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## COMPILATION OF RESULTS IN CONTROL OF POTATO RING ROT IN 1941

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Industry, U. S. Department of Agriculture, Washington, D. C.*

### COMMITTEE REPORT

The committee appointed by the American Potato Association for the purpose of stimulating and coordinating research on ring rot caused by *Phytophthora septonica* has again made a survey to determine the extent of the occurrence of this disease in the United States in 1941. On the basis of this survey, the committee submits the following report:

Replies received from 38 states reveal that in 17 states the disease was less prevalent than a year ago, whereas eight states reported the disease more widespread than in 1940. In one state 22 per cent of the carload lots shipped in 1940 contained ring rot, whereas in 1941 only 6 per cent showed the disease. Another state mentioned that during the last 2 years the losses in one county alone were \$200,000 whereas this year, with greater care in handling the seed, the loss did not exceed \$10,000. Most of the states attributed the decrease in ring rot to the campaigns that were conducted during the winter through meetings and publicity on control measures; to change in seed stocks; and

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<sup>1</sup>Pathologist.

to the low price of certified seed in 1940, which enabled more growers to plant certified seed.

The disease is most serious in home gardens and on farms where little attention is paid to the source of seed. Although in many cases the planting of certified seed has given satisfactory results, in some instances the results were disappointing. One state reported the distribution of several carloads of certified seed potatoes that contained enough ring rot to produce from 10 to 50 per cent plant infection. The consensus of opinion is that the outlook for control is good as long as the certifying agencies in the seed-producing states maintain rigid control.

Attention was called to the difficulty of accurate diagnosis of the disease in the field,—especially after an early frost. In such cases and in some others, the inspector will have to rely on examination of the tubers in the bin. In order that a more complete and thorough inspection may be made it is recommended that in addition to field inspection at least four 25-pound random samples for each 1,000 bushels stored be examined by cutting each individual tuber. Suspected tubers should be examined under fluorescent light or smears should be made for examination under the microscope. Because the tuber symptoms become more distinct later in the season, it is desirable to make the bin inspection as late in the winter as possible.

More detailed experiments on the damage caused by planting seed containing a trace of ring rot should be conducted. More information is needed on methods of disinfecting bags, crates, storage houses, and farm machinery. The educational campaign to inform the growers of the seriousness of this disease and its occurrence in non-certified potatoes should be continued.

T. P. DYKSTRA, *Chairman*

R. W. GOSS

J. G. LEACH

COMMITTEE TO COORDINATE RESEARCH ON NEW AND UNUSUAL POTATO DISEASES.

The ring-rot disease is being studied in different parts of the country, and, on the recommendation of the committee, the potato project of the U. S. Department of Agriculture is continuing its function of compiling the reports on this disease as submitted by the different states. Many of the states that are investigating the ring-rot disease of potatoes have agreed to take part in this nation-wide program and have submitted reports. These are not necessarily complete and

conclusive, and contain generally only the results obtained during the 1941 season.

The cooperating states and investigators who have submitted reports are as follows:

Colorado:	D. P. Glick, R. Manuel, J. G. McLean, W. A. Kreutzer, and G. M. List
Florida:	A. H. Eddins
Idaho:	J. M. Raeder
Kansas:	O. H. Elmer
Maine:	R. Bonde
Michigan:	J. H. Munice
Minnesota:	Carl J. Eide
Montana:	G. H. Starr, W. A. Riedl
West Virginia:	V. E. Iverson and F. M. Harrington
Wyoming:	J. G. Leach
U. S. Department of Agri- culture, Beltsville, Md.	Lillian C. Cash

A report on investigations of the ring rot of potatoes in Canada was prepared by H. N. Racicot of the Central Experimental Farm, Ottawa, Canada, and is also incorporated in this compilation.

#### DETERMINING THE MOST EFFECTIVE METHOD OF INOCULATION

Since it is important in studies on ring rot to be able to produce infection at will, the Wyoming Experiment Station conducted a test to determine which method of inoculation gave the most reliable results. The plants inoculated were tested for the presence of the ring-rot organism at approximately 2-week intervals to get the rate of ring-rot development, as well as the amount of ring rot at the final check-up.

The results (table 1) indicate that ring-rot symptoms appeared earlier where the hypodermic needle was used in inoculation through sprouts. It was immaterial whether a contaminated knife with a stainless steel or a non-stainless steel blade was used. Smearing the cut surface of tubers with a ring-rot-infested tuber did not increase the amount of ring rot. The bacterial suspension method produced later symptoms and slightly less ring rot than any of the other methods. Less disease was produced with whole than with cut tubers.

TABLE I.—*Methods of inoculation and the rate and amount of ring-rot development. Wyoming.*

METHODS OF INOCULATION	PLANTS SHOWING RING-ROT SYMPTOMS AT DIFFERENT DATES (PLANTED MAY 21, 1941)			
	JULY 24	AUG. 6	AUG. 20	SEPT. 4
	<i>Per Cent</i>	<i>Per Cent</i>	<i>Per Cent</i>	<i>Per Cent</i>
1. Tubers cut and dipped in bacterial suspension .....	8.3	40.0	75.0	91.7
2. Tubers cut and smeared on cut surface with ring-rot tuber .....	13.7	60.8	95.0	98.3
3. Hypodermic needle through short sprouts .....	49.7	88.5	94.0	97.0
4. Contaminated cutting knife (stainless steel) .....	20.0	71.0	88.5	100.0
5. Contaminated cutting knife (not stainless steel) .....	26.1	82.7	98.3	100.0
6. Whole tubers dipped in bacterial suspension .....	5.0	25.0	55.0	70.0

The effect of four methods of inoculation was tested in Beltsville on plants growing in the greenhouse, using as inoculum a suspension in 5 cc, sterile tap water of a 4-day agar slant culture on Burkholder's special medium.

1. Hypodermic inoculations were made 2 mm. from the eyes of the seed pieces, and the uninoculated eyes were removed.
2. Inoculations of cut seed pieces were made by soaking the cut surface immediately upon cutting in the bacterial suspension for 15 minutes and planting immediately.
3. Whole tubers were treated in the same manner as in No. 2.
4. Shoots 8 to 10 inches high were inoculated by hypodermic injection of a suspension of the ring-rot organism, inoculating one shoot of a plant.

Two months after inoculation the stems were examined microscopically for the ring-rot organism, using the gram stain. Method

2 gave the greatest percentage of infection; No. 1 gave a high percentage of infection; No. 4 gave a low percentage, and No. 3 produced none.

The effect of different concentrations of bacterial suspension used in hypodermic inoculations in the eyes of tubers was tested. Forty plants were used in each series. Bacterial suspensions were made by adding 5 cc. of sterile tap water to each of four agar slant cultures and pouring the suspension into a single beaker. This suspension was used to inoculate series A, after which the remainder was diluted with an equal amount of water for series B thus making the dilution equivalent to 10 cc. per culture. For series C the inoculum used for B was again diluted with an equal amount of water to make the dilution equivalent to 20 cc. per culture. The concentration of the bacteria in the original 5 cc. suspension was determined by the color chart for white precipitate methods.<sup>2</sup> It checked with the third place for white precipitate, or 150 parts per million chlorides.

The percentage of infection was high in the stalks but low in the daughter tubers. It was only possible to run this test 2½ months, which may account for the slight amount of tuber infection.

TABLE 2.—*Results of inoculation with varying concentrated suspensions of Phytomonas sepedonica. Beltsville, Md.*

SERIES	DILUTIONS	INFECTION IN STALKS	INFECTION IN TUBERS
		<i>Per Cent</i>	<i>Per Cent</i>
A	5 cc. H <sub>2</sub> O per agar slant.....	45.6	1.7
B	10 cc. do	72.0	8.3
C	20 cc. do	31.5	0.0

COMPARISON OF CAUSAL ORGANISM FROM INFECTED TUBERS COLLECTED FROM DIFFERENT STATES, AND THE USE OF THESE IN INOCULATION STUDIES

The Wyoming Station collected ring-rot tubers from California, Kansas, North Dakota, Montana, Minnesota, Maine, New Jersey, and Oregon. again 3 weeks later from the same tubers. About 3 weeks later plant-

<sup>2</sup>Spurway, C. H. 1935. Soil testing, a practical system of soil diagnosis. Mich. Agr. Exp. Sta. Tech. Bull. No. 132, pp. 1-125.

ings were made with healthy tubers (five in each case) and inoculated as follows: ((1) Directly from the infected tubers by the smear method on the cut surface and the needle stab through the eyes, (2) from cultures isolated 18 days before and inoculated by the needle stab method, and (3) from cultures isolated about 40 days before by the same method of inoculation.

The plants were inspected in the field for ring-rot symptoms which were recorded for each lot. No difference in symptoms could be observed in the infected plants inoculated by the organism isolated from tubers obtained from different states. The infection was 100 per cent from the tubers whether the inoculations were by the smear or the needle-stab method. The average infection from cultures 18 to 20 days old was 56 per cent, and from those 40 days old, 12 per cent. Slides were made from all the cultures and tubers at the time of inoculation. These showed that in cultures approximately 18 days old the ring-rot bacteria were very numerous, accompanied usually by few soft rot bacteria. Cultures approximately 40 days old contained few ring-rot bacteria and proportionately more soft rot bacteria. This indicates that the purification of cultures is important when isolations from tubers are involved. It was therefore recommended that healthy tubers should be inoculated with ring-rot cultures and grown in sterilized soil in pots under greenhouse conditions so that infected tubers may be available for use in inoculation. Infected tubers can be kept for relatively long periods and have the advantage over artificial cultures in that the bacteria will remain pathogenic as long as the tubers persist.

#### TESTING MEDIA FOR CULTURING *Phytomonas sepedonica*

Various modifications of culture media were tested in Beltsville to find one favorable for the growth of *Phytomonas sepedonica*. A modified potato-dextrose medium gave the most satisfactory results. This is prepared as follows: To approximately 300 grams pared and sliced potatoes add 1,000 cc. of water. Cook in a steamer until the potatoes break up. Then pour water off and on the potatoes several times in order to extract as much nutrient as possible. Filter through cheese cloth and make up to 1,000 cc. After adding 12 grams agar, 5 grams peptone, 6 grams dextrose, and 1 gram yeast extract, heat in a steamer until dissolved. Adjust with N/5 NaOH to 6.8 or 6.9. Finally, filter through cotton, tube and sterilize. This medium has certain definite advantages: it is comparatively easy to make colonies appear

in 3 to 4 days on agar plates; there is copious growth in 4 days on agar slants; and the organism retains its typical pathogenic rod form for at least 1 month.

DETERMINING THE EFFECT OF A MIXED CULTURE OF *Phytophthora septedonica* AND SOFT-ROT ORGANISMS

The effects of mixed cultures of *Phytophthora septedonica* containing respectively each of several contaminants often present in ring rot tubers were compared with *P. septedonica* alone in inoculation studies in Beltsville. Seed pieces were inoculated hypodermically and planted in lots of 20 pots. There was little difference in the amount of infection on stalks; but the mixed cultures produced increased infection in the tubers, in comparison with the pure culture.

TABLE 3.—Results of infection with *Phytophthora septedonica* alone and in mixture with other organisms. Beltsville, Md.

CULTURES	AMOUNT OF INFECTION	
	STALKS	TUBERS
	<i>Per Cent</i>	<i>Per Cent</i>
<i>P. septedonica</i> alone .....	45.6	1.7
<i>P. septedonica</i> + <i>B. subtilis</i> .....	68.4	65.2
<i>P. septedonica</i> + <i>B. mesentericus</i> .....	52.4	No tubers formed
<i>P. septedonica</i> + green fluorescent organism .....	56.7	20.6
<i>P. septedonica</i> + culture No. 31 .....	51.8	44.4

RATE OF MOVEMENT OF RING ROT ORGANISM AFTER INOCULATION

At Beltsville, tubers were inoculated hypodermically with a suspension of the ring-rot bacteria, held at room temperature, and examined to determine the progress of the organisms in the vascular bundles. After 2 months infection had spread 5 mm. to 10 mm.; after 3 months in 67 per cent of the tubers positive readings were obtained at 1 cm.

from the needle track, and in 23 per cent of the tubers infection had spread 2 cm.

Cut surfaces of seed pieces were placed in contact with bacterial suspension 10 minutes before planting. In 3 weeks infection occurred as follows: Shoot base, positive 4, negative 9; shoot at 5 mm. above base, positive 0, negative 13; tuber, at a depth of 5 mm. from inoculated surface, positive 1, negative 4. In 6 weeks all shoots were found to be infected. The distance of the organism from the point of inoculation varied in different shoots from 1 to 14 cm. All shoots examined emerged from the seed piece less than 5 mm. from the inoculated surface.

#### LATENT INFECTION OF RING ROT

Inoculation experiments conducted in West Virginia in 1940 gave apparently negative results, since none of the inoculated tubers produced visible infection on the plants in the field or in the harvested tubers on inspection late in December. In 1941 as many as 57 per cent infected hills resulted when the stock was planted as whole tubers. Because there was no opportunity for transmission by the cutting knife, this would indicate that as high as 57 per cent of some of the seed lots were infected.

The significant point in these results is that insofar as ring rot was concerned, this experimental plot would have passed all requirements for certification, yet there was a very high percentage of potential infection in the seed stock. This indicates that under certain conditions the usual field inspection, coupled with bin inspection shortly after harvest, will not be sufficient to give a true picture of the amount of ring rot present.

#### NO SPREAD OF RING ROT FROM HILL TO HILL OR FROM ROW TO ROW

It was found in Florida that although 80 per cent of the plants in rows planted with diseased seed were affected with the ring rot in 1941, none of 358 plants originating from healthy seed and grown in the adjacent rows developed the disease. None of 722 plants comprising the progeny of tubers produced similarly in 1940 in rows adjacent to affected plants showed any symptoms of the disease in 1941. No signs of ring rot were detected in 28 plants originating from healthy seed and grown in the same row in hills 1 foot distant from 14 hills that were planted with diseased seed pieces, 10 of which rotted and 4 of which produced plants affected with ring rot.



In Michigan ring-rot-infected tubers were planted every fourth hill along the row with healthy tubers. The healthy tubers were planted first and the ring-rot tubers inserted in the proper place. The rows were replicated three times, and in no case was there any spread from the ring-rot-infected hill to the healthy adjacent hill. This field was irrigated with an overhead sprinkling system once during the season.

#### RING ROT SPREAD BY HANDLING SEED AFTER CUTTING

In a test in Florida healthy seed was cut with a contaminated knife and planted immediately. Of 250 plants 9.2 per cent were affected with ring rot. But when a similar lot of seed was cut and stored in a jute bag for 36 hours before planting, 23 per cent of the plants developed the disease. In another test, 148 tubers from a stock affected with ring rot were cut into two pieces each. One piece from each tuber was planted immediately, and the other pieces were placed in a basket and later transferred to a jute bag where they were kept for 24 hours before planting. Seed pieces planted immediately after cutting produced plants of which 10 per cent were affected with ring rot, seed pieces stored in the jute bag for 24 hours produced plants of which 31 per cent were affected with the disease. Possibly the additional handling of the cut seed caused the bacteria to be spread over a greater number of seed pieces and thus increased the amount of infection.

#### NO OVERWINTERING OF THE RING-ROT ORGANISM IN SOIL

No ring rot was detected in tops and tubers of 552 potato plants originating from healthy seed and grown in Florida in 1941 in soil where 25 to 64 per cent of the plants were affected with ring rot in 1938, and later seeded with 5 barrels of diseased tubers to increase the infestation. None of the 2,700 plants grown in the soil during the 3 years since 1938 has shown any signs of ring rot. Furthermore, no ring rot developed in 784 plants of the first generation progeny and in 341 plants of the second generation progeny of tubers produced in this infested soil in 1939. There has been no loss from ring rot at Hastings in potato crops planted with healthy seed in a number of fields where the disease was present the preceding year.

For three successive seasons experiments were conducted at Ottawa, Canada, on the overwintering of *Phytophthora septentrionalis* in the soil. The soil was contaminated in the fall by incorporating a heavy

application of decayed and partly rotted tubers from ring-rot-infected plants. In the spring, freshly cut healthy sets were planted in this soil. At no time did any of the plants from these sets ever become infected, although one year some of the partly decayed tubers overwintered and produced diseased plants the next season; therefore, it is believed that the ring-rot organism does not overwinter in the soil under Ottawa conditions in such way as to cause new infection.

In Wyoming ring-rot-infected tubers were placed approximately 4 inches under the soil and on top of the soil and left there during the winter to determine whether the bacteria would survive. Also, burlap sacks were contaminated with ring-rot bacteria, and one lot of these was placed outside and another inside the storage cellar during the winter months. At planting time each lot of bags was soaked in water and healthy cut seed pieces were dipped in the wash water just before being planted. The infected tubers left in the soil during the winter were also used, as well as the soil around these tubers, to make suspensions in which healthy cut seed pieces were dipped. These were planted and the resulting plants checked for the presence of ring rot. (See table 4).

TABLE 4.—*Overwintering test of ring rot bacteria from different sources. Wyoming.*

SEED INOCULATED	SOURCES OF INOCULUM	RING ROT
		<i>Per Cent</i>
Healthy seed pieces	Contaminated sacks left outdoors during winter .....	45
Do	Contaminated sacks kept in storage cellar	10
Do	Tubers kept in outside soil .....	2.5
Do	Soil surrounding infected tubers left outside during winter .....	2.5

Considerable ring rot resulted from the test in which washings from the burlap sacks left outdoors were used as the inoculating agent. There was much less ring rot in the test where burlap sacks left in the storage cellar were used as the source of inoculum. There was some evidence that the organism may overwinter in the tubers left outside in the soil. The same was true of the soil taken from around these tubers. Thus, there is some evidence from this test that the bacteria are able to

live over in the soil or in the tubers remaining in the soil under the Wyoming conditions.

In continuing the study of contaminated sacks as a source of ring-rot infection, in Minnesota, seed was planted on the 19th of May, 1941, which had been shaken in sacks emptied on the 25th of March. The sacks contained 1.5 to 27 per cent of ring-rot tubers at the time they were emptied and were kept in a cool, dry place until they were used for the cut seed pieces. Plants from the seed shaken in nine sacks were found to be infected at digging time.

The survival of *Phytophthora septentrionalis* in soil was studied in Minnesota under controlled conditions involving the following variables:

1. Soil sterilized and non-sterilized.
2. Source of bacteria used to contaminate soil: pure cultures and yellow material from the vascular ring of diseased tubers.
3. Place of storage—
  - a. In a laboratory at 65° to 75° F.
  - b. In a refrigerator at about 35° F.
  - c. Out of doors.

Pint fruit jars of soil involving all possible combinations of the above conditions were prepared during the winter of 1940, and successive samples were tested for the presence of *P. septentrionalis* at intervals of a month or 6 weeks. This was done by pouring the soil over freshly cut seed pieces and allowing them to grow in pots in the greenhouse. The presence of the disease and gram-positive bacteria in the plants was taken as evidence of the survival of the bacteria in the soil. Table 5 shows the maximum time in which the bacteria lived in the soil as determined by these methods.

Apparently *P. septentrionalis* survives less than a month in non-sterile soil at temperatures high enough for the growth of most organisms. Freezing or near-freezing temperatures protect it for some time. These results raise doubts about the ability of *P. septentrionalis* to survive for any length of time anywhere, it must live saprophytically, and where the temperature and moisture conditions permit the growth of competing saprophytic bacteria and fungi.

Seed harvested with a contaminated digger in the fall of 1940, picked in a contaminated basket, and stored in a contaminated sack produced a few ring-rot plants.

TABLE 5.—*Survival of P. sepedonica in sterile and non-sterile soil under different conditions. Minnesota.*

STORAGE CONDITIONS	SOURCE OF INOCULUM	LATEST SURVIVAL DATE <sup>1</sup>	
		IN STERILE SOIL	IN NON-STERILE SOIL
Outside .....	Pure culture <sup>2</sup> .....	1941 March 13	1941 February 11
	Diseased tissue <sup>3</sup> .....	March 13	March 13
35° F. ....	Pure culture .....	April 11	January 1
	Diseased tissue .....	March 13	March 13
Laboratory .....	Pure culture .....	February 11	0 <sup>3</sup>
	Diseased tissue .....	February 11	c

<sup>1</sup>Last test made.

<sup>2</sup>Soil was contaminated with the diseased tissue on November 29, 1940; with the pure culture on December 4, 1940.

<sup>3</sup>The non-sterile soil stored in the laboratory was apparently free from ring rot bacteria in a month (Jan. 1, 1941) after the experiments were started.

#### EFFECT OF DATE OF PLANTING ON THE DEVELOPMENT OF RING ROT

Experiments were conducted in Wyoming to determine the effect of date of planting on the occurrence of ring rot. Two rows, of 40 hills each, were planted at four planting dates with 10-day intervals to study ring-rot development. The cut seed was inoculated by dipping it in a suspension of the ring-rot bacteria just before planting.

In the earliest planting (May 11) the stand, as well as the ring-rot percentages, were considerably reduced as compared with the later plantings. The time required for visible ring-rot symptoms was much longer than in the case of the later plantings, due in part to the longer time required for germination of the seed. However, ring rot actually showed up in the second planting (May 21) before it did in the earliest planting (May 11). Ring rot appeared in the latest planting (June 11) sooner after planting than it did in any of the other lots, although the difference was slight when compared with the planting of the 1st of June.

#### DISINFECTING CUTTING KNIVES

During the season of 1941 numerous experiments were conducted in Wyoming to obtain practical information on the control of ring rot.

An almost disease-free lot of Bliss Triumphs was used in the tests. The treatment rows consisted of 10 hills planted 18 inches apart in the row, with four replications arranged in a random manner. Ring-rot readings were made on the basis of plant symptoms.

TABLE 6.—*Effect of date of planting on the development of ring rot. Wyoming.*

DATE PLANTED	STAND	RING-ROT	TIME ELAPSING BETWEEN PLANTING DATE AND FIRST VISIBLE RING-ROT SYMPTOMS
	<i>Per Cent</i>	<i>Per Cent</i>	<i>Days</i>
May 11	77.5	40.3	88
May 21	97.5	98.7	67
June 1	95.0	90.8	63
June 11	98.5	100.0	59

In an attempt to find suitable disinfectants to replace mercuric chloride, 24 compounds were tested, some in as many as five concentrations, making a total of 62 treatments. These tests were made by dipping a cutting knife for 10 seconds into the disinfectant to be tested, and immediately using it to cut a healthy tuber in half. This dipping was repeated before each tuber was cut. The seed pieces were planted on the Agronomy Farm at Laramie, Wyo.

As in the past, mercuric chloride and acidulated solution of mercuric chloride were highly effective; iodine was almost as effective; and Semesan Bel. Du Bay, Cinnex 20, and copper sulfate were relatively ineffective in the 10-second exposure. Formaldehyde (1:10) was also relatively less effective, except when used as both solution and vapor, when it was highly efficient. Ethyl alcohol gave poor results for this length of exposure. The following compounds, tried for the first time, were relatively ineffective at the concentrations used: Gentian violet, proliferol, furfural, and cuprocide. Ethyl alcohol plus mercuric chloride was slightly less effective than the mercurial alone. The chlorinated compounds, H.T.H., Steri-chlor, Gasklor (except that Gasklor was effective at 8,000 p.p.m.), were relatively ineffective with the 10-second exposure; while B-K was quite effective at the tested strength greater than 2,000 p.p.m. in the test. Lysol, 4-per cent, gave

good results, whereas Kreso dip was less effective. Boiling water in exposures of 5, 10, and 15 seconds gave good results and offers possibilities in the control of ring rot.

A number of chemicals were tested in Ottawa, Canada, as disinfectants for the knife used in cutting sets. A stainless steel paring knife was thoroughly contaminated by cutting into diseased tubers known to carry large numbers of *P. sepedonica*, dipped into the solution of the chemical to be tested for 10 seconds, then used immediately to cut small healthy tubers longitudinally into two sets. Two-per cent Lysol (a saponified cresol) was not effective, while 4-per cent and 10-per cent solutions were. Formalin (commercial 40-per cent formaldehyde) at 1/10, 1/2, and full strength was not effective. Semesan and Semesan Bel, 2-per cent solutions, were very ineffective. Mercuric chloride, 1:1,000 in water, was not effective; but the 1:500 dilution with either 1-per cent hydrochloric acid or 0.35-per cent hydrochloric acid (equivalent to Mercurinol at the rate of 32 fluid ounces to 20 gallons) was completely effective.

#### DETERIORATION OF CUTTING KNIFE DISINFECTANTS

Experiments were conducted in Colorado to determine at what rate disinfectants deteriorate under practical conditions. When a rotary knife is used, the disinfectant is subject to contamination by tuber pulp, and soil from the surface of the tubers. Standard bacteriological methods were used to measure the strengths of numerous disinfectants at selected intervals during the actual cutting operations. When 1 pint of disinfectant was used a mercuric chloride solution (1:500 in water) became ineffective after two sacks had been cut; acidulated mercuric chloride (1:500 in 1-per cent hydrochloric acid) after two sacks; and a 1-per cent solution of iodine after four sacks. When 1 gallon of disinfectant was used, a solution containing 2,000 p.p.m. of chlorine lasted through eight sacks, 5-per cent phenol was not exhausted after 12 sacks (but severely damaged the seed pieces), and 2-per cent cresol was effective after 24 sacks. When amounts greater than 1 gallon were used, the efficiency increased slightly but not in proportion to the amount of disinfectant.

When the cost and rate of deterioration of the more common disinfectants were taken into consideration, it was tentatively concluded that the best cutting knife disinfectant would be boiling water, because the effectiveness of this disinfectant is merely a function of the temperature and because the cost is low in comparison with that of the chemical solutions.

## TREATING INOCULATED TUBERS BEFORE CUTTING AND AFTER CUTTING

In Wyoming 30 different treatments were tested to find a disinfectant to replace mercuric chloride in the treatment of seed potatoes for control of ring rot. Few effective disinfectants are available that can be used to treat seed potatoes after cutting, although a number of them can be used previous to cutting the tubers.

All tubers were inoculated by dipping them in a suspension of the causal organism. A portion of these was dipped for the required time in the disinfectant and later cut into halves. Another portion was cut into halves and then dipped in the disinfectant to be tested. The tubers were then spread out and the surfaces were allowed to dry before planting.

From the standpoint of both ring-rot control and yield, the 1:500 solution of mercuric chloride gave some of the best results. The same was true of Mercurinol. Iodine in treatments of 15 minutes reduced ring rot to a minimum and gave good yields when used either before or after cutting. Lysol, 2 per cent, gave good yields in both cases but did not control ring rot so well as stronger solutions, which reduced yields when used on cut seed. In a general way, numerous disinfectants gave satisfactory control of ring rot, as well as good yields when used on whole seed, but few of them gave satisfactory yields when used on cut seed.

In Michigan in tests with chlorinated phenol at strengths of 1:1,000 and 1:2,000, corrosive sublimate at 1:500, and acidulated corrosive sublimate solution for tuber treatments, the acidulated corrosive sublimate severely injured the seed pieces and only 37 per cent of the tubers produced plants. There was no evident injury apparent from the other treatments. The acidulated corrosive sublimate was the only material used which seemingly prevented ring rot. The stand, however, was so reduced that using this material seems out of the question.

In Florida 100 large tubers were cut into four seed pieces each with a knife contaminated with *Phytomonas sepedonica* and one piece from each tuber was planted in each of our lots. Lot 1 was not treated, lot 2 was soaked in water at 124° to 126° F. for 4 minutes; lot 3 was soaked in 1:30 formalin solution at 124° to 126° F. for 4 minutes; and lot 4 was held for 24 hours and then soaked in 1:30 formalin solution at 124° to 126° F. for 4 minutes. All lots were covered with canvas for 1 hour after treating and then spread out and allowed to dry before

planting. The seed was not injured by the hot water treatment, but this treatment spread ring rot as 22.5 per cent of the plants grown from this seed were infected whereas only 6.5 per cent of the plants grown from the non-treated seed developed the disease. The hot formalin solution gave no control of ring rot because infection was 6.6 and 8.6 per cent in lots 3 and 4, respectively. The hot formalin injured the cut seed and reduced the stand of plants 54 per cent in the seed treated immediately after cutting and 30 per cent in seed treated 24 hours after cutting.

In Minnesota disinfectants were used on cut seed to prevent the spread of ring rot. Seed was cut with a knife drawn through a ring-rot tuber before cutting each seed tuber. These results are shown in table 7.

Because acetic acid has given promising results in the control of bacterial canker of tomatoes, and *Phytophthora septentrionalis* is related in some ways to *P. michiganensis*, investigations were conducted at the Ottawa Central Experiment Station in Canada to determine if the ring rot organisms might be sensitive to low concentrations of acetic acid. Sets were cut from healthy tubers under a suspension of the ring-rot organism, allowed to stand 20 minutes, and dipped 1 minute into acetic acid solutions varying in concentrations from 0.25 to 5 per cent, allowed to dry completely, and then planted in the field. After 1 week some sets were dug to study the effect of the acid on cork formation and set decay. The acid greatly inhibited cork formation, particularly in the higher concentrations used, resulting in the rotting of sets and a high percentage of misses. Moderate disease control was obtained with the 5-per cent solution, but very low yields; the other concentrations gave very poor disease control, and also reduced yields. Acetic acid did not prove at all practical for the control of ring rot in cut sets.



TABLE 7.—Percentage of ring-rot-infected hills from seed variously treated after cutting. Minnesota, 1941.

TREATMENT <sup>1</sup>	TREATED IMMEDIATELY		TREATED 15 MINUTES AFTER CUTTING	
	AT HARVEST <sup>2</sup>	TOTAL	AT HARVEST	TOTAL
	Per Cent	Per Cent	Per Cent	Per Cent
Acid mercury	8.0	38.0	9.0	28.0
Cinnex Special <sup>3</sup>	8.0	25.0	4.0	8.7
Semesan Bel	45.3	71.7	20.0	46.0
Dust Treatment <sup>4</sup>	22.3	47.7	18.7	49.3
Check	54.7	85.0	56.3	84.3

<sup>1</sup>Seed was dipped about 2 minutes in acid mercury or 15 to 20 seconds in Cinnex or Semesan Bel. The dust was shaken over the cut seed as recommended by the manufacturer.

<sup>2</sup>Percentages are based on the number of hills with visibly rotted tubers at harvest time. Tubers from apparently healthy hills were stored until February and examined for ring-rot then. These figures were added to those found at harvest time to give the records.

<sup>3</sup>Cinnex Special contains yellow oxide of mercury and 1 per cent of iodine.

<sup>4</sup>The dust contained powdered mercuric chloride as the active ingredient.

Tests were conducted in Canada both in the greenhouse and in the field with various chemicals as disinfectants for cut sets. The sets were cut from healthy tubers with a knife contaminated by cutting through diseased tubers. In a few cases the knife was contaminated by dipping it into a suspension of a virulent culture of *P. sepedonica*. The sets were allowed to stand about 30 minutes before treating them, and then planted on the following day. The results are briefly as follows:

One-per cent copper sulphate solution used as a dip or as a 5-minute soak, and a 2-per cent solution as a dip, gave very variable results both in injury to sets and in control of ring rot. Formalin, 0.5-per cent, as a 10-minute soak, and 1-per cent as a dip, gave poor results and caused serious injury to sets. Semesan and Semesan Bel, 0.25-per cent and 2-per cent as dips, gave practically no control, although they caused no injury to sets. Lime-sulphur, 1.4-per cent, as a 1-minute dip and as a 5-minute soak, gave poor control, but no injury to sets. Iodine, 0.5-per cent in 1-per cent potassium iodide, and 0.5-per cent potassium

permanganate, gave no control of ring rot and caused no injury to sets. Mercuric cyanide, 0.2-per cent as a dip, and 0.2-per cent mercuric chloride in 1-per cent hydrochloric acid used as a dip, gave 97 per cent and 95 per cent control, respectively, and caused no injury to the sets.

No appreciable injury to sets and no appreciable control were secured with the use of a 5-per cent solution and 30 minutes of soaking of the following chemicals: Aluminum potassium sulphate, lead acetate, magnesium sulphate, manganese sulphate, sodium thiosulphate, calcium hypochlorite, and sodium thiocyanate. Sodium borate gave no injury to sets but gave variable control. Zinc chloride gave varying amounts of injury and moderate control. Potassium pyrosulphite and potassium dichromate killed practically all the sets. Potassium ferrocyanide and sodium sulphate killed 50 per cent or more of the sets, and gave only moderate control.

In Idaho the effect of various treatments was tested on Netted Gems, which contained 5 per cent infected tubers, using approximately 200 seed pieces in each treatment. After this seed was cut, without disinfecting either knife or cut seed, and planted with a 2-man planter, 88 per cent ring-rot plants developed; the seed pieces planted with a picker planter developed 81 per cent diseased plants. When the seed was cut with a knife disinfected with B-K (8,000 p.p.m.), 4.5 per cent diseased plants developed; when acidulated mercuric chloride solution was used 6.7 per cent ring rot developed. Five-per cent Lysol reduced the amount of diseased plants to 13 per cent; but 10-per cent formalin proved to be quite ineffective as a disinfectant, as 26 per cent of the plants showed ring-rot symptoms.

None of the various materials tested as a disinfectant for the cut seed showed any promise of control. In the checks where no disinfectant was used 89 per cent ring rot was evident. Acidulated mercuric chloride dip for 10 minutes gave the best results, but 24 per cent of the resulting plants showed ring-rot symptoms. Very little reduction in infection or none at all was caused by Semesan Bel, 1:1,000, Brilliant Green, or B-K at 8,000 p.p.m. dilution.

In Kansas seed pieces were cut with a knife previously contaminated by insertion into a ring-rot-infected tuber between every cut. One-half of the seed pieces of sack A was dipped 10 minutes in corrosive sublimate at 6 ounces to 25 gallons of water plus hydrochloric acid to give a 1-per cent concentration. One-half of the seed pieces of sack B was treated for 10 minutes in calcium hypochlorite at a concentration of 19 grams of 50-per cent calcium hypochlorite to 5 gallons of water. The other two halves of sacks A and B were left untreated. The two

untreated and the two treated lots of seed pieces were planted while still wet with a commercial potato planter in adjoining rows, approximately 550 feet long, in a field of commercial potatoes. A similar lot was planted on a different farm.

The results showed that a considerably higher percentage of the plants from the two treated lots of seed pieces were alive at harvest time than from the corresponding two untreated lots. There was little difference in the number of plants with decaying tubers at harvest time following either treatment or in the corresponding untreated controls. Yields were much greater from the treated than from the untreated lots. Higher yields were obtained from the lot treated with acidulated corrosive sublimate than from the lot that had been treated with calcium hypochlorite.

These tests like those conducted in Kansas in 1940 indicate the value of treating ring-rot-contaminated cut seed pieces with acidulated corrosive sublimate or calcium hypochlorite before they are planted. The treatment with acidulated corrosive sublimate is apparently the more desirable of the two methods tested, because it was followed by higher yields in both plots. On the basis of these tests, the corrosive sublimate treatment is recommended because it is also effective for the prevention of *Rhizoctonia* infection and seed piece decay.

#### EFFICIENCY OF COMPOUNDS AS STORAGE DISINFECTANTS

In Wyoming tests were conducted to determine different compounds as storage-cellar disinfectants by simulating conditions comparable to actual storage conditions. Short pieces of boards were inoculated by rubbing them with tubers infected with ring rot. These boards were left in the storage cellar. The various solutions were then sprayed on them at intervals of 30 minutes and 16 hours, and healthy cut tubers (10 for each test) were rubbed over these surfaces to pick up the ring-rot bacteria.

To test the effectiveness of the vapors, 2-quart fruit jars were used each with 250 cc. of the solution in the bottom of the jar. A narrow board was inoculated, as above, and put in each jar, this resting in each case above the solution and exposed to the vapors of the closed jar. At intervals of 5 hours and 24 hours the boards were removed and likewise rubbed with the cut portion of healthy seed pieces (10 for each treatment).

These seed pieces were planted and the plants examined later for ring rot to determine whether the treatments were effective. The results are shown in table 8.

TABLE 8.—*The effectiveness of chemicals as storage cellar disinfectants. Wyoming.*

DISINFECTANTS USED	RING-ROT RESULTING			
	CONTAMINATED BOARDS SPRAYED		CONTAMINATED BOARDS EXPOSED TO FUMES	
	TESTED 30 MIN. AFTER INOCULA- TION	TESTED 16 HOURS AFTER INOCULA- TION	TESTED 5 HOURS AFTER INOCULA- TION	TESTED 24 HOURS AFTER INOCULA- TION
	<i>Per Cent</i>	<i>Per Cent</i>	<i>Per Cent</i>	<i>Per Cent</i>
B-K, 2,000 p.p.m. ....	0.0	0.0	50.0	20.0
B-K, 1,000 p.p.m. ....	10.0	10.0	60.0	20.0
Furfural, 1:60 ....	30.00	20.0	90.0	20.0
Furfural, 1:120 ....	80.0	20.0	60.0	10.0
Steri-color, 1,000 p.p.m. ....	10.0	0.0	80.0	20.0
Steri-clor, 1,000 p.p.m. ....	10.0	0.0	100.0	50.0
Formaldehyde, 1:120 ....	30.0	0.0	60.0	40.0
Copper sulfate, 1 lb. to 50 gallons ....	10.0	0.0	1	1
Control, none ....	60.0	10.0	100.0	100.0

<sup>1</sup>Copper sulfate not tested for action of fumes.

In general, the fumes alone, even after 24 hours, were not so effective as the solutions acting for 5 hours. When tested after 16 hours the following solutions, when sprayed on the contaminated boards, gave good control of ring rot: B-K, 2,000 p.p.m.; Steri-chlor, 500 and 1,000 p.p.m.; formaldehyde 1:120; and copper sulfate 1 pound to 50 gallons of water. None of the fumes acting for 24 hours completely controlled ring rot.

#### ACCURACY OF THE ULTRAVIOLET-LIGHT METHOD FOR SELECTING RING-ROT-FREE POTATO SEED STOCK

To obtain further evidence regarding the ultraviolet-light method extensive greenhouse experiments were conducted in Montana during 1941. These were designed primarily to compare the accuracy of the ultraviolet-light method with the gram-stain method for selecting disease-free seed stocks. From a stock of Netted Gem potatoes that had shown considerable ring rot in previous gram-stain tests, 148 tubers were selected at random for ultraviolet-light examinations, 148 for gram-stain tests, and 25 for a check. All uncut tubers were surface sterilized by soaking for 3 minutes in a solution of mercuric chloride,

1:1000, and the knives used in cutting the tubers were soaked for 10 seconds in a solution of mercuric chloride, 1:500. After examination two seed pieces approximately 2 ounces in size, one designated as A and the other as B, were cut from the area nearest the stem end of each tuber. Each seed piece was then planted in a pot in the greenhouse, which was kept at a temperature of 60° F.

By using the ultraviolet light method the tubers examined were classified into three groups: (1) Those that were considered to be free from ring rot, (2) those that were thought to be infected with ring rot, and (3) those that seemed doubtful because of fluorescence somewhat different from that in the second group.

The gram-stain test was made according to the method outlined by Savile and Racicot<sup>1</sup>, but modified by taking the sample around the entire vascular ring.

Approximately 1 month after planting, examinations were made for plant emergence. A few of the plants did not emerge because the seed piece rotted. In no case, however, was bacterial ring rot found in the non-sprouting seed pieces in a group determined as disease-free by either method. All the plants were allowed to grow to maturity, but before this time ring-rot symptoms developed in most of the plants in the presumably infected groups. Just prior to harvesting, gram-stain tests were made of every stem from the plants in each group, but no stem infection was found in either one of the two groups considered to be free from ring rot.

After harvest, every tuber grown in the pots and many of the old seed pieces remaining in good condition were carefully examined by the gram-stain method for the presence of bacterial ring rot. Again, none of the disease was found in the groups selected as healthy by either the ultraviolet-light examinations or the gram-stain tests. Most of the old seed pieces from the diseased groups were too badly decomposed to permit further examination. The results obtained indicate that (1) the accuracy of the ultraviolet-light method was just as effective as the gram-stain method, (2) the ultraviolet light examinations were made in less than one-tenth of the time necessary for the gram-stain tests, and (3) the ultraviolet-light method had additional value in detecting and thus reducing the incidence of other vascular disorders.

Tests with fluorescent light in Colorado confirmed the fact that the light is more effective in detecting ring-rot symptoms at 40° F. than at 70°. Of a lot of 534 tubers known to contain 29 per cent ring

<sup>1</sup>Savile, D. B. O., and Racicot, H. N. 1937. Bacterial wilt and rot of potatoes. *Sci. Agr.* 17: 518-522.

rot, tubers classified as healthy on the basis of light examination at 70° F. were rechecked by microscopic examinations using the gram-stain, and by this method 3.4 per cent infected tubers were found. Another lot of 527 tubers containing 24 per cent ring rot was examined by the light at 40° F. Those classified as healthy were again examined by the gram-stain method and were found to contain 0.44 per cent ring rot.

In Colorado several species of insects were tested as possible carriers of ring rot. It was found that several of these are capable of carrying the ring-rot organism from the foliage of infected plants to the foliage of healthy plants. This was determined by petiole smears. Whether or not the migration of the bacteria in the plants is rapid enough to infect the tubers is still under study.

#### SUMMARY

The results in these reports show that potato ring-rot does not spread from hill to hill or from row to row.

The causal organism, *Phytophthora septentrionalis*, may overwinter in infected tubers in the soil, but experiments on the overwintering of the organism in the soil itself gave negative results in practically all tests. The exception was a test in Wyoming, which indicated that under some conditions the organism may overwinter in the soil, but in relatively small numbers.

As in the past, mercuric chloride and acidulated mercuric chloride solutions were highly effective, and iodine was almost as effective in disinfecting cutting knives, whereas practically all other chemicals tested gave unsatisfactory results. Injury to the seed pieces due to these treatments varied in different areas from serious to practically none. Boiling water in exposures of 5, 10, and 15 seconds gave good results and offers possibilities as an aid in the control of ring-rot.

Corrosive sublimate, 1:500, gave some of the best results in controlling the spread of ring-rot between healthy and infected seed pieces.

Acidulated mercuric chloride and Cinnex Special also gave very satisfactory control. Injury to seed pieces due to those treatments varied in different areas from serious to practically no injury at all.

Many disinfectants when used as a spray gave highly satisfactory results in disinfecting pieces of wood contaminated with the ring-rot organism. Fumes of chemicals tested were in practically all cases ineffective.

The ultraviolet-light method for the detection of affected tubers continues to give satisfactory results, but this process must be operated under proper conditions.