ABSTRACT OF THESIS

IDENTITY, CULTURAL CHARACTERISTICS AND PATHOGENICITY OF THE CAUSAL AGENT OF THE "WESTERN LEAK" DISEASE OF POTATOES.

Submitted by

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INTRODUCTION

In recent years the potato industry in Colorado has been subject to a tuber-rot complex. A leak-type disease known as "Western Leak" has been one contributing factor to this complex. No satisfactory control has been established. In order to effect such control, basic fundamental information is necessary concerning the pathogen causing "Western Leak".

The problem

What causal agent is responsible for the "Western Leak" of potatoes in Colorado, what are some of the factors influencing its growth, and what symptoms may be expressed by the affected host plant?

Analysis of problem.--1. Is the same pathogen involved in all diseased tissue of potato tubers manifesting symptoms of "leak"?

2. What are the cultural characteristics of the pathogen? Do they vary between isolates?

3. Do different isolates of the pathogen vary in their pathogenicities?

4. What symptoms may be expressed by the host plant?

Delimitation.--This study has been limited to morphologic, physiologic, and pathogenicity studies of 11 pure hyphal-tip isolates of the organism causing a leak-type disease of potato tubers.

Definition of terms.--1. Amphigynous antheridium. "Antheridia are considered amphigynous when they are apparently penetrated by the oogonial stalk, becoming a permanent 'collar' enclosing it."

2. "Leak type of disease", as used here, means a wet-rot of the host. When tubers are affected a watery exudate is given off.

3. A paragynous antheridium attaches itself to the oogonium but is not penetrated by the "budding" oogonium, and is not persistent around the oogonial stalk.

4. Room temperature, as used in these studies, means a range between 20° and 22° C.

METHODS AND MATERIALS

The isolates of the pathogen causing "Western Leak" of potatoes were obtained from field-grown diseased potato tubers. Isolates were obtained by surface sterilizing the tuber, cutting through a diseased zone, and then removing sound but infected tissue to dextrose agar plates. From the original isolations, pure hyphal-tip isolations were made. Eleven isolates were selected which showed the greatest differences between cultures in temperature reactions, morphology, and tuber pathogenicity.

Solid and liquid media were both used in this study. The solid media were: potato dextrose agar, dextrose agar, oatmeal agar, corn meal agar, prune agar, cooked pumpkin, peanut hulls and wheat grain, and hydrogen-ion buffered dextrose media. The liquid media were: Knop's solution, dextrose solution, and distilled water.

Tubers from the Red McClure, Yampa, Irish Cobbler, Teton, and Russet Burbank varieties of potatoes were used as hosts in the pathogenicity studies.

Irish Cobbler potato plants were grown in the greenhouse to test leaf, stem, and soil inoculations, and to study symptomology.

ANALYSIS OF DATA

Morphological studies.--The objective of the sexual sporulation study was to determine whether there were differences in the diameters of the oogonia and oospores of the 11 test isolates as a basis for their identification.

Statistical analyses showed that differences between each isolate were highly significant. It was also shown that the isolates could be grouped into two distinct classes. One class, with two isolates had spore sizes which fell within the range of Phytophthora drechsleri Tucker, while the remaining isolates fell within the spore sizes for Phytophthora erythroseptica Peth.

The results of the asexual sporulation study showed that a liquid medium was necessary for the production of sporangia and that Knop's solution was a better medium for asexual spore formation than distilled water or water-soil extract.

When variance analyses were made of the lengths and widths of the sporangia, differences between the isolates were found to be highly significant.

An effort was made to correllate sexual and asexual spore measurements with negative results.

The results of the substrata observations on potato dextrose agar, showed that the test isolates could be put into two groups. One group of isolates produced a color in the substrata which was lighter in color than the control (uninoculated media), and the other darker.

An effort was made to correlate the results with data on sexual and asexual spore sizes with negative results.

Since three of the eleven test isolates used in this study did not produce sexual bodies in pure culture, a test of homo- versus heterothallism was made. This was done by making crosses of each of the isolates two at a time in all possible combinations. Results were not conclusive, but four of the isolates behaved as if they were heterothallic.

Physiological studies.--The effect of temperature on the mycelial growth of the 11 isolates showed similarity between the isolates when they were cultured at 20° and 25° C. When the isolates were cultured at temperatures nearing the extremes, i. e. at either 5° or 40° C., large differences between isolates were measured. At 40° C. none of the isolates grew. At 35° C. five isolates grew, while at 30° C. some growth was observed in all the 11 isolates with one isolate growing only slightly. At 25° C. growth was observed for all the isolates. The optimum temperature for most isolates was between 20° and 25° C. About 5° C. was the minimum temperature for growth for all the test isolates.

The hydrogen-ion concentration study showed that the pH tolerance for the isolates in this study was between pH 4.0 and pH 7.0. Three of the eleven isolates grew at a pH of 4.0 and all the isolates grew at pH's 5.0, 6.0, and 7.0.

Pathogenic studies.--The results of the tuber invasion studies showed that tubers became infected more easily through the eyes and artificial wounds than through apparently unbroken skin.

When isolations were made from the nonfluorescent tissue of the tubers, it was apparent that the pathogen invaded the tissue in advance of any symptoms. The study showed that host and pathogen relationship varied between the different isolates of the pathogen as well as between host varieties.

Cut potato tuber tissue, affected with the test isolates, exhibited pinkness when exposed to air. Irish Cobbler and Yampa varieties developed the pinkness within 30 minutes for all the test isolate inoculations. Russet Burbank and Red McClure tubers showed pinkness of the affected tissue within 2 hours, but in 30 minutes only some of the infected tubers showed pinkness.

DISCUSSION

In 1941, a field and storage wet-rot of potatoes appearing in Colorado was found to be caused by a Phytophthora species of soil fungi. Because of the close resemblance to a disease occurring in the eastern United States caused by Pythium debaryanum and known as leak, Kreutzer and Lane (16) called the Phytophthora disease "Western Leak".

A more detailed description of the causal agent, symptoms, and factors of growth of "Western Leak" than that presented by Kreutzer and Lane is reported herein.

Causal agent.--Previously, it was stated that mycelium of the young fungus was coencytic and often became septate only in older cultures. This would class the organism as a Phycomycete. Additional inspection

showed that the organism produced oogonia and the amphigynous type of antheridia. According to Middleton (18):

> The amphigynous type of antheridium predominant in the genus Phytophthora is unique and not encountered in the genus Pythium; that the antheridium is a component part of the sexual phase can be considered a valid criterion for separation. (18:19)

This then, would automatically support the report by Kreutzer and Lane (16) that the organism causing "Western Leak" was a species of Phytophthora. Since the test fungus was coenocytic and eight isolates employed in this investigation possessed the amphigynous type of antheridium, it was reasonable to believe that the test organism was a species of Phytophthora. In Tucker's Table 1 (27), the following species of Phytophthora are listed as having the potato as its host: P. erythroseptica, P. parasitica, P. drechsleri and P. infestans. Leonian (17) also listed P. erythroseptica and P. drechsleri as having the potato as its host. By using these four species of Phytophthora as criteria, the isolates studied herein are compared with the cultural characteristics of this group.

Since all the cultures investigated in this paper grew profusely on potato dextrose agar and P. infestans did not, it was eliminated.

Tucker (27) and Rosenbaum (25) reported sporangia in cultures of P. parasitica on oatmeal agar. They also reported that this species might be distinguished by its ability to yield papillate sporangia and chlamydospores on solid media. When solid media cultures were inspected, in this study, asexual sporulation was rarely observed. Nonpapillate sporangia were rare, while no chlamydospores were seen.

Tucker (27) reported that P. drechsleri was morphologically similar to P. erythroseptica. However, he stated that P. erythroseptica may be separated from P. drechsleri by its large oogonia and oospores. Rosenbaum (25), in his comparative studies of oospore measurements, also showed that P. erythroseptica oospores were larger than other species of Phytophthora. Tucker (27) gave the following measurements of oogonia and oospores on oatmeal cultures: oogonia and oospores respectively; P. erythroseptica, 36.3 μ and 31.4 μ; P. Parasitica, 26.4 μ and 22.8 μ; P. drechsleri, 31.3 μ and 25.6 μ. Rosenbaum (25) reported that the greatest number of oospores measured for P. erythroseptica were concentrated in classes between 33.5 to 39.49 μ. The mean for all the oospore measurements was 35.78 ± .127. The standard deviation was 3.77 ± .090.

The mean oogonia and oospore measurements in this investigation fell between 32.323 μ and 43.617 μ for the oogonia, and 23.197 μ and 36.004 μ for the oospores. The standard deviation for the oospores was 4.1262 . It would appear from these results that the sexual structures of many of the test isolates have mean diameters close to those reported for P. erythroseptica. Isolates 8 and 9, whose mean oospore diameters differ most from those reported for P. erythroseptica correspond more closely in this characteristic with P. drechsleri.

Since the organism P. debaryanum, which has been named as the pathogen causing potato leak (2, 11, 23), was not included in the studies reported here, diagnostic differences between it and our isolates of

Phytophthora cannot be pointed out at this time. A more extensive and parallel study of the two fungi might lead to a better understanding of both organisms and their symptoms. As Pethybridge (22) pointed out, potato tubers affected with P. erythroseptica yielded a watery exudate. The liquid did not evaporate rapidly if the tubers were enclosed. Thus a "wet-rot" of the tubers was the result. This might be confused with the leak-type symptoms of P. debaryanum. Since both P. debaryanum and P. erythroseptica caused a leak-type disease, it was understandable why Kreutzer and Lane (16) chose to call the Phytophthora wet-rot, "Western Leak".

Symptoms.--In previous studies by White (28), it was shown that a potato tuber partly invaded with P. erythroseptica had three distinct zones: First, of invaded dead tissue which became pink when exposed to air; second, living tissue where the organism was a true parasite; and third, a zone of healthy, uninvaded tissue.

The investigations here showed that tuber tissue inoculated with the test isolates did not always show visible symptoms of the disease under white light. It was for this reason that ultraviolet light was used. Under ultraviolet light the affected tuber tissue fluoresced clearly, thus making it possible to isolate from the "healthy", nonfluorescent zone. The 2-percent dextrose solution, used for culturing the nonfluorescent tissue, proved to be an excellent medium for growing the fungus contained within the isolated tissue.

In the apparently healthy tissue, between 0-5 mm, the fungus was obtained from all the affected tubers. These results were in harmony with White's report (28).

Since four tuber varieties were used in this study, it was desirable to know whether different test isolates caused different reactions in the tuber tissue. Therefore, further isolations were made in zones between 5-10, 10-15, and 15-20 mm away from the fluorescent tissue. It was shown that differences were present between isolates within varieties, and between varieties within isolates. Through these comparisons it was shown that a pathogenic fungus could move to various distances into tubers without showing disease symptoms. The variation of penetration exhibited by the different isolates among the potato varieties tested, indicated that the isolates were not physiologically alike, and that host reactions to the isolates were different.

Goss (9) reported that Bliss Triumph tubers infected with P. erythroseptica and P. drechsleri turned pink in 20 to 30 minutes after the tubers were cut and exposed to the air. Tubers that he inoculated with P. debaryanum showed less rot, were slightly darker -- almost brown; and the affected tissue did not turn pink when exposed to the air.

The results reported here showed that the Irish Cobbler and Yampa tubers infected with any one of the 11 test isolates turned pink in 30 minutes. Two other varieties, Russet Burbank and Red McClure, did not produce pinkness within 30 minutes, for all the 11 test isolates. This difference in varietal response suggests a need for naming the potato variety when reporting pinkening.

It has been demonstrated that potato plants grown in the greenhouse will produce aerial symptoms from leaf, stem and soil inoculations. It was suggested that further study should be conducted along these lines. When the soil was inoculated around the growing plant, top symptoms were very striking. The symptoms resembled those of black leg, but the discoloration was not as dark and lacked the characteristic sheen of black leg. Isolations from these plants yielded the original fungus. However, as reported by Goss (9) these plant symptoms could be easily confused with the bacterial disease.

Factors of growth.--The temperature and growth relation studies of the 11 isolates on dextrose agar, showed the minimum temperature for growth was near 5° C.; the optimum temperature was between 20° and 25° C.; and the maximum temperature for some of the isolates was between 30° and 35° C., and between 35° and 40° C. for others. No growth was observed at 40° C.

The production of asexual spores in a liquid medium and none on a solid medium, indicates that the fungus might reproduce asexually more readily in vivo, in wet surroundings. The ability of the fungus to produce asexual bodies when soil was an ingredient in the liquid culture and when Knop's solution was used, indicates that the natural substances in the soil plus a liquid medium stimulates asexual reproduction. The fungus, therefore, would be expected to be more active in wet soils than in well-drained and aeriated soils.

Suggestions for further study

1. Are tubers that are bruised and wounded at harvest time more susceptible to the "Western Leak" disease than are other tubers?

2. Pathogenicity tests on other potato varieties should be made to obtain more information on host range.

3. What effect would oxygen and carbon dioxide in varying concentrations have on the breakdown of tubers infected with the "Western Leak" isolates?

4. What effects would varied proportions of carbon and nitrogen have on the growth of the "Western Leak" fungus in culture media?

5. Would different vitamin media stimulate sexual sporulation in a greater number of the 11 isolates?

SUMMARY

Investigations made in this study were concentrated under morphologic, physiologic, and pathogenicity studies of 11 pure hyphal-tip isolates of Phytophthora species causing potato leak.

1. Oogonia and antheridia were produced in cultures by employing various temperatures, media and age of cultures. Size of oospores and oogonia showed variability between isolates.

2. Asexual spores were found to sporulate profusely in liquid media, with variability noticeable between isolates.

3. Crossing of isolates did not stimulate production of sexual bodies.

4. The test isolates could be grouped by the color and fluorescence of their substrata.

5. Cultural characteristics included a description of the mycelium, sexual bodies, and asexual bodies.

6. At controlled temperatures on dextrose agar the optimum and maximum temperatures were obtained for the test isolates. The minimum temperatures were not obtained. Differences in growth between isolates was significant within periods.

7. Variation between isolates within pH and variation between pH within isolates showed differences between isolates and differences between hydrogen-ion concentrations.

8. Tubers were more easily infected through eyes and wounds than through "unbroken" skin.

9. Affected areas of inoculated tubers could be measured and observed in ultraviolet light.

10. Isolations made from apparently healthy tissue adjacent to the visibly affected areas of tuber tissue yielded the pathogen.

11. The cut surfaces of affected tubers became pink but the time required differed between potato varieties. Symptoms of both leak and pink rot were produced by the test isolates.

12. In all tuber inoculation tests, differential pathogenicity between isolates was observed.

13. Leaf and stem inoculations showed that aerial symptoms could be produced under greenhouse conditions.

14. Soil inoculations indicated that stem and underground parts are invaded and may cause death to the host.

15. On the basis of morphological studies the test isolates more nearly resembled Phytophthora erythroseptica and Phytophthora drechleri than any other described species.

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY VERNON E. WILSON

ENTITLED IDENTITY, CULTURAL CHARACTERISTICS AND PATHOGENICITY OF THE CAUSAL AGENT OF THE "WESTERN LEAK" DISEASE OF POTATOES.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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Vernon E. Wilson has acceptably read passages of scientific German in the botanical literature, selected at random from both familiar and unfamiliar material.

Bruno Klinger

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Chapter I

INTRODUCTION

The potato industry in Colorado has been subject in recent years to high losses due to a tuber-rot complex. Contributing to this complex has been a leak type of disease known as "western leak" (16). The sporadic losses resulting from it in the San Luis Valley have been sufficiently great to justify its ranking as one of the major potato diseases of the state. The symptomology, the pathogen, and its physiology have not been fully determined. No satisfactory control for the disease is known. In order to effect such control, basic fundamental information concerning the pathogen and the disease, itself, is necessary. Therefore, it is the purpose of this thesis to provide some pertinent information concerning the pathogen involved in the leak type disease occurring in potatoes in Colorado.

The problem

What causal agent is responsible for the "western leak" of potatoes in Colorado, what are some of the factors influencing its growth, and what symptoms may be expressed by the affected host plant?

Problem analysis.--1. Is the same pathogen involved in all diseased tissue of potato tubers manifesting symptoms of "leak"?

2. What are the cultural characteristics of the pathogen? Do they vary between isolates?

3. Do different isolates of the pathogen vary in their pathogenicity?

4. What symptoms may be expressed by the host plant?

Delimitation.--This study has been limited to morphologic, physiologic, and pathogenicity studies of 11 pure hyphal-tip isolates of the organism causing a leak type disease of potato tubers.

Definition of terms.--1. Amphigynous antheridium. "Antheridia are considered amphigynous when they are apparently penetrated by the oogonial stalk, becoming a permanent 'collar' enclosing it." (27:43).

2. "Leak type of disease", as used here, means a wet-rot of the host. When tubers are affected, a watery exudate is given off.

3. Paragynous antheridium. An antheridium is considered paragynous when it attaches itself to the oogonium but is not penetrated by the "budding" oogonium, and is not persistent around the oogonial stalk.

4. Room temperature means a temperature range between 20° and 22° Centigrade.

Chapter II

REVIEW OF LITERATURE

Leak

The leak type disease of potatoes has been known in the United States and widely separated parts of the world for many years. One of the first reports of such a disease was given by Orton (19), who attributed a watery breakdown of potato tubers in San Joaquin County, California, to be due to infection by Rhizopus nigricans Ehr. Orton referred to this disease as "leak". Since then numerous investigators have reported similar maladies, but the descriptions of the symptoms and causal agents have been divergent.

In 1915, severe losses were suffered in the delta region of the San Joaquin River, California. The following year, Hawkins (11) presented a report in which he discussed the tuber rot of potatoes known as "potato leak" disease. The report did not support completely Orton's conclusions. Although it was shown that R. nigricans would give a typical tuber rot, typical in appearance to that of a "leak" disease, isolations from other tubers with similar symptoms also yielded the fungus Pythium debaryanum Hesse. The extensive studies by Hawkins bore out the fact that both R. nigricans

and P. debaryanum could produce a typical rot. However, the former organism was not isolated from tubers showing leak symptoms in field experiments while the latter fungus was isolated forty-nine times out of sixty-one trials. This indicated the prevalence of P. debaryanum as the causal organism.

Pethybridge and Smith (23) in 1930 reported a watery wound-rot which caused serious damage in England. The rot appeared to have its origin at a wound or an abrasion of the skin. Outwardly the affected tubers were dark and discolored, and often the skin over the diseased tissue was moist. Sometimes a dark line separated the healthy from the diseased tissue. However, the outward appearance did not indicate the extent of decay within the tuber. An entire tuber sometimes was in a state of decay with only one or two small surface wounds or breaks in the skin. "Not infrequently the skin over the decayed portion is stretched flat and taut, owing to the internal shrinkage of the rotten tissue, and when it becomes split, a considerable quantity of a thin watery liquid gradually exudes." When the tuber was cut, exposing the diseased tissue to air, a gradual discoloration resulted. It became gray first, then brown, finally black with an occasional faint pink tinge. The rotten tissue was pulpy, and in the early stages gave off a faint fishy odor.

All isolations from the diseased tubers yielded a fungus which was believed by Pethybridge and Smith to be P. debaryanum. It was similar in all essential features to that fungus, with the exception that no zoospores were observed. Tests proved that the fungus was a pathogen causing a rot to potato tubers, and that tubers in an incubator at 22° C. rotted much more rapidly than did tubers at room temperature. It was shown also that the fungus was incapable of invading the undamaged skin of the tuber.

In 1931, Dowson (7) reported heavy losses sustained in early, immature potato tubers in Tasmania. The causal organism, P. debaryanum, caused a soft rot by entering the tuber following mechanical injury. The affected tubers completely disintegrated into a watery mass during transit.

A watery wound-rot of potato tubers was observed in New Zealand for many years. Brein (3) reported in 1940 that Pythium ultimum Trow. was the cause of the leak-like disease. The tubers showed discoloration, and the interior rotted rapidly, followed by a watery exudation. At temperatures between 20° and 30° C. the disease caused serious losses in storage.

Later, in 1944, Person (20) made isolations from potatoes in Louisiana which were attacked by a sudden wilt of the aerial parts and a tuber rot. The isolations were made from tubers having firm

but smokey gray-colored interior tissue. P. debaryanum was obtained. The Pythium produced a tuber rot which differed in appearance from other forms of potato tuber break-down in the United States. The symptoms in some ways resembled the disease caused by Pythium butleri Subr. in Cyprus, as was reported by Natrass (6) in which about 80 percent of a potato field in Famagusta was invaded by a disease that resulted in a shriveling of the main stem, and eventually a collapse of the entire plant.

Blodgett and Ray (2) reported that P. debaryanum was widely prevalent in Idaho, causing a leaky potato tuber rot. Due to the manner in which the tuber was affected in storage, the popular name "shell rot" was designated for the disease. However, during the months immediately following digging, the outstanding symptom of the diseased tubers was that of leak, ultimately involving the entire tuber in a watery decay. Isolations from tubers from different localities repeatedly produced P. debaryanum.

Pink rot

The literature indicates that "leak" might be caused by a number of different pathogens. Kreutzer and Lane (16) have stated that "western leak" is caused by a species of Phytophthora, first reported in Colorado. Since a species of Phytophthora (Phytophthora

erythroseptica Peth.) has been reported repeatedly to cause pink rot of potatoes in many potato-growing regions over the world, a review of the literature on pink rot is pertinent to this thesis.

Pethybridge (22), in 1913, reported a disease of potatoes, in Ireland, caused by a new species of Phytophthora. "For this new fungus the name of Phytophthora erythroseptica is proposed, while for the disease caused by it in the tubers the trivial name of 'pink rot' may be suggested." The rot began at the proximal or "heel" end of the tuber and rapidly proceeded toward the "rose" end. It was found that in only two instances did the infection start at any other point. In one of those instances it was evident that the fungus entered through a skin wound.

Often the external portion of the tuber was darker in color and showed an irregular blackish band of varying width around the tuber. The lenticels in the affected tuber became black, and not infrequently formed various types of pustules. As the tuber rotted a watery exudate was frequently given off through the lenticels.

The skin over the decayed region of the tuber was loosely attached and could be pulled off easily. Sometimes gas would form between the decaying tissue and skin, causing blisters to arise.

The diseased tuber was fairly firm, but if squeezed a watery exudate would be produced. When the tubers were left

uncovered the exudate evaporated rapidly, and the skin became wrinkled, but when the tuber was enclosed, the skin remained intact and the exudate did not evaporate. This caused a "wet rot".

The internal portion of the tubers, when cut, usually showed some contrast between the water-soaked, dirty-white diseased tissue and the healthy tissue. Upon being exposed to air the diseased tissue went through a series of color changes. A few minutes after exposure a tinge of pink appeared which usually became a deep salmon-pink within a half hour. After a few hours of exposure to air the diseased tissue turned from pink to dark purplish-brown, and finally became nearly black.

Drechsler (8) obtained a species of Phytophthora from potato tubers in Oklahoma with a watery decay, as well as from tubers in Kentucky showing a mealy pink rot. When the fungus was inoculated into tubers, a rot appeared characterized by a purplish discoloration of the eyes and death of the buds. Upon cutting and exposing the diseased tissue to air the affected area rapidly became pink, gradually blackening with time. Similar symptoms had been produced by various other species of Phytophthora, e. g., P. erythroseptica. The organism under study showed energetic diplanetism of the primary zoospores, later forming secondary zoospores through an evacuation tube 1 to 7 μ long and 3 μ wide, or indirectly by terminal production of a papillate germ-sporangium, limited by a basal septum.

Pethybridge (21), in 1926, reported pink rot of potato tubers caused by the fungus he described in 1913 as P. erythroseptica, causing a rot of potatoes in Ireland. The disease was first recorded as occurring in England in 1921 where it was causing tuber rot sporadically, but was without serious economic importance.

Cairns and Muskett (4) isolated a fungus from decayed potato tubers in northern Ireland which was identified by S. F. Ashby as Phytophthora megasperma Drechs., the pathogen of crown rot on hollyhocks in the United States. P. megasperma differed from P. erythroseptica in the production of larger oospores and a preponderance of paragynous antheridia, yet its effect on the tuber was indistinguishable from the pink rot caused by the latter fungus.

Another report by Cairns and Muskett (5), in 1933, showed that pink rot in northern Ireland was caused by both P. erythroseptica and P. megasperma. Their investigations showed that at times the disease might be systemic in the plant, and might cause poor stands and "pink-rot wilt". In improper storage (bad ventilation and high humidity) heavy losses (up to 50 percent) might be caused by "pink rot". Before harvest the tuber normally was infected through the mother stolon. However, the tuber could be infected directly, generally through the eyes. It was pointed out that P. cryptogea Peth. & Laff. and P. cactorum (Lebert & Cohn) Schrodter

also could cause a typical pink rot of potato tubers under experimental conditions.

Blodgett (1) recently reported a water rot of potatoes occurring in Idaho from which P. erythroseptica was isolated repeatedly. The affected tubers appeared to be frost-injured. The diseased tissue was dark and water-soaked, while the skin tended to slip easily. At first the interior diseased tissue was creamy-white, later darkening. The affected tubers turned pink when exposed to air by cutting.

Investigations by Jones (13) in 1945 revealed that P. erythroseptica caused a pink rot of potatoes in British Columbia. The external symptoms included a dark discoloration of the lenticels, dull skin, and purple eyes. When cut and exposed to the air, the internal tissue appeared dirty-white, becoming salmon-pink within a half hour. When plants were grown in soil inoculated with P. erythroseptica, they developed a necrosis of the stolons, sprouts, and basal parts of the stems. Near the end of the growing period some leaves wilted. The optimum temperature for the growth of the fungus was about 24° C., the maximum below 34° C., and the minimum between 4° and 8° C.

Laboratory studies by White (28) showed that a potato tuber partly invaded by P. erythroseptica consisted of three distinct zones:

One of living uninvaded tissue, a second of invaded living tissue where the organism was a true parasite, and a third zone of invaded dead tissue which was characterized by pink coloration when exposed to oxygen. In the latter tissue the fungus lived as a saprophyte.

Goss (9) in 1949 made a comparative study of P. erythroseptica, P. drechsleri Tucker, and P. debaryanum. However, most of his investigations were limited to pink rot caused by P. erythroseptica. In describing the pink-rot vine symptoms, Goss observed that stem lesions below the ground line were somewhat similar in appearance to Rhizoctonia lesions. Above the soil surface, lesions appeared soft and water soaked 8 inches up the stem. The stem symptoms displayed by the plant infected with P. erythroseptica were difficult to distinguish from symptoms on plants infected with black-leg. Goss pointed out that P. drechsleri and P. debaryanum were not parasitic to growing potato plants and these organisms caused, primarily, post-harvest diseases.

Summary.--It is evident, therefore, that several factors and organisms may cause a "leak" disease of potato tubers. However, it appears that the genera Rhizopus, Pythium, and Phytophthora comprise the main causal fungi, Rhizopus being of little importance. Except for the report by Kreutzer and Lane (16), leak generally has been reported to be caused by some species of Pythium, while Phytophthora has been accredited for pink rot.

Chapter III

METHODS AND MATERIALS

Tubers of the following potato varieties were used as hosts in studies of pathogenicity: Yampa, Russet Burbank, Irish Cobbler, Teton, and Red McClure[1].

Numerous isolations were made from field samples of potato tubers with symptoms of "Western Leak", obtained from different potato-growing areas in Colorado. Tubers were washed in tap water to remove soil and disintegrated tissue, and allowed to dry. They were then surface-sterilized by dipping them in 95-percent alcohol and flaming. The diseased tissue was removed by a scalpel surface-sterilized between each cut by the same method used in sterilizing the tuber. Figure 1 shows a tuber infected with "Western Leak".

In preliminary studies it was found that dextrose agar was unfavorable for bacterial growth while the fungus from the tubers grew uninhibited. Therefore, the medium used for the original isolation was dextrose agar.

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[1]The potato varieties were generously supplied from Colorado-grown stocks by Dr. Robert Kunkel, Horticulturist, Colorado A & M College; and Dr. Cecil W. Frutchey, Extension Potato Specialist and Manager of Potato Certification for Colorado.

Fig. 1.--Red McClure potato tuber showing "Western Leak" symptoms.

The original isolates were grouped as to temperature reaction, morphologic and pathogenicity differences in preliminary studies. From these, a representative isolate from each group was transferred and incubated at room temperature. The mycelium was allowed to grow until single strands of hyphae were approximately 1 inch away from the transplant. Single hyphal tips were then transferred to fresh dextrose agar plates. This method was repeated five times for each isolate in order to insure obtaining as pure a culture as possible. Isolates 1, 3, 8, 9, 10, 15, 17, 21, 23, 80c, and 81c were used in subsequent experiments.

Culture media

The following media were used in studying the organisms under consideration: Potato dextrose agar, dextrose agar, oatmeal agar, prune agar, yellow corn meal agar, canned pumpkin, wheat grain and peanut hulls, Knop's solution, and dextrose solution.

Potato dextrose agar.--1000 g of unpeeled potato tubers were washed in tap water, sliced, and added to 1500 ml of distilled water. A preparation of 60 g of agar and 60 g of dextrose was dissolved in 1500 ml of distilled water. The sliced potatoes and the agar dextrose preparation were then autoclaved together for 45 minutes at 15 pounds pressure. Before flasking and sterilizing,

the watery potato liquid was strained into the other ingredients and distilled water was mixed with the solution to make up to a volume of 3000 ml.

Dextrose agar.--15 g of dextrose and 17 g of agar were mixed with 1000 ml of distilled water and sterilized.

Oatmeal agar and corn meal agar.--Oatmeal agar and corn meal agar were prepared according to Tucker (27): Oatmeal agar, 60 g of ground oatmeal and 17 g of agar in 1000 ml water; corn meal agar, 60 g of yellow corn meal and 17 g of agar in 1000 ml of water. These were sterilized but not filtered.

Prune agar.--As described by Rawlins (24), 40 g of dried prunes were steamed in 500 ml distilled water for 1 hour. 25 g of agar were dissolved in 500 ml distilled water and mixed with the prune water extract, flasked and sterilized for 15 minutes at 10 pounds pressure.

Pumpkin medium.--Del Monte brand pumpkin was sterilized and used without adding other ingredients.

Peanut hulls and wheat grain.--Peanut hulls and wheat grain, as used by Snieszko, et al (26), were prepared by mixing 1 g wheat grain, 2 g peanut hulls and 2 ml distilled water and sterilizing.

Knop's solution.--Knop's solution was prepared by adding to 1000 ml distilled water a trace of ferric phosphate, 0.2 g

magnesium sulphate, 0.2 g monopotassium phosphate, 0.2 potassium nitrate, and 0.8 g of calcium nitrate.

Hydrogen-ion media.--The media used in the hydrogen-ion concentration experiment were prepared by using Clark and Lub's pH buffer solutions.

pH 2.0	50	cc	0.2 M	KCl		plus	10.6	cc	1.0 M	HCl
pH 2.5	50	cc	0.2 M	KH phthalate		plus	36.0	cc	0.2 M	HCl
pH 3.0	50	cc	0.2 M	KH phthalate		plus	20.4	cc	0.2 M	HCl
pH 3.5	50	cc	0.2 M	KH phthalate		plus	8.0	cc	0.2 M	HCl
pH 4.0	50	cc	0.2 M	KH phthalate		plus	0.4	cc	0.2 M	NaOH
pH 5.0	50	cc	0.2 M	KH phthalate		plus	23.65	cc	0.2 M	NaOH
pH 6.0	50	cc	0.2 M	KH_2PO_4		plus	5.64	cc	0.2 M	NaOH
pH 7.0	50	cc	0.2 M	KH_2PO_4		plus	29.54	cc	0.2 M	NaOH
pH 8.0	50	cc	0.2 M	H_3BO_3, 0.2 M	KCl	plus	4.0	cc	0.2 M	NaOH
pH 8.5	50	cc	0.2 M	H_3BO_3, 0.2 M	KCl	plus	10.0	cc	0.2 M	NaOH
pH 9.0	50	cc	0.2 M	H_3BO_3, 0.2 M	KCl	plus	21.4	cc	0.2 M	NaOH
pH 9.5	50	cc	0.2 M	H_3BO_3, 0.2 M	KCl	plus	34.0	cc	0.2 M	NaOH

The buffer solutions were made in the above proportions and diluted to 200 ml with distilled water. The pH concentrations were adjusted on a Beckman glass electrode pH meter, before they were used as one-half the liquid in making dextrose agar. The agar media were tested before and after sterilization (30 minutes at 10 pounds pressure). No change in the hydrogen-ion concentration was observed after the sterilizing.

Dextrose solution.--The dextrose solution used in isolation work was suggested by Mr. George H. Lane[2], for isolating phycomycetes

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[2]Associate Plant Pathologist, Colorado A & M College.

from the soil. A 2-percent dextrose solution was prepared by dissolving the dextrose in distilled water and sterilizing at 15 pounds pressure for 15 minutes.

Except where otherwise indicated, all studies were made in 100 x 15 mm Petri dishes, containing approximately 20 ml of medium.

Media were made in bulk quantities in tests to insure uniformity throughout each experiment. The media were put into 250-ml Erlenmeyer flasks, stoppered with cotton stoppers, and sterilized in a steam-type autoclave for 15 minutes at 15 pounds pressure, unless otherwise indicated.

Chapter IV

ANALYSIS OF DATA

Morophological studies

Sexual sporulation.--The sexual sporulation of the 11 isolates was studied to determine if they differed in this respect. The studies were based on the presence or absence of oogonia, oospores and their respective measurements. The type of antheridium was recorded as being amphigynous or paragynous in character.

Three treatments, replicated five times, consisting of seven different temperature studies and seven different media, and arranged according to the ages of cultures, were applied to each isolate. The temperature studies were conducted in electric thermostatically regulated incubators. The dextrose agar cultures used in the temperature and growth relation studies were also inspected for sporulation in this experiment.

Besides dextrose agar, cultures were incubated on potato dextrose, oatmeal, prune, and corn meal agars, canned pumpkin, and wheat grain and peanut hulls.

Cultures were inspected for spore production at 2-week intervals for a period of 8 consecutive weeks.

Due to limitations in the amount of space and time required, this experiment could not be performed with the desired continuity. Therefore, the isolates were cultured on potato dextrose agar and oatmeal agar at 10° and 20° C.; on corn meal agar, canned pumpkin, and wheat grain and peanut hulls at 20° C., and on prune agar at 10° C. All tests were allowed to incubate at a given temperature for 8 weeks, except cultures on dextrose agar, at temperatures 15° and 20° C. The cultures on dextrose agar at 15° and 20° C. were not allowed to incubate for more than 4 weeks.

At 5° C. no sexual spores were observed on dextrose agar. Between 10° and 25° C. sexual organs were produced on each medium at each temperature. The effects of temperature on sexual sporulation are shown in Table 1. At 10° C. isolate 9 sporulated around the edge of the culture after 6 weeks, and isolate 8 after 8 weeks. At 15° C. isolates 3, 9, and 15 showed sporulation after 4 weeks. At 20° C. sporulation was produced in isolates 1, 3, 9, and 15 at the end of 2 weeks, and in isolate 10 after 4 weeks. The latter two temperatures were used for a period of 4 weeks with dextrose agar. At 25° C., on dextrose agar, isolate 1 sporulated in 2 weeks, and isolate 10 after 4 weeks. Isolates 3, 9, and 10, which sporulated in 4 weeks at 20° C., did not sporulate in 8 weeks at 25° C. At 30° and 35° C. sexual fruiting bodies were not observed in any of the cultures.

Table 1.--EFFECTS OF AGE OF CULTURE, CULTURE MEDIA, AND TEMPERATURE ON THE PRODUCTION OF OOGONIA AND ANTHERIDIA BY 11 ISOLATES OF PHYTOPHTHORA SPECIES.

Medium	Temperature	Age of Culture (Weeks)	Isolates 1	3	8	9	10	15	17	21	23	80c	81c
			Production of oogonia and Antheridia										
Dextrose agar	5° C	2	-	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-	-
		6	-	-	-	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-	-	-	-
	10° C	2	-	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-	-
		6	-	-	-	+	-	-	-	-	-	-	-
		8	-	-	+	+	-	-	-	-	-	-	-
	15° C	2	-	-	-	-	-	-	-	-	-	-	-
		4	-	+	-	+	-	+	-	-	-	-	-
		6	...	...	...	...	...	...	...	...	...	...	...
		8	...	...	...	...	...	...	...	...	...	...	...
	20° C	2	+	+	-	+	-	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	...	...	...	...	...	...	...	...	...	...	...
		8	...	...	...	...	...	...	...	...	...	...	...
	25° C	2	+	-	-	-	-	-	-	-	-	-	-
		4	+	-	-	-	+	-	-	-	-	-	-
		6	+	-	-	-	+	-	-	-	-	-	-
		8	+	-	-	-	+	-	-	-	-	-	-

The sign + means both oogonia and antheridia produced; - means neither kind of sexual body produced; ... signifies no data.

(Continued on following page)

Table 1.--EFFECTS OF AGE OF CULTURE, CULTURE MEDIA, AND TEMPERATURE ON THE PRODUCTION OF OOGONIA AND ANTHERIDIA BY 11 ISOLATES OF PHYTOPHTHORA SPECIES.
(Continued)

Medium	Temperature	Age of Culture (Weeks)	Isolates 1	3	8	9	10	15	17	21	23	80c	81c
			Production of oogonia and antheridia										
Potato dextrose agar	10° C	2	+	+	-	+	+	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	+	+	-	+	+	+	-	-	-	-	-
		8	+	+	-	+	+	+	-	-	-	-	-
	20° C	2	+	+	-	+	+	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	+	+	-	+	+	+	-	-	-	-	-
		8	+	+	-	+	+	+	-	-	-	-	-
Oatmeal agar	10° C	2	+	+	-	+	+	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	+	-
		6	+	+	-	+	+	+	-	-	-	+	-
		8	+	+	+	+	+	+	-	-	+	+	-
	20° C	2	+	+	-	+	+	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	+	+	-	+	+	+	-	-	-	-	-
		8	+	+	-	+	+	+	-	-	-	+	-

The sign + means both oogonia and antheridia produced; - means neither kind of sexual body produced; ... signifies no data.

(Continued on following page)

Table 1.--EFFECTS OF AGE OF CULTURE, CULTURE MEDIA, AND TEMPERATURE ON THE PRODUCTION OF OOGONIA AND ANTHERIDIA BY 11 ISOLATES OF PHYTOPHTHORA SPECIES. (Continued)

Medium	Temperature	Age of Culture (weeks)	Isolates 1	3	8	9	10	15	17	21	23	80c	81c
			Production of oogonia and antheridia										
Prune agar	10° C	2	+	-	-	-	-	-	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	+	+	-	+	+	+	-	-	-	-	-
		8	+	+	+	+	+	+	-	-	-	-	-
Cornmeal agar	20° C	2	+	+	-	+	+	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	+	+	-	+	+	+	-	-	-	-	-
		8	+	+	-	+	+	+	-	-	-	-	-
Pumpkin (cooked)	20° C	2	+	+	-	+	+	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	+	+	-	+	+	+	-	-	-	-	-
		8	+	+	-	+	+	+	-	-	-	-	-
Wheat grain and peanut hulls	20° C	2	+	+	-	+	+	+	-	-	-	-	-
		4	+	+	-	+	+	+	-	-	-	-	-
		6	+	+	-	+	+	+	-	-	-	-	-
		8	+	+	-	+	+	+	-	-	-	-	-

The sign + means both oogonia and antheridia produced; - means neither kind of sexual body produced; ... signifies no data.

Isolates 1, 3, 9, 10, and 15, incubated at 10° and 20° C. on potato dextrose and oatmeal agars, produced oogonia and antheridia before the 2-week inspection. On oatmeal agar at 10° C. isolate 80c produced sexual organs within 4 weeks. On the 8th-week inspection isolates 8 and 23 were observed to sporulate. At 20° C. isolate 80c did not sporulate until the 8th-week inspection.

Cultures on corn meal agar, canned pumpkin, and wheat grain and peanut hulls, incubated at 20° C. for 8 weeks, produced sexual spores in 5 isolates: 1, 3, 9, 10, and 15. Spores were observed in 2 weeks on each of the media.

On prune agar, at 10° C., sexual spores were present on isolate 1 within 2 weeks, and on isolates 1, 3, 9, 10, and 15 within 4 weeks. At 8 weeks isolate 8 had produced sexual spores.

While the media used supported sexual body production between 10° and 25° C., oatmeal agar and an 8-week incubation stimulated more sporulation by isolates than other combinations. Isolates 17, 21, and 81c were not observed to yield sexual fruiting bodies under the controlled conditions used.

Sexual spore measurements.--Rosenbaum (25) considered the sexual bodies of Phytophthora to be the most important criteria in delimiting the species. Therefore, to determine differences between the test isolates in this study, diameters of the oogonia and

oospores were measured, and statistical analyses were made.

All fruiting bodies were measured with an eyepiece micrometer in a Bausch and Lomb 15x ocular with a Bausch and Lomb 8 mm 21x objective.

Because the oogonia and oospores were generally round, the greatest diameter was measured through the center of the organ, perpendicular to the side of the hyphae attachment. Using cultures grown on oatmeal agar for 8 weeks at 10° C., a minimum of 50 measurements of each respective organ was made for each individual isolate producing sexual bodies. The oogonia selected for measurement had antheridia attached.

Variance analyses of the measurements of the oogonia and the oospores showed the variability between isolate means to be highly significant. When the variability due to the oogonia was removed by means of a covariance analysis the true variability between the oospore means had been affected to some extent by that between the oogonia means. Consequently, adjusting the isolate means of the oospores to the experimental mean for the oogonia gave quite different values to the oospore means. See Tables 2 and 3. In the sexual sporulation studies, the measurements were well correlated for the totals between treatments and within treatments categories, Figure 2.

Table 2.--VARIANCE AND COVARIANCE ANALYSES OF THE OOGONIA AND OOSPORE DIAMETERS OF 8 ISOLATES OF PHYTOPHTHORA SPECIES.

Variance Analyses

Variability due to:	D/F	x-oogonia Sums of squares	x-oogonia Mean square (MS)	x-oogonia Obs. F	x-oogonia Req. F 0.05	x-oogonia Req. F 0.01	y-oospores Sums of squares	y-oospores Mean square	y-oospores Obs. F
Totals	399	223.588					245.212		
Between isolates	7	108.696	15.528	52.98	2.03	2.69	135.973	19.4247	69.71
Within isolates	392	114.892	0.293				109.239	0.2787	

Covariance Analysis

Variability due to:	D/F	Sums of squares and products (x^2)	(xy)	(y^2)	r	D/F	Adjusted sums of squares Sums of squares	Mean square	Obs. F	Req. F 0.05	Req. F 0.01
Totals	399	223.588	209.050	245.212	0.893	398	49.754				
Between isolates	7	108.696	119.866	135.973	0.986	7	9.745	1.3921	13.60	2.03	2.69
Within isolates	392	114.892	89.184	109.238	0.796	391	40.010	0.1023			

Table 3.--COMPARISON OF OOGONIA AND OOSPORE DIAMETERS OF 8 ISOLATES OF PHYTOPHTHORA SPECIES GROWN ON OATMEAL AGAR AT 10° C. FOR 8 WEEKS.

Isolate No.	Mean oogonia diameter (x)	Mean oospore diameter (y)	Mean oospore diameter adjusted
	Microns	Microns	Microns
1	37.86	28.80	27.92
3	37.21	28.97	28.61
8	32.32	24.20	27.63
9	32.74	23.20	26.30
10	33.32	26.10	28.75
15	34.51	25.71	27.44
17	-----	-----	-----
21	-----	-----	-----
23	43.62	36.00	30.67
80c	42.34	35.47	31.13
81c	-----	-----	-----

Minimum significant difference (for adjusted y)
at 0.05 level = 0.98 microns
at 0.01 level = 1.29 microns

Standard deviation for x = 4.22 microns
Standard error for x = 0.60 microns

Standard deviation for y = 4.13 microns
Standard error for y = 0.58 microns

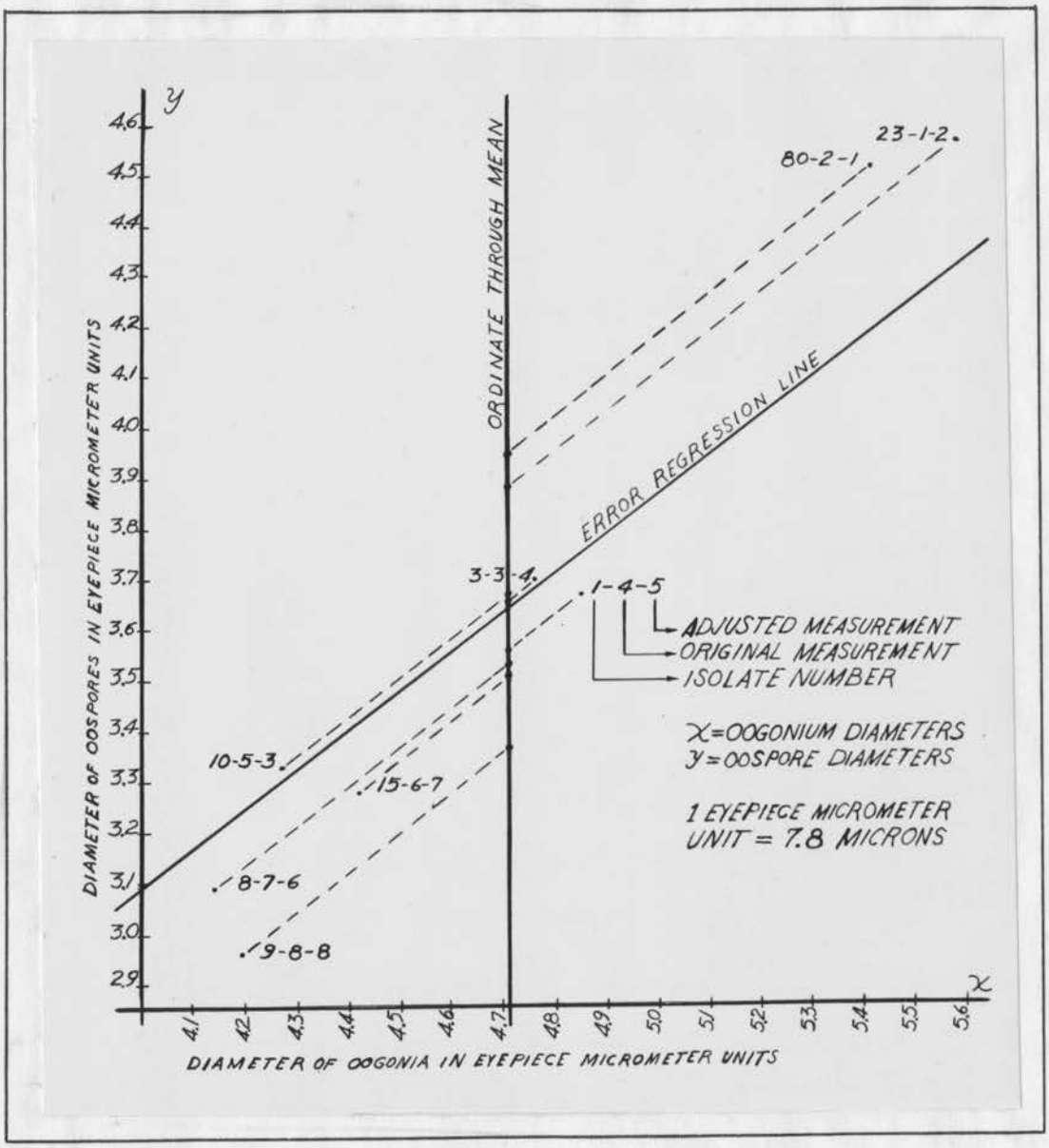

Fig. 2.--The regression of oogonia diameters (x) on oospore diameters (y) of eight isolates of Phytophthora.

Asexual sporulation.--Kreutzer and Bryant (14) reported that sporangial production by Phytophthora capsici was stimulated by mixing ground barley culture of the fungus with unsteamed soil.

In an attempt to induce the production of asexual spores, the test isolates were grown on different media. In preliminary experiments, asexual spores were produced by cultures grown in Knop's solution. Therefore, to induce asexual sporulation in test isolates, unsteamed soil, green potato leaves, and sterilized Knop's solution were mixed with distilled water and mats of aerial mycelium. The mycelial mats were from fungus colonies grown on potato dextrose agar for 4 weeks. The materials were used in approximately the same quantities for two experiments. The first experiment was in test tubes with the mycelium completely submerged in liquid. The second experiment was in Petri dishes with the mycelium arranged partly submerged in liquid medium. Each experiment was made up of six triplicate tests.

Each test of the experiment was composed as follows:

Test 1. 20-30 ml distilled water and mycelial mats.

Test 2. 20-30 ml distilled water, mycelial mats, and potato leaves.

Test 3. 20-30 ml distilled water, mycelial mats, and 5 g of unsteamed soil.

Test 4. 20-30 ml sterilized Knop's solution and mycelial mats.

Test 5. 20-30 ml sterilized Knop's solution, mycelial mats, and potato leaves.

Test 6. 20-30 ml sterilized Knop's solution, mycelial mats, and 5 g of unsteamed soil.

The experiments were performed at room temperature. After 2 days the mycelium was inspected each consecutive day until asexual spores were produced abundantly or until contamination by other organisms made observations for asexual fruiting impossible.

When the secondary organisms began to collect around the asexual bodies, the mycelial mats were removed from the medium and placed in flasks containing fresh tap water. Within a few hours, glycerin jelly slides and water mounts were prepared and measurements were made of the greatest width and the length of the sporangia.

Conidia, measured in this experiment, were selected from test number 5. A minimum of 30 conidia were measured for each isolate which produced asexual fruiting bodies.

When the isolates were cultured on solid media in other experiments, asexual sporulation was never observed. Since the organisms under study were Phycomycetes, it seemed likely that

asexual sporulation might be stimulated by using a liquid medium: water and Knop's solution. In the first experiment, in which the mycelium was completely submerged in the medium, no sporangia were observed. A few chlamydospores were produced.

The second experiment, which was conducted in Petri dishes, produced both sporangia and chlamydospores. In the Petri dishes in which the mycelium was arranged so that part of the mycelium was completely submerged, the greatest concentration of sporangial production was near the liquid surface: either just below the surface, at the surface, or just above the surface of the medium.

As shown in Table 4, a water solution was not a good medium for producing asexual spores. Very few sporangia were produced by isolates 3, 10, 17, and 21 when distilled water and mycelium were used, while other isolates in the same test remained unaffected. Isolates 3, 10, 17, and 80c produced very few sporangia when the leaf was added to the water with the mycelium. However, distilled water, unsteamed soil and mycelium resulted in a limited production of sporangia by all isolates except 81c. It was apparent that sporangia and a few chlamydospores were stimulated by the liquid medium. Again, isolate 81c was an exception in that only chlamydospores were observed.

By comparative inspection, Knop's solution was a much

Table 4.--A COMPARISON OF THE EFFECTS OF WATER, KNOP'S SOLUTION, POTATO LEAF AND SOIL EXTRACTS ON THE ASEXUAL SPORULATION OF 11 ISOLATES OF PHYTOPHTHORA SPECIES.

Medium	Isolate Number										
	1	3	8	9	10	15	17	21	23	80c	81c
Water	...	1	...	...	1	...	2	3	...	...	...
Water and leaf	...	1	...	...	1	...	2	...	...	1	...
Water and soil	1	1	1	1	1	1	1	1	1	1	3
Knop's solution	1	1	1	1	1	1	1	1	1	1	3
Knop's solutions and leaf	1	1	1	1	1	1	1	1	1	1	3
Knop's solution and soil	1	1	1	1	1	1	1	1	1	1	3

1 signifies chlamydospores and sporangia were produced.
2 signifies only sporangia were produced.
3 signifies only chlamydospores were produced.
... signifies no data.

better medium for sporangial and chlamydospore production. A few chlamydospores were produced by all isolates and sporangia were present in every instance, except with isolate 81c. It cannot be stated that isolate 81c did not produce sporangia, however, as chlamydospores were so abundant that all attempts to distinguish sporangia were questionable.

Although it was shown that liquid media would stimulate asexual spores, it was also indicated that air was important to the development of asexual reproductive bodies.

Variance analyses which were made on the length and the width of the conidia, Table 5, showed differences between isolate means to be highly significant for both experiments. A covariance study, using the length as the independent variable x and the width as the dependent variable y, indicated that removal of the variability due to the length made very little difference in the variability due to the width alone. However, when the isolate means for the widths were adjusted to the experimental mean for the length, the relative values of these adjusted isolate means changed appreciably. See Figure 3 and Table 6.

On the whole, the widths of the asexual spores were well correlated with the lengths. However, the correlation between the measurements for the isolate means was significant at the 0.05 level only.

Table 5.--VARIANCE AND COVARIANCE ANALYSES OF SPORANGIA WIDTHS AND LENGTHS OF 10 ISOLATES OF PHYTOPHTHORA SPECIES.

Variance Analyses

Variability due to:	D/F	x-Lengths					y-Widths		
		Sums of squares	Mean square	Obs. F	Req. F 0.05	Req. F 0.01	Sums of squares	Mean square	Obs. F
Totals	299	573.109					146.064		
Between isolates	9	82.223	9.136	5.40	1.91	2.48	33.705	3.745	9.67
Within isolates	290	490.886	1.693				112.360	0.387	

Covariance Analysis

Variability due to:	D/F	Sums of squares and products (x^2)	(xy)	(y^2)	r	D/F	Adjusted sums of squares: Sums of squares	Mean squares	Obs. F	Req. F 0.05	Req. F 0.01
Totals	299	573.109	195.313	146.064	0.675	298	79.502				
Between isolates	9	82.223	37.933	33.705	0.721	9	17.600	1.9555	9.13	1.91	2.48
Within isolates	290	490.886	157.380	112.360	0.670	289	61.902	0.2142			

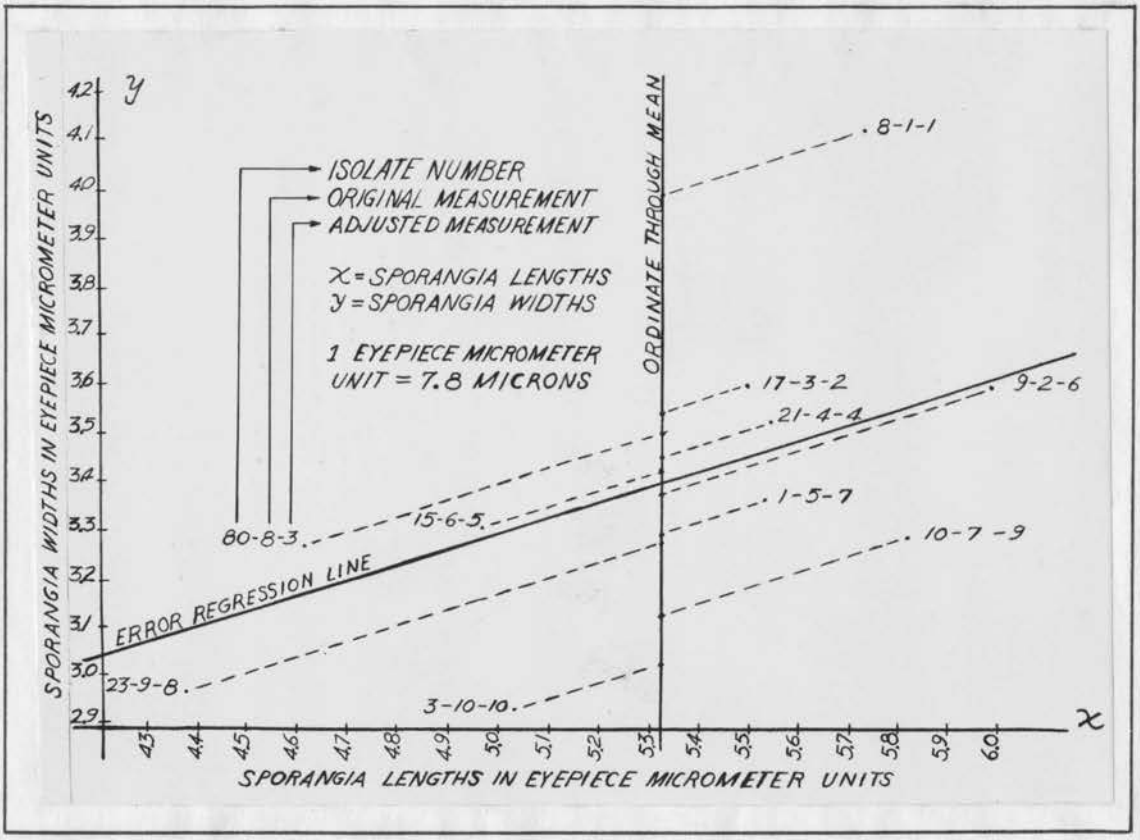

Fig. 3.--The regression of sporangia lengths (x) on sporangia diameters (y) of ten isolates of phytophthora

Table 6.--COMPARISON OF SPORANGIA WIDTHS AND LENGTHS OF 10 ISOLATES OF PHYTOPHTHORA SPECIES GROWN ON POTATO LEAVES (IRISH COBBLERS) IN KNOP'S SOLUTION.

Isolates	Lengths (x)	Widths (y)	Widths (adjusted y)
	Microns	Microns	Microns
1	43.157	26.262	25.747
3	39.257	22.932	23.673
8	46.176	32.502	31.020
9	46.620	28.103	26.481
10	45.372	25.716	24.492
15	38.766	25.794	26.691
17	42.876	28.080	27.658
21	43.188	27.534	27.011
23	34.140	23.142	25.521
80c	36.059	25.482	27.245
81c	------	------	------

Minimum significant difference (for adjusted y)
at 0.05 level = 4.493 microns
at 0.01 level = 5.920 microns

Standard deviation for x = 10.148 microns
Standard error for x = 1.853 microns

Standard deviation for y = 4.852 microns
Standard error for y = 0.886 microns

Genetic studies.--During the course of this study, some difficulty was encountered in obtaining oospores from some of the test isolates. To stimulate oospore production, an experiment was conducted in which all the 11 isolates were crossed in all possible combinations. This study was repeated three times at a temperature of 20° C. on potato dextrose agar. Twenty ml of sterile potato dextrose agar were allowed to solidify in Petri dishes for 2 days before the agar was separated into halves. The medium was divided by passing a 2-mm, sterile, steel wire through the agar, completely separating the two halves by a deep groove. The plates were then inoculated. One 4-mm cube of inoculum (mycelium plus potato dextrose agar medium on which the fungus was cultured) was placed on each separated half of the medium about 10 mm from the groove. This located the two inoculations about 18 mm apart near the center of the Petri dish. The placement of the inoculum in this particular manner made it possible for the hyphal tips to meet as they crossed the area separating the two agar portions.

In Table 1, it is shown that isolates 1, 3, 8, 9, 10, 15, 23, and 80c produce sexual spores in pure culture. Therefore, it might be expected that sexual spores would be observed in any combination involving the above isolates. However, in some isolate crosses, this did not prove to be true when individual crossed culture observations were made.

The results of this experiment indicated that all combinations involving isolates number 1, 3, 9, 10, and 15 produced both antheridia and oogonia. Isolate 8 crossed with 8, 17, 21, 23, or 80c did not produce sexual spores, nor did isolate 17 crossed with itself, 21, 23, 80c or 81c produce sexual spores. The same was true when isolate 21 was crossed with itself, 23, 80c, and 81c. When 23 was crossed with 23 or 80c, no sexual spores were produced, but when 23 and 81c were crossed, both antheridia and oogonia were produced. When 81c was crossed with 81c, and 80c crossed with 80c no fruiting bodies were seen; yet 80c crossed with 81c showed the formation of both male and female fruiting bodies. See Table 7.

When the results of this experiment were viewed independently of other studies it was noticed that isolates 8, 17, 23, 80c, and 81c did not sporulate when crossed with themselves. However, when 8, 23, and 80c were crossed with 81c, sexual spores were produced. Since spores were produced in the crossed cultures and not in the pure cultures, heterothallism in isolates 8, 23, 80c and 81c was indicated. However, by its unexpected inconsistence, the genetic study indicated that a more extensive study should be conducted along this line in order to obtain clear-cut results.

Table 7.--THE PRODUCTION OF OOGONIA AND ANTHERIDIA BY CROSSING 11 ISOLATES OF PHYTOPHTHORA SPECIES IN ALL POSSIBLE COMBINATIONS.

Isolate Numbers	1	3	8	9	10	15	17	21	23	80c	81c
1	+	+	+	+	+	+	+	+	+	+	+
3		+	+	+	+	+	+	+	+	+	+
8			-	+	+	+	-	-	-	-	+
9				+	+	+	+	+	+	+	+
10					+	+	+	+	+	+	+
15						+	+	+	+	+	+
17							-	-	-	-	-
21								-	-	-	-
23									-	-	+
80c										-	+
81c											-

Isolate Numbers

+ = oognoia were produced

- = no sexual bodies were produced

Substrata observations.--For an additional comparison of isolates, substrata observations of the test isolates were made on different media. Using 125-ml Erlenmeyer flasks, stoppered with cotton stoppers and containing 40 ml of sterile medium, the 11 isolates were cultured on potato dextrose agar, oatmeal agar, and dextrose agar. While incubating for 6 weeks, the cultures were in darkness at room temperature. After the incubation period the cultures were examined under two General Electric 40-watt 4500 white fluorescent lamps and an ultraviolet light yielding wave lengths in the vicinity of 3650 A.

Three replications of each isolate per medium were prepared. Three uninoculated flasks per medium were used as comparing standards, and are referred to as checks. The substrata were grouped as to whether they were darker or lighter than the checks.

When the white light was concentrated on the substratum, a different color was observed between isolates. Isolates cultured on potato dextrose agar and observed with white light yielded a color ranging from light amber to dark amber or brown. The same cultures under ultraviolet fluoresced yellow or cream. Under white light, isolates 1, 3, 9, 10, 15, and 81c on potato dextrose agar had a darker substratum than the checks, while they fluoresced yellow. Isolates 8, 17, 21, 23, and 80c were lighter than the checks under

white light, and fluoresced cream under ultraviolet light.

When the isolates were cultured on dextrose agar there was no apparent difference between the isolates or between the isolates and the checks.

Isolates cultured on oatmeal agar gave no clear-cut differences between isolates, although under white light the isolates were more yellow than the checks. The ultraviolet light caused no fluorescence in the checks, while the substrata on which the fungus was grown fluoresced yellow.

Different isolates, cultured on potato dextrose agar, changed the color of the substrata between isolates. There were no marked differences between substrata of isolates cultured on either dextrose or oatmeal agars.

The substrata observations showed the six isolates darker in color than the checks, to be identical with the isolates which gave a yellow fluorescence. The five isolates, lighter in color than the checks, fluoresced with a cream color. From this comparison, it was shown that the isolates could be separated into two color groups.

Cultural characteristics.--Based on aerial mycelial growth characteristics on potato dextrose agar, the isolates studied here were grouped into two readily distinguishable types. The first type (Figure 4A) yielded cultures which had fine, white, fluffy aerial mycelial forming ripples at the margin of the colony. As the

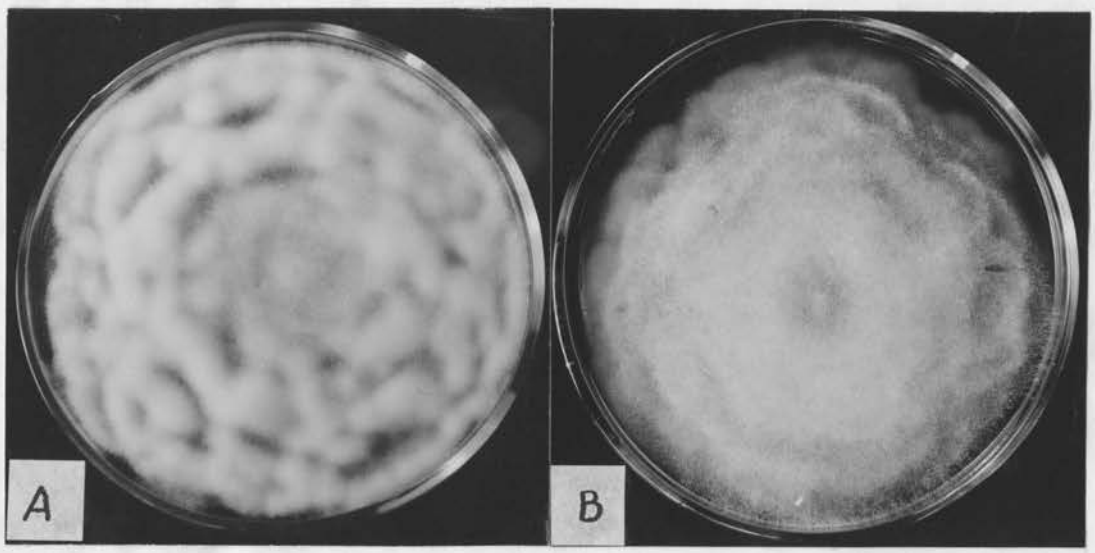

Fig. 4.--Mycelial growth characteristics of "Western Leak" isolates on potato dextrose agar. A. This type was characteristic of all isolates except isolate No. 8. B. This type was characteristic of only isolate No. 8.

cultures aged the ripples developed into white tufts of "islands" over the surface of the agar. The second type, Figure 4B, produced aerial mycelium which appeared coarse and wirey. The fluffiness was less cottony than the first type and the mycelium was dirty-white or gray. The top contour, made by the aerial strands, was "smooth" and even, with less pronounced ripples. The colony margin was more even. As the cultures aged the general characteristics persisted. Both types of cultures yielded profuse mycelial growth, with the mycelium of the latter type appearing less delicate than the first.

Microscopic examination of the hyphae in both types of cultures showed that young filaments were branched in many directions, coenocytic and filled with a dense, granular, semi-liquid substance containing large dense protoplasmic materials. In the older cultures in which protoplasmic streaming had stopped, cross walls were often observed.

Sexual bodies.--The description of the sexual organs was made from old cultures and well-developed structures. The oogonia and antheridia were apparently developed on separate hyphae or at widely separate origins. A continuous hyphal strand joining the two sexual organs was not traced in these studies.

Paragynous type of antheridium was not seen in cultures of the 11 "Western Leak" isolates. The antheridium was amphigynous,

located at the base of the oogonia and persistent around the stalk of the female organ, Figure 5. The antheridium which was almost clubbed or flattened-sphere shaped, was terminal. On the end of usually long hypha, the male internal material was separated from the "stem" or hyphal contents by a transverse septum. Although the antheridium was located tight against the base of the oogonium, sexual connection between the two sex organs was not observed.

The oogonium was borne singly on a short stalk. The stalk was not noticeably differentiated from the vegetative hypha except that it was usually short. At the base of the oogonium a cross wall separated the oospore from the internal mass of the supporting structure.

The oospore contained within the oogonium was spherical, smooth, and hyaline to yellowish or straw-colored.

Asexual bodies.--The number of sporangia found on a conidiophore was limited to one terminal organ, Figure 6. The conidia were of various sizes and shapes; ovate to globose, with the apical end of the reproductive structure usually non-papillate. The contents of the organs were finely granulated and usually filled the sporangium. When the protoplasmic material did not fill the conidium, it had the general shape of the case. Conidia were uniform in color, buff-gray to gray.

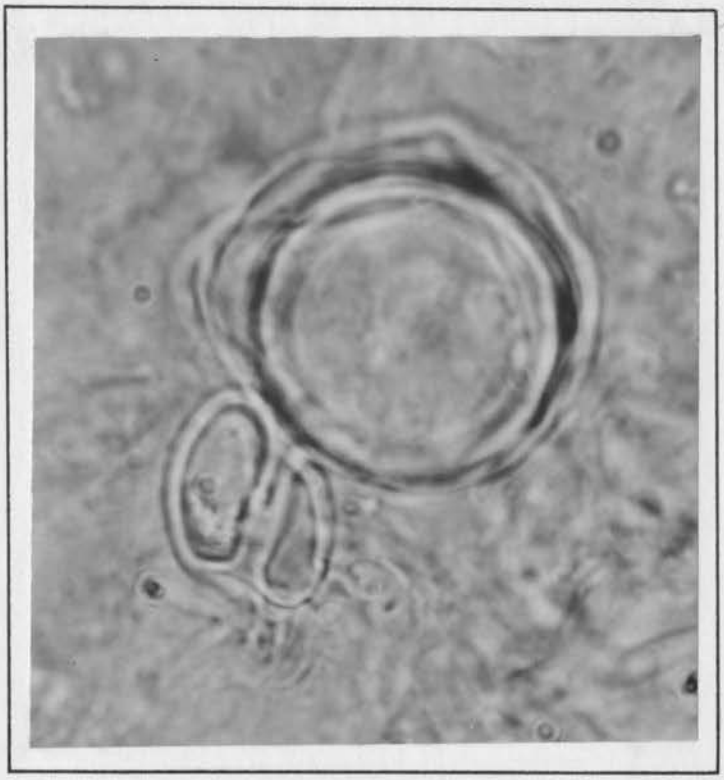

Fig. 5.--Sexual organs of the "Western Leak" fungus. An oogonium with an amphigynous type of antheridium surrounding the oogonial stalk.

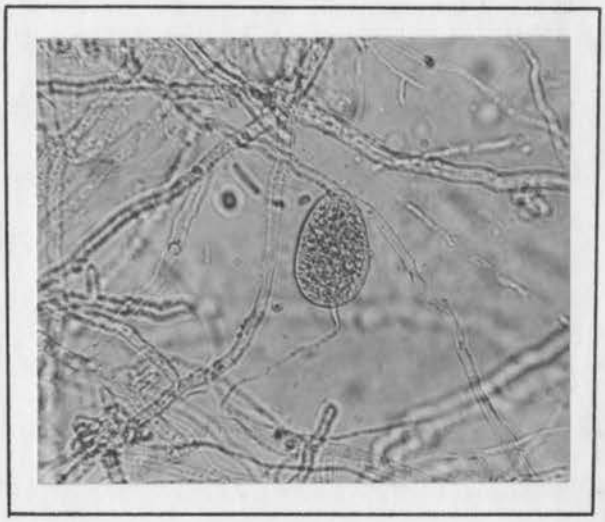

Fig. 6.--A sporangium of the "Western Leak" fungus.

Although germination of conidia was not observed, conidia stored in water for about 8 weeks were frequently observed to be empty. A germ tube arose from the apex of some of the germinated structures. It was questionable whether swarm spores were recognized in this study. Figure 7.

Chlamydospores were observed to be produced by all 11 isolates. The thick-walled spores formed either terminally or intercalarily, and were straw-colored, smooth, and spherical. They were usually greatly branched. The spores were linked together by short tubes. In the asexual tests, isolate number 81c produced chlamydospores so quickly and so profusely that sporangia were not distinquishable. The conditions which stimulated the formation of other asexual spores, induced the production of chlamydospores.

Physiologic reactions

Temperature and growth relations.--The 11 test isolates were grown under a series of controlled temperatures, in electric thermostatically-regulated incubators to determine their growth responses. They were cultured on dextrose agar and incubated at eight different temperatures: 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° C.

Inoculum, for temperature tests, was maintained on dextrose agar for not more than 14 days. Within that period, blocks of the

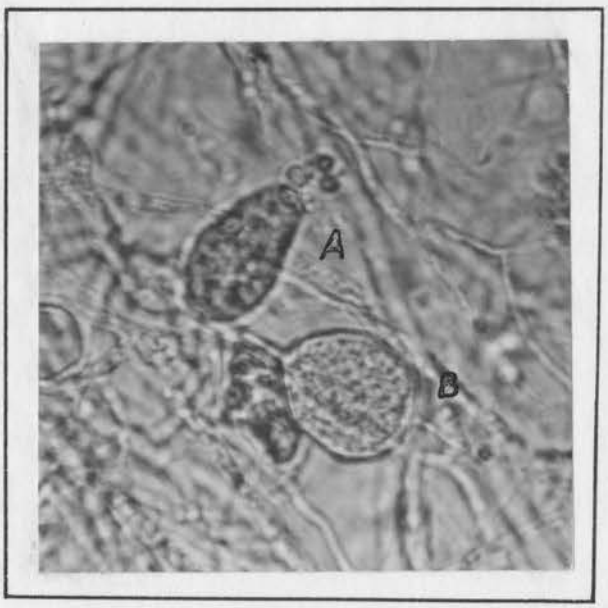

Fig. 7.--Two sporangia of the "Western Leak" fungus. A. Sporangium with possible swarm spores. B. Sporangium forming a germ tube.

inoculum approximately 4 mm square were transferred to one side of fresh solidified dextrose agar plates. The cultures were kept at room temperature until mycelial growth was observed in each. A minimum of five growing cultures per isolate was selected for each temperature study. The selected cultures were placed in their respective incubators at a constant temperature (± 2° C.) for 48 hours preceding the first measurements. After the first measurements, all cultures were inspected and measured every 24 hours except cultures at 5° C. which were studied after each 48-hour period. Cultures at 10°, 15°, 20°, and 25° C. were measured over a period of 7 days; at 30° and 35° C. for 6 days; and cultures at 5° C. for 8 days.

The inoculum was placed on the medium close to the wall of the Petri dish. The greatest radial distance between the block of inoculum and the margin of the colony was used as a measure of growth. All measurements were cumulative and were recorded in millimeters.

Tucker (27) studied the mycelial growth and various temperature relationships of many species of Phytophthora. He reported that different species of the genus varied widely in their temperature relations. He also regarded the temperature relations to be of taxonomic value in that different isolates of the same species reacted similarly, indicating that the behavior of organisms to temperature was a specific character.

When isolates were cultured at temperatures nearing the extremes of the fungal tolerance, differences between isolates were observed. At 40° C. none of the isolates grew. At 30° C. and 35° C. isolates ceased to grow at various periods.

At 35° C. isolates 8, 17, 21, and 23 were growing after 9 days. Except for isolate 15, some growth was obtained by all 11 isolates at this temperature. However, isolates 1, 3, 9, 10, and 81c grew only slightly and for a short period of time.

Growth of isolate 1 was inhibited at 30° C. Isolates 1, 3, 9, and 15 ceased to grow at this temperature before the 7 inspection days were completed. Although isolate 10 was growing on the last inspection date, its growth was only 2 mm larger than isolates 9 and 15.

The 11 isolates incubated at 25°, 20°, 15°, 10°, and 5° C. were growing after 10 days.

Variance analyses were made of the results of each of the seven temperature tests and of the six temperatures together where the readings were made daily. Table 8. First, the variability between periods, which was not of particular interest in these studies, was removed. Then the variability within periods was analyzed. This variability was broken into the variability between isolates within periods and that within isolates within periods.

Table 8.---VARIANCE ANALYSES OF THE EFFECT OF TEMPERATURE ON GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES.

Variability due to:	D/F	35° C			30° C		
		ss	ms	Obs. F	ss	ms	Obs. F
Totals	439	50,082.00			152,388.80		
Between periods	7	4,212.18	601.74	5.67	49,017.20	7,002.46	29.26
Within periods	(432)	(45,869.82)	106.18		(103,371.60)	239.29	
Between isolates within periods	80	41,483.02	518.54	41.61	101,821.60	1,272.77	28.90
Within isolates within periods	352	4,386.80	12.46		1,550.00	44.03	

Variability due to:	D/F	25° C			20° C		
		ss	ms	Obs. F	ss	ms	Obs. F
Totals	439	134,524.72			97,589.18		
Between periods	7	124,349.66	17,764.24	754.21	94,974.13	13,567.73	2241.35
Within periods	(432)	(10,175.06)	23.55		(2,615.05)	6.05	
Between isolates within periods	80	7,024.26	87.80	9.81	990.25	12.38	2.68
Within isolates within periods	352	3,150.80	8.95		1,624.80	4.62	

(Continued on following page)

Table 8.--VARIANCE ANALYSES OF THE EFFECT OF TEMPERATURE ON GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES. (Continued)

Variability due to:	D/F	15° C			10° C			Required F (35° - 10° C)	
		ss	ms	Obs. F	ss	ms	Obs. F		
Totals	439	94,352.89			41,819.00				
Between periods	7	83,198.20	11,885.46	460.30	16,880.56	2411.51	41.77	2.03	2.69
Within periods	(432)	(11,154.69)	25.82		(24,938.44)	57.73			
Between isolates within periods	80	8,019.49	100.24	11.25	22,702.44	283.78	44.67	1.34	1.51
Within isolates within periods	352	3,135.20	8.91		2,236.00	6.35			

Variability due to:	D/F	5° C			Required F (5° C)	
		ss	ms	Obs. F		
Totals	329	2,395.91				
Between periods	5	969.36	193.87	44.03	2.24	3.08
Within periods	(324)	(1,426.55)	4.40			
Between isolates within periods	60	974.15	16.24	9.47	1.38	1.56
Within isolates within periods	264	452.40	1.71			

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Table 8.--VARIANCE ANALYSES OF THE EFFECT OF TEMPERATURE ON GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES. (Continued)

Combined temperature analysis: 10°, 15°, 20°, 25°, 30°, and 35° C.

Variability due to:	D/F	ss	ms	Obs. F	Required F .05	Required F .01
Totals	2639	656,589.64				
Between temperatures	5	85,833.06	17,166.61	79.22	2.22	3.04
Within temperatures	(2634)	(570,756.58)	216.69			
Between periods within temperatures	42	372,631.92	8,872.19	116.07	1.40	1.60
Within periods within temperatures	(2592)	(198,124.66)	76.44			
Between isolates within periods within temperatures	480	182,041.06	379.25	49.80	1.13	1.19
Within isolates within periods within temperatures	2112	16,083.60	7.62			

The F value between periods measured the variability of the periods with respect to that within periods, whereas the F value for the variability between isolates within periods used the within isolates within periods variability for its measure.

Table 9 lists the standard deviations and standard errors for the various studies, with the criteria of significance for the between isolates within period (and in the composite study, the between isolate within period within temperature) differences. Table 10 lists the cumulative means of isolates per period within temperatures. The results of the statistical analyses beginning with data taken at 35° C., and going down to 10° C., showed F values for the between periods as being highly significant but the F value at 35° C. was the smallest. The highest value was for 20° C., after which the trend was downward. The F value never became as small for any of the other temperatures as it did for 35° C.

However, the F value for the between isolates within periods showed a trend quite the opposite from that for the between periods. Beginning with 35° C., the F value of 41.61 dropped to 2.68 for the 20° C., and then proceeded upward again to 44.67 for 10° C. Again the differences between the means for all the isolates within periods were highly significant.

When the six temperatures, 10°, 15°, 20°, 25°, 30°, and

Table 9.--THE EFFECT OF TEMPERATURE ON THE MEAN CUMULATIVE MYCELIAL GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES DURING 7 SUCCESSIVE 24-HOUR PERIODS.

Temperature	Mean mycelial growth — Periods							Standard deviations (s)	Standard error ($s_{\bar{x}}$)	Minimum significant difference	
	1	2	3	4	5	6	7			at 0.05	at 0.01
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
5° C.[1]	2.236	3.545	4.691	5.382				1.309	0.585	1.630	2.148
10° C.	12.782	15.545	17.709	20.200	22.818	26.673	28.709	2.520	1.127	3.135	4.129
15° C.	13.891	19.909	25.255	31.782	37.891	43.873	49.909	2.984	1.335	3.713	4.489
20° C.	5.455	12.764	18.745	25.727	32.564	38.655	43.691	2.148	0.961	2.673	3.519
25° C.	4.964	13.691	20.090	27.764	35.109	43.255	48.600	2.992	1.338	3.722	4.901
30° C.	12.200	18.491	23.709	29.436	33.800	38.382	42.600	6.646	2.968	8.255	10.870
35° C.	11.691	14.818	17.509	18.491	19.600	20.018	21.127	3.530	1.579	4.392	5.783

[1] Due to slowness of mycelial growth, measurements at 5° C. were made each 48 hours instead of each 24 hours.

Table 10.--CUMULATIVE MYCELIAL GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES AT TEMPERATURES OF 5°, 10°, 15°, 20°, 25°, 30°, AND 35° C.

Temperature	Isolate No.	24-hour periods						
		1	2	3	4	5	6	7
		mm	mm	mm	mm	mm	mm	mm
5° C.[1]	1	2	3	3	4	6	7	...
	3	3	6	7	8	11	12	...
	8	1	2	3	...	...	...	...
	9	2	3	4	5	6	7	...
	10	3	3	4	4	6	7	...
	15	3	5	6	7	8	9	...
	17	2	3	4	...	...	...	...
	21	2	2	4	4	5	...	...
	23	3	5	6	6	7	...	...
	80c	2	4	6	6	7	8	...
	81c	3	4	6	8	10	11	...
10° C.	1	14	16	18	21	23	26	28
	3	20	23	25	28	30	37	40
	8	8	10	12	13	15	18	19
	9	14	17	19	22	24	29	30
	10	17	20	23	26	29	33	36
	15	16	20	22	25	30	32	34
	17	3	5	8	11	13	15	18
	21	6	6	7	8	9	12	13
	23	12	14	17	20	23	24	29
	80c	13	17	19	21	24	28	30
	81c	19	23	26	29	33	38	41
15° C.	1	10	15	21	26	32	37	42
	3	9	15	20	28	37	43	49
	8	13	18	23	29	35	40	46
	9	17	24	30	36	41	48	55
	10	18	26	32	38	43	50	57
	15	8	15	19	25	35	41	48
	17	16	21	27	33	39	44	48
	21	15	21	25	30	34	40	46
	23	15	20	25	32	38	44	50
	80c	13	19	25	31	36	44	51
	81c	19	26	31	39	47	53	59

[1] Due to slowness of mycelium growth, measurements at 5° C. were made each 48 hours instead of each 24 hours.

... signifies no data.

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Table 10.--CUMULATIVE MYCELIAL GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES AT TEMPERATURES OF 5°, 10°, 15°, 20°, 25°, 30°, AND 35° C. (Continued)

Temperature	Isolate No.	24-hour periods						
		1	2	3	4	5	6	7
		mm	mm	mm	mm	mm	mm	mm
20° C.	1	5	12	18	25	31	38	43
	3	5	12	17	25	32	37	42
	8	6	13	15	25	32	38	43
	9	4	12	18	25	32	38	43
	10	4	11	17	24	31	37	41
	15	7	14	20	23	33	40	45
	17	7	13	19	25	31	35	40
	21	6	14	19	26	34	41	47
	23	6	13	19	26	34	40	45
	80c	6	13	20	27	34	40	46
	81c	5	13	20	27	34	40	45
25° C.	1	6	13	18	27	33	40	45
	3	4	13	19	26	33	40	45
	8	5	14	20	29	37	46	52
	9	3	12	18	25	33	41	46
	10	1	8	13	20	27	34	40
	15	7	15	21	28	38	43	48
	17	4	14	20	26	32	39	43
	21	7	17	24	32	40	48	54
	23	7	17	23	32	40	49	56
	80c	6	16	23	32	40	49	54
	81c	5	13	21	29	38	47	53
30° C.	1	6	8	...	...	...	...	...
	3	10	13	14	19	21	...	...
	8	13	23	31	42	51	62	70
	9	11	16	18	23	25	...	...
	10	10	15	19	20	21	25	26
	15	9	12	15	19	20	23	...
	17	15	22	30	38	42	48	55
	21	14	20	29	33	39	43	50
	23	19	31	40	51	61	69	79
	80c	17	25	33	41	48	54	63
	81c	12	19	25	32	38	45	49

... Signifies no data.

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Table 10.--CUMULATIVE MYCELIAL GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES AT TEMPERATURES OF 5°, 10°, 15°, 20°, 25°, 30°, AND 35° C. (Continued)

Temperature	Isolate No.	24-hour periods						
		1	2	3	4	5	6	7
		mm	mm	mm	mm	mm	mm	mm
35° C.	1	7	7	8	...	...	...	...
	3	11	11	12	...	...	...	...
	8	13	18	22	27	30	32	34
	9	10	12	...	...	...	...	...
	10	6	7	8	...	...	...	...
	15	11	...	...	...	...	...	...
	17	13	19	25	25	26	28	30
	21	13	21	27	28	30	31	33
	23	16	22	28	28	29	29	32
	80c	18	24	30	35	38	39	...
	81c	11	12	...	...	...	...	...

... Signifies no data.

35° C., were combined into one study, the F values for the differences for the temperatures, for the periods within temperatures, and for the isolates within periods within temperatures were all highly significant.

The eleven isolates, cultured at 10°, 15°, 20°, and 25° C. were growing after 7 days incubation in their respective temperatures. At 5° C. all isolates were growing after 8 days. At 30° C. only isolates 1, 3, 9, and 15 ceased to grow before the 7-day observation period was over. At 35° C. only isolates 8, 17, 21, 23, and 80c grew for 7 days.

Relation of hydrogen-ion concentration to mycelial growth.--In order to ascertain further differences between the test isolates, the effect of various hydrogen-ion concentrations on the growth of the isolates was studied.

The dextrose agar for these tests was prepared and hydrogen-ion concentrations maintained by Clark and Lub's pH buffer solutions as was previously described. The cultures were replicated five times per isolate for each pH test and the entire study was conducted in a electric thermostatically-regulated incubator at 25° C. (± 2° C.). Except for the buffered dextrose agar and the constant temperature, this experiment was conducted as described in the temperature and growth relation studies.

Results showed that isolates cultured on buffered dextrose agar were limited in growth to four hydrogen-ion concentrations. No growth was observed with isolates cultured at pH 3.5 and below, or with isolates cultured at pH 8.0 or above.

At pH 4 only isolates 1, 23, and 80c showed mycelial growth. In 7 days, the radial mycelial growth for the three isolates was as follows: Isolate 1, 3 mm; 23, 5 mm; 80c, 7 mm. The number of replicates living per isolate at the end of 7 days observation was as follows: For isolate 1, two cultures; 23, two; for 80c all five replicates.

At pH's 5.0, 6.0, and 7.0 the 11 isolates were growing on the seventh inspection date. The amount of radial mycelial growth for each isolate per respective hydrogen-ion concentration was tabulated in millimeters.

The results of the hydrogen-ion concentration experiment was analyzed. In all studies the F values were highly significant. In the pH 5 studies the variability between periods appeared to be less than that between isolates within periods.

In the composite studies all the F values were highly significant. When the three pH's with the 11 isolates were used, the greatest variability was between the pH's. Next was the variability between the periods within the pH's, with the least variability between the isolates within the periods within the pH's. Table 11.

Table 11.--VARIANCE ANALYSES OF THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE RATE OF GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES.

Variability due to:	D/F	pH 5			Req. F	
		ss	ms	Obs. F	0.05	0.01
Totals	439	48,589.67				
Between periods	7	19,118.11	2731.16	40.03	2.03	2.69
Within periods	(432)	(29,471.56)	68.22			
Between isolates within periods	80	28,773.96	359.67	181.49	1.34	1.51
Within isolates within periods	352	697.60	1.98			

Variability due to:	D/F	pH 6			Req. F	
		ss	ms	Obs. F	0.05	0.01
Totals	439	152,210.18				
Between periods	7	133,165.78	19,023.68	431.53	2.03	2.69
Within periods	(432)	(19,044.40)	44.08			
Between isolates within periods	80	17,733.20	221.66	59.51	1.34	1.51
Within isolates within periods	352	1,311.20	3.72			

(Continued on following page)

Table 11.--VARIANCE ANALYSES OF THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE RATE OF GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES.

(Continued)

Variability due to:	D/F	pH 7			Req. F	
		ss	ms	Obs. F	0.05	0.01
Totals	439	70,136.80				
Between periods	7	46,403.52	6629.08	122.76	2.03	2.69
Within periods	(432)	(23,733.28)	54.94			
Between isolates within periods	80	21,284.08	266.05	38.24	1.34	1.51
Within isolates within periods	352	2,449.20	6.96			

(Continued on following page)

Table 11.--VARIANCE ANALYSES OF THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE RATE OF GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES.
(Continued)

Combined analysis of variance at pH's of 5.0, 6.0, and 7.0

Variability due to:	D/F	ss	ms	Obs. F	Req. F 0.05	Req. F 0.01
Totals	1319	351,688.62				
Between pH's	2	80,751.97	40,375.98	196.26	3.00	4.62
Within pH's	(1317)	(270,936.65)	205.72			
Between periods within pH's	21	198,687.41	9,461.31	169.72	1.57	1.87
Within periods within pH's	(1296)	(72,249.24)	55.75			
Between isolates within periods within pH's	240	67,791.24	282.46	66.91	1.18	1.27
Within isolates within periods within pH's	1056	4,458.00	4.22			

Table 12 lists the means with standard deviations and standard errors, and also the criteria of significance for the 11 isolates. Table 13 tabulates the cumulative mean radial mycelial growth of the 11 isolates as they grew on their respective buffered media.

Pathogenicity

— Tuber inoculations.--Yampa, Irish Cobbler, Teton, Russet Burbank and Red McClure potato varieties were used to determine the type and rates of tuber invasion made by the 11 isolates of the fungus under study. The experiments were conducted in a moist chamber with a water-saturated atmosphere at 24° C. (± 1° C.). Tubers of approximately 3-inch diameter were thoroughly washed in running tap water and surface sterilized for 30 minutes by submerging them in 1:1000 mercuric chloride ($HgCl_2$). To remove the disinfectant each tuber was placed under running tap water for not less than 30 minutes. They were then dried at room temperature.

— The inoculum was grown on potato dextrose agar for 14 days prior to inoculation.- Each culture was tested for viability within 24 hours preceding its use, by transfers to fresh potato dextrose agar plates and observing mycelial growth. - They were cut into approximately 5-mm squares immediately before the tubers were inoculated. -

Table 12.--THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE MEAN CUMULATIVE MYCELIAL GROWTH OF 11 ISOLATES OF PHYTOPHTHORA SPECIES DURING 8 SUCCESSIVE 24-HOUR PERIODS.

Hydrogen-ion concentration	Mean mycelial growth								Standard deviation (s)	Standard error ($s_{\bar{x}}$)	Minimum significant difference	
	Periods										at 0.05	at 0.01
	1	2	3	4	5	6	7	8				
	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
pH 5.0	1.945	5.055	7.818	10.382	13.473	16.564	19.127	22.218	1.408	0.630	1.751	2.306
pH 6.0	5.055	12.182	19.291	27.818	34.527	41.855	50.127	58.400	1.930	0.863	2.401	3.162
pH 7.0	4.255	8.691	13.673	18.455	22.255	26.836	31.545	35.509	2.638	1.180	3.282	4.321

Table 13.--THE RELATION OF HYDROGEN-ION CONCENTRATION TO THE CUMULATIVE RADIAL MYCELIAL GROWTH AT 25° C. OF 11 ISOLATES OF PHYTOPHTHORA SPECIES DURING 8 SUCCESSIVE 24-HOUR PERIODS.

pH	Isolate No.	24-hour periods							
		1	2	3	4	5	6	7	8
		mm	mm	mm	mm	mm	mm	mm	mm
4	1	1	2	2	2	3	4	...	...
	23	10	11	12	12	13	15	...	...
	80c	5	7	8	8	9	12	...	...
5	1	1	4	6	8	12	15	17	21
	3	2	4	5	6	7	8	9	10
	8	1	5	9	14	21	26	32	36
	9	2	4	7	8	9	12	13	15
	10	2	4	6	7	8	9	10	12
	15	1	3	4	5	6	7	8	11
	17	2	3	4	7	8	10	12	14
	21	1	3	4	5	6	8	10	12
	23	5	11	17	23	30	37	42	49
	80c	5	10	16	21	26	31	35	40
	81c	1	4	7	10	15	19	21	25
6	1	5	11	18	26	34	40	49	57
	3	5	12	18	25	31	37	45	54
	8	6	15	24	35	44	53	63	73
	9	4	11	18	26	33	40	48	55
	10	5	11	15	22	25	29	36	42
	15	2	5	11	17	22	28	35	43
	17	7	15	24	35	44	52	63	72
	21	6	13	19	28	33	43	49	57
	23	6	15	23	32	38	46	56	64
	80c	5	13	20	30	37	46	55	65
	81c	6	14	22	30	38	45	52	61
7	1	1	6	11	17	20	26	32	37
	3	3	5	9	11	12	14	15	16
	8	8	15	21	28	32	38	43	47
	9	3	7	12	14	17	21	24	27
	10	0	2	4	8	12	17	20	26
	15	1	4	8	12	16	20	24	27
	17	8	14	21	28	34	39	46	50
	21	4	10	17	23	28	34	39	46
	23	7	12	18	22	26	30	34	37
	80c	6	10	16	21	26	31	36	40
	81c	5	9	14	18	22	27	32	36

... Signifies no data

To test the type and rate of fungal invasion, three inoculation loci were employed: Over the eyes, over an area devoid of any visible skin wounds or eyes, and over an artificial skin wound. Each test was in triplicate. The artificial wounds were made by inserting a wooden toothpick about 3 mm into the tuber and removing immediately prior to the inoculation.

Mats of fungal mycelium were inverted and placed over each inoculation locus. Moist, sterile cotton mats, large enough to cover the inoculum mat, were pressed firmly over the inoculum and saturated with sterile tap water. Following each treatment, the potato tubers were placed in the moist chamber for 120 hours.

The tubers were removed from the moist chamber following incubation, and prepared for fungal invasion measurements. In order to measure the infection area, the tubers were quartered; using the center of inoculation as the point of origin, two perpendicular slices were made through the tuber in the direction of the third perpendicular plane. By slicing the tuber in this manner the greatest penetration of the affected tissue was exposed.

Fluorescence of the diseased tubers.--The cut surface of potato tubers, affected with certain diseases, will fluoresce under ultraviolet light (10, 12, 15). Therefore, preliminary studies were initiated to determine whether or not the organism under study would

also cause tissue fluorescence when inoculated into the tuber. With ultraviolet radiation with wave lengths in the vicinity of 3650 A, the affected tissue fluoresced distinctly from the "healthy" tissue, thus making it possible to draw a line separating the two areas with an indelible pencil. This line is referred to as the "front line", and served as the zero value for the invasion tests. As the exposed tissue of tubers in the final test did not show the depth of the affected area immediately, and sometimes not for hours, the tubers were inspected with ultraviolet radiation. Figure 8. The greatest distance from the point of inoculation to the "front line" of any tuber was recorded as the greatest penetration depth of the disease.

Previous work by White (28) indicated that the true distance of the fungous penetration probably was not observed under the ultraviolet light. To find the relationship of the fungus to the fluorescent area, a series of isolations, 5 mm square and 2 mm thick, were made from the front line into the normal tissue. Designating the line between the fluorescent and normal tissue as zero, the cubes of isolations were prepared in a consecutive order as: 0-5, 5-10, 10-15, and 15-20 mm, into tissue perpendicular to the front line. Figure 9.

Triplicate series of cubes from each potato variety were surface sterilized in 1:1000 mercuric chloride for 15 seconds,

Fig. 8.--Healthy and infected potato tubers showing zones of fluorescence and pinkening. A. Healthy tuber. B. Infected tuber. Black area shows the zone in which diseased tubers fluoresce in ultra violet light and turn pink in white light. The diseased zone was blackened with ink for photographing.

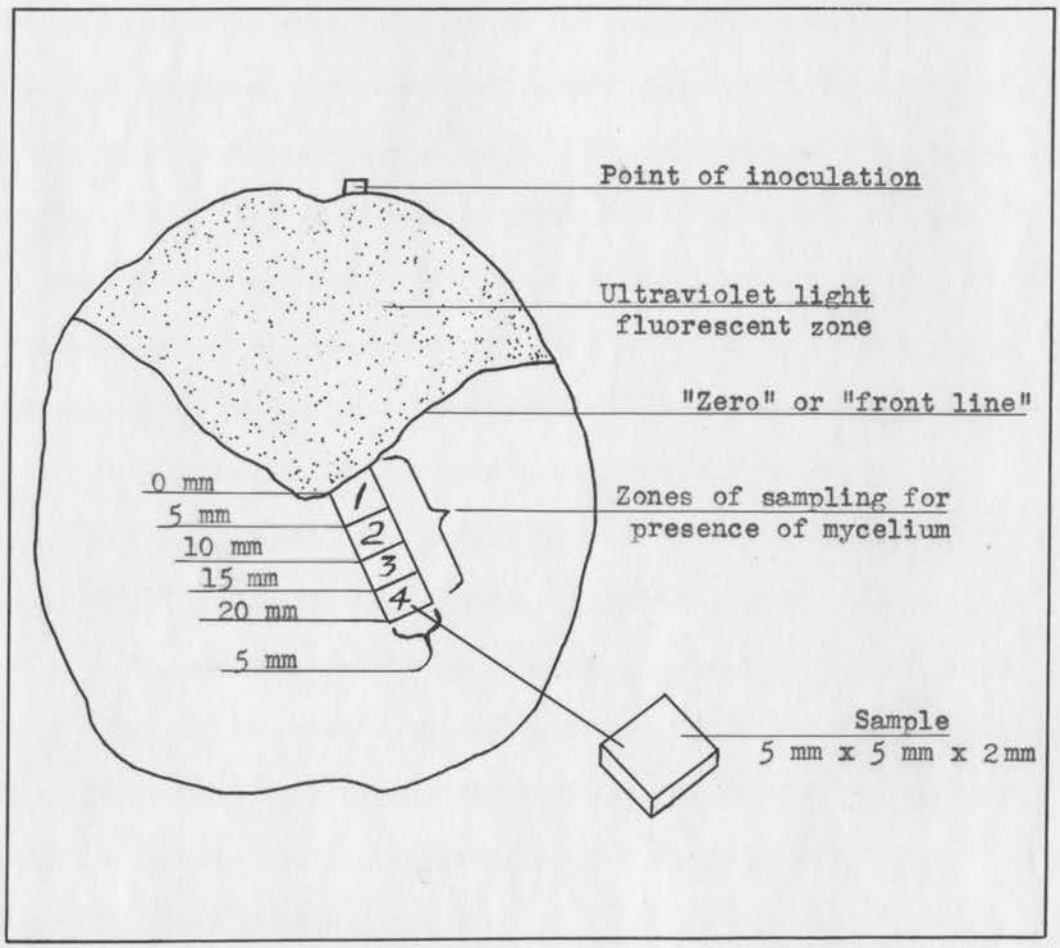

Fig. 9.--Diagramatic cross section of a potato tuber infected with an isolate of Phytophthora illustrating pathogenic sampling technique.

removed and washed immediately in three baths of sterile tap water. After 5 minutes in each water bath, the isolated tissue was placed in cotton-stoppered test tubes which were three-fourths filled with sterile 2-percent dextrose solution. The cultures were incubated at room temperature for 4 to 6 days after which they were examined for the presence of mycelium. When mycelium was observed growing from the cube isolations, small amounts were transferred to fresh potato dextrose agar plates for observation of colony characteristics.

The results of the tuber invasion studies showed that tubers become infected more easily through the eyes and artificial wounds than they do through apparently unbroken skin. Table 14.

Inoculation over "unbroken" skin revealed that isolates 23 and 80c invaded Irish Cobbler; isolates 15, 23 and 81c penetrated Yampa; Red McClure was invaded by isolates 3, 10, and 23; isolates 9 and 81c invaded Russet Burbanks, while none of the isolates penetrated Teton tubers.

When the tubers were inoculated over the eyes, isolates 1 and 9 entered all five varieties of tubers. Each tuber of Teton was invaded by all 11 isolates, and each tuber of Yampa was penetrated by each isolate except 81c. Irish Cobbler was infected by isolates 1, 3, 9, 10, 15, and 80c. Only isolates 8 and 17 failed to enter Red McClure. Isolates 1, 9, and 81c invaded Russet Burbank tubers.

Table 14.--PATHOGENICITY TEST OF 11 ISOLATES OF PHYTOPHTHORA SPECIES ON IRISH COBBLER, TETON, RED McCLURE, YAMPA, AND RUSSET BURBANK POTATO TUBERS.

Potato variety	Point of inoculation	Depth of fluorescence of the host tissue below the point of inoculation										
		Isolate No.										
		1	3	8	9	10	15	17	21	23	80c	81c
		mm	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
Irish Cobbler	Over "unbroken" skin	0	0	0	0	0	0	0	0	9	22	0
Teton		0	0	0	0	0	0	0	0	0	0	0
Red McClure		0	24	0	0	27	0	0	0	9	0	0
Yampa		0	0	0	0	0	8	0	0	11	0	7
Russet Burbank		0	0	0	17	0	0	0	0	0	0	22
Irish Cobbler	Over eyes	5	29	0	20	32	37	0	0	0	15	0
Teton		19	32	25	35	19	29	10	27	47	30	50
Red McClure		20	21	0	20	20	15	0	33	32	34	17
Yampa		15	24	37	37	27	32	27	16	10	20	0
Russet Burbank		32	0	0	46	0	0	0	0	0	0	6
Irish Cobbler	Over skin wound	27	37	13	31	23	32	22	17	47	37	35
Teton		0	36	30	0	33	39	0	0	35	0	0
Red McClure		20	50	43	38	31	36	37	36	34	37	0
Yampa		25	16	51	22	32	17	35	32	21	0	25
Russet Burbank		0	23	18	0	35	35	13	0	6	0	25

All 11 isolates entered Irish Cobbler through an artificial wound, while all isolates but number 81c infected Red McClure. Isolate 80c was the only isolate which did not enter Yampa tubers. Isolates 3, 8, 10, 15, and 23 invaded Teton, but isolates 1, 9, 21, and 80c did not invade Russet Burbank.

The results of isolations from the "healthy" nonfluorescent area of the affected tubers are shown in Table 15. As the mycelium was observed growing from the isolations, transfers were made which yielded colony growth characters similar to those of the original inoculum. Each isolate was obtained from Irish Cobbler, Teton, Red McClure and Yampa tubers, 5 mm from the fluorescent area. Mycelial growth was obtained for each isolation made between 5 to 10 mm from the front line in Teton tubers. No mycelium of isolates 1, 10, 17, 21, 80c, and 81c was obtained from the isolations made between 5 to 10 mm in the healthy tissue in the Irish Cobbler tubers, nor isolates 9, 21, 80c, and 81c in Red McClures. Isolations from 5 to 10 mm in Yampa did not yield mycelium of isolates 1, 15, 17, and 23.

Isolations made from 10 to 15 mm into the nonfluorescent region produced mycelium of isolates 8, 17, 23, and 80c in Tetons. Yampas yielded fungal growth for isolate 8; Red McClure isolations also produced fungal growth for isolate 17, while no mycelium was obtained from Irish Cobblers at this location.

Table 15.--MYCELIAL PENETRATION OF 11 ISOLATES OF PHYTOPHTHORA SPECIES COMPARED TO THE DEPTH OF FLUORESCENCE OF TUBER TISSUE IN 4 POTATO VARIETIES.

Potato variety	Sampling zones	Isolate Numbers										
		1	3	8	9	10	15	17	21	23	80c	81c
		Production of mycelium										
Irish Cobbler	(1) 0 - 5 mm	+	+	+	+	+	+	+	+	+	+	+
Teton		+	+	+	+	+	+	+	+	+	+	+
Red McClure		+	+	+	+	+	+	+	+	+	+	+
Yampa		+	+	+	+	+	+	+	+	+	+	+
Irish Cobbler	(2) 5 - 10 mm	-	+	+	+	-	+	-	-	+	-	-
Teton		+	+	+	+	+	+	+	+	+	+	+
Red McClure		+	+	+	-	+	+	+	-	+	-	-
Yampa		-	+	+	+	+	-	-	+	-	+	+
Irish Cobbler	(3) 10 - 15 mm	-	-	-	-	-	-	-	-	-	-	-
Teton		-	-	+	-	-	-	+	-	+	+	-
Red McClure		-	-	-	-	-	-	+	-	-	-	-
Yampa		-	-	+	-	-	-	-	-	-	-	-
Irish Cobbler	(4) 15 - 20 mm	-	-	-	-	-	-	-	-	-	-	-
Teton		-	-	-	-	-	-	-	-	-	-	-
Red McClure		-	-	-	-	-	-	-	-	-	-	-
Yampa		-	-	-	-	-	-	-	-	-	-	-

Mycelium was not obtained from any isolations made in the healthy tissue between 15 to 20 mm from the front line.

Russet Burbank tubers demonstrated the greatest resistance to fungal invasion in the inoculation tests over the eyes and artificial wounds. However, with inoculations on "unbroken" skin the indication was that Russet Burbank and Cobbler ranked second in resistance while Teton was completely unaffected by the organisms.

When isolations were made from the nonfluorescent area in the tubers, it was apparent that the pathogen invaded the tissue in advance of visible symptoms. This study proved that host and pathogen relationships vary between the different isolates of the pathogen as well as between host varieties.

Pinkening of the cut surfaces of diseased tubers.-- Pethybridge (22), and others (1, 8, 13), have reported that exposing the diseased areas of potato tubers affected with a leak-type disease will cause a pinkening of the tissue. Figure 10.

Irish Cobbler, Russet Burbank, Red McClure, and Yampa tubers were inoculated with 11 isolates to determine whether these varieties would turn pink when infected by the test "leak" isolates.

The tubers were prepared as for the invasion studies. A small "V"-shaped cut, about 5 mm deep, was made into duplicate series of tubers, in which inoculum from each isolate was placed after which the cut piece was replaced. After inoculation the

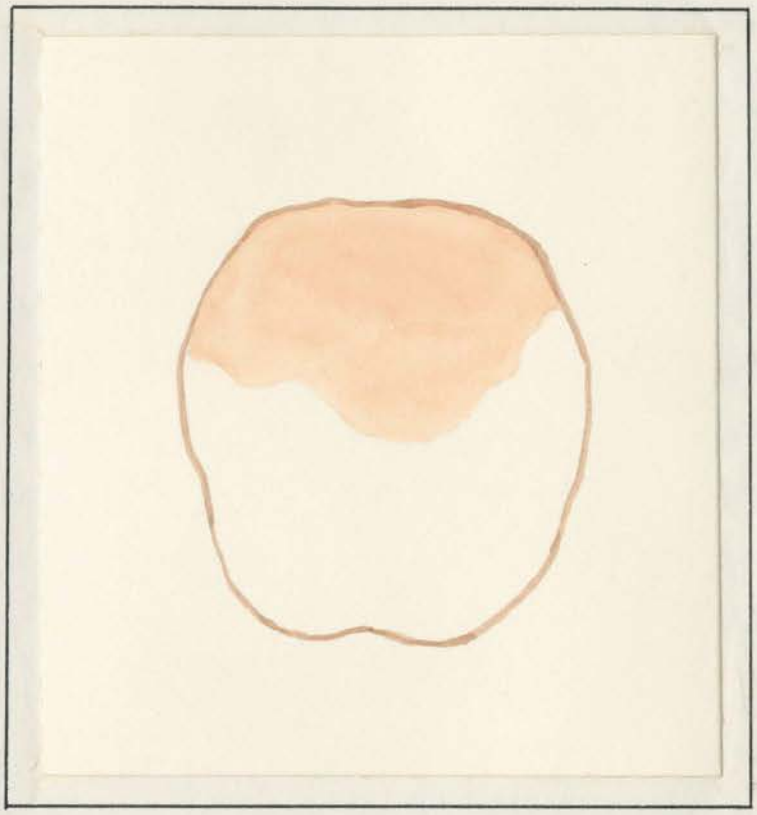

Fig. 10.--Sketch of a "Western Leak" infected tuber, in water color, showing diagnostic pinkness when diseased tissue is cut and exposed to air.

tubers were placed in the moist chamber. Five days after inoculation the tubers were removed from the moist chamber and cut to expose the affected surfaces. Observations were made after the tubers were exposed for 30 minutes and 2 hours.

Cut potato tubers affected with 11 isolates of the "leak" fungus, exhibited pink discolored tissue when exposed to the air but the time required varied. Table 16. Irish Cobbler and Yampa developed the pink color with all the isolates within 30 minutes. Russet Burbank and Red McClure showed pink tissue for each isolate within 2 hours, but not in 30 minutes. From these results, it was evident that potato varieties react differently between varieties within isolates and between isolates within varieties.

Leaf and stem inoculation.--The aerial symptoms of the disease causing tuber leak have been discussed to great lengths by Colorado potato growers, and varied opinions have resulted. Therefore, an experiment was conducted to determine whether aerial symptoms would be expressed by plants inoculated with the organisms being studied in this investigation.

Twenty-eight Irish Cobbler potato plants were grown from surface-sterilized tubers in sterilized pots of soil in the greenhouse between 10° and 20° C. While young (about 12 to 14 inches tall), the potato plants were inoculated with inoculum which had

Table 16.--COMPARATIVE RATES OF PINKENING OF CUT TUBER SURFACES IN 4 POTATO VARIETIES INOCULATED WITH 11 ISOLATES OF PHYTOPHTHORA SPECIES.

Isolate No.	Irish Cobbler		Russet Burbank		Red McClure		Yampa	
	30 min.	2 hrs.	30 min.	2 hrs.	30 min.	2 hrs.	30 min.	2 hrs.
1	+[1]	+	T[2]	+	0[3]	+	+	+
3	+	+	T	+	T	+	+	+
8	+	+	T	+	0	+	+	+
9	+	+	T	+	0	+	+	+
10	+	+	+	+	T	+	+	+
15	+	+	T	+	T	+	+	+
17	+	+	T	+	+	+	+	+
21	+	+	T	+	0	+	+	+
23	+	+	T	+	0	+	+	+
80c	+	+	T	+	0	+	+	+
81c	+	+	T	+	0	+	+	+

[1] + = Pinkness in well defined area.
[2] T = Trace of pinkness not well defined.
[3] 0 = No pinkness.

been growing on potato dextrose agar for 14 days. The cultures were cut into 5-mm squares immediately prior to administration to the plant parts. The stems and leaflets were inoculated by inverting and placing a mycelial mat in direct contact with the plant tissue and covering the mat with enough saturated, sterile cotton to cover it. The inoculum on leaflets was placed as near the center of the tip leaflets as possible. The inoculation of the stem was in the axil of the leaf. After inoculation, the entire stem was inclosed within a cellophane bag for 4 days. Not less than five leaves and five stems were inoculated per isolate in the test.

The experiment was arranged so that one pot of plants was designated to test one isolate, thus making it possible to test 11 isolates while using 11 pots for host plants and 2 pots of plants as controls. A second set of controls was set up for each pot to determine whether or not the moist cotton on the leaflets and stems would cause aerial symptoms which might be confused with those produced by the inoculum.

Isolations were made from diseased tissue to check the presence of the test fungus. This was done by selecting portions of the stem, petiole, and leaf that showed both healthy and diseased tissue. The tissue was submerged into 1:1000 mercuric chloride for 15 seconds, removed, and then washed in three baths of sterile water

for 5 minutes each. The tissue was then deeply submerged into cotton-stoppered test tubes containing a sterile 2-percent dextrose solution. When mycelium was observed growing from the infected tissue, it was transferred to dextrose agar and potato dextrose agar plates.

The results of the leaf and stem inoculations showed that aerial symptoms could be induced artificially under greenhouse conditions. All of the leaflets and stems that had been inoculated became infected to some degree, the necrotic areas ranging in size from minute, black pin points to large brown and black lesions. There were no symptoms that were consistent in or between individual isolates. The plants were not recovered with cellophane bags, but were allowed to grow under normal greenhouse conditions. On the fifth day after inoculations, and on each day thereafter, the lesions on the leaves increased to larger dark green patches surrounded by a darker green margin with borders running into the normal green of a healthy leaflet. Some leaves became dry with a green sheen. Often the discoloration migrated from the tip leaflet down the rachis and to other leaflets. Figure 11. The black discoloration migrated faster in the vascular region than in other tissues of the rachis. In extreme cases, the entire leaf and a small area below the leaf scar were affected by the migration. As the disease moved along the rachis,

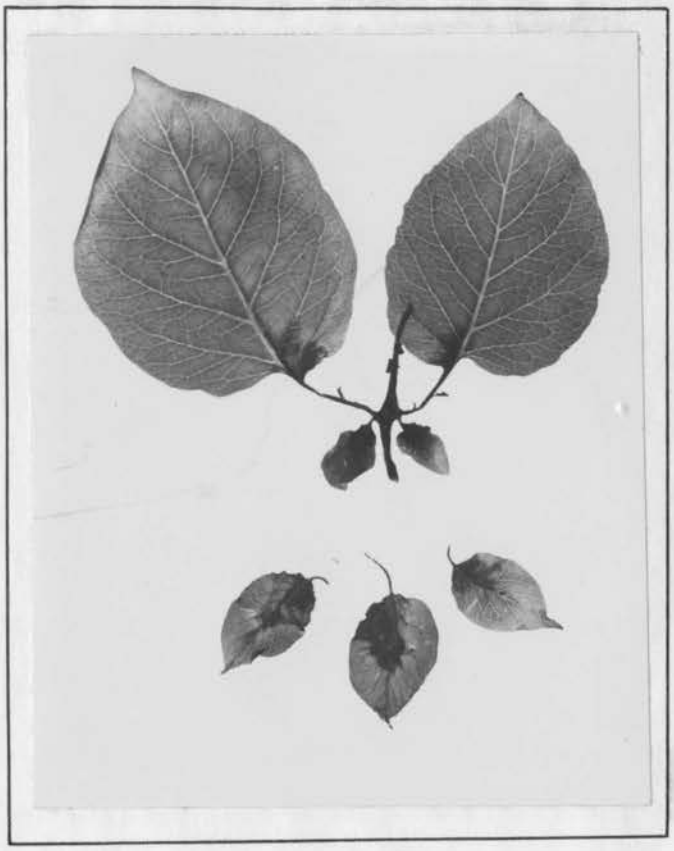

Fig. 11.--Potato leaflets infected with "Western Leak" showing dark discolored areas symptomatic of the disease.

three distinct regions were generally observed. These regions were composed of tissue which was normal and not yet affected, tissue having a glossy, water-soaked appearance, graduating into a tissue which was hard, dry, tough and black. On reaching the main stem, the symptoms would stop, in most cases, and the leaf would dry and fall from the plant. When the discoloration did enter the main stem, it was concentrated in a very small area immediately below the leaf scar and about as wide as the scar. A slightly sunken, black, dry canker formed just below the leaf scar which was never more than three-fourths inch long. In this condition it persisted.

The results of the stem inoculations indicated that the main stem was more resistant to fungal invasion than were the leaves. Only black pin point lesions, which never enlarged, could be observed on the stem; the leaf blade became infected in every case. The symptoms on the rachis were the same as those described as the result of leaflet infection except that the entire leaf quickly wilted and usually dropped from the plant when the petiole became infected near the axil.

The results of the isolations made from the affected plant parts produced mycelium in 247 cultures in 260 trials. Negative isolations were most numerous from leaflets. When the mycelium was cultured on potato dextrose agar, the growth characteristics were the

same as those of the original isolate except in two cases in which species of Fusarium were cultured. Microscopic examination of the surface of affected tissue failed to disclose mycelium or fructifications.

In a greenhouse where the temperature ranged between 10° and 20° C., aerial symptoms were produced artificially on growing potato plants. Although the main stem of the host plant was not much affected by the fungus, the leaves of the plants were often completely killed.

Soil inoculations.--Since the stem and leaf inoculation tests apparently affected only the stem and leaves inoculated, it was possible to use the same plants in additional tests. This time the soil around each plant was inoculated with the same respective isolates that had been used to inoculate the aerial parts. Before the soil was inoculated, the potato plants were allowed to grow in the greenhouse for 2 weeks following the completion of tests for aerial symptoms. After this elapsed time, no additional aerial symptoms were observed.

The inoculum was from isolates that had been cultured on corn meal agar for 3 weeks. Without additional handling the corn meal agar was mixed into the top 2 inches of soil around the plants. The culture and 5 tablespoons of soil were mixed in a morter with

a spoon and then mixed with the soil in the pot. This procedure was carried out for each individual pot except the two controls which were cultivated for a depth of about 2 inches. After the inoculations the pots were watered daily and the plants allowed to grow normally under greenhouse conditions between 10° and 20° C.

The presence of the fungus in the underground parts of the diseased plants was checked by the same means as was used for the aerial portions.

The results 2 and 4 weeks after inoculation showed that all of the organisms would attack the stem of the potato plant when grown in the greenhouse. The symptoms expressed by the plants were first observed near the ground level. Figure 12. The stems of the plants began to darken and gradually became black. This discoloration extended up the stem only slightly. However, following the initial stem symptoms, chlorosis of the entire plant soon followed. The pathogen also was noticed to attack some of the roots and stolons, causing them to become water-soaked, dark brown, and often mushy.

As tubers had not begun to form from any stolons observed, it could not be discerned that young tubers were attacked by this fungus.

Isolations from under-ground parts of stems, roots, and stolons repeatedly yielded a fungus which produced the same growth

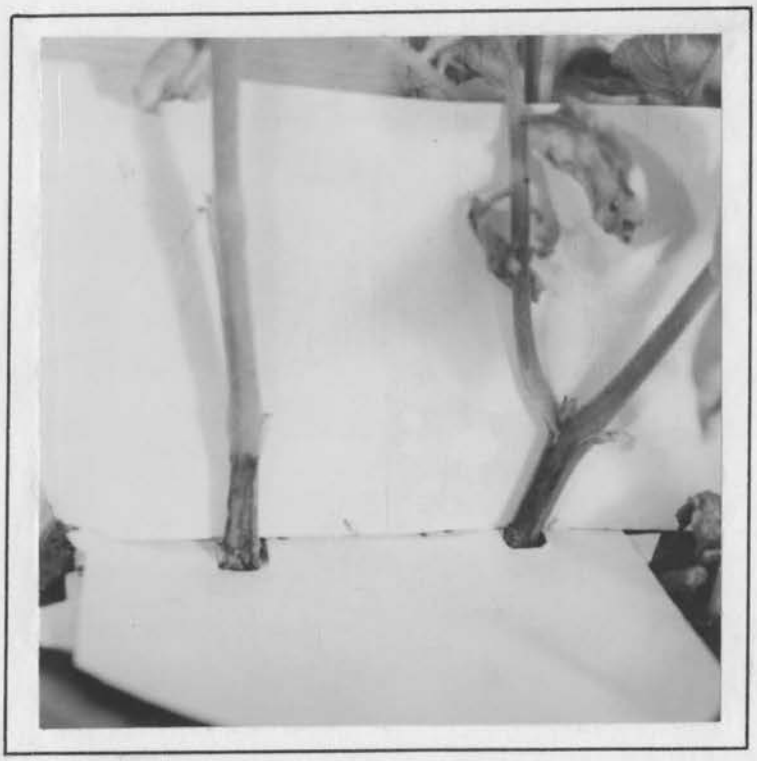

Fig. 12.--Basal stem symptoms of potato plants infected with "Western Leak".

characteristics, on potato dextrose agar, as did the isolate providing the inoculum.

In the greenhouse, symptoms in potato plants could be induced by the 11 isolates when there was a high concentration of the pathogen in the soil.

Later soil inoculation tests, conducted similarly to the earlier tests, also yielded top symptoms. Figure 13. This later test showed that the fungus entered the stem of the plants in 8 days. Within 2 or 3 days after the stem symptoms appeared, a quick wilt resulted. The leaflets rotted and wilted without losing much natural color. A few of the leaflets became yellow around the margin. On the fifth day, following the first symptoms, the plants appeared to be dead from the lack of water. First symptoms were noticed on the stems at the soil line. Brownish-black discolorations extended from this level up the plant about 1 inch and the lesions seldom migrated more than 2 inches. In 5 days the stems showed atypical symptoms of black leg, the disease caused by Erwinia carotovorus Jones. The leak infection differed from black-leg infection in that the discoloration was not as dark and lacked the characteristic sheen of black leg.

Fig. 13.--Aerial symptoms of "Western Leak".

Summary of data presented

In the study about the causal agent of "Western Leak" of potatoes in Colorado, data have been presented in three phases: Morphological studies, physiologic reactions, and pathogenicity tests.

The morphological studies presented data on sexual and asexual sporulation, sexual and asexual spore measurements, genetic studies, substrata observations and cultural characteristics.

The physiologic reactions presented data on temperature and growth relations, and relations of the hydrogen-ion concentration of media to mycelial growth.

The pathogenicity tests presented data on tuber inoculations, fluorescence and pinkening of diseased tubers, leaf and stem inoculations, and soil inoculations.

Location of original data

The original data on which this study is based are filed in the office of the Botany and Plant Pathology Department, Colorado A & M College, Fort Collins, Colorado.

Chapter V

DISCUSSION

In 1941, a field and storage wet-rot of potatoes appearing in Colorado was found to be caused by a Phytophthora species of soil fungi. Because of the close resemblance to a disease occurring in the eastern United States caused by Pythium debaryanum and known as leak, Kreutzer and Lane (16) called the Phytophthora disease "Western Leak".

A more detailed description of the causal agent, symptoms, and factors of growth of "Western Leak" than that presented by Kreutzer and Lane is reported herein.

Causal agent.--Previously, it was stated that mycelium of the young fungus was coencytic and often became septate only in older cultures. This would class the organism as a Phycomycete. Additional inspection showed that the organism produced oogonia and the amphigynous type of antheridia. According to Middleton (18):

> The amphigynous type of antheridium predominant in the genus Phytophthora is unique and not encountered in the genus Pythium; that the antheridium is a component part of the sexual phase can be considered a valid criterion for separation. (18:19)

This then, would automatically support the report by Kreutzer and Lane (16) that the organism causing "Western Leak" was a species of Phytophthora. Since the test fungus was coenocytic and eight isolates employed in this investigation possessed the amphigynous type of antheridium, it was reasonable to believe that the test organism was a species of Phytophthora. In Tucker's Table 1 (27), the following species of Phytophthora are listed as having the potato as its host: P. erythroseptica, P. parasitica, P. drechsleri and P. infestans. Leonian (17) also listed P. erythroseptica and P. drechsleri as having the potato as its host. By using these four species of Phytophthora as criteria, the isolates studied herein are compared with the cultural characteristics of this group.

Since all the cultures investigated in this paper grew profusely on potato dextrose agar and P. infestans did not, it was eliminated.

Tucker (27) and Rosenbaum (25) reported sporangia in cultures of P. parasitica on oatmeal agar. They also reported that this species might be distinguished by its ability to yield papillate sporangia and chlamydospores on solid media. When solid media cultures were inspected, in this study, asexual sporulation was rarely observed. Nonpapillate sporangia were rare, while no chlamydospores were seen.

Tucker (27) reported that P. drechsleri was morphologically similar to P. erythroseptica. However, he stated that P. erythroseptica may be separated from P. drechsleri by its large oogonia and oospores. Rosenbaum (25), in his comparative studies of oospore measurements, also showed that P. erythroseptica oospores were larger than other species of Phytophthora. Tucker (27) gave the following measurements of oogonia and oospores on oatmeal cultures: oogonia and oospores respectively; P. erythroseptica, 36.3μ and 31.4μ; P. parasitica, 26.4μ and 22.8μ; P. drechsleri, 31.3μ and 25.6μ. Rosenbaum (25) reported that the greatest number of oospores measured for P. erythroseptica were concentrated in classes between 33.5μ to 39.49μ. The mean for all the oospore measurements was 35.78 ± .127. The standard deviation was 3.77 ± .090.

The mean oogonia and oospore measurements in this investigation fell between 32.323μ and 43.617μ for the oogonia, and 23.197μ and 36.004μ for the oospores. The standard deviation for the oospores was 4.1262 . It would appear from these results that the sexual structures of many of the test isolates have mean diameters close to those reported for P. erythroseptica. Isolates 8 and 9, whose mean oospore diameters differ most from those reported for P. erythroseptica correspond more closely in this characteristic with P. drechsleri.

Since the organism P. debaryanum, which has been named as the pathogen causing potato leak (2, 11, 23), was not included in the studies reported here, diagnostic differences between it and our isolates of Phytophthora cannot be pointed out at this time. A more extensive and parallel study of the two fungi might lead to a better understanding of both organisms and their symptoms. As Pethybridge (22) pointed out, potato tubers affected with P. erythroseptica yielded a watery exudate. The liquid did not evaporate rapidly if the tubers were enclosed. Thus a "wet-rot" of the tubers was the result. This might be confused with the leak-type symptoms of P. debaryanum. Since both P. debaryanum and P. erythroseptica caused a leak-type disease, it was understandable why Kreutzer and Lane (16) chose to call the Phytophthora wet-rot, "Western Leak".

Symptoms.--In previous studies by White (28), it was shown that a potato tuber partly invaded with P. erythroseptica had three distinct zones: First, of invaded dead tissue which became pink when exposed to air; second, living tissue where the organism was a true parasite; and third, a zone of healthy, uninvaded tissue.

The investigations here showed that tuber tissue inoculated with the test isolates did not always show visible symptoms of the disease under white light. It was for this reason that ultraviolet

light was used. Under ultraviolet light the affected tuber tissue fluoresced clearly, thus making it possible to isolate from the "healthy", nonfluorescent zone. The 2-percent dextrose solution, used for culturing the nonfluorescent tissue, proved to be an excellent medium for growing the fungus contained within the isolated tissue.

In the apparently healthy tissue, between 0-5 mm, the fungus was obtained from all the affected tubers. These results were in harmony with White's report (28).

Since four tuber varieties were used in this study, it was desirable to know whether different test isolates caused different reactions in the tuber tissue. Therefore, further isolations were made in zones between 5-10, 10-15, and 15-20 mm away from the fluorescent tissue. It was shown that differences were present between isolates within varieties, and between varieties within isolates. Through these comparisons it was shown that a pathogenic fungus could move to various distances into tubers without showing disease symptoms. The variation of penetration exhibited by the different isolates among the potato varieties tested, indicated that the isolates were not physiologically alike, and that host reactions to the isolates were different.

Goss (9) reported that Bliss Triumph tubers infected with P. erythroseptica and P. drechsleri turned pink in 20 to 30 minutes after the tubers were cut and exposed to the air. Tubers that he inoculated with P. debaryanum showed less rot, were slightly darker -- almost brown; and the affected tissue did not turn pink when exposed to the air.

The results reported here showed that the Irish Cobbler and Yampa tubers infected with any one of the 11 test isolates turned pink in 30 minutes. Two other varieties, Russet Burbank and Red McClure, did not produce pinkness within 30 minutes, for all the 11 test isolates. This difference in varietal response suggests a need for naming the potato variety when reporting pinkening.

It has been demonstrated that potato plants grown in the greenhouse will produce aerial symptoms from leaf, stem and soil inoculations. It was suggested that further study should be conducted along these lines. When the soil was inoculated around the growing plant, top symptoms were very striking. The symptoms resembled those of black leg, but the discoloration was not as dark and lacked the characteristic sheen of black leg. Isolations from these plants yielded the original fungus. However, as reported by Goss (9) these plant symptoms could be easily confused with the bacterial disease.

Factors of growth.--The temperature and growth relation studies of the 11 isolates on dextrose agar, showed the minimum temperature for growth was near 5° C.; the optimum temperature was between 20° and 25° C.; and the maximum temperature for some of the isolates was between 30° and 35° C., and between 35° and 40° C. for others. No growth was observed at 40° C.

The production of asexual spores in a liquid medium and none on a solid medium, indicates that the fungus might reproduce asexually more readily in vivo, in wet surroundings. The ability of the fungus to produce asexual bodies when soil was an ingredient in the liquid culture and when Knop's solution was used, indicates that the natural substances in the soil plus a liquid medium stimulates asexual reproduction. The fungus, therefore, would be expected to be more active in wet soils than in well-drained and aeriated soils.

Suggestions for further study

1. Are tubers that are bruised and wounded at harvest time more susceptible to the "Western Leak" disease than are other tubers?

2. Pathogenicity tests on other potato varieties should be made to obtain more information on host range.

3. What effect would oxygen and carbon dioxide in varying concentrations have on the breakdown of tubers infected with the "Western Leak" isolates?

4. What effects would varied proportions of carbon and nitrogen have on the growth of the "Western Leak" fungus in culture media?

5. Would different vitamin media stimulate sexual sporulation in a greater number of the 11 isolates?

Chapter VI

SUMMARY

Investigations made in this study were concentrated under morphologic, physiologic, and pathogenicity studies of 11 pure hyphal tip isolates of Phytophthora species causing potato leak.

1. Oogonia and antheridia were produced in cultures by employing various temperatures, media and age of cultures. Size of oospores and oogonia showed variability between isolates.

2. Asexual spores were found to sporulate profusely in liquid media, with variability noticeable between isolates.

3. Crossing of isolates did not stimulate production of sexual bodies.

4. The test isolates could be grouped by the color and fluorescence of their substrata.

5. Cultural characteristics included a description of the mycelium, sexual bodies, and asexual bodies.

6. At controlled temperatures on dextrose agar the optimum and maximum temperatures were obtained for the test isolates. The minimum temperatures were not obtained. Differences in growth between isolates was significant within periods.

7. Variation between isolates within pH and variation between pH within isolates showed differences between isolates and differences between hydrogen-ion concentrations.

8. Tubers were more easily infected through eyes and wounds than through "unbroken" skin.

9. Affected areas of inoculated tubers could be measured and observed in ultraviolet light.

10. Isolations made from apparently healthy tissue adjacent to the visibly affected areas of tuber tissue yielded the pathogen.

11. The cut surfaces of affected tubers became pink but the time required differed between potato varieties. Symptoms of both leak and pink rot were produced by the test isolates.

12. In all tuber inoculation tests, differential pathogenicity between isolates was observed.

13. Leaf and stem inoculations showed that aerial symptoms could be produced under greenhouse conditions.

14. Soil inoculations indicated that stem and underground parts are invaded and may cause death to the host.

15. On the basis of morphological studies the test isolates more nearly resembled Phytophthora erythroseptica and Phytophthora drechleri than any other described species.

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