INVESTIGATIONS ON THE BLACKLEG DISEASE OF THE POTATO

Thesis for Degree of M. S.

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THESIS

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INTRODUCTION

For years serious losses from potato tuber rots, both in the field and in storage, have been reported from various sections of the Upper Peninsula of Michigan. The diagnoses of specimens sent to various plant pathological laboratories resulted in considerable difference of opinion as to the causal organism, and it was to clear up this matter, as well as to make a more thorough study of the disease, with especial reference to its manner of dissemination, and host relations, the better to recommend control measures, that the present investigations were undertaken.

SIGNS OF THE DISEASE

The disease in Michigan presents a picture similar to the Blackleg disease of potato as described by Morse[1*] in Maine, Harrison[2] in Canada, Pethybridge and Murphy[3] in Ireland, van Hall[4] in Holland, and Appel[5] in Germany. This disease, as the name indicates, is characterized by an inky blackening of the stem usually at the ground region. This character is not, however, a primary symptom of the disease, but on the other hand, is the

* The numbers following names of authors mentioned refer to the papers listed in the bibliography at the close of this paper.

characteristic which confirms the diagnosis. The first signs are the gradual fading of the deep green color of the foliage into yellow-green, and later, a yellow color. This sickly appearance is usually accompanied by an upturning of the leaflets and a tendency of the branches to grow upward forming a more or less compact top. The plant gradually dies as the base of the stem is rotted away by the bacteria. The diagnosis of these abnormal plants is easily confirmed by pulling them up. Such stems already having been considerably rotted offer no resistance to being pulled up and show an inky black appearance, the extent of which depends upon the stage of the disease. (Plate I.) Under weather conditions more favorable to rapid bacterial growth, the disease presents some different aspects. When the disease progresses rapidly the stems are rotted in a very short time and the plants fall over before the leaves have had time to assume a light green or yellow color. In these cases the stems may show a water-soaked appearance up to eight or ten inches above the surface of the ground. This condition is immediately followed by blackening. Under less favorable conditions when the disease progresses very slowly aerial tubers may be formed as in the case of Rhizoctonia disease of potatoes. The Blackleg germ makes rapid progress in the pith area of the stem and this in many cases results in hollow stalks.

In tubers the disease is usually characterized by rotting which proceeds from the stem end, the infection

having spread from the diseased seed piece to the plant stem, and from the latter to the stolen and finally to the new tuber. The writer has observed but one case of lateral infection of a tuber while in the soil and this was brought about by the immediate contact with a diseased stem. The skin of the tubers which are being rotted in the soil presents a gun-metal appearance over those portions which are rotting. Under favorable conditions new tubers are very quickly rotted, whereas if unfavorable conditions follow, the rotted portions dry up and give the tubers a truncated appearance. In this condition the disease remains dormant until more favorable conditions allow further rotting to progress. Tubers rotting from Blackleg when cut open show a white, creamy bacterial growth surrounded by a black line or border. There is also a tendency toward canal formation. (Plate II.)

PREVIOUS HISTORY

Stem and tuber rots of potatoes have been investigated since a comparatively early date. Among the earlier investigators were Hallier (1878), Reinke and Berthold (1879), Prillieux and Delacrois (1890), Kramer (1891) and Laurent (1899), but from their published accounts we cannot be certain of the exact nature of the rots dealt with because of the meagerness of their reports and because most of their experiments, due to the faulty methods of pathological technique of their time,

must of necessity have been performed with mixed cultures or, at least, poorly described organisms.

Frank[6] (1899) was the first to associate the stem disease of potatoes which he designated as "Schwarzbeinigkeit" and the accompanying rot of tubers with a definite bacterial organism. To this organism he gave the name Micrococcus phytophthorus but did not publish a complete description of it.

Appel[5] (1903) in his monographic report upon the Blackleg disease of the potato gave in considerable detail results of his investigations as to the causal organism, its host relations, its loss of virulence, resistance and susceptibility of varieties. His work was a continuation of that begun by Frank. In this work, it was found that the causal organism is a bacillus and because Frank failed to give a complete description of the organism with which he worked and left no cultures, the former renamed the causal organism Bacillus phytophthorus. Smith[7] having received a culture of the organism from Appel gives the most complete description of Bacillus phytophthorus available today. This description was made according to bacterial standards. Harrison[8] (1906) reported in a complete manner the results of his two years' study of the Blackleg disease of Canada. In this report he confirmed the bacterial nature of the rot, but assigned the disease to Bacillus solanisaprus. He also took up the effects upon varieties of potatoes and inoculated other plants.

Morse[1] (1917) in summarizing his nine years' work on the Blackleg disease in Maine gave a review of the literature, the geographical distribution as well as ecomomic aspects of the disease, control measures, and a comparative study of the causal organisms. He also reported as a result of his observations on the sources of infection and wintering over of the organism, that diseased tubers and not soil was the spring source of infection.

Rosenbaum and Ramsey[8] (1918) in investigating the influence of temperature and precipitation on the Blackleg disease of potato, found that the severity of the disease was closely correlated with temperature and precipitation and was dependent upon them. These investigators conducted experiments on wintering over of the disboth in Maine and Virginia, confirming the previous views of Morse.

Ramsey[9] (1919) reported studies on the viability of the potato Blackleg organism after being subjected to various climatic and environmental conditions. These experiments supplemented in a minor way the more extensive experiments of Rosenbaum and Ramsey. This work consisted in subjecting infected tubers to low temperatures, which invariably proved fatal. He also obtained negative results from attempts to inoculate plants by the watering method.

MICHIGAN OBSERVATIONS AND EXPERIMENTS

Occurrence

Edson as a result of field observation and subsequent laboratory work was led to believe that the "Blackleg" disease in Michigan differed from the disease as seen in Maine and other parts of the United States. In his opinion, the disease found in the Upper Peninsula of Michigan more nearly resembled a Fusarium disease of potatoes which he observed in Minnesota, and he explained the presence of bacteria in the tissues by considering them of the soft rot type which were merely secondary invaders.

In order to determine definitely whether the Blackleg disease of the Upper Peninsula of Michigan was a Fusarium trouble or whether it was due to a bacterial parasite as is the case of Blackleg diseases reported from other states of the United States and from other countries, the writer poured dilution plates from diseased material obtained from two different fields in Alger County located about eighteen miles apart. From these plates nine bacterial colonies were isolated, five of which, when inoculated into raw tubers and into healthy, growing plants, produced characteristic symptoms of the disease. In no case were any of the molds which developed upon the plates found to be Fusarium. The writer has repeatedly isolated bacteria from diseased plants from a great many Upper Peninsula points which when tested out with authentic strains of Bacillus phytophthorus obtained through the courtesy of the

Wisconsin Experiment Station produced similar Blackleg effects. There seems no chance for question as to the etiology of the Blackleg disease of the Upper Peninsula of Michigan, and certainly Fusarium is not concerned. The organism is a bacillus which is similar, if not identical, with that described as the causal organism of the Blackleg disease of potato in other sections of America and Europe.

Economic Losses

The losses resulting from Blackleg vary with the seasons. During seasons which are cool and wet conditions are very favorable for the disease and much greater losses follow than is the case during dry seasons of little rainfall. As to the importance of the disease in this and different countries, the various investigators report as follows:

Germany: 6 5
Frank and Appel: The disease is common, widespread and often destructive.

France: 9
Delacroix: Perhaps wider spread than this investigator supposed because of the confusion between this disease and "brunissure".

Ireland: 3
Pethybridge: 94% of diseased plants produced by selecting sound seed tubers from an infected crop of the previous season.

Canada: 2
Harrison: Estimates total loss through rot 10-75% of the crop in Province of Ontario in 1905.

Canada: 10

Murphy[1]: In parts of the Maritime Provinces of Canada 10% of total crop in 1915. In some cases percentage of Blackleg plants higher than that, and total misses were as high as 80% and more.

United States:

Morse[1]: Extensive observations in Aroostook County, Me., covering a period of five years revealed in many fields only scattered Blackleg plants and not infrequently these amount to 1 or 2 percent, 5 percent of diseased plants being considered by the growers as representing a severe attack. Losses of 10 or 15 percent or more are by no means unknown, however.

Coons[11]: Reports that losses of from 25 to 75 percent were not uncommon in the Upper Peninsula of Michigan in 1915.

The writer studying the disease during the 1919 season, which was very dry and unfavorable to the disease, visited a number of fields which showed 10% of infected plants. The average for the entire Upper Peninsula during that season, however, was considerably less and 1-3% would be a fair average.

From the above reports it can be concluded that Blackleg is indeed a serious disease of the potato and that in the Upper Peninsula of Michigan it is one of the limiting factors of the potato growing industry.

Wintering over of parasite:

In Tubers:

It has been generally conceded by most investigators that diseased tubers kept in storage over winter are the main source of infection. These tubers under favorable conditions rot and cause the rotting of adjoining healthy tubers. The bacterial slime may also spread over the surface and into the eyes, cracks, or bruises of otherwise

normal tubers. Although Morse[1] and others have noted that the organism is readily killed by dessication when it is dried on a smooth glass surface, the writer has found that under other conditions the organism will retain its vitality for a much longer time. For instance, Morse found that the organism when dried on small glass discs at room temperature was dead in less than half an hour, whereas the writer has found that the organism will retain its vitality for four days when dried on a silk thread under similar conditions. (See Table I.)

Table I.

Dessication of the Blackleg Organism

Medium	Where kept	Tested on raw tubers and nutrient broth: 1 day	2days	3days	4days	5days	6 days
Cover Glass	Desk drawer	dead	dead	dead	dead	dead	dead
" "	Desiccator	"	"	"	"	"	"
Silk Thread	"	-	+ alive	+ alive	+ alive	- dead	- dead
" "	Desk drawer	alive	"	"	"	"	"

From these results it is seen that the organism is able to live for a considerable period under conditions of very little moisture and sufficient protection from dessication to tide the organism over until more favorable conditions arise.

In regard to wintering over of the disease in

tubers which remain in the soil over winter, Morse[1] basing his opinion upon field observations makes the following statement: "On fields which are planted with potatoes the second time in succession there is usually quite a percentage of volunteer plants which spring from tubers which remained in the soil over winter. These plants are frequently easily recognized by their irregular occurrence on the sides of the rows or between hills, The writer,(Morse) has never seen such plants affected by Blackleg. This observation was quite unexpected, for it seems not unreasonable to suppose that, if the tubers were sufficiently protected from frost to be able to germinate, the bacteria causing the disease might live over in the soil in such infected tubers as well as in storage." Ramsey[9] in studying the problem of over-wintering of the bacteria, inoculated healthy disinfected potato tubers with a pure culture of a strain of B. atrosepticus van Hall. After being left in a moist chamber for 48 hours the tubers showed marked rotting at the point of inoculation and were then packed in crushed ice in a refrigerator. Every twenty-four hours one of these tubers was taken out and planted. Out of the eleven tubers which were planted only two germinated but the sprouts were immediately killed by Blackleg. All other tubers decayed without showing any sprouts above ground. Plates which were poured from the tubers before planting showed that the number of bacteria was reduced to a minimum after 264 consecutive hours. Ramsey offers this as an explanation as to the

reason why Blackleg is never found in volunteer plants in Maine, and states: "Evidently the infected tubers which remain buried in the fields at digging time, decay to such an extent that they are unable to germinate, or if they do germinate, the sprouts are immediately killed by the organism. Therefore, all volunteer plants which make their appearance in the spring are free from Blackleg."

Rosenbaum and Ramsey[8] in more extensive experiments on the wintering over of the Blackleg organism in naturally infected and artificially inoculated tubers found that: "In no case was the blackleg organism found to live over a winter in the soil or in the tubers remaining in the soil."

The experiments of Ramsey and of Rosenbaum and Ramsey may be criticised in that they were not sufficiently extensive to warrant definite conclusions to be drawn therefrom. Then, again, it is very probable that the wintering over might occur in tubers which in the fall show very slight, if any outward indication of infection. In the experiments conducted by the above investigators only badly infected tubers were used, and these no doubt account for the results obtained. Soil conditions, or at least those of the Upper Peninsula of Michigan during the winter cannot be compared to conditions represented by Ramsey's cracked-ice experiment, because in ordinary years the ground is not frozen before it is covered with snow and permits the wintering over of potatoes at a comparatively low but not freezing temperature. The writer[12] has also observed from experiments on chilling and frost injury, that potatoes which have been exposed to

freezing temperatures decay very rapidly through the invasion of secondary organisms, and it is possible that these secondary bacteria swamped and crowded out the Blackleg organisms in Ramsey's experiments.

In order to determine the question of wintering over, under conditions existing in the Upper Peninsula of Michigan, a plot of approximately one-quarter of an acre was left undug in the fall of 1918. Blackleg was present in this plot during the 1918 growing season. In the spring of 1919 this plot was dug and a portion of the crop was spread out upon a clean barn floor to greensprout. The writer carefully selected six bushels of this green-sprouted stock, discarding all tubers which showed evidence of rotting. Two bushels were disinfected with mercuric chloride, and two bushels with formaldehyde in the usual manner, while the remaining two bushels were left untreated as a check. The potatoes were planted with a machine planter, the Corrosive Sublimate treated tubers being planted first, followed by the formaldehyde treated and untreated tubers, respectively. To avoid the possibility of soil infection, the tubers were planted on virgin soil which had borne no previous crop. Careful examination was made of these plots twice a week throughout the entire growing season and each diseased hill was staked as found in order to avoid duplication in recording the number of diseased hills in each plot. The results obtained from this experiment are shown in the following table:

Table II.

Overwintering of Blackleg in Tubers remaining in the soil at the U. P. Exp. Sta., Chatham, Mich., 1918-1919.

Treatment	No. diseased hills found during season	Percent
Corrosive Sublimate, 4 oz. to 30 gal., 30 min.	2	0.4
Formaldehyde, 1 pint to 30 gal., 15 min.	7	1.4
Untreated	35	7.2

Plates were poured from diseased plants, the causal organism isolated, and its pathogenicity proved by inoculation into raw tubers and into healthy growing plants. Further evidence of Blackleg was found in three other plots of the same spring-dug seed stock which were planted in another field. The above experiment and observations indicate that the Blackleg disease can winter over in tubers which remain in the soil under conditions existing in the Upper Peninsula of Michigan. The relation of treatment to percentage of Blackleg appearing is also significant.

In the Soil:

As in the case of wintering over of the organism in tubers, there is also difference of opinion as to the wintering over of the organism directly in the soil. Appel 5

and Harrison[2] both believed in the organism remaining in the soil and in their control measures recommend rotation of crops. Morse[1], drawing conclusions from observations made of fields planted to potatoes two years in succesion which during the second year showed either not any or reduced numbers depending upon, in his opinion, the thoroughness of seed treatment, states: "--infected seed potatoes are the sole source of infection and distribution and that the disease does not live over the winter in the soil." Rosenbaum and Ramsey[8] arrived at the same conclusion after attempting to isolate the organism from soil in various parts of their wintering over test plots. These investigators poured many series of plates and made a great number of isolations, all of which proved to be non-pathogenic when inoculated into raw tuber slices and into growing plants. The difficulty experienced in endeavoring to pick up colonies of the Blackleg organism from plates poured from soil samples is, of course, obvious and militates against the force of their conclusions. There is such a great number of soil organisms which develop upon agar plates very similar to the Blackleg organism in appearance, that the chances of picking up the right organism are very small.

The following experiment is noteworthy in view of the positive statements of other workers: In this experiment three kinds of soils were used, Clay, Sand, and Muck. Sufficient soil of each kind was used to fill two

large wooden pails and one series was kept in the greenhouse, while the other was allowed to winter over outdoors. Each of these was thoroughly mixed after receiving two liters of a decoction made up of severely rotted tubers and water, the soils being literally saturated with the organism. In the spring each soil was thoroughly mixed and an average sample taken according to the methods followed in soil analysis. Instead of attempting the almost futile task of isolating the organism from the soil, raw-tuber slices were inoculated with portions of the soil, thus allowing the tuber slices, the natural host, to do the selecting. One hundred and eight raw tuber slices in Soyka dishes were then inoculated with small quantities of soil, eighteen being used for each of the six samples taken. The data obtained from the experiment are recorded in the following table:

Table III.

Wintering over of the Blackleg Organism in various Soils. 1919-1920.

No.	Kind of Soil	Where kept	Number slices blackened	Number slices not blackened	Total slices rotted
1	Clay	Greenhouse	7	9	16
2	Sand	"	9	7	16
3	Muck	"	11	6	17
4	Clay	Outdoors	10	5	15
5	Sand	"	8	3	11
6	Muck	"	13	2	15

It should be noted that the soils which were used as inoculum in the above experiment were mixed cultures of bacteria and fungi, and not all of the rotting which followed can be attributed to the Blackleg organism. Fut some of the tuber slices showed discoloration not entirely dissimilar to the blackening which appears on tubers rotting from Blackleg. Direct inoculation from one of these discolored tuber slices into a healthy growing potato plant resulted in a brown black discoloration similar to the discoloration of potato stems following inoculation with pure cultures of the blackleg organism. Isolations are now in progress from this inoculated stem.

Although the securing of a pure culture of the Blackleg organism from the soil was not attempted directly, there is sufficient evidence brought forward in the overwintering experiment reported above to lead the writer to believe that it is possible for the organism to withstand the ravages of Michigan winters. If in several cases and with several types of soil, organisms capable of producing typical Blackleg rot of potato slices are found, and Smith holds this capacity as a decisive proof of identity, it is evident that under the multiplicity of conditions presented in nature, a great deal of overwintering does take place, and this may be of some little importance in control recommendations.

Other hosts for the parasite:

Appel[5] was the first to attempt to ascertain whether

the organism which was responsible for "Schwartzbeinigkeit" in Germany was parasitic on any other hosts. This investigator succeeded in getting infection from inoculations of B. phytophthorus on Cucumber, Winsor Bean, Carrot, Teltower Beet, Lupine and Tomato, but was not successful in inoculations on Sugar Beets, Mangels, Pelargonium, and the grains.

Harrison[2] also reports successful results from inoculating other hosts with his B. solanisaprus. Positive results were obtained from inoculating tomatoes, Capsicum annum, and cucumbers, while negative results were obtained from inoculations into egg plant, S. Melongena, Physalis, Datura stramonium, Schizanthus pinnatus, Collinsia verna, Salpiglossis sinuata, and Impomoea purpurea.

In a similar experiment the writer obtained positive results from inoculations into tomato and Nicotiana rustica, negative results being obtained from inoculations into Petunia, Bittersweet, Datura stramonium, and Physaloides. (Plate III.)

From the above experiments it is evident that the causal organism of Blackleg of potato is also parasitic on a number of other hosts and under favorable conditions might cause serious damage. This fact is also of importance in recommendation of control measures. Appel[5] states that potatoes should not follow in rotation such crops as cucumbers, carrots, lupines, or tomatoes, nor should these immediately follow potatoes.

Relation of Host and Parasite:

Uninjured Roots:

There has been in the past some question as to whether or not the Blackleg organism, in the event that it is able to live over winter in the soil, is able to penetrate healthy roots. Ramsey[9] conducted several experiments to determine this question and secured negative results from attempts to inoculate healthy growing plants by a watering method. As a result of his studies he concludes that unless the seed piece is infected at planting time, there is little chance that uninjured plants will contract the disease even though the causal organism is washed about the stem and root system. The writer also has carried on experiments in this regard. Inoculation of healthy roots of potato plants growing in water cultures has been attempted. Tubers were allowed to sprout in wet excelsior and when the roots were approximately 3 cm. long, a piece of the tuber about 1 cubic centimeter in size containing the sprout and roots was cut and placed in a quart Mason jar containing a nutrient solution made up of the following:

Magnesium Sulphate	60.0 cc
Acid Potassium Phospate	72.0 cc
Calcium Nitrate	20.8 cc
Water	1000.0 cc

The stock solutions of the salts used were of one-fourth molecular concentrations. Shives[13] found that this nutrient solution produced optimum growth of wheat and it was thought probable that it would also prove a good medium for potato-growth. Two such cultures were prepared. About two months after the cultures were started, they were about 10 cm tall and had about seven leaves each.

One was inoculated by pouring about 5 cc of a 4-day-old broth culture of B. phytophthorus into the solution. Nine days after inoculation it was noted that the plant had made no further growth and that the leaves presented a lighter color than the check. The check had a good root system, healthy green leaves, and had begun to form new tubers, the largest of which at this time was about the size of a marble. A month after the addition of the broth containing the Blackleg organism, the water culture potato was dead. Contrary to what occurs in Blackleg infected plants in the soil, there was no blackening of the roots or stem of the water culture potato either above or below the surface of the solution. It appears that the organism was not able to penetrate the uninjured roots, but the toxins produced by the organism, being absorbed by the roots resulted in the death of the plant. The check remained normal throughout the experiment. (Plate IV.)

The water culture experiment, therefore confirms Ramsey's results in that it shows that the bacteria are unable to penetrate uninjured roots . It may be well to point out that too general a conclusion must not be drawn from this fact. In nature the roots are often broken by cultivation, or are injured by other diseases as Rhizoctonia or Fusarium and thus ample opportunities for the entrance of bacteria are presented. It would not seem justifiable, therefore, to give the significance as Ramsey has done, from experiments conducted on uninjured roots and to deny all

effects from washings of bacteria to the roots of plants in the field. Further field experimentation is necessary before any definite conclusions can be drawn in regard to the transmission of Blackleg from hill to hill through the soil.

Amount of Inoculum:

It has been noted by Smith[7] " -- that in culture-media not exactly adapted to the needs of an organism, a scanty inoculation may not give any growth - not even after a long time - whereas a copious one will lead to a growth which gradually clouds the fluid or covers the solid. -- The only explanation I can think of is that a multitude of bacteria is stronger than a few, and thus by union are able to overcome obstacles too great for the few. The same fact comes repeatedly to the attention of the animal pathologist as a result of his inoculations. The animal body, we must assume, is often able to oversome and destroy a few hostile organisms, where it would not be able to defend itself against many; otherwise whole races would be exterminated by natural infections. The same is undoubtedly true in plants. The modus operandi in plants is not altogether clear. We may advance several hypotheses:(1) The formation of a resistant cork-layer before the bacteria have multiplied to such an extent as to prevent cell-division; (2) the destructive action of antiseptic plant-substances, e.g., acids, before these can be neutralized or otherwise destroyed by the substances produced by the multiplying bacteria.

In some instances, the introduction of a very considerable mass of bacteria seems to be necessary to induce disease; in other cases a very few are sufficient. It would be extremely interesting to know the minimum number capable in any given case of inducing disease."

In order to determine the effect of amount of inoculum in the case of the Blackleg disease of potato, the following experiment was performed. Dilutions of 1:100, 1:10,000, 1:100,000, and 1:1,000,000 were made from a virulent 72-hour broth culture of B. phytophthorus. Bliss Triumph tubers were disinfected with corrosive sublimate and cut into small pieces by means of a sterile knife. These slices were then placed in Soyka dishes upon pieces of absorbent cotton saturated with distilled water. By means of sterile pipettes one drop of the broth, full strength, and one drop of each dilution was placed upon each of ten tuber slices. One drop of each dilution was also placed in tubes of melted nutrient agar and plates poured. Data obtained from this experiment are recorded in the following table:

Table IV.

Effect of Amount of Inoculum

Dilution	No. cols. on Plates	Incubation period	Growth on individual slices 1:	2:	3:	4:	5:	6:	7:	8:	9:	10:
Broth, Full strength	very numerous:	2 da.:	+:	+:	+:	+:	+:	+:	+:	+:	+:	+:
1:100	19,650:	2 da.:	+:	+:	+:	+:	+:	+:	+:	+:	+:	+:
1:10,000	556:	3 da.:	+:	-:	+:	+:	+:	+:	+:	+:	+:	+:
1:100,000	96:	5 da.:	-:	+:	-:	-:	+:	+:	+:	+:	+:	-:
1:Million	8:	5 da.:	-:	+:	-:	+:	-:	-:	-:	+:	-:	-:

The data tabulated in table IV reveal several interesting facts. First, the period of inoculation is increased as the dilution is increased. Whereas, in the cases of full strength broth and 1:100 dilution , growth appeared after the usual incubation period of 2 days, growth in the 1:10,000, 1:100,000 and 1:1,000,000 dilutions was not evident until 3 days in the case of the 1:10,000 dilution and not until 5 days in the last two. That the growth in the two greatest dilutions, that caused by 96 and 8 bacteria, respectively, was considerably weaker than in the others is also significant. The 96 bacteria per drop out of a possible ten, produced six successful inoculations, while the 8 bacteria per drop were able to overcome the resistance of only three slices which shows that this number is about the smallest which can successfully cause the rotting of potato tubers.

The effect of various quantities of inoculum on slices of raw tubers was also tested out in another experiment. Raw-tuber slices of Bliss Triumph variety were inoculated by placing varying quantities of a virulent 3-day-old broth culture on the cut surfaces by means of a 3 mm platinum wire loop. The tuber slices were not pricked with a needle as is the method ordinarily practiced in making inoculations. After inoculation the slices were placed in moist chambers, and 48 hours later showed marked differences. Those slices which were inoculated with very thin films of broth culture, instead of showing creamy bacterial growth as in the cases of inoculations with one or more loopsful of the cultures,

showed the effect of the inherent resistance of tubers, which was exemplified by a wart-like protuberance, or an oedema formation. (Plate V.) It was first thought that this phenomenon might possibly be due to rapid division of cells, the minute quantity of inoculum being the exciting factor. Microscopic examination, however, revealed the fact that these protuberances instead of being composed of great numbers of minute cells were made up of giant cells, and that these cells contained very few starch grains as compared to the number found in normal cells. As a probable explanation of the phenomenon it is thought that as a result of the effect of the organism or its products upon the cells the starch grains are changed into sugar, thus increasing the osmatic pressure which resulted in greater hydration of t the colloids, hence the increased size of cells.

These experiments confirm the belief of Smith that a multitude of bacteria is stronger than a few, and shows that whereas in the cases of minute quantities of inoculum potato tubers are able to resist the attack of the Blackleg bacteria, larger amounts are able to break down this resistance and cause the rotting of the tubers.

Enzyme Studies:

Harrison[2] was able to produce typical Blackleg lesions by the use of the chemical products (enzymes) of the growth of his bacillus, and states that the solvent action of the enzyme on the cement substance of the cells was quite marked. The writer was able to cause the softening of raw-tuber slices by placing small quantities of a dry enzymic extract, by means of a sterile scalpel and platinum

needle, upon the freshly cut surfaces. This enzymic extract was made by mashing 12 tuber slices, which were severely rotted by Blackleg, in 50 cc. of water, the mixture being then filtered through filter paper and precipitated with 90% alcohol, and the precipitate dried on glass plates.

In order to ascertain whether the enzyme produced by Blackleg was similar to that produced by Bacillus carotovorus, as described by Jones[13], and to compare bacterial with enzymic action, the following experiment was performed; Six tuber and six carrot slices were inoculated with the dry enzymic extract, as described in the preceeding paragraph; while one tuber and one carrot slice each were inoculated with 24-hour broth cultures of P. phytophthorus, B.spickermani, and distilled water, the inoculations being made by placing one drop of inoculum on the surface of each tuber and carrot slice. The data obtained are tabulated below:

Table V.

Effect of Enzymic Extract on Raw Potato and Carrot

Inoculum	On Potato			On Carrot			
	No. Inocs	24 Hours	48 Hours	No. Inoc	24 Hours	48 Hours	72 Hours
Enzymic Extract	6	Soft-ening	Marked soft-ening	6	Liquid; surf. sli. soft	Slight soften-ing	Marked soften-ing
P.phytoph-thorus	1	-	Slight creamy growth	1	-	Slight discol-oration	ditto
B. spickermani	1	-	-	1	-	"	Slight creamy growth
Distilled water	1	-	-	1	-	-	-

Microscopic examination of the softened tuber and carrot slices revealed many free cells, showing that the enzyme acts on pectin of the middle lamella, which would suggest that the enzume was pectase. The fact that carrots were also softened, though not as rapidly as were potatoes, signifies that the enzyme produced by B. phytophthorus, if not identical, is very closely related to that produced by B. carotovorus.

The data recorded in Table V also show that the enzymic activity was much more vigorous than in the case of the bacterial inoculations. Whereas, in 24 hours, in the former there was marked evidence of activity, in the latter there was none. There were also several other points of difference between enzymic and bacterial activity. The tuber slices softened by the former were not blackened as is the case when tubers are rotted by the Blackleg germ. Then again, neither the softening of the tuber slices nor those of the carrot was accompanied by the disagreeable odor which is produced when tuber slices are inoculated with the live organism. On the other hand, the odor was rather pleasant, and especially so in the case of the carrot slices.

Toxin Studies:

It was also observed from the above enzyme experiments that there was no discoloration of tubers as a result of the enzymic activity, and as blackening both in tubers and on the stems of infected plants is such a fixed character of Blackleg, the question was raised as to what caused the discoloration. As the enzyme was unable to produce dis-

coloration, it was thought very probable that the organism must in addition produce a toxic substance which causes the blackening. This theory was readily confirmed by placing a virulent 24 hour broth culture of Bacillus phytophthorus into a thin sterile collodion sack about 8 mm. in diameter and 4 cm. long and then placing the sack on sterile raw potato plug in a sterile test tube. Substances of colloidal nature, the enzyme, etc., which are produced in cultures were thus kept inside the sack while the crystalloidal substances were able to diffusethrough to the surface of the potato plug. This effect produced a brownish discoloration resembling typical Blackleg staining in its first stages of development. There is therefore produced a crystalloid toxic substance.

The presence of a toxic substance was also shown by placing leaves from healthy vigorously-growing plants in beef and potato broth. That part of the petioles which was kept in the broth was enveloped in a thin collodion sack. In three days these leaves were wilted while the check, which was enveloped in a similar sack and kept in distilled water, was still turgid. Plate VI.

From the above studies of the relations of the host and parasite, it seems extremely likely that two agencies operate in the mechanism of the attack by the Blackleg organism upon potato. The Blackleg germ produces a pectin-dissolving enzyme and also a toxic substance which causes discoloration, and the two working in conjunction, under favor-

able conditions, produce the symptoms by which the disease is recognized. Viewed in the light of these two factors, the rotting effects of heavy dosage and the oedema formation with light dosage are very clear. The difference in pathogenicity between B. carotovorus and B. phytophthorus is thus found to rest upon differences in toxic substances.

Resistance and Susceptibility of Varieties:

Practically all of the investigators who have studied the Blackleg problem have noted the difference in resistance and susceptibility of varieties of potatoes. Appel[5] was the first to note this difference, and Harrison[2] also found that some varieties seemed to be more susceptible than others. The latter investigator, however, in variety tests found out that many varieties which showed comparative resistance in the field were comparatively susceptible when subjected to artificial inoculation. Morse[1] in studying the disease in Maine observed that the Irish Cobbler, an early maturing variety, was more susceptible to Blackleg than the Green Mountain which is a late maturing variety, and goes on to state that: "As long as Maine potato growers planted this variety almost exclusively, Blackleg was of minor consequence." Field observations of the writer during the 1919 season, supplemented by laboratory and greenhouse tests has led the writer to conclude that the Green Mountain is not as resistant as one would be apt to believe after reading the obser-

vations of Morse. Laboratory tests on tubers of eight varieties were conducted and from the data obtained a difference in resistance and susceptibility was noted. These showed that the Bliss Triumph, a very early maturing variety, was more resistant to Blackleg than was the Green Mountain, which proved to be only slightly more resistant than the Russet Rural, another late variety.

To determine whether resistance and susceptibility as expressed by tuber inoculations was correlated with similar resistance and susceptibility of stems, healthy succulent plants of the same eight varieties were inoculated with the same quantity of a virulent 24-hour broth culture of B. phytophthorus, the inoculations being made on the stems just above the surface of the soil. The plants were of the same age and were grown in the greenhouse. After inoculation the plants were placed in a Wardian chamber where a very humid condition was kept. Ten days after inoculation the plants were examined and, as in the case of tuber inoculations, a difference in resistance and susceptibility was apparent. The Green Mountain and Bliss Triumph varieties, instead of being resistant, however, were the first varieties to succumb to the attack of the disease, while the Irish Cobbler and Rural New Yorker types proved to be more resistant.

A comparison of the resistance and susceptibility of varieties as expressed by tuber and stem inoculations may be made from the following table:

Table VI.

Resistance and Susceptibility of varieties to Blackleg

Tubers	Growing Plants
Resistant	Resistant
1. Bliss Triumph	1. Sir Walter Raleigh
2. Green Mountain	2. Carmen #3
3. Russet Rural	3. Irish Cobbler
4. Irish Cobbler	4. Russet Rural
5. Early Ohio	5. Early Ohio
6. Early Rose	6. Early Rose
7. Sir Walter Raleigh	7. Bliss Triumph
8. Carmen #3.	8. Green Mountain.
Susceptible	Susceptible

From the above experimentation it is evident that there is some difference in resistance and susceptibility of varieties of potatoes to the Blackleg disease, but the contradictory evidence presented also makes it clear that conclusions in regard to such resistance or susceptibility cannot be drawn from tuber or stem inoculations alone. Extensive trials in the field and greenhouse as well as in the laboratory must be made before definite conclusions in regard to varietal resistance can be drawn.

GENERAL DISCUSSION

These investigations having been undertaken to attempt to clear up the question of the causal organism of the Blackleg disease of potato in Michigan as well as to make a more thorough study of the trouble, have brought

forward the following facts:

1. The Blackleg disease of the potato as found in the Upper Peninsula of Michigan is caused by a bacterial organism, identical so far as behavior is concerned, with B. phytophthorus.

2. The organism is able to winter over in tubers which remain in the soil under conditions existing in the Upper Peninsula of Michigan.

3. It is also able to winter over directly in the soil.

4. The disease is not limited to the potato. Tomato, Nicotiana, and other plants are susceptible to attack.

5. The organism is unable to attack healthy uninjured roots, but injures them by its soluble toxic substances.

6. The rapidity of the progress of the rotting of tubers is dependent upon the amount of inoculum, and the type of reaction of the host varies with the mass applied.

7. Two products are formed abundantly by the organism in rotting tubers and in pure cultures, an enzyme which causes softening, and a toxic substance which causes discoloration.

8. There is some difference in resistance and susceptibility of varieties.

9. The disease is readily controlled by careful selection and disinfection of seed tubers in the spring before planting.

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PLATES

Plate I.

Characteristic blackening of stem.

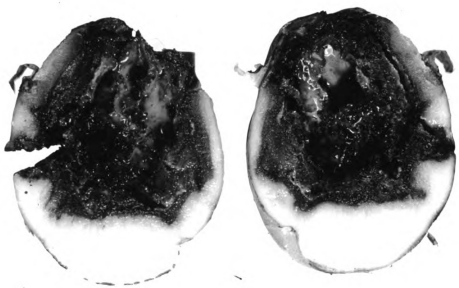

Plate II.

Rotting tuber, showing creamy growth, blackening, and canal formation.

Plate III.

Nicotiana rustica L. inoculated with
Bacillus phytophthorus.

Plate IV.

Effect produced by addition of 5 cc. of broth culture of B. phytophthorus to uninjured roots of potato plant growing in water culture.

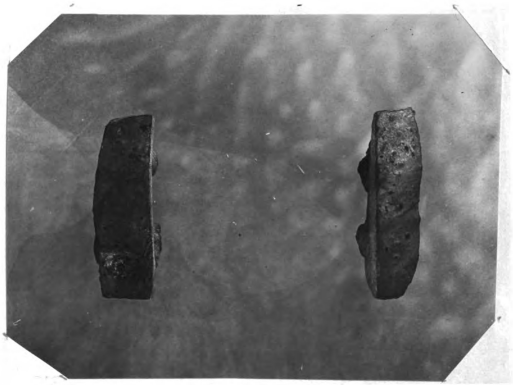

Plate V.

Oedema formation, produced when tuber slices were inoculated with small amounts of inoculum.

Plate VI.

Effect of toxic substance on potato leaves.