1) COLORADO

3) TECHNICAL BULLETIN 30 4) FEBRUARY 1943

Location and Movement OF THE Causal Agent of Ring Rot IN THE Potato Plant

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Location and Movement of the Causal Agent of Ring Rot in the Potato Plant[1]

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Contents

SINCE THE first report of the occurrence in Colorado of the bacterial ring-rot disease of potato (*Solanum tuberosum L.*) caused by *Phytomonas sepedonica* (Spieck.) Magrou (11, 14)[3], the emphasis has been on the prevention of spread of the malady (5, 11, 15 16). Within the past year, however, it became evident that the use of certified seed, developed from apparently disease-free foundation stock, and the automatically disinfected rotary knife in cutting seed had checked the ravages of ring rot. Consequently more attention has been given to the location and movement of the pathogen in the host tissue and the possible effect of these functions on the spread of the ring-rot disease.

Certain findings are presented in this bulletin regarding (a) the initial infection of potato seed pieces by *Phytomonas sepedonica,* (b) the movement of the bacteria from seed pieces

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[1] Cooperative investigations of the Sections of Botany and Plant Pathology and Horticulture.

[2] The writers gratefully acknowledge the assistance of Mr. E. W. Bodine of the Section of Botany and Plant Pathology during the course of certain phases of this work. They also wish to thank Mr. Ralph Manuel, formerly of the Section of Horticulture, for his assistance in the ultraviolet-light studies presented herein. In addition they wish to express appreciation to Professors L. W. Durrell and A. M. Binkley of the Sections of Botany and Plant Pathology and Horticulture respectively and Dr. D. P. Glick of the Pathology and Bacteriology Section for their helpful suggestions and cooperation.

[3] Numbers in parentheses refer to bibliography, page 27.

into the potato plant, (c) the tissue elements involved in such movement, (d) the incidence of infection in tubers harvested from diseased plants, (e) the use of ultraviolet light to detect infection of tubers, (f) the complete breakdown (soft rot) of tubers originally infected with *Phytomonas sepedonica*, and (g) the movement of *P. sepedonica* after artificial inoculation of the above-ground portion of the plant.

The criterion upon which all disease readings recorded herein was based was the presence of bacteria of uniform and characteristic morphology showing a positive reaction to Reed's Gram stain (17). Only smears showing a relatively general distribution of such bacteria were considered as definite proof of infection. The presence of a very few Gram-positive bacteria, or the presence of both Gram-positive and Gram-negative bacteria, or the presence of Gram-positive bacteria showing considerable variation in morphology in an entire smear were not considered as proof of infection. Relatively few such smears were encountered. In the great majority of smears, the presence or absence of *P. sepedonica* was quite definite. These arbitrary standards have been tested in the greenhouse and field and have been found to be reliable.

PENETRATION OF *Phytomonas sepedonica* INTO POTATO SEED PIECES

According to Bonde (3) and Starr and Riedl (20) infection of potato tubers by *P. sepedonica* occurs readily through bruises or injuries. Bonde introduced the organism into wounds in tubers in the fall of the year. The bacteria remained viable and when the tubers were planted the following spring they produced diseased plants. A study by Starr (19) showed that although tuber infection by *P. sepedonica* did not occur through the unbroken skin between the eyes, infection apparently took place readily through the uninjured eyes.

In order to study the penetration of *P. sepedonica* into potato seed pieces, investigations were conducted both in the greenhouse and in the field. Certified Bliss Triumph tubers were thoroughly washed and dried and then halved with a knife which was disinfected before each cutting operation. One half of each tuber was then subjected to a given type of inoculation, while the corresponding half was planted as a control for possible tuber-borne infection.

In one series of inoculations, a suspension of ring-rot bacteria was made by using the necrotic tissue of vascular cylinders of diseased tubers as a source of inoculum.[4] Drops of the inoculum were placed on slides, dried, and stained to verify the presence of ring-rot bacteria. With a clean camel-hair brush, inoculum was then painted on the freshly cut surfaces of tubers, on cut surfaces which had been allowed to stand for periods of 24 hours and 48 hours, on the uninjured skin surfaces, on "scuffed" surfaces, and on skin surfaces on which the lenticels had been forced by exposure to high humidity.

In a second series of inoculations, tuber halves in one lot which previously had been cut aseptically were then recut with a knife which was contaminated by cutting a diseased tuber prior to cutting each healthy tuber. These tubers were allowed to stand for 48 hours; then two-thirds of them were washed in warm soap suds, rinsed in water, then in 95 percent ethyl alcohol, and allowed to dry. The unwashed third was held as an inoculated control. With a disinfected knife, slices of tissue approximately 2 millimeters in thickness were removed from the previously cut surfaces of one-half the washed seed pieces and 5-millimeter sections were removed from the inoculated ends of the remainder.

All seed pieces were numbered and wrapped separately in clean paper to prevent contact spread of ring-rot bacteria. In planting, the hands of the operator were washed in running water before planting each series. All field readings were made after 85 days, while greenhouse readings were made at the end of a 75-day period. Separate smears were made for microscopic examination from all vines of all plants; these smears were taken in each case from that portion of the stem just at or slightly below the ground line, according to the method described by Metzger and Glick (15).

In the field a total of 480 plants were tested, from which 1,279 vine smears were stained and examined. In the greenhouse 70 plants and 261 vines were tested. For the sake of completeness, both field and greenhouse results are presented in table 1.

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[4] It was found that pure cultures of *P. sepedonica* were not always reliable sources of inoculum because of an apparent loss in pathogenicity. Further, the use for inoculum of potatoes which showed complete breakdown following ring-rot infection was abandoned because of failure to obtain infection. Since the necrotic material forced from the vascular rings of tubers showing early ring-rot symptoms proved reliable in obtaining uniform ring-rot infection, this material was used throughout the work reported herein as a standard source of inoculum.

TABLE 1.—PENETRATION OF *Phytomonas sepedonica* INTO THE POTATO SEED PIECE.

Type of seed-piece inoculation	Field results			Greenhouse results		
	Plants diseased	Vines infected	Vines infected per diseased plant	Plants diseased	Vines infected	Vines infected per diseased plant
	percent	percent	percent	percent	percent	percent
Inoculum painted on freshly cut surfaces (half tubers)	33.0	19.4	56.0	——	——	——
Control (half tubers)	0.0	0.0	0.0	——	——	——
Cut surfaces allowed to stand for 24 hours before painting with inoculum (half tubers)	6.6	4.2	50.0	——	——	——
Control (half tubers)	0 0	0.0	0.0	——	——	——
Cut surfaces allowed to stand for 48 hours before painting with inoculum (half tubers)	0.0	0.0	0.0	0.0	0.0	0.0
Control (half tubers)	0.0	0.0	0.0	0.0	0.0	0.0
Inoculum painted on uninjured skin (half tubers)	13.8	7.0	54.5	0.0	0.0	0.0
Control (half tubers)	0.0	0.0	0.0	0.0	0.0	0.0
Skin surfaces "scuffed" and painted with inoculum (half tubers)	32.1	17.1	64.7	——	——	——
Control (half tubers)	0.0	0.0	0.0	——	——	——
Lenticels forced and painted with inoculum (half tubers)	——	——	——	0.0	0.0	0.0
Control (half tubers)	——	——	——	0.0	0.0	0.0
2 mm. removed from cut ends 48 hours after knife inoculation (half tubers)	47.8	25.0	57.1	83.3	42.1	57.1
Control (half tubers)	0.0	0.0	0.0	0.0	0.0	0.0
5 mm. removed from cut ends 48 hours after knife inoculation (half tubers)	3.3	1.3	50.0	——	——	——
Control (half tubers)	0.0	0.0	0.0	——	——	——
Seed pieces knife inoculated (half tubers)	84.6	56.1	69.5	90.0	71.0	84.3
Control (half tubers)	3.6	1.4	50.0	0.0	0.0	0.0

The data shown in table 1 indicate that suberization and cork formation are directly responsible for reduction in contact infection by ring-rot bacteria, in that inoculated freshly cut surfaces or injured surfaces gave 33.0 percent and 32.1 percent infection respectively. Inoculation after 24 hours reduced infection to 6.6 percent, while painting surfaces which had been allowed to suberize for 48 hours gave no infected plants. Painting expanded lenticels or uninjured skin surfaces in greenhouse trials likewise gave no infection. However, in the field studies 13.8 percent infection was obtained by painting uninjured skin surfaces.

There are several possible explanations for this discrepancy between greenhouse and field trials. The eyes of the seed pieces used in the greenhouse studies were quite dormant when planted, while in field plantings the tubers had begun to sprout. Since the skin and eye surfaces were inoculated in both cases, injury to the sprouts might account for infection in the field trials. Further, injury to any portion of the seed piece would be far more likely to occur in the field than in the greenhouse. The low percentage of infection (3.6) obtained in one of the control series can be explained on the basis of natural tuber-borne infection.

Inoculation with a contaminated knife gave the greatest number of infected plants. Removal of a layer of tissue 2 mm. in thickness from the inoculated surfaces reduced infection from 84.6 percent (inoculated control) to 47.8 percent. Removal of 5 mm. further reduced infection to 3.3 percent. These results indicated that to treat effectively seed pieces that have been cut with a contaminated knife, the disinfecting agent would need to penetrate the tuber tissue more than 5 mm. from the cut surface.

Of 240 diseased plants grown from artificially inoculated seed pieces (table 1), only 57.9 percent of the vines produced by these plants were infected.

SEED TREATMENT STUDIES.—Attempts to control ring-rot infection by treatment of the seed pieces following cutting with contaminated knives have not been particularly successful. California workers (10, 1) using iodine (0.5 percent in 1 percent KI) potassium permanganate, acidified $HgCl_2$ solution, and certain organic mercurials to treat knife-inoculated seed pieces failed to control ring rot effectively. In addition studies in Colorado during the past 3 years have shown that iodine (0.5 percent in 1 percent KI), 1 to 500 $HgCl_2$, and organic mercurials such as hydroxymercurinitrophenol were ineffective in controlling ring rot when used as seed treatments.

Since it was believed that the penetrating properties and toxicity of solutions of mercuric chloride might be increased by the addition of other chemicals, such a study was conducted in the greenhouse. Subjecting knife-inoculated seed pieces to 5-minute treatments of 1 to 500 $HgCl_2$ gave 83 percent infection in the resulting plants. The same solution containing 1 and 5 percent of glacial acetic acid, 5- and 10-percent ethyl alcohol (95 percent), and 1- and 3-percent tergitol, gave 50 to 75 percent infection in the plants developing from these seed pieces. Untreated inoculated seed pieces gave 90 to 100 percent infection in the resulting plants. Solutions of 1 to 500 $HgCl_2$ containing glacial acetic acid proved more effective (50 to 60 percent infection) as disinfecting agents than did other solutions tested. No solution used, however, effectively reduced infection by *P. sepedonica* following knife inoculation. Although these results indicate that seed treatments could not be substituted for the use of the automatically disinfected rotary cutting knife, it should not be inferred that seed treatments are valueless in preventing contact spread of infection (11).

MOVEMENT OF *P. sepedonica* INTO THE POTATO PLANT FROM INFECTED SEED PIECES

It was felt that the position of the ring-rot bacteria in the potato plant and the time of their appearance in different portions of the plant were important in any consideration of spread and control of the disease. With that in mind, comparative studies on the rate of migration and the relative numbers of bacteria present in different parts of the plant were made with both naturally infected and artificially infected plants.

MIGRATION OF *P. sepedonica* FROM INFECTED SEED PIECES INTO SPROUTS.—In the study of the movement of the ring-rot organism from the seed piece into the potato plant, certified Bliss Triumph tubers first were halved with a disinfected knife. Half of each tuber was then placed immediately in moist sphagnum moss as a control, while the remaining half was held for inoculation. Inoculations were made either by needle or contaminated knife. Needle inoculations were made by stabbing a needle through drops of inoculum placed in several eyes of the seed piece.

The naturally infected tubers used in the study had been harvested the previous fall from plants that were known to be diseased. The stem ends of these tubers were removed first, a disinfected knife blade being used for each cutting operation, and tubers showing symptoms of ring rot were separated from those showing no symptoms. Both tuber lots were then placed in separate flats and embedded in moist sphagnum.

All tubers were held in flats of moistened sphagnum at temperatures approximating 75° F. for 75 days. At intervals of 10, 20, 30, 45, 60, and 75 days the developing sprouts from the tubers were tested by the Gram-stain method. Smears in all instances were made from the bases of the sprouts. The results of this study are shown in table 2.

TABLE 2.—THE RAPIDITY OF MIGRATION OF *P. sepedonica* FROM NATURALLY AND ARTIFICIALLY INFECTED TUBERS INTO DEVELOPING SPROUTS.

Type of inoculation	Tuber symptoms of ring rot	Number tubers or seed pieces planted	Number tubers failing to grow	Vine examinations (Gram stain) — Number of tubers producing diseased and healthy shoots in											
				10 days		20 days		30 days		45 days		60 days		75 days	
				d	h	d	h	d	h	d	h	d	h	d	h
Natural infection	visible	40	22	—	—	—	—	17	1	—	—	18	0	—	—
Natural infection	visible	25	10	1	14	10	5	11	4	—	—	14	1	—	—
Natural infection	Not visible	31	5	—	—	—	—	0	26	—	—	13	13	14	12
Knife inoculation	—	16	0	0	16	2	14	—	—	10	6	13	3	—	—
Eye stab	—	12	0	1	11	1	11	—	—	5	7	10	2	—	—
Control	—	15	0	0	15	0	15	—	—	0	15	0	15	—	—

The tubers showing visible ring rot (table 2) produced sprouts in which early detection of the ring-rot bacteria was possible. In one lot the bacteria were observed in stem smears from two-thirds of the plants at the end of 20 days, while in a similar lot of seed 17 out of 18 plants were infected after 30 days. Plants from naturally infected tubers showing no visible symptoms failed to produce stems showing infection at 30 days, but one-half the plants gave positive smears after 60 days. The amount of stem infection obtained was generally as great from the knife or eye-stab inoculated tubers as from naturally infected tubers showing no ring-rot symptoms. Although a few infected stems were found at 20 and 10 days respectively, the number of infected plants was not great until after 45 days.

It has been found that tubers showing ring-rot symptoms carry many more bacteria than infected tubers which show no symptoms. Accordingly the data in table 2 indicate that there is a direct relation between the relative numbers of bacteria and the rapidity of their movement into the sprouts. In addition, although it would appear that inoculations of the eyes should bring about a more rapid movement of bacteria in the developing sprouts than knife inoculations, such was not the case. A possible explanation is that a far greater amount of bacterial inoculum was introduced into the tuber by the knife than by the needle.

MIGRATION OF *P. sepedonica* INTO MATURING VINES.—A study was conducted next to obtain information regarding movement and distribution of *P. sepedonica* in maturing potato vines. In trials carried out in both the greenhouse and the field, both apparently disease-free and naturally infected Bliss Triumph tubers were used as a source of plant material. The disease-free tubers were cut in half with a disinfected knife and one half of each tuber was planted as a control. The other half was then inoculated by recutting with a contaminated knife. All naturally infected tubers used in the study showed the characteristic tuber symptoms of ring rot. In addition, tubers showing such symptoms were always tested as a precautionary measure by use of the Gram stain.

The stems of all vines of all plants developing from these tubers were cut and smeared, beginning 1 inch above the point of the seed-piece attachment and continuing at 3- to 4-inch intervals to the apical growing point. In addition, the bases of all leaf petioles were smeared and examined. Representative roots, stolons, and tubers were also tested in like fashion for each vine studied. Complete smear studies were made of 30 plants, and from 20 to 45 smears were made from roots, stolons, stems, and leaves of each plant. All smears were made within a 60- to 80-day interval following planting.

Because of the variation of the distribution of the organism in the resulting infected plants, diagrams were made of representative situations. These are shown in figure 1. The results of this study show that in plants developing from both artificially and naturally infected seed pieces, infection at the end of 60, 70, or 80 days was more marked in the subterranean parts (roots, stolons, and underground stem) than in the aerial por-

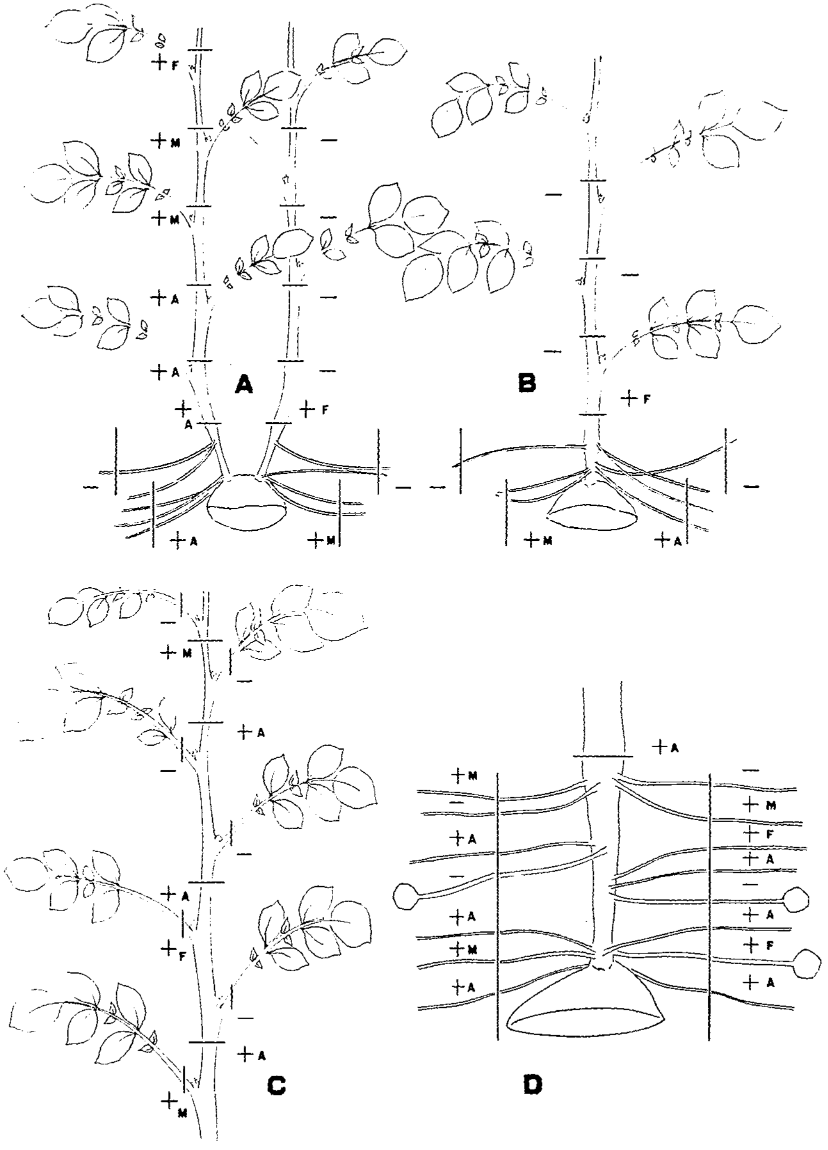

FIGURE 1.—The movement and distribution of *Phytomonas sepedonica* in the tissues of the potato plant. A.—Infected 80-day-old plant showing one diseased vine and the other almost free of bacteria. Subterranean infection is greater nearest the infected vine. B.—Diseased 70-day-old plant showing light aerial infection and more marked subterranean infection. C.—Complete and severe stem infection showing leaves with light to moderate to no infection. D.—Subterranean infection showing more severe root infection than stolon infection. Symbols: + = presence of *P. sepedonica*; — = absence of *P. sepedonica*; a = organism abundant; m = organism in moderate numbers, and f = organism few in number.

tions. Infected plants grown in the greenhouse invariably showed a greater degree of aerial (stem and leaf) infection than corresponding plants grown in the field within the same time duration. By the same token, wilt symptoms were much easier to detect in greenhouse material than in the corresponding field material. Seventy days after artificial tuber inoculations, infection in several instances was found to be quite marked in the roots and underground stems, but no infection could be detected in the stem 2 inches or more above the ground line (fig. 1B). These findings in general agree with those of Haasis (6) who observed in infected volunteer plants that there were greater numbers of bacteria near the seed piece than in the upper portions of the plant.

Figure 1A illustrates one heavily infected vine and one with slight infection developing from the same infected seed piece. It may be observed that root and stolon infection is greater in the severely infected vine than in the lightly infected vine. While most of the roots and stolons were infected, there were always a few found in which no bacteria were observed. Figure 1D illustrates a condition of common occurrence with regard to subterranean infection. Roots were usually more severely infected than stolons. Figure 1C shows relatively heavy infection in the stem with moderate to no infection in the leaf petioles. In general in such cases lower petioles were more likely to be infected than upper petioles.

TISSUE ELEMENTS INVOLVED IN THE MOVEMENT OF *P. Sepedonica*.—Savile and Racicot (18) and Racicot, Savile, and Connors (17) first indicated that *P. sepedonica* moves within the vessels of the potato plant. Savile and Racicot also observed that the causal bacteria gradually escape from the vessels into the intercellular spaces. Larson et al (12) observed *P. sepedonica* in the xylem of artificially infected tomato plants and noted intercellular progress of the bacteria into the parenchyma of the pith and cortex.

Results of studies by the writers on both infected potato and tomato plants have agreed with these observations. Stained sections of diseased potato and tomato stems show that *P. sepedonica* moves in the plant principally in the conducting elements of the xylem (figs. 2A and B). Further, the causal bacteria appear to escape from the vessels in the xylem parenchyma (fig. 2B).

Indications of escape of the bacteria into the parenchyma of the cortex and pith have also been observed. This phenomenon has been observed not only in mature tissues but in the primordial tissues near the apical meristem. In addition it appears that the primary tuber symptom of the ring-rot disease, which is a disintegration of the principal vascular system, is due to the escape of *P. sepedonica* from the vessels of the vascular ring into the adjoining parenchymatous tissues which results in a necrotic breakdown of the invaded areas. Further, it has been observed that this breakdown occurs over a period of time in storage.

A study of plants grown from naturally infected and artificially inoculated tubers in the greenhouse has shown that under this environmental condition aerial symptom manifestation (wilt) is largely dependent upon the degree of vascular invasion. Pronounced wilt and death of leaves is invariably accompanied by the presence of bacterial masses in many of the xylem conducting elements in the vascular bundles of the stem, petioles, and leaves. Sections taken from the stems of plants which were infected but exhibited no symptoms frequently showed but one or two vessels invaded.

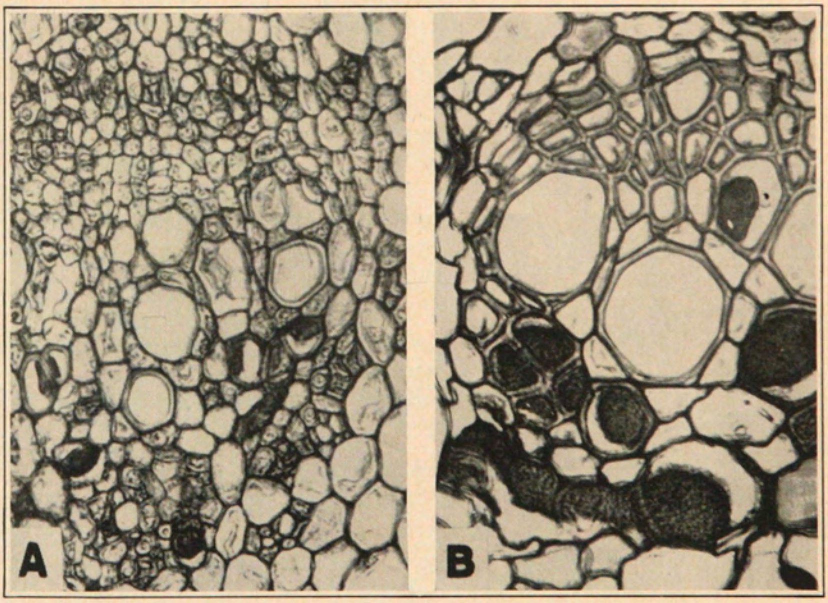

FIGURE 2.—*Phytomonas sepedonica* in the tissues of its hosts. A.—Cross section of the young potato stem near the growing point showing bacterial invasion of some of the conducting elements of the xylem (natural infection). B.—Cross section of tomato petiole showing bacteria in the vessels (artificial inoculation). Note in particular the bacterial masses in the xylem parenchyma adjoining the vessels.

INCIDENCE AND LOCATION OF INFECTION IN TUBERS HARVESTED FROM DISEASED PLANTS.—In order to determine whether all of the tubers produced by a given diseased plant were infected by *P. sepedonica,* all tubers were harvested from seven infected Bliss Triumph plants in the field. One or more tubers from each of these seven plants showed typical secondary soft rot. Such tubers were discarded. Tubers were numbered and kept in separate lots corresponding to the plant from which they were harvested.

A total of 71 sound tubers obtained from the seven plants were stored 112 days in a cool cellar. At the end of this time the stem end of each tuber was removed with a sterile knife. An observation was then made to determine whether symptoms of ring rot were evident. In addition, smears were made from at least four equidistant portions of the vascular system of each tuber in such a way as to include approximately one-half of the exposed vascular ring. Following this, the tubers were planted in the greenhouse in sterile pots containing steamed soil. Developing sprouts and vines were smeared at the end of 30-, 60-, and 75-day intervals. The results of this study are shown in table 3.

Of the 71 tubers studied, 40 showed ring-rot symptoms. All 40 were Gram-positive. Of these 40 tubers, only 18 produced plants, all of which were infected. The balance of the tubers studied, totaling 31, showed no visible symptoms of ring rot. When smeared and stained, 28 were negative, while only 3 were positive. Of the 26 plants which developed from these tubers, 14 were found to be infected. The data presented in table 3 indicate that uninfected tubers may be produced by an infected plant; tubers showing no symptoms and giving no positive Gram-stain reactions may still be infected and produce infected vines, and any tuber showing the typical soft-yellow-ring symptom will invariably give a Gram-positive stain reaction, and if it does not rot it will produce infected vines.

In a study of infected tubers, Haasis (6) found that bacteria were not always present in all parts of the vascular ring. Metzger and Glick (15) expressed the belief that the vascular rings of tubers so slightly infected as to exhibit no vascular discolorations might not have been totally invaded by *P. sepedonica.*

In order to obtain an indication as to the degree of bacterial invasion into tubers harvested from infected plants, tubers were selected at random from the yield of five diseased plants. One-half inch at the stem end of each tuber was removed with a knife blade which had been disinfected. Four to seven equidistant

TABLE 3.—THE INCIDENCE OF INFECTION IN TUBERS HARVESTED FROM DISEASED VINES.

Parent plant No.	Total sound tubers harvested per plant	Tuber symptoms present: Tuber readings (Gram-stain) No. +	No. —	Resultant vine growth from tubers (Gram-stain) No. +	No. —	No. tubers failing to grow	Tuber symptoms absent: Tuber readings (Gram-stain) No. +	No. —	Resultant vine growth from tubers (Gram-stain) No. +	No. —	No. tubers failing to grow
166-15	12	8	0	5	0	3	2	2	1	1	2
166-11	9	5	0	1	0	4	0	4	0	3	1
166-21	11	4	0	2	0	2	0	7	4	2	1
166-13	4	3	0	2	0	1	1	0	0	0	1
166-9	8	1	0	0	0	1	0	7	5	2	0
166-17	13	8	0	1	0	4	0	5	3	2	0
166-10	11	11	0	1	0	7	0	3	1	2	0
Totals	71	40	0	18	0	22	3	28	14	12	5

points in the vascular ring of each tuber were then tested for *P. sepedonica*. The tissue of approximately 5 millimeters of the vascular ring was crushed and smeared for each point tested.

P. sepedonica invariably was present in all portions of the of the vascular rings of tubers showing recognizable symptoms of ring rot. It was sometimes found, however, that tubers which did not show symptoms of ring rot yielded *P. sepedonica* from only one out of four or one out of seven points in the vascular ring. No symptomless tuber was found which showed *P. sepedonica* in all parts of the vascular ring.

USE OF ULTRAVIOLET OR "BLACK" LIGHT TO DETECT TUBER INFECTION.—From a practical standpoint the introduction of the use of ultraviolet light by Iverson and Kelly (8) for the detection of tuber infection by *P. sepedonica* was of interest to Colorado investigators and potato growers alike. However, there has been considerable speculation as to the accuracy of this method either for use in the laboratory or by individual potato growers. Harvey (7) indicated that ring rot could be determined definitely by characteristic fluorescence, when an infected tuber was exposed to ultraviolet light. On the other hand, Flint and Edgerton (4) considered the ultraviolet light to be unreliable in diagnosing tuber infection by *P. sepedonica*. Recently Iverson and Harrington (9) reported a study in which they concluded that the ultraviolet-light method is as effective as the Gram-stain method in detecting ring-rot infection in tubers.

For a study to evaluate the efficiency and determine the possible limitations of "black" light in the detection of tuber infection by *P. sepedonica* in Colorado, 120 tuber lots containing both red- and white-skinned varieties were harvested from naturally and artificially infected as well as healthy plants. Varieties used in the study were Bliss Triumph, Red McClure, Irish Cobbler, Katahdin, and Rural. The individual lots harvested ranged from no infection to all tubers showing symptoms of the disease.

Approximately one-half of the tubers were stored at 70° F. and the remainder at 40° F.[5] During a period of 2 to 5 months in storage all tubers were examined in a darkened room at the temperature at which they were stored. This was done by cutting away a portion of each tuber near the stem end and immediately exposing the cut surface to the ultraviolet light

[5] Iverson and Kelly (8) state that, "fluorescence appears best at low temperatures. Even a short exposure of tubers to higher temperatures reduces somewhat the effectiveness of the work."

emitted by a 220-volt, alternating current, H4-type mercury vapor lamp. The potatoes were next segregated according to their appearance under both ordinary and "black" light into the following arbitrary classes.

1. LIGHT-NEGATIVE. No visible symptoms when exposed to ordinary light. Very faint dull purple hue of the entire cut surface when exposed to ultraviolet light. This was not considered as fluorescence.

2. LIGHT-POSITIVE. No visible symptoms when exposed to ordinary light. Yellow to yellow-green or white to cream fluorescence under ultraviolet light in the vascular ring ranging in extent from an area of 1 millimeter to a fluorescence of the entire vascular ring.

3. VISIBLE RING ROT. Vascular discoloration (11) and breakdown visible under ordinary light. Typical tuber symptoms of ring rot.

Examinations by the Gram-stain technique were made of vascular tissues of representative tubers from each of these three categories. In the light-negative tubers the entire vascular ring was probed for smearing, while in the light-positive tubers only those areas showing fluorescence were examined. The results of 60 random lots read at 70° F. and 60 lots at 40° F., representing a total of 4,071 tubers examined, are shown in table 4.

There was considerable variation in the types of fluorescence shown by infected tubers. These reactions were influenced by both the variety and temperature at which the tubers were stored. At 70° F. a few of the white tubers (varieties Katahdin and Rural) showing marked visible ring-rot symptoms did not fluoresce. In such tubers mealy necrotic tissues forced from the vascular area either were light-negative or only faintly light-positive. No tubers manifesting such reactions were found in the red-skinned varieties. At 40° F. all visibly infected tubers of both red- and white-skinned varieties showed definite light-positive reactions. The fluorescences of infected tubers varied in color from white to cream to canary yellow at 70° F., with the yellow hue predominating. At 40° F. the fluorescences were uniformly pale-yellow to green-yellow.

The data indicate that temperature exerts a considerable influence on the efficiency of ultraviolet light in the detection of tuber infection by *P. sepedonica*. When potatoes were stored and examined at 70° F. an average of 13.9 percent of the light-nega-

TABLE 4—THE ACCURACY OF ULTRAVIOLET LIGHT IN DETECTING RING ROT INFECTION IN FIVE POTATO VARIETIES STORED AT TWO DIFFERENT TEMPERATURES AS DETERMINED BY THE GRAM-STAIN TECHNIQUE.

Variety	Storage temps. degrees F.	Light readings			Total No. tubers examined	Microscopic readings				Total No. smears examined
		Tubers showing visible ring-rot symptoms	Tubers light-positive	Tubers light-negative		Light-positive microscopically Positive	Light-positive microscopically Negative	Light-negative microscopically Positive	Light-negative microscopically Negative	
		Percent	Percent	Percent		Percent	Percent	Percent	Percent	
Katahdin	70	14.2	27.0	58.7	429	42.0	58.0	20.0	80.0	124
	40	12.8	35.6	52.0	202	16.7	83.3	0.0	100.0	69
Irish Cobbler	70	0.8	24.6	74.6	252	38.7	61.3	26.1	73.9	54
	40	4.4	58.0	37.6	338	7.1	92.9	0.0	100.0	78
Rural	70	4.1	17.1	78.9	246	54.6	45.5	21.7	78.3	56
	40	5.3	33.6	61.1	113	27.3	72.8	4.0	96.0	36
Bliss Triumph	70	1.8	17.5	77.8	1132	30.8	56.9	13.9	86.1	202
	40	6.9	39.3	53.7	661	11.1	88.9	0.0	100.0	138
Red McClure	70	5.3	22.9	71.8	433	32.6	67.4	0.0	100.0	103
	40	7.6	47.6	44.9	265	0.0	100.0	0.0	100.0	65
Average of	70	4.7	22.1	73.2	2492	42.1	57.9	13.9	86.1	539
5 varieties	40	7.1	43.8	49.1	1579	11.7	88.3	0.4	99.6	386

tive group were found to be microscopically positive. These tubers would have been regarded as being ring-rot free on the basis of ultraviolet-light examinations alone. Examination of similar lots at 40° F., however, allowed only 0.4 percent of the infected tubers to escape detection.

Several types of fluorescence were observed other than those listed. Brown vascular discolorations found associated with Fusarium infection showed a bluish-white fluorescence under the "black" light. Tubers manifesting this reaction were considered light-positive but were not subjected to smear tests. Surface injuries which showed fluorescence such as those caused by scab, worm track, or bruises were not considered light-positive if the injury could be cut away and if the vascular ring showed no fluorescence.

COMPLETE TUBER BREAKDOWN FOLLOWING INFECTION BY *Phytomonas sepedonica*.—In 1938, Racicot, Savile and Connors (17) stated that "when the bacteria (*P. sepedonica*) have spread through the tuber to an eye or eyes, the tissues in that region are often killed, rendering the tuber liable to invasion by soft-rotting organisms." Ark and Bodine (2) also expressed the belief that infection by *P. sepedonica* "is the precursor of the bacterial soft rot which has caused heavy losses." Later Kreutzer, Glick, and McLean (11) reported that evidence was obtained which agreed with these contentions.

The first step in investigating the soft-rotting phases of the ring-rot disease was to remove the stem ends of clean Bliss Triumph and Cobbler potato tubers with a disinfected knife. The stem ends were then tested for the presence of *P. sepedonica* by use of the Gram-stain technique. Following this the tubers were cut with a disinfected knife into slices approximately one-fourth inch thick and then transferred to moistened sterile Petri dishes. Each of the various inocula was prepared by mixing it in a few milliliters of sterile water in order to obtain a uniform suspension. A drop of inoculum then was transferred to the center of an exposed tuber slice by means of a loop needle. The inoculated slices were incubated at a uniform temperature of 25° C.

Inoculation of tuber slices with pure cultures of *P. sepedonica* or material taken from the necrotic yellow-ring areas of tubers showing early ring-rot symptoms invariably failed to produce soft rot.

When necrotic tissue was taken from the interior of tubers which had undergone complete breakdown and used as inoculum, marked rotting of inoculated slices was observed in from 24 to

48 hours. Cultures of *Erwinia carotovora* (Jones) Bergey et al (both soft-rotting and black-leg strains)[6] produced approximately the same type of rotting within the same time period. When tuber slices were allowed to stand in a moist Petri dish for from 12 to 24 hours before inoculation with *E. carotovora*, rotting was markedly reduced. However, when such slices were scraped prior to inoculation, rot progressed from three to four times more rapidly than on the suberized slices.

The use of mixed inoculum prepared by combining *P. sepedonica* with either the black-leg or soft-rot strains of *E. carotovora* gave no increase in the severity of the rotting of tuber slices over the use of strains of *E. carotovora* alone. However, where slices of tubers naturally affected with *P. sepedonica* and healthy tubers were used and inoculated with either of the two strains of *E. carotovora,* the indications were that slices from tubers affected with *P. sepedonica* showed a greater degree of rotting than those which were free of infection by *P. sepedonica* (fig. 3). It is felt that the degree of vascular invasion induced by *P. sepedonica* in a large measure determines the predisposition of a given tuber to attack by *E. carotovora.*

MOVEMENT OF *P. sepedonica* INTO THE POTATO PLANT FROM ARTIFICIAL AERIAL INOCULATIONS

In order to determine how readily *P. sepedonica* may be introduced into the leaf and aerial stem tissues of both potato and tomato plants, varying types of inoculation trials were conducted. The pathogenicity of *P. sepedonica* to the tomato (*Lycopersicum esculentum* Mill.) was reported by Larson et al (12) in Wisconsin studies in 1941. Since it was found that tomato plants reacted more rapidly to artificial inoculation with *P. sepedonica* than did potato plants and also showed subsequently a more rapid wilt manifestation, they were included with the potato as host material in this study.

Bonny Best tomato seedlings approximately 2 inches in height, when inoculated by halving a cotyledon on each plant with scissors previously dipped in a suspension of *P. sepedonica* showed 50-percent infection as based on wilting symptoms within an 11-day period. The stems of 25 plants showing symptoms all proved to be infected when subjected to the Gram stain.

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[6] Several of these cultures were isolated from rotted potato tubers which had been harvested from vines affected with the ring-rot disease. Others were obtained through the courtesy of Drs. P. A. Ark and J. G. Leach of the California and West Virginia Agricultural Experiment Stations respectively.

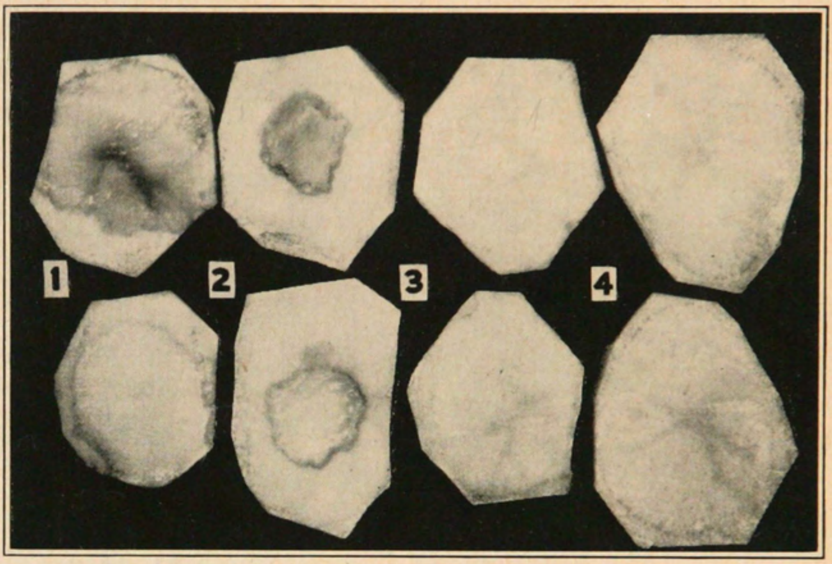

FIGURE 3.—The effect of infection by *Phytomonas sepedonica* on tuber rotting caused by *Erwinia carotovora* in two experimental trials. 1.—(Upper) potato tuber slice infected with *P. sepedonica* showing rot due to *E. carotovora* (black-leg type); (lower) slice infected with *P. sepedonica* showing rot due to *E. carotovora* (soft-rot type). 2.—(Upper) healthy potato tuber slice showing rot due to *E. carotovora* (black-leg type), and (lower) healthy potato slice showing rot due to *E. carotovora* (soft-rot type). 3.—Control noninoculated slices infected with *P. sepedonica* (upper and lower). 4.—Control noninoculated healthly slices (upper and lower).

When 6- to 8-weeks-old Earliana tomato plants were inoculated at the apical growing point symptoms became apparent in from 12 to 20 days (fig. 4). Three days after inoculation, bacteria could be found 3 to 4 inches below the point of inoculation and in 14 days the organism could be found throughout the plant. Where the middle of the stems of such plants were inoculated by stabbing a needle into the pith, the movements of bacteria were slower, moving 4 to 6 inches down the stem from the point of inoculation in 17 days. When tomato plants were inoculated at the ground line bacteria could be found after 14 days in the tap and secondary roots. Few cases were found where *P. sepedonca* was detectable at any appreciable distance above the point of inoculation.

In the study of the effect of aerial inoculations on potato plants, 48 plants of the Bliss Triumph variety averaging 8 to 10 inches in height and approximating 30 days in age were subjected to varying types of leaf and stem inoculations. Smear studies were made on each plant during an interval of 15 to 60 days following the inoculations. In each instance sufficient smears were made on each plant to determine the extent of the migration of *P. sepedonica*.

Leaf inoculations were successful only 57 percent of the time, whereas stem inoculations were successful 86 percent of the time. Removing the petiole by cutting with scissors carrying *P. sepedonica* (fig. 5 A2) proved to be the best method of leaf inoculation, while halving the terminal leaflet at a right angle with the midrib proved the least effective (fig. 5 A1). Leaf inoculations by stabbing the midrib (fig. 5 A3) or removing the petiole in many cases produced general plant infection, while halving the terminal leaflet never produced stem infection. In general bacteria which were introduced into the upper leaves or the stem were not observed in the underground parts of the plants in less than 45 days. It was also noted that inoculating upper leaves proved more effective than inoculating lower leaves. It was found that inoculation of the stem with a needle, scissors, or scalpel was effective in producing infection.

FIGURE 4. — Tomato plant (variety Earliana) infected with *P. sepedonica*. This plant was inoculated by the removal of the apical growing point with scissors which had previously been dipped into a suspension of *P. sepedonica*. Photograph taken 17 days after inoculation. Note the marked symptoms of wilt.

As in the case of the tomato study, it was noted that the movement of *P. sepedonica* following an aerial inoculation was almost entirely downward (figs. 5 B, D, and E). Few exceptions to this were found. The only upward movement of any appreciable distance observed was when the inoculation was made by cutting off the stem with scalpel or scissors. As a result of this injury a new terminal shoot or leader developed. Bacteria were invariably found in such a new leader or terminal shoot (fig. 5 C).

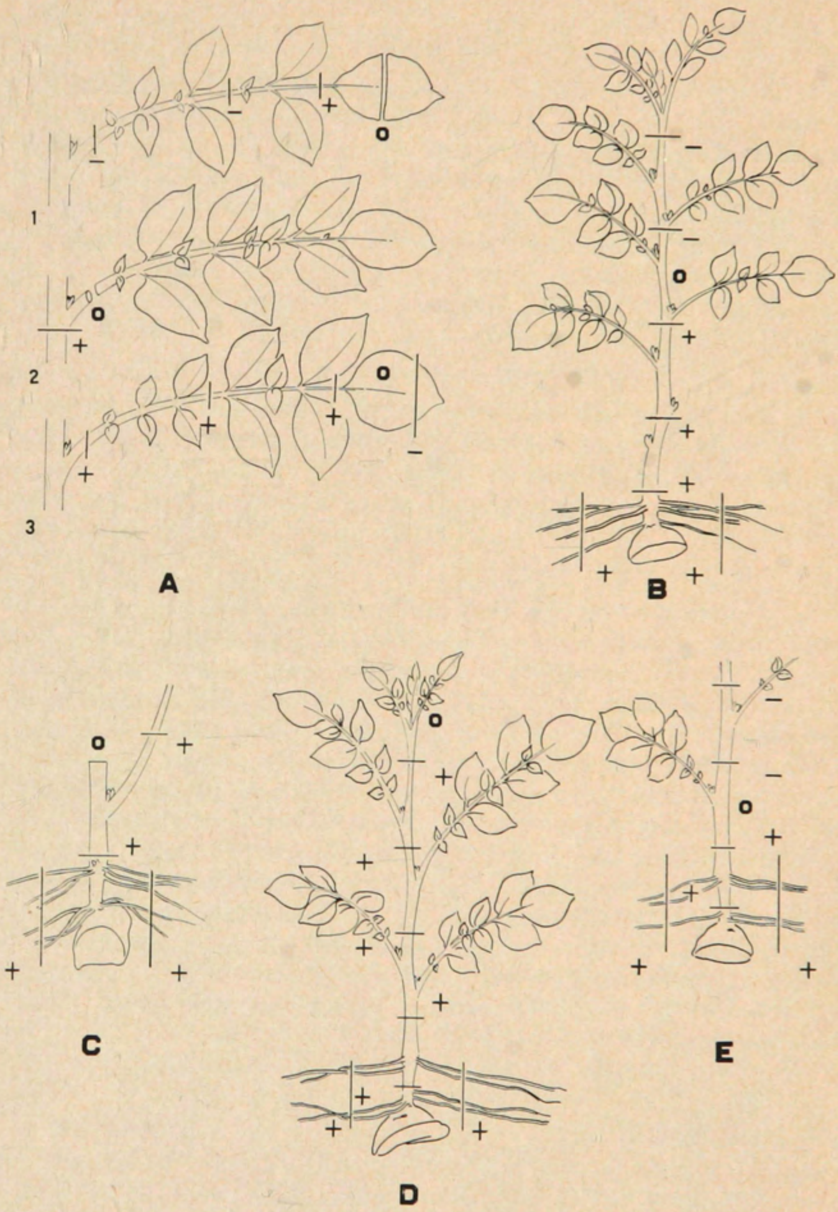

FIGURE 5.—Movement and distribution of *P. sepedonica* following artificial aerial inoculations as shown by representative diagrams. In each diagram "O" indicates the point at which the bacteria were introduced. A1.—Leaf inoculated by halving terminal leaflet, showing point of farthest movement. A2.—Leaf inoculated by cutting petiole. Stem and in many cases subterranean infection resulted in not less than 45 days. A3.—Leaf inoculated by stabbing terminal leaflet. All movement of *P. sepedonica* confined in direction of the stem. B, D, and E.—The result of inoculating different portions of the stem. In all cases the movement of the organism is downward. C.—Inoculation by removing the stem. Upward movement is observed here in the newly formed terminal growth.

DISCUSSION

By utilization of the information obtained in the migration studies, methods were employed in the field which enabled List and Kreutzer (13) to learn that the black blister beetle (*Epicauta pennsylvanica*), potato beetle (*Leptinotarsa decimlineata*), and grasshopper (*Melanoplus differentialis*) were capable of transmitting *Phytomonas sepedonica* from diseased to healthy plant tissues. Because of the slow migration of bacteria both upward from infected seed pieces and downward from mechanical inoculations it is doubtful that field spread of the disease by insects will be a problem.

From the work presented herein it appears that infection by *P. sepedonica* occurs principally through freshly cut or injured surfaces on the tuber seed pieces. Entry of the causal bacteria through natural openings such as eyes, lenticels, or unbroken skin appears to be so slight as to warrant little consideration from an economic standpoint.

Studies have shown that although the aerial parts of an infected plant may not be invaded by *P. sepedonica* the underground portions and hence the tubers may be infected. It has also been found that if the bacteria have migrated into the aerial portions, not all the vines of a diseased plant may be infected. In one study it was found that of 240 plants grown from artificially inoculated seed pieces only 57.9 percent of the vines produced by these seed pieces were actually carrying *P. sepedonica*. Further, under the same environmental conditions the presence of wilt symptoms appears to be largely dependent upon the degree of bacterial invasion in the vessels of the affected plant. If many vessels were invaded, wilt symptoms were almost invariably present. On the other hand, if only one or two vessels were invaded in the entire stem no wilt systems could be recognized. This latter condition has been frequently observed.

It follows that the zero tolerance requirement in Colorado for bacterial ring rot should be strictly adhered to in the certification of seed potatoes. If a trace or fraction of a percentage of ring rot is allowed as tolerance, a field showing such a condition may have a high percentage of infection. If no symptoms of ring rot can be found it does not necessarily follow that there is no ring rot present, but the chances are good that the field is entirely free of the disease.

Research to develop shortcut methods as an aid in the development of healthy foundation stock has been disappointing. Studies previously described have shown that infection in stems produced by naturally infected seed pieces showing no ring-rot symptoms and knife-inoculated seed pieces cannot be detected in less than 45 to 60 days. The results of this work indicate that sprout smearing could not be safely used as a dependable method of tuber indexing. Although sprouts from tubers showing recognizable ring-rot symptoms (11) frequently showed abundant Gram-positive bacteria in from 10 to 15 days, such information is of little value since tubers such as these obviously could be discarded readily.

In indexing work the use of the Gram stain as a test for infection in suspected tubers showing no ring-rot symptoms is not always a safe procedure. Studies reported herein have agreed with the work of Haasis (6) in showing that bacteria may not be in all parts of the vascular ring of infected tubers. To be safe the entire vascular ring must be probed for smearing.

Use of the ultraviolet light appears to be of some practical value in indexing tuber lots. In addition to the experimental work previously described in this connection, 20,000 seed pieces from 40 lots were subjected to the light previous to planting in pots in the greenhouse. In one lot ring rot which was not previously suspected was detected and eliminated. In the hands of an experienced operator "black" light has proved of value to several Colorado foundation stock growers who collectively examined 600 sacks of seed potatoes under the "black" light previous to planting. On the basis of the study reported herein and previous work (11) they were advised to discard any tuber which showed a fluorescence. This operation, which cost approximately $0.35 per sack, easily paid for itself by the reduction of ring-rot infection, fusarium infection, and black leg. In addition there was a decrease in the amount of roguing required for the elimination of these diseases in the resultant seed plot, together with a greater yield and uniformity of stand.

It is not considered that the ultraviolet lamp is as reliable a scientific instrument as the microscope in detecting ring-rot infection. If this were so, there should be a greater correlation between the light-positive and microscopically positive readings in table 4, page 18, and between the light-negative and microscopically negative readings, while the light-positive microscopically-negative column should be completely blank. The evidence presented in this study shows too much variation from expected conditions to make the lamp alone an accurate instrument for the diagnosis of ring-rot infection. It must be remembered, however, that the number of tubers that can be examined is many times greater than could be examined microscopically. It is felt that as an aid to indicating tubers from which smears should be made the light is of considerable importance to the laboratory worker.

SUMMARY

Infection of cut surfaces of potato tubers by *Phytomonas sepedonica* was reduced by apparent suberization and cork formation occurring in a 24- to 48-hour period. The removal of a layer of tissue 5 millimeters in thickness from the cut ends of knife-inoculated tubers greatly reduced resultant infection. Painting the unbroken surfaces of tubers with a suspension containing *P. sepedonica* gave no infection in resultant plants in greenhouse trials and only slight infection in the field, while painting injured tuber surfaces gave a relatively high degree of infection. Subjecting knife-inoculated tubers to various disinfectants gave no effective control of ring rot.

Tubers in which typical ring-rot symptoms were evident produced infected sprouts earlier than did artificially inoculated tubers or than did those naturally infected which showed no symptoms. The lower portions of diseased vines developing from both naturally and artificially infected tubers usually contained greater numbers of the causal bacteria than did the upper portions. In addition, *P. sepedonica* was more frequently found in the roots than in the stolons. Some vines of severely infected plants showed only slight to no infection and tubers were harvested from such plants which produced growth free from infection. Migration of *P. sepedonica* occurred principally in the xylem elements. Such movement was accompanied frequently by the escape of the organism into adjoining parenchymatous tissues.

Infected tubers with or without recognizable symptoms of ring rot and uninfected tubers were produced by plants affected by *P. sepedonica*. In addition tubers showing no symptoms frequently failed to reveal the presence of *P. sepedonica* when tested by the Gram-stain technique but later produced infected vines (table 3.) In other tubers showing no symptoms the organism was encountered frequently at one point only in the vascular cylinder. However, tubers showing ring-rot symptoms gave a Gram-positive stain reaction and if they did not rot invariably produced diseased vines.

Infection in tubers stored at 40° F. was more accurately detected by the use of ultraviolet light than those stored at 70° F.

Both pure cultures of *P. sepedonica* and material taken from the necrotic, yellow rings of affected tubers (the characteristic early ring-rot symptom) when used as inoculum failed to bring about soft rot in healthy tubers. Soft rot, however, was induced by using material as inoculum taken from tissues of tubers which had undergone complete breakdown. Slices from tubers affected with *P. sepedonica* when inoculated with *Erwinia carotovora* tended to rot more rapidly than similarly inoculated slices from tubers which were free from infection by the ring-rot organism. Mixed inocula obtained from cultures of both *P. sepedonica* and *E. carotovora* did not increase soft rotting of healthy tuber slices over the use of *E. carotovora* alone.

In artificial aerial inoculation studies it was observed that *P. sepedonica* moved more rapidly in tomato plants than in potato plants. In all cases the movement of the organism was downward with the exception of cases where the stem was completely removed and a new terminal shoot was produced. In such latter cases, bacteria were usually found in the tissues of the new shoot. The movement of bacteria both upward in the plant from infected seed pieces and downward from aerial inoculations was extremely slow.

LITERATURE CITED

1. ARK, P. A.
1941. The use of iodine in the control of potato ring rot and scab. Phytopath. 31:954-956.

2. ———, AND E. W. BODINE.
1939. Bacterial ring rot in Kern County, California. Plant Disease Reporter 23:199.

3. BONDE, REINER.
1939. Bacterial wilt and soft rot of the potato in Maine. Maine Agr. Exp. Sta. Bul. 396.

4. FLINT, L. H., AND C. W. EDGERTON.
1941. Fluorescence of diseased potatoes. Phytopath. 31:569.

5. GLICK, DUDLEY PETERS.
1941. Results of attempted eradication of bacterial ring rot from potatoes. Amer. Potato Jour. 18:140-143.

6. HAASIS, FERDINAND W.
1940. The distribution of *Phytomonas sepedonica* in potato seed pieces, plants and tubers, and its significance. Bul. Dept. Agr. State of California. 29:16-20.

7. HARVEY, R. B.
1941. Fluorescence of potatoes under ultraviolet light for detecting ring rot. Phytopath. 31:10.

8. IVERSON, V. E. AND H. C. KELLY.
1940. Control of bacterial ring rot of potatoes with special reference to the ultraviolet light method for selecting disease-free seed stock. Montana Agr. Exp. Sta. Bul. 386.

9. IVERSON, V. E. AND F. M. HARRINGTON.
1942. Accuracy of the ultraviolet-light method for selecting ring-rot free potato seed stock. Amer. Potato Jour. 19:71-74.

10. KENDRICK, JAMES B., P. A. ARK, AND C. EMLEN SCOTT.
1941. Potato-ring-rot studies in California. Phytopath. (Abst.) 31:14.

11. KREUTZER, WILLIAM A., DUDLEY P. GLICK, AND JOHN G. MCLEAN.
1941. Bacterial ring rot of potato. Colo. Agr. Exp. Sta. Press Bul. 94.

12. LARSON, R. H., J. C. WALKER, AND S. O. FOGELBERG.
1941. Bacterial ring rot in relation to the tomato. Phytopath. (Abst.) 31:14.

13. LIST, GEO. M. AND W. A. KREUTZER.
1942. Transmission of the causal agent of the ring-rot disease of potatoes by insects. Jour. of Econ. Ent. 35:455-456.

14. METZGER, C. H.
1938. A new potato disease in Colorado. Amer. Potato Jour. 15:225-230.

15. ————, AND DUDLEY PETERS GLICK.
1940. A promising method for eradicating bacterial wilt and ring rot from the potato. Amer. Potato Jour. 17:45-53.

16. ————, D. P. GLICK, AND W. A. KREUTZER.
1939. Station directing efforts on production of seed potatoes free of bacterial wilt. Colo. Agr. Exp. Sta. Farm Bul. 1 (4):15-16.

17. RACICOT, H. N., D. B. O. SAVILE, AND I. L. CONNORS.
1938. Bacterial wilt and rot of potatoes—some suggestions for its detection, verification, and control. Amer. Potato Jour. 15:312-318.

18. SAVILE, D. B. O., and H. N. RACICOT.
1937. Bacterial wilt and rot of potatoes. Sci. Agr. 17:518-522.

19. STARR. G. H.
1940. Experimental work for the control of ring rot of potatoes. Amer. Potato Jour. 17:318-322.

20. ————, AND W. A. RIEDL.
1941. Bacterial ring rot of potatoes. Wyo. Agr. Exp. Sta. Bul. 244.

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