INVESTIGATIONS ON BACTERIAL RINGROT OF POTATOES CAUSED BY PHYTOMONAS SEPEDONICA (SPIECKERMANN AND KOTTHOFF) BERGEY ET AL

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INVESTIGATIONS ON BACTERIAL RINGROT OF POTATOES CAUSED BY PHYTOMONAS SEPEDONICA (SPIECKERMANN AND KOTTHOFF) BERGEY ET AL

by

JOSEPH ANTHONY RUPERT

A THESIS

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THESIS

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NOTE

Due to the writer's eligibility under the Selective Service Act of 1940 these studies were cut short. Consequently the material herein prepared represents incomplete data.

Contents

INTRODUCTION

The potato ranks sixth among the crops grown in the United States as determined by acreage and value of the crop produced (1). If it is considered on the basis of a table food product, it ranks second only to wheat.

In Michigan the potato crop ranks fourth in total value being exceeded only by corn, hay, and oats as determined by the 1930-1939 average. During this ten-year period the average annual yield for the state was 25,778,000 bushels with an average annual income of $14,789,000 (2). As a cash crop in the state, the potato is second to none (3).

A serious new disease of the potato was reported in this country in 1937 (4). Recently this malady, bacterial ringrot of potato, has spread into Michigan and is causing grave concern among the growers. The swiftness with which this disease has spread to all potato growing states is unique in the annals of potato diseases.

Name of the Disease

Savile and Racicot (5) first suggested the name "Bacterial Wilt and Rot". Bonde (6) proposed "Bacterial

Wilt and Soft Rot". This led to some confusion as "bacterial wilt is the common name associated with a disease of potatoes caused by Phytomonas solanacearum while "soft rot" is associated with the organism Erwinia carotovora. Hence the Committee to Coordinate Research on New and Unusual Potato Diseases recommended that the name "Bacterial Ringrot" or "Ringrot", a direct translation of the German "ringfäule", be adopted (7).

History and Distribution

Spieckermann of Germany was the first to report bacterial ringrot of potatoes in 1906 (8). In this country E. F. Smith received some affected tubers from Massachusetts in 1918 which he believed to be related to Spieckermann's ringrot disease (9). His bacterial isolates yielded no definite results on inoculations into the potato and tomato. His excellent photographs of these tubers show no close resemblance to bacterial ringrot today. In conclusion he states, "When the plates were made, I believed the disease due, probably, to bacteria, but now I have no definite opinion as to its cause." The name of "net necrosis" was given this disease.

In 1931 Conners (10) reported the presence of ringrot in Canada where it has since become widely distributed.

The first report of the disease in this country was made by Bonde of Maine in 1937 (4) although he states that he had observed the disease as early as 1932. Burkholder (11) in 1938 established the identity of the causal agent of potato ringrot as Phytomonas sepedonica (Spieckermann and Kotthoff) Bergey et al.

In Michigan the disease was first reported in 1939 by Muncie (12). However, the first serious outbreak occurred on the Knoblauch farm near Blissfield in the summer of 1940. When this field of Chippewas was examined in July, approximately 40% of the hills had failed to emerge. At digging time this plot yielded 80 bushels per acre; this same grower's adjacent field of Cobblers produced 240 bushels per acre.

In September of the same year a severe infection was found on the Schoenfeld Brothers' farm near Imlay City. A careful checkup by random sampling in the field revealed that 64% of the hills were diseased. This 20 acre field of Chippewas was grown on poorly-drained muck soil. The disease here was much more severe than on the well-drained sandy loam of the Knoblauch farm.

Both of these 1940 outbreaks were in fields grown for table stock planted to Maine select Chippewa seed stock. In 1941 in Oakland County another lot of the same

kind of seed stock showed ringrot.

Aside from these two cases no other destructive outbreaks have been observed. Several mild instances of the disease were found. In all cases these growers have agreed to dispose of their present seed stock and to obtain a fresh supply of reliable seed.

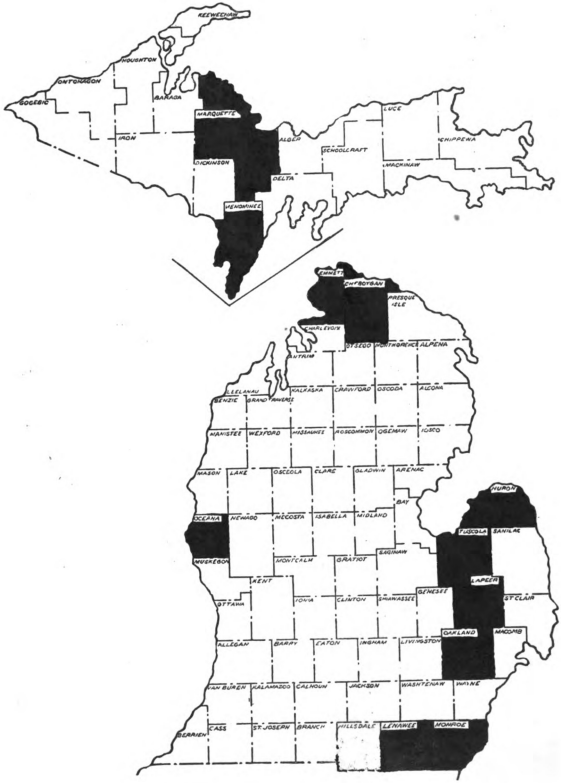
At present the disease has been found either in the field, in storage, or in seed stock in eleven widely scattered counties in the state as follows: (see map)

Lapeer
Lenawee
Monroe
Oakland
Tuscola
Oceana
Emmet
Menominee
Cheyboygan
Marquette

Economic Importance

Losses from ringrot may vary from a trace to almost total loss. Growers of Maine (6) and Florida (13) have suffered heavy losses. In Michigan losses have been small to date but the importance of the disease cannot be judged alone from the losses that have been sustained. This disease constitutes a potential threat to the increasingly effective potato certification program that has been built up by decades of untiring effort and to the to the produc-

DISTRIBUTION OF BACTERIAL RINGROT IN MICHIGAN
MAY 1941



tion of quality table stock potatoes. The certification program has taken on an added significance since the appearance of ringrot and is playing a decisive role in the fight to control the disease.

Hosts

All commercial varieties of the Irish potato, Solanum tuberosum, are susceptible to ringrot infection (14) and (15). In addition the tomato, Lycopersicum esculentum, is susceptible upon artificial inoculation and shows typical ringrot symptoms of the foliage (5).

The following plants were tested in the greenhouse for susceptibility to the ringrot organism:

1. Black nightshade (Solanum nigrum)
2. Jimson weed (Datura stramonium)
3. Wild tobacco (Nicotiana rustica)
4. Sticky tobacco (Nicotiana glutinosa)
5. Red pepper (Capsicum annuum)
6. Egg plant (Solanum melongena)
7. Pyrathorn solanum (Solanum pyracanthus)
8. Tomato (Lycopersicum esculentum)

With the exception of tomato, no definite infection developed (Fig. 1).

Symptoms

Foliage

The first symptom to appear, aside from the general unthriftiness of the plant, is a slight inward rolling

Fig. 1A. John Baer tomato plant showing wilting of lower leaves 32 days after inoculation at base of stem with inoculum from ringrot infected potato. Healthy plant on the right.

Fig. 1B. Same plant as in Fig. 1A (left) 59 days after inoculation.

of the leaves somewhat resembling the leaf-roll virous disease at first glance. The rolled leaves are soft and pliable in contrast to the leathery and brittle leaves typical of leaf-roll. Partial wilting follows during the hottest part of the day, the plant recovering during the night. The leaves become chlorotic and marginal necrosis develops followed by death of the shoot (Fig. 2). On splitting the diseased stem, no characteristics of diagnostic value can be seen; bacterial ooze is absent and the tissues appear normal in color in contrast to the typical brown vascular discoloration of Fusarium Wilt or the black discoloration of blackleg. Plants infected with blackleg may be further distinguished from ringrot infected plants by their characteristic black basal stem rot. This is entirely absent in ringrot infected plants.

It has been observed that in some instances the plant infected with ringrot does not wilt, although upon microscopic examination of a stem smear, a rich abundance of the causative bacteria is found. Moreover, not infrequently one shoot may become severely diseased while the remainder of the plant grows normally to maturity.

The field symptoms are variable and come late in the season when late and early blights and insect in-

Fig. 2A. A naturally infected Chippewa tuber (Schoenfeld stock) planted in the greenhouse showing wilting typical of ringrot.

Fig. 2B. Chippewa potato plant showing wilt symptoms 47 days after stem inoculation using a pure culture of Phytomonas sepedonica. Control plant on the left.

juries may be prevalent and when the vines begin to show signs of maturity. Fusarium wilt and blackleg also may cause confusion of diagnosis. Consequently this masking of the true symptoms makes field detection of ringrot extremely difficult

Tuber

On digging an infected plant, usually all stages of decay are present in the tubers - from complete decomposition to apparent soundness (Figs.3,4,& 5). In the early stages only the vascular tissue may be affected by a soft, yellow creamy, or more often, crumbly rot. Upon clipping and squeezing the stolon end of such a tuber, the decaying but odorless vascular tissue crumbles, showing the consistency of a cooked potato. At the same time pressure separates the cortex from the vascular ring.

When secondary soft-rotting bacteria gain entrance, the whole tuber rapidly disintegrates into a foul-smelling, slimy mass (Fig. 6). Numerous less destructive secondary invaders such as the Fusaria and saprophytic bacteria, enter the ringrotted tuber in the later stages, more or less obliterating any typical ringrot symptoms (Fig.5). At times the vascular decay spreads to the center of the tuber, leaving only the outer shell, a portion of which may be decayed. This is particularly true of potatoes in storage.

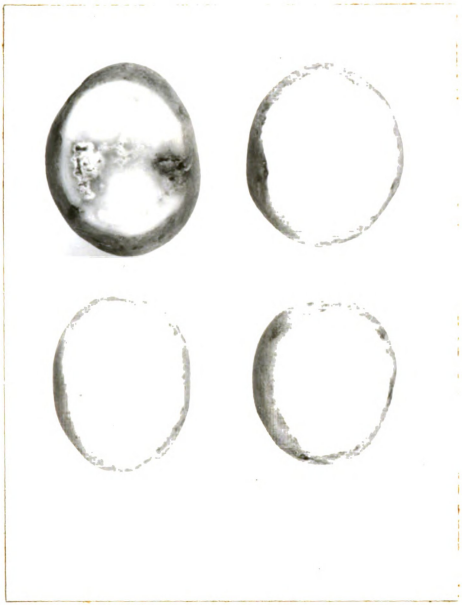


Fig. 3. Cut tubers showing light infection of the vascular tissue. Such lightly infected tubers easily escape detection by visual examination.

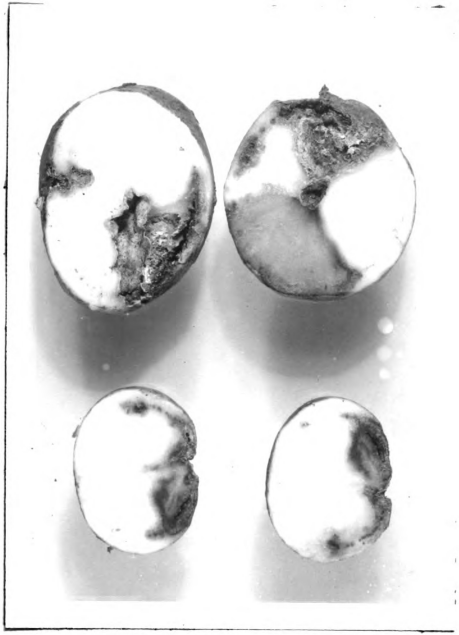


Fig. 4. Advanced decay following ringrot infection.

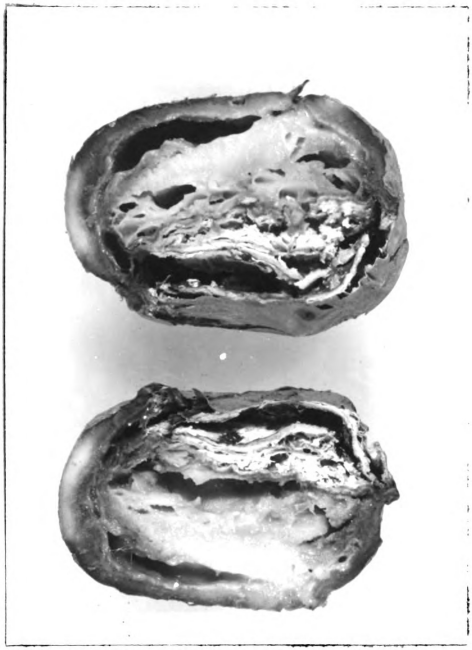


Fig. 5. Internal tuber decay following severe ringrot infection and invasion by secondary organisms.

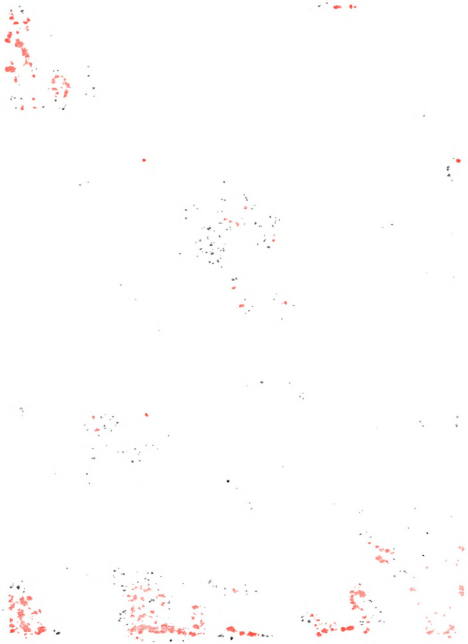


Fig. 6. Outward appearance of a tuber showing advanced decay of the stem end due to ringrot infection. Cracking of the tuber permits the entrance of various secondary soil organisms which hasten decay.

In the spring the more severely infected tubers in the bin fail to sprout, the eyes appearing blackened and dried (Fig. 7)

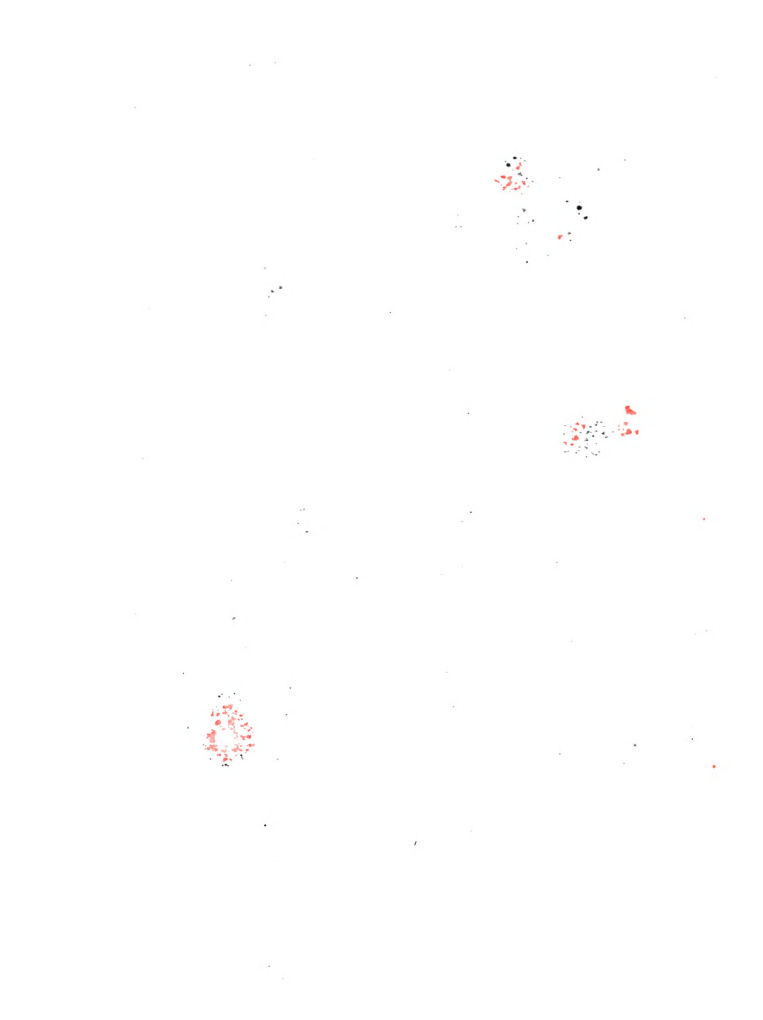
Diagnosis

Gram's Method of Staining

Since diagnosis by field inspection is difficult, it is important that some reliable laboratory method be available. The Gram stain answers this purpose. The suspected tuber or stem tissue is smeared on a microscopic slide, stained by Gram's method and examined under the oil immersion objective. If the smear reveals an abundance of Gram positive rods appearing singly, in pairs, and, less often, in short chains, 0.3 - 0.5 x 0.6 - 1.0 microns in size, then the presence of ringrot in the stock has been established. Gram positive bacteria larger than one micron are of no significance in the diagnosis as they are harmless soil bacteria. This procedure, as outlined by Racicot, Savile, and Conners (16) has been found to be highly satisfactory.

Often badly decomposed samples are sent to the laboratory by growers who request a diagnosis for ringrot infection. On staining a sample of this kind, such a mixed bacterial flora is seen under the microscope that accurate diagnosis is quite impossible

Fig. 7. Outward appearance of ringrot decay. Infection begins at the stem end and gradually progresses toward the eye end of the tuber.



as is also the isolation of the pathogen. If care is taken in collecting samples to select, insofar as is possible, only slightly infected tubers, a clear-cut picture of the pathogen is obtained on staining.

The Ultra-Violet Light

The introduction by Iverson and Kelly (17) of the ultra-violet light as a method of identifying potato tubers free from ringrot has facilitated tuber examinations. The method is rapid and permits the inspection of the entire vascular ring rather than a small portion as in the case of the staining method. The greenish fluorescence emitted by ringrotted tuber tissue on exposure to a G.E. BH4 ultra-violet lamp constitutes the diagnostic basis for the detection of the disease. Examinations are made at storage temperature (40°F. or lower) in a totally dark room.

In limited tests conducted by the writer, several tubers which had passed the macroscopic examination as "healthy" showed a slight silvery-green fluorescence of the vascular tissue under the ultra-violet light. To these tubers the Gram stain test was applied and gave positive results, indicating that ultra-violet light is successful in detecting infections that are easily overlooked by examination under ordinary light. In other instances tubers showing a suspicious greenish

fluorescence under the ultra-violet lamp gave negative results upon Gram staining.

Unfortunately, satisfactory results can be obtained only after considerable experience and even then it is difficult to distinguish the greenish fluorescence of ringrot from other similar fluorescences which are encountered (15). This method is valuable in making rapid preliminary checks but it cannot replace the staining method.

Etiology

Proof of Pathogenicity

The writer's first isolation of the causal organism of ringrot was from tubers furnished by C. H. Metzger of Colorado. Proof of pathogenicity was established as demanded by Koch's postulates. Young potato plants growing in the greenhouse were inoculated by placing a drop of the bacterial suspension on the base of the stem and pricking it in with a needle.

Six weeks later the first symptoms, that is, slightly upward rolling of the leaves and partial wilting of the shoot, appeared and the organism was isolated both from the stem and from the daughter tubers using the dilution plate method. These reisolates were used to inoculate another set of potato

plants and typical symptoms were again produced, this time in seven weeks.

Description of the organism

Phytomonas sepedonica, the causal agent of potato ringrot, is a short, Gram positive, non-motile, non-encapsulated, non-spore-forming rod, averaging 0.3 - 0.5 x 0.6 - 1.0 micron in size. It is aerobic, occurs singly, in pairs, and in short chains, and grows best at 21°C. On potato dextrose agar small pin-point colonies appear in about 10 to 12 days, while on the special medium devised for this organism by Burkholder (11) colonies appear a light yellow; 1 to 2 mm. in diameter, smooth, glistening, circular, and slightly raised. Gelatin is not liquified and growth in Burkholder's broth is cloudy, producing a granular sediment, but not producing a pellicle. Growth on cooked potato is slimy and brownish. In litmus milk there is growth with slight reduction of litmus but no clearing. Starch hydrolysis is slight. No gas is produced.

Life history of the causal organism.

The pathogen overwinters normally in diseased tubers, in storage. In the spring the slightly infected tubers easily escape detection and are planted, giving rise to a diseased crop of daughter-tubers. All available evidence appears to indicate that the

organism cannot overwinter in the soil in Maine (18), North Dakota, Florida, and California (15). Eide (15) has shown that the ringrot organism may overwinter on the sacks. It is likewise probable that storage bins and implements which come in contact with infected stock may still be contaminated with the virulent pathogen even after three to four months.

Cultural Characteristics

In pure culture the bacterium was found to grow poorly on all ordinary media. Nutrient agar gave no growth, beef-extract-peptone agar supported a sparse growth, while potato-dextrose agar supported a fair growth but the organism was found to lose its viability rapidly on this medium. Growth was materially enhanced on addition of .2% asparagine. Burkholder's (11) medium, developed specifically for this organism, supported a luxuriant growth and viability was maintained by transferring the cultures every two weeks. Virulence, however, was found to diminish to a greater or lesser extent on all media, including Burkholder's. Workers in Colorado, Oregon, and West Virginia agree that in pure culture the organism loses some of its disease-producing power (15). On the other hand, workers in California and Beltsville (15) report a high percentage of successful pure-culture inoculations.

Metzger, Glick, and Kreutzer (20) report that a mixed culture from partly decomposed tubers appears to be more virulent than a pure culture. In the field experiment conducted by the writer in the summer of 1940, but 17 infections out of 150 pure culture inoculations were obtained. Dorrel, Marten, and Leach (19) report the same experience in their 1940 field experiments. They conclude that "the results raise some question as to the methods by which seed stocks become infected."

Two factors which may be in part responsible for the small percentage of infection resulting from pure culture inoculations are 1, diminished virulence caused by growth on artificial medium; and 2, a synergistic relationship existing between Phytomonas sepedonica and the various secondary soft-rotting organisms.

Inoculation tests carried out in the greenhouse seem to indicate that both factors may affect the virulence of the pathogen. Eight young tomato plants and an equal number of potato plants were inoculated with an actively-growing, week-old agar slant suspension by pricking the base of the stem. Some time later another set of these plants was inoculated as previously except that a suspension of the soft, cheesy vascular tissue from an infected tuber was used as the inoculum. The results are reported as follows:

Table 1. Inoculations of Potato and Tomato Using Pure Culture and Inoculum from Infected Tuber

Host	Number of Plants Infected					
	Pure Culture			Infected Tuber		
	Severe	Medium	None	Severe	Medium	None
Tomato	0	0	8	5	3	0
Potato	2	5	1	6	2	0

It should be stated that in subsequent inoculations of tomato plants using freshly isolated cultures infection resulted. However, in the above inoculations no tomato infection was obtained. The first symptoms appeared on the potato plants after 42 days when pure cultures were used. On the other hand, when inoculum from an infected tuber was used, all of the tomato plants became infected, the first symptoms appearing after only 23 days; the potato plants in this series showed symptoms after 35 days.

Experiments conducted in Colorado (15) have shown that when the soft-rotting organism, Erwinia carotovora was used to inoculate a tuber already infected with ringrot, a serious and rapid decay followed; whereas when this soft-rotting organism was used to inoculate a healthy tuber, no infection developed.

In order to observe this phenomenon and to test the Gram negative contaminant frequently occurring in

the isolation plates, the following experiment was run:

Healthy and ringrotted tubers were disinfected, sliced aseptically, and the slices placed in sterile petri dishes. The treatments and results are given in the table below:

Table 2. Effect of Several Organisms on Healthy and Ringrot Tuber Tissue

	Amount of Decay Produced after 5 Days	
Inoculum	Healthy Tuber Slices	Ringrotted Tuber Slices
1. Erwinia carotovora*	Slight	Complete Disintegration
2. Phytomonas sepedonica*	None	Slight
3. Gram negative Contaminant*	Slight	Complete Disintegration
4. Check (5 c.c. water sterile)	None	Slight

* 5 cc. of 5 day old broth culture

The results show a pronounced acceleration of decay when the soft-rotting bacteria were added to ringrotted tubers. The cause of this phenomenon is not clearly understood. It may be that a combination of enzymes is essential to produce the severe type of tuber decay.

The idea of synergistic associations among plant disease pathogens is by no means a new one. Erwin F. Smith (21) points out that Phytomonas campestris, the causal agent of black-rot of crucifers, and Phytomonas solanacearum, causal agent of brown rot of potatoes, are often aided by soft white rots.

Fawcett (22) has appealed to plant pathologists to investigate more thoroughly mixtures of organisms. He states, "This necessary insistence on pure cultures of single organisms has led, perhaps unconsciously, to a feeling that to allow the use of a mixed culture in plant pathological work is extremely unscientific if it is not actually a 'deadly plant pathological sin'... We are not, it would seem, getting the whole story by working with pure cultures of single organisms. Nature does not work with pure cultures alone but most frequently with associations...Pure cultures we must, of course, continue to use as a basis for the known mixtures and as controls on the activity of the mixtures."

Pathological Histology

Histological sections of infected stems were prepared using chrom-acetic killing solution. Dehydration and infiltration were carried out in the usual manner. Sections 10 microns thick were cut on the microtome and stained according to Jones' method (26).

The sections prepared from infected stems revealed that the entire vascular bundle ring is seldom invaded by the bacteria. In cross section there are frequently one or more bundles which appear to be free of the invading bacteria. The number of bacteria present in the vessels varies greatly. Some of the vessels con-

tain only a small number of organisms while others are occupied by masses that completely occlude the lumen (Figs. 8,9). Moreover, completely occluded vessels exist side by side with vessels which to all appearances are entirely free of bacteria. None of the slides show any infection of the parenchymatous tissue adjacent to the vessels of the stem.

Invasion of the tuber is primarily vascular. In time the bacteria escape from the vessels into the adjoining intercellular spaces of the parenchyma, causing a separation and breakdown of this tissue (Fig. 10). As this breakdown continues, the tissue of the vascular ring assumes a characteristic yellow, cheesy aspect.

Often the infection does not extend for more than 1 or 2 centimeters in depth from the stem end of the tuber. When such a tuber is planted, sprouts originating from the non-infected end may develop their own root system and by rotting of the seed piece may become independent of the mother tuber before the infection of the seed piece advances to the point where the young sprouts become infected. This apparently explains the occurrence of healthy and diseased stems and tubers in the same hill.

Fig. 8A. Cross section of a potato stem in the vascular region showing vessels occupied by Phytomonas sepedonica.

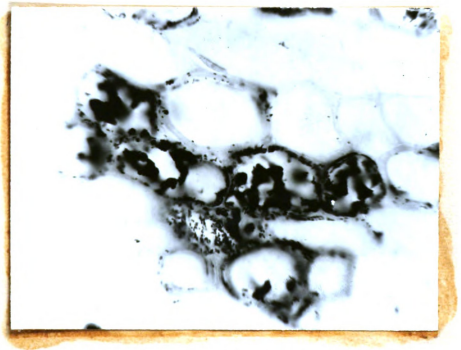


Fig. 8B. A portion of the same section as in Fig. 10A as viewed under oil immersion showing various degrees of bacterial occlusion of the vessels.

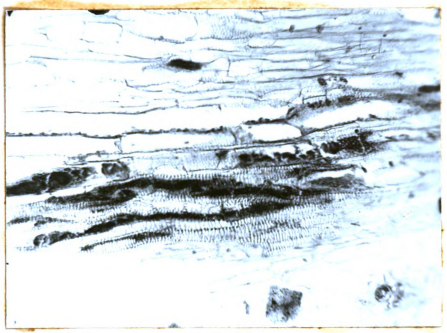


Fig. 9A. Longitudinal section thru an inoculated potato stem showing the dense masses of Phytomonas sepedonica restricted to the vessels.

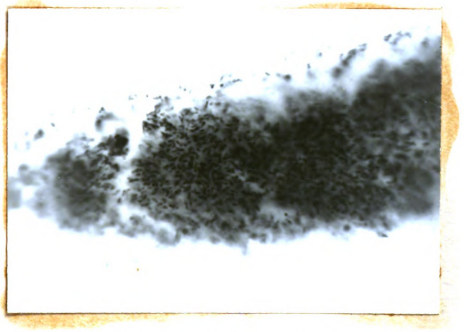
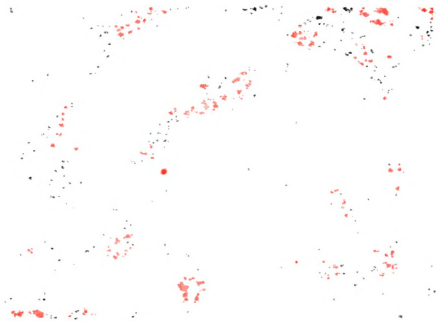


Fig. 9B. A detail from Fig. 9A showing the individual bacteria of the occluded vessel.



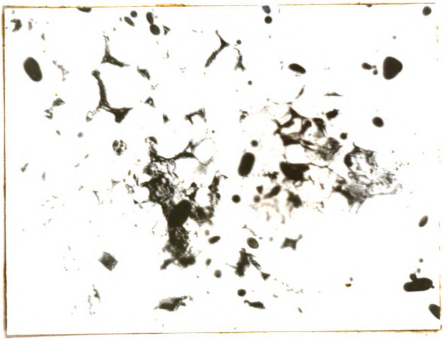


Fig. 10A. Section of a tuber infected with ringrot showing masses of bacteria in the intercellular spaces of the parenchyma.

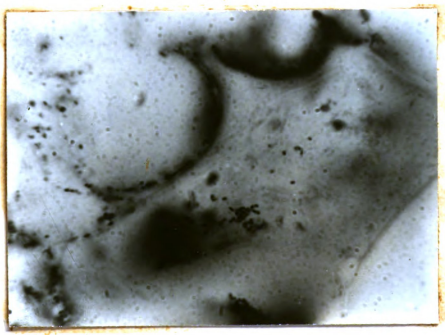


Fig. 10B. A portion of the same section as in Fig. 10A but under oil immersion to show the individual bacilli.

1940 Field Experiments

I. Disinfectants and Control

A field experiment was run for the purpose of determining the comparative bactericidal value of several disinfectants. Because no naturally infected stock was available at the time, pure cultures of the causal organism were used to inoculate healthy tubers. The pathogenicity of these cultures had been established several months previously by stem inoculations of potato plants growing in the greenhouse.

To week-old sub-cultures on agar slants 5 c.c. of sterile tap water were added to make a turbid bacterial suspension. The tubers were inoculated by dipping the flamed scalpel into this suspension and then cutting the seed piece.

One lot of inoculated tubers was treated in various disinfectants and planted the day after inoculation, while a second lot was stored for four days before treating to determine the effect of suberization of the cut surface on the incidence of the disease.

The plots were under observation for foliar symptoms throughout the summer. Stems of suspected plants were cut from time to time, smears made on clean slides, stained, and examined. Only two diseased hills were detected in this manner. At harvest time the hills

were dug by hand and the tubers were examined by clipping the stem end.

Because of the small number of infected hills, the tubers from the individual plots were bagged and stored for approximately four months to allow any slightly infected tubers to develop a more advanced decay.

At this second examination no increase in the amount of infection could be observed after a careful scrutiny in which all suspicious tubers were stained and examined under the microscope.

The results are indicated in the following table:

Table 3. Seed Treatment Experiment with Lot of Inoculated Chippewa Potatoes Treated Immediately after Cutting and 4 Days after Cutting.

Disinfectant	Number of Hills Infected	
	Treated Immediately	Treated After 4 Days
Improved Semesan Bel*	0	0
Dowicide A. 1-1000	0	0
Dowicide A. 1-2000	0	0
Corrosive sublimate 1-500	0	0
Acid mercury**	0	0
Check - inoculated	8	5
Check - not inoculated	0	0
Total no. inoc. checks	50	50
Total no. infected checks	8	5
% successful inoculations	16	10

* 1 pound to 7½ gallons water
** $HgCl_2$ 1-500 + 1% Hcl

All treatments applied for 15 minutes

Because of the small amount of successful inoculation, the comparative value of the disinfectants tested or the effect of suberization could not be established. It is interesting to note that in a similar experiment run concurrently in West Virginia by Dorrel, Marten, and Leach (19), the same experience is reported. They state that the amount of successful inoculation was "extremely small."

However, the results raise some question concerning the effect of artificial culture upon the virulence of the organism. Likewise the question of virulence in mixed versus pure culture is brought to the fore. These questions are discussed further under Cultural Characteristics.

Penetration of Dowicide A into Tuber Tissue

Because the infection of the ringrot-diseased tuber often affects the vascular ring tissue of the entire tuber, it was decided to determine the depth to which Dowicide A will penetrate into the cut surface of halved tubers, and to determine also whether the disinfectant is germicidal at different depths of penetration.

Accordingly, healthy and diseased tubers were cut in half and soaked in the disinfectant. Depth of penetration was established by using a spot test technique as outlined by G. R. Anderson of The Dow Chemical Com-

pany. The following formula was applied:

Reagents (A) Dissolve 0.03 grains of 2,6 Dibromo quinone chloroimide in 10 ml. ethyl alcohol. Prepare fresh daily.

(B) Buffer. Dissolve 15 grams anhydrous sodium tetraborate in 900 ml. of distilled water; add 3.27 grams sodium hydroxide in the form of a strong solution, and make up to one liter.

Procedure Put a drop of solution "A" on the cut surface of the potato. Then put a drop of buffer on the same spot. A blue stain shows the presence of Dowicide A.

Successive thin slices were cut from the surface of the treated tubers and the spot test applied until the freshly exposed surface no longer gave a positive reaction.

In addition, diseased tissue from various depths of the treated infected tubers was plated to determine germicidal action of the disinfectant. The results follow:

Table 4. Depth of Penetration of Dowicide A into Tuber Tissue of Potato

Dilution	Time (min.)	Penetration (mm.)	Plate Readings Depth (mm.)	Growth
1-1000	15	5	Surface	-
1-1000	30	7	Surface	-
1-1000	45	7	2	+
1-1000	60	10	5	+
1-2000	15	2	Surface	+
1-2000	30	4	2	+
1-2000	45	5	2	+
1-2000	60	8	5	+
1-3000	15	1	Surface	+
1-3000	30	2	1	+
1-3000	45	2	1	+
1-3000	60	5	2	+
1-4000	15	Less than 1	Surface	+
1-4000	30	Less than 1	Surface	+
1-4000	45	1	Surface	+
1-4000	60	3	2	+

The results indicate that the usefulness of this disinfectant is limited to surface disinfection. The lowest dilution, 1-1000, was found to be effective at the surface but the pathogen was not destroyed at a depth of 7 mm. even though the spot test showed that the disinfectant had penetrated to a depth of 10 mm. It appears that the action of the disinfectant may have been neutralized by the organic matter of the potato tissue, or diluted by the water content of the tuber.

Penetration into the diseased vascular tissues of infected tubers was not perceptibly greater than

in the case of healthy vascular tissue. In no case was any penetration through the intact periderm observed.

II. Spread of the Disease in the Field

To gain some idea as to the amount of spread in the field and the manner in which this spread takes place, alternate hills of healthy and inoculated tubers, 50 of each, were planted in a row on well-drained sandy loam. On each side of this row healthy check rows were planted. No above-ground symptoms could be observed during the growing season. At harvest time the hills were dug by hand and the tubers were examined macroscopically by clipping the stem end. Out of the 50 inoculated seed pieces which were planted, only four diseased hills were produced or 8%. As in the preceding experiment the healthy and inoculated tubers were bagged separately and stored for four months. Again no increase in disease could be ascertained on this second examination after a careful check, indicating that the inoculations did not produce infection. No disease was found in any of the tubers originating from healthy seed pieces.

It is concluded that, insofar as the experiment is capable of revealing, no field spread took place.

Dissemination

Undoubtedly the chief means of dissemination of ringrot from one state to another is through shipment of infected seed stock. Both of the serious outbreaks in Michigan in the summer of 1940 originated from Maine seed. Drastic steps are being taken by the certification industry to insure the shipment of ringrot-free seed to other states by eliminating ringrot from their own seed plots.

Spread in the field occurs in alarming amounts by means of the cutting knife. Experiments in California have demonstrated that a knife contaminated by cutting through a diseased tuber is capable of transmitting ringrot to the 24th healthy tuber cut immediately thereafter. When the picker type planter is used, two to three times the original amount of ringrot may result (15).

Contaminated bins, containers, and graders are known to be responsible for spreading infection (14).

Bonde (23) and Metzger and Brinkley (24) have advanced experimental evidence showing that some spread occurs through the agency of soil water.

Contact of healthy and diseased tubers in the bin, in sacks, or in crates likewise has been shown to result in substantial spread (15).

All attempts to transmit the disease by insects have been unsuccessful. In North Dakota (25) carefully planned experiments to determine possible dissemination by grasshoppers yielded negative results.

Varietal Resistance

In Wyoming (15) 23 commercial varieties were tested for resistance to ringrot and all were found to be susceptible. However, Russet Rural was found to be significantly more resistant than the Katahdin or Chippewa. Irish Cobbler and Bliss Triumph were among the most susceptible.

These workers tested 340 seedlings for resistance and out of this number 28 seedlings showed no symptoms of ringrot. These were saved for further testing.

Bonde (20) tested 145 seedlings but found all of them to be susceptible.

The development of resistant varieties is an important phase in the control program. Preliminary reports of resistance are distinctly encouraging.

Control

Primary controls

I. Eradication of ringrot infected stock appears to be the most promising method of complete control.

Since the main source of infection is the infected seed tuber, and since there is little evidence of the organism overwintering in the soil, it is apparent that the weakest link in the life cycle of this pathogen is the overwintering phase, and that an attack on the disease from this angle offers the most hopeful prospect of control. Exclusive use of ringrot free seed stock is, therefore, of supreme importance as is also the strictest observance of the zero toleration by all certifying agencies.

The following method is proposed for selecting a disease-free lot of seed:

1. Disinfect the whole tubers by dipping in 1-1000 $HgCl_2$ for 10 minutes.

2. Clip the stolon end of each tuber and examine macroscopically. Discard all tubers exhibiting any vascular discoloration and disinfect the cutting knife in $HgCl_2$ 1-500 for 5 seconds after each cut.

3. Examine under the ultra-violet light all tubers passing the macroscopic inspection. This should be done at temperatures of 40°F or lower and in a totally dark room. Discard all tubers showing a greenish fluorescence in the vascular region and also all suspicious or doubtful tubers.

4. From each of the tubers surviving the first two tests cut a slice containing a portion of the vascular ring and press it lightly on a numbered slide. Place the tuber into a correspondingly numbered paper sack.

Disinfect the knife with each new cut and wipe the blade off with a small wad of absorbent cotton, using fresh cotton each time.

5. Fix smears by passing the slide through the flame several times and then stain by Gram's method as outlined by Racicot, Savile, and Conners (16).

6. Examine slides under microscope using the oil immersion objective. Discard all tubers which yield slides displaying the morphologically typical Gram positive bacilli.

7. Tubers yielding smears which appear to be negative may be considered "ringrot-free". These should be planted in tuber units in a field which is well isolated from other potato fields and which has not been planted to potatoes the previous year. Inspect and rogue the plot at frequent intervals during the summer.

8. At the time of digging cut a stem at the base from each hill and press the cut surface on a numbered microscopic slide. Dig each unit separately and tag to correspond to the slide number.

9. Fix, stain, and examine smears. Discard all units from which any smear reveals morphologically typical bacteria.

10. The remaining tubers may be used for increase, the following season under supervision of the certification service.

On farms where ringrot is present, all equipment, such as cutting knives, containers, planter, etc., should be disinfected before handling clean seed.

Two pounds of copper-sulphate to ten gallons of water or one pint of 40% formaldehyde to fifteen gallons of water may be used.

This is admittedly a laborious and time-consuming process but pending the development of a more rapid and less cumbersome method the production of ringrot-free seed stock should be expedited with the existing methods.

II. The breeding of resistant varieties has indications of becoming an important factor in eventual control as was pointed out under Varietal Resistance. This is a long-time proposition and should be carried on to supplement the eradication program and to hasten the realization of the objectives of eradication.

Secondary Controls

Under this heading are controls which do not eliminate ringrot from seed stock but which merely prevent further spread. These methods should be of interest only to growers of table stock who have purchased the best seed possible but in which some ringrot is present. Seed treatment cannot be recommended to certified seed growers to control this disease unless it is to prevent accidental infection from outside sources.

1. Treat cut seed for 10 minutes with 1-1000 $HgCl_2$, Acid mercury ($HgCl_2$ 1-500 + 1% Hcl), or formaldehyde, 1 pint to 30 gallons of water.

2. Plant whole tubers. This eliminates the spread of the disease by the cutting knife but obviously does not eliminate the disease from the seed stock.

SUMMARY

Bacterial ringrot is a serious new disease of potatoes. It was first reported in the United States in 1937 by Bonde of Maine, and since then has been reported from 37 other states. It has been known in Germany since 1908.

In Michigan, where the disease has been reported in 11 counties, losses have been small. However, the disease constitutes a potential threat to the potato industry and the potato certification program, hence its importance cannot be overestimated.

All commercial varieties of Irish potato are susceptible. In addition, the tomato develops typical symptoms upon artificial inoculation.

Foliar symptoms are variable and come late in the season when early and late blight and insect and drought injury may be prevalent. Masking of ringrot by symptoms of other diseases and injuries makes detection difficult.

Of more diagnostic value is the soft, yellow, crumbly vascular ring tissue of the infected tuber.

The Gram stain serves as a reliable laboratory method for identifying diseased tubers. The recent introduction of the ultra-violet light test has facilitated detection of infected stock.

Phytomonas sepedonica, the causal agent, is a short, non-motile, Gram positive rod, measuring 0.3 - 0.5 x 0.6 - 1.0 microns.

The bacteria overwinter primarily in diseased tubers in storage. Available evidence indicates that the organism does not overwinter in the soil except on volunteer plants.

In field experiments conducted during the summer of 1940, inoculation of healthy tubers with pure cultures resulted in a surprisingly small percentage of infection. Diminished virulence of the bacteria following growth on artificial media and a possible synergistic relationship between Phytomonas sepedonica and secondary soft-rotting bacteria which, of course, were absent from the pure culture, may in part account for the small amount of successful infection.

The chief means of geographical dissemination is through the shipment of infected seed stock from one

state to another. Spread in the field occurs mainly by means of the cutting knife and the picker-type planter. No insect vector has been found.

Eradication of infected stock appears to be the most promising method of control. Minor controls such as seed treatment and planting whole tubers serve to prevent spread but obviously do not eliminate the disease from the stock.

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