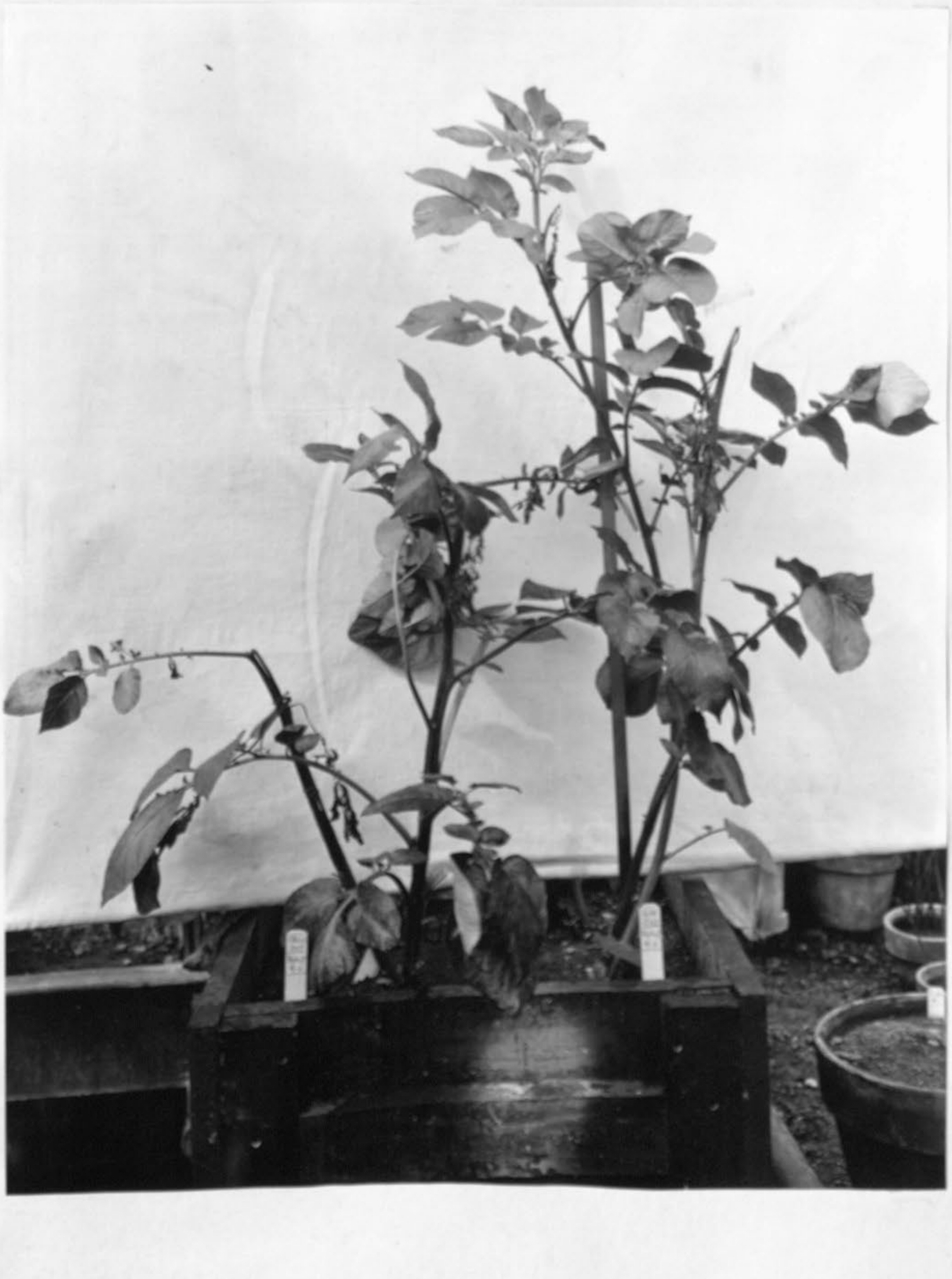


Plate XIII.