BACTERIAL SPOT OF NATIVE GOLDEN CURRANT (RIBES AUREUM)[1]

By G. W. BOHN, *associate pathologist*, and J. C. MALOIT, formerly *agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

Since 1939 a bacterial spot has caused severe defoliation of native golden currant (*Ribes aureum* Pursh) in experimental plantings at the United States Department of Agriculture Cheyenne Horticultural Field Station, Cheyenne, Wyo. In 1940 and 1941 the disease was severe enough to cause the loss of all leaves except those produced on new shoots late in summer. This disease has been observed in ornamental plantings in Cheyenne and in plants which occur naturally in the Pole Mountains west of Cheyenne. It causes plants to be unsightly in ornamental and windbreak plantings and reduces yields directly by fruit infections and probably indirectly by weakening the plants through the loss of leaves.

A review of the literature on diseases of currants revealed several leaf spots (*6, 11, 12, 14, 17*),[3] [4] but none that was reported to be caused by bacterial pathogens. So far as the writers know the only reports of parasitic bacteria on *Ribes* are those concerned with crown gall (*3, p. 22; 10, p. 38; 13, p. 586; 20*) and a blossom blight (*16*), both on gooseberry. No other information has been found to add to the statement "two bacterial diseases have been reported on Ribes" made in 1920 by Smith (*18, footnote p. 7*). It is probable that Smith referred to crown gall and blossom blight of gooseberry. Since no description of the bacterial spot of golden currant has appeared in the literature, it and its causal organism are described in this paper.

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[2] The writers wish to thank H. A. Edson, formerly principal pathologist in charge, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, for assistance in obtaining references on bacterial diseases of *Ribes*, and W. H. Burkholder, professor, Department of Plant Pathology, Cornell University, Ithaca, N. Y., for helpful criticism of the technical description of the pathogen and for the data on its effects on lipoids, sodium tartrate, and tyrosine. Thanks are extended also to the following members of the staff of the United States Department of Agriculture Cheyenne Horticultural Field Station for the services indicated: A. C. Hildreth, formerly principal plant physiologist and superintendent, and A. W. Krofchek, formerly agent, for supplying plants of *Ribes;* Gerald Brown, junior chemist, for determinations of the H-ion concentrations of carbohydrate and other media; and J. V. Hastings, formerly clerk, for making the photographs.

[3] Italic numbers in parentheses refer to Literature Cited, p. 289.

[4] WEISS, F. Check list revision. U. S. Bur. Plant Indus., Plant Dis. Rptr. 26: 176–189. 1942. [Processed.] (See pp. 179–185.)

THE DISEASE

Bacterial leaf spots on native golden currant are usually 2 to 4 mm. in diameter, but isolated spots are frequently larger. Spots in the blade are circular; those near large veins are irregular in shape. Well-developed spots have dark-brown, slightly depressed centers and reddish-brown, water-soaked, slightly raised margins. The raised margins are surrounded by narrow halos with distinct, entire or minutely undulate edges or by broad halos with indistinct edges (fig. 1, *A*). Under some conditions the margins grade into slightly

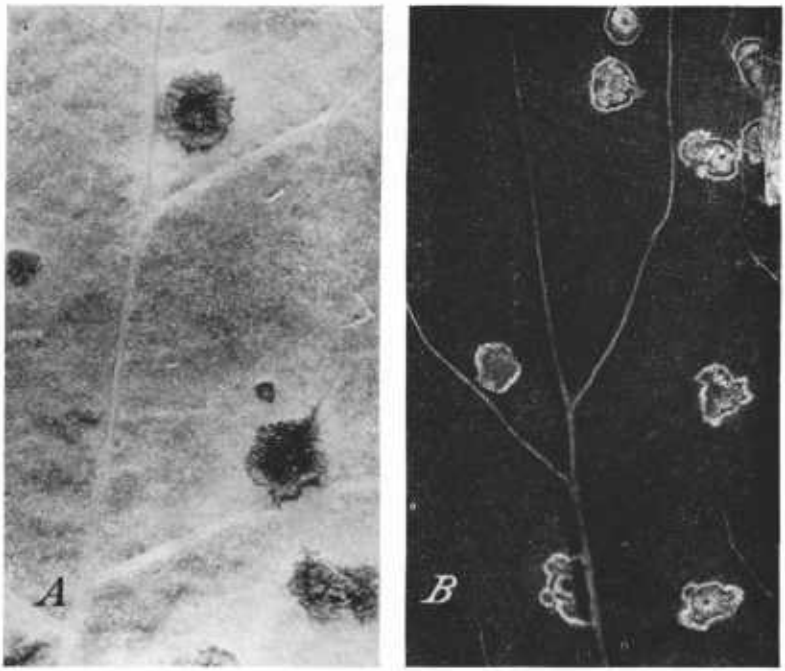

FIGURE 1.—Leaf spots on *Ribes aureum* resulting from brush inoculation with *Pseudomonas ribicola*, photographed on panchromatic film: *A*, In reflected tungsten light with a green Wratten (No. 58) filter; *B*, in transmitted tungsten light without a filter. *A*, × 6; *B*, × 3.

raised, dark-green, water-soaked areas with irregular, indistinct margins. Small veins within spots are discolored reddish brown and shriveled. This discoloration may extend short distances beyond the spot margins in the blade. Spots involving veins sometimes coalesce; those in the blade rarely do.

The spot shows a definite pattern by transmitted light (fig. 1, *B*). Bacterial masses at the inoculation point in the center and at the inner edge of the raised margin stop light. The remainder of the depressed center is moderately translucent, and the remainder of the raised margin is more translucent. If a piece of a leaf containing a spot is placed on a glass slide, cut through the center of the spot, covered with water and a cover slip, and examined with a microscope, masses of

bacteria can be seen to exude from the cut edges of the central point and the raised margin.

Infection occurring early in the ontogeny of the leaf results in distortion and tearing. Leaves with several spots turn various shades of yellow and red with green zones remaining around the spots (fig. 2, *A*). Severely spotted leaves soon turn brown, dry, and fall. Leaves

FIGURE 2.—*Ribes aureum* naturally infected by *Pseudomonas ribicola*, photographed on panchromatic film in daylight with a yellow (Eastman No. K2) filter: *A*, Spotted leaves showing chlorosis and green zones around spots; *B*, young shoots infected after hail injury (from an original on Kodachrome); *C*, leafy shoots showing a prematurely ripened infected fruit (*a*), uninfected spots caused by hail injury on fruit (*b*) and leaves (*c*), and infected spots on leaves (*d*). *A*, × 1.6; *B*, × 1.2; *C*, × 0.7.

infected near the petioles fall when less spotted than leaves infected near the margins.

On petioles and tender shoots spots may occur after hail injury (fig. 2, *B*). Girdled petioles break off, or the leaves dry from lack of water and fall. Spots rarely are large enough or numerous enough to kill shoots.

On the surface of fruits small (1 mm. or less), raised, brown spots may appear. Spotted fruits are small and occasionally deformed, and they ripen prematurely (fig. 2, *C*, *a*).

At Cheyenne the spots occur principally on leaves that develop early in the season (fig. 2, *C*, *d*). Leaves that develop on new shoots late in the season usually are not affected. Infected bushes are unsightly and unthrifty, especially after severe attacks during successive seasons. Some fruits are lost because of fruit infections, and yields are probably reduced also because the plants are weakened by the loss of leaves.

ISOLATION AND PROOF OF PATHOGENICITY

The organism that causes bacterial spot was isolated first in June 1940. Portions of leaves including single typical leaf spots were surface-sterilized in a 1 : 500 solution of mercuric chloride in 50-percent ethyl alcohol (and water) for 30 seconds, washed separately in three changes of sterile tap water, and crushed in tubes of sterile tap water. They were left to diffuse in the water for 10 to 20 minutes, when a series of dilution plates (beef-extract dextrose agar) was made from each spot. Similar series of dilution plates were made from bacterial spots that followed hail injury on green shoots.

A white bacterium making moderate, opaque growth on beef-extract dextrose agar and scant, translucent growth on beef-extract agar was obtained in apparently pure culture from all spots. Few colonies of other organisms grew in any of the plates. Six isolates, including two from infected hail spots, were selected for pathogenicity tests.

Repeated inoculation tests in the field, greenhouse, and laboratory by methods described in detail elsewhere (*2*) demonstrated that all six isolates were virulently pathogenic in leaves of *Ribes aureum* but dependent on wounds or forced spray to gain access to susceptible tissues. Typical spots developed on young leaves after they were rubbed with a brush or a cloth pad wet with suspensions of the organism. Spots were more numerous on leaves that were dusted with 300-mesh carborundum before the suspensions were applied. Spots rarely developed on leaves of plants kept in moist chambers and sprayed with the suspensions. All six isolates were recovered in pure culture from inoculated leaves and found to be identical with their parent cultures, thus completing Koch's rules of proof.

DESCRIPTION OF THE PATHOGEN

ISOLATION AND PURIFICATION

Cultures of six single-colony isolates, after reisolation from inoculated currant leaves and purification by series of dilution plates, were used in preliminary cultural studies in 1942. The cultural studies

were repeated in 1943–44 with three isolates obtained by second reisolations from inoculated leaves and purification by series of dilution plates. Although mechanical methods of single-cell isolation were not used, each isolate passed through at least four single-colony transfers from dilution plates and the cultures appeared to be pure. Among several thousand colonies observed only two appeared to differ from the others. Cultures from these two were identical with the others in pathogenicity tests and in critical cultural studies in which they were compared with their parent cultures. They differed only in morphological characters as will later be shown (p. 286).

MATERIALS AND METHODS

Unless otherwise stated, the formulas and techniques used in the cultural studies were those described by the Society of American Bacteriologists (*19*). If more than one method or formula were recommended, all were used. Other formulas used are described, or the sources are cited. Except where indicated otherwise, cultures were incubated at 25° C. Growth in liquid media was compared by visual observation and by the reduction of light transmission in a Klett-Summerson photoelectric colorimeter. Hydrogen-ion concentrations of media were determined with a Beckman pH meter. Utilization of sugars was determined by the amount of growth and the change in the pH value in flask cultures with 1 percent sugar as compared with blanks and with beef-extract broth check cultures. Sugars utilized in preliminary tests with steam-sterilized media were retested twice or more in media containing sugars sterilized by filtration. Sugars not utilized in preliminary tests were retested in media containing sugars sterilized by steam. Except for potato-dextrose agar, certain synthetic media, and media used in studies on pH range, the media were adjusted to pH 7.0 before sterilization.

MORPHOLOGY AND STAINING REACTIONS

The bacterium is rod-shaped; cells occur singly, in pairs, and in hyphalike chains. Chains have cross walls difficult to distinguish even in stained mounts. The bacterium varies in size within single mounts; the dimensions of 200 cells in nonflamed, nigrosin mounts from 12-hour, beef-extract dextrose agar cultures were 0.9μ to 1.7μ long by 0.4μ to 0.9μ wide; those of 200 cells in flamed, Gram-stain mounts were 1.1μ to 1.4μ by 0.3μ to 0.6μ. Mounts from cultures of several ages grown on several kinds of media failed to show endospores or capsules. The bacterium is motile usually by one polar flagellum or under certain conditions by a tuft of two to five flagella at one end of the cell. Cells in pairs frequently have flagella at the distal end of each cell. Suspensions in tap water yield better flagella mounts than do suspensions in distilled water. The bacterium is Gram-negative and not acidfast, but it stains readily with ordinary bacterial stains.

CULTURAL CHARACTERISTICS

The bacterium is a facultative anaerobe with the temperature growth cardinals: Minimum less than 3.5° C., maximum between 30° and 32.5°, and optimum between 20° and 25°. It grows more lux-

uriantly on solid media containing 1 percent or less agar than on media with greater concentrations. Its pH tolerance is greater than the range 5.6 to 7.5; the optimum is 7.0. One hundred and sixty determinations indicated that the pH values of peptone media containing dextrose are altered in 10 days at 25° to pH 6.7 ±0.5[5] by growth of the organism. The pH value attained varies in different batches of media, particularly as a result of variation in proportions and concentrations of nutrients and amounts of inoculum. The pH values of peptone media lacking sugar are altered to pH 8.1 ±0.4.

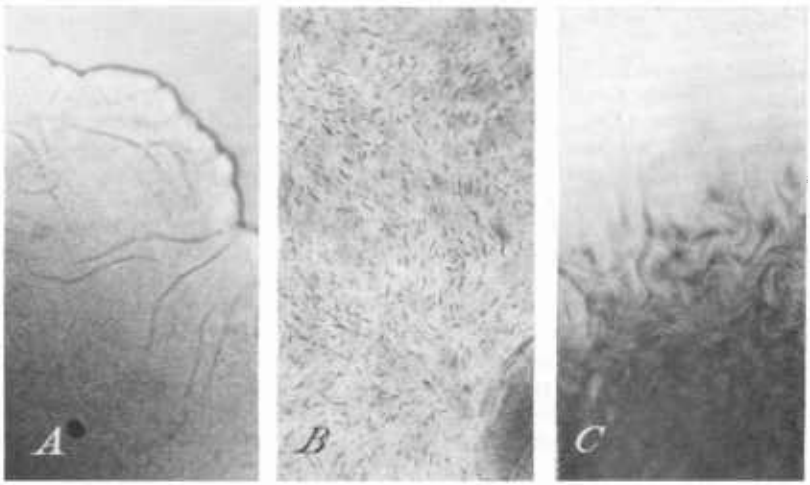

FIGURE 3.—Cultures of *Pseudomonas ribicola* on beef-extract dextrose agar in Petri dishes, photographed on panchromatic film with a green Wratten (No. 57A) filter: *A*, Lower surface of a colony showing reticulation characteristic of most isolates; *B*, upper surface at the center of a colony showing crystallike excrescences; *C*, lower surface of an atypical isolate showing curly striations. All × 80.

Beef-extract media.—On beef-extract agar 4-day-old surface colonies are punctiform, smooth, translucent, and white and have entire edges; subsurface colonies are discus-shaped. Slants yield scanty, filiform, glistening, translucent, white, slightly viscid growth. Beef-extract broth is made slightly turbid; no ring or pellicle is formed.

Beef-extract dextrose media.—On beef-extract dextrose agar 4-day-old surface colonies are circular, convex, smooth, glistening, opalescent, and white and have entire or minutely undulate edges; subsurface colonies are discus-shaped. Colonies with smooth edges have a uniform, finely granular structure; those with undulate edges have a reticulate structure near the edges. The reticulate structure is more easily observed in colonies 8 to 10 days old (fig. 3, *A*). This structural difference has not been segregated by the dilution technique of isolation and purification; it may result from differences in moisture at the agar surface. Centers of older colonies have crystallike excrescences on their surfaces (fig. 3, *B*). These are made up of hyphalike chains of cells.

Dilution plates have yielded two colonies with excessive proportions of hyphalike chains; these colonies had smooth edges and a curly striate structure (fig. 3, *C*). Cultures from these two colonies were like their parent cultures in other cultural characters and in pathogenicity, but the curly striate structure was retained after reisolation from currant leaves.

[5] Range is indicated by the ± signs in this paragraph.

Beef-extract dextrose agar slants yield moderate, filiform, glistening, opalescent white, butyrous growth. Beef-extract dextrose broth is densely clouded and made turbid; a slight ring, but no pellicle, is formed; the scanty sediment is turbid.

Potato-dextrose agar.—On potato-dextrose agar [6] 4-day-old surface colonies are circular, 6.5 mm. in diameter, convex, smooth, glistening, opalescent, and white, and their edges are entire or minutely undulate. Subsurface colonies are discus-shaped. Slants yield moderate, filiform, glistening, butyrous to slightly viscid, grayish-white growth. A dirty-pink pigment is produced in the lower parts of old cultures. The medium is slightly yellowed.

Potato plugs.—Growth on steamed potato plugs is moderate, filiform, raised, viscid, glistening, and pale yellow to cream-colored. The surface and edges are undulate.

Synthetic media.—Good growth occurs in Fermi's solution; [7] a pellicle is formed and the medium is yellowed. No growth occurs in Cohn's solution [8] or in Ashby's mannitol solution (*19*). Good growth occurs in Uschinsky's solution (*15*); a flocculent sediment is formed and the medium becomes yellowish green.

Nitrogen relations.—Milk is slightly darkened and made more alkaline; litmus is slowly reduced; slight peptonization occurs if 0.5 percent dextrose is added.

Gelatin in cultures incubated at 18° C. is liquefied slowly or in occasional transfers not at all. The slight liquefaction is infundibuliform. Growth is filiform and best at the surface; the color of the medium is unchanged.

Asparagine is utilized as a sole source of carbon and nitrogen in Starr, Weiss, Klein, and Sisselman's synthetic asparagine medium (*23*).

Burkholder found this organism to be very active in oxidizing tyrosine, producing a dark-brown pigment in a medium containing this amino acid.[9]

The currant spot pathogen produces moderate growth in peptone-nitrate broth and in peptone-nitrate agar. Tests for nitrites in cultures of different ages yield erratic results; no isolate or method has given a strong positive reaction. Although growth is more abundant in such media if dextrose is added, the intensity of the reaction is not increased in carbohydrate media. Feeble growth occurs in synthetic media which contain potassium nitrate as the sole source of nitrogen. Tests for nitrites in cultures of different ages in such media also yield erratic results.

Moderate growth occurs in organic media recommended for tests for the production of ammonia, indole, and hydrogen sulfide. All tests for the production of these compounds give negative results.

Carbon relations.—The bacterium grows luxuriantly and produces moderate amounts of acids in organic liquid media containing dextrose, galactose, levulose, xylose, or mannitol. The bacterium grows moderately and produces an alkaline reaction in organic liquid media lacking carbohydrates or containing maltose, lactose, dextrin, glycogen, inulin, or starch. No gas is produced in any of these media.

The bacterium does not hydrolyze starch, but faint trace reactions occur in agar media containing this carbohydrate. Starch agar stained with iodine shows traces of red and purple coloration near the edges of streak colonies in Petri dishes.

Burkholder [10] found this organism to be inert to lipoids in spirit blue agar (*5*, *21*, *22*) and to sodium tartrate.

HABITAT

The bacterium is pathogenic on *Ribes aureum*, causing necrotic spots on leaves, tender shoots, and fruits and defoliation. It is a wound parasite in leaves, shoots, and fruits, and it probably occurs in soils.

[6] Filtrate from 200 gm. of nonpeeled boiled potato, 10 gm. of technical dextrose, 10 gm. of agar, 1 liter of distilled water.

[7] Fermi's solution: 0.2 gm. of magnesium sulfate, 1 gm. of monobasic potassium phosphate, 10 gm. of dibasic ammonium phosphate, 45 gm. of glycerol, 1 liter of distilled water.

[8] Cohn's solution: 5 gm. of monobasic potassium phosphate, 5 gm. of magnesium sulfate, 0.5 gm. of potassium chloride, 10 gm. of ammonium tartrate, 1 liter of distilled water.

[9] The formula for Starr's tyrosine medium can be obtained from Starr or from W. H. Burkholder, Cornell University, Ithaca, N. Y.

[10] Personal correspondence.

TAXONOMY OF THE PATHOGEN

The bacterium which causes necrotic spots on *Ribes aureum* is related to the green-fluorescent bacterial plant pathogens included by Bergey and others in Appendix I of *Phytomonas* (*1, pp. 169–206*). More recently this group has been included in the genus *Pseudomonas* (*4, 7, 8, 9, 22*).[11] The currant pathogen described in this paper is unlike any organism now included in that genus; hence, the name *Pseudomonas ribicola* n. sp. is proposed.

TECHNICAL DESCRIPTION

Pseudomonas ribicola n. sp.

A bacterium parasitic on *Ribes aureum* causing necrotic spots on leaves, tender shoots, and fruits. Short rod, 0.9μ to 1.7μ long by 0.4μ to 0.9μ wide in nonflamed negatively stained mounts; cells occur singly, in pairs, and hyphalike chains; organism motile by one or a tuft of two to five polar flagella; endospores or capsules not demonstrated; Gram-negative and not acidfast. Facultative anaerobe with minimum temperature for growth less than 3.5° C., maximum between 30° and 32.5°, and optimum between 20° and 25°. Grows best in culture media containing 1 percent or less agar and having an initial active acidity of pH 7.0. Colonies on beef-extract agar punctiform, translucent, white; those on beef-extract dextrose agar and potato-dextrose agar circular, convex, smooth, glistening, opalescent, white, and butyrous; internal reticulations and crystallike surface excrescences occur in dish cultures. Insoluble dirty-pink pigment produced in old tube cultures on media containing sugar. Soluble yellow or yellowish-green pigment produced in media containing sugar or asparagine. Growth on steamed potato plugs filiform, raised, glistening, pale yellow to cream-colored, and viscid. Gelatin liquefied very slowly or not at all. Good growth in beef-extract dextrose broth, Uschinsky's solution, and Fermi's solution; growth feeble in beef-extract broth, and none in Cohn's solution or Ashby's mannitol solution. Asparagine utilized as the sole source of carbon and nitrogen. Milk slightly darkened and made more alkaline; no curd or clearing; slight peptonization in dextrose milk. Inorganic nitrogen used with difficulty; nitrates reduced to nitrites weakly (erratic results); ammonia, indole, and hydrogen sulfide not produced; tyrosine oxidized. Utilizes dextrose, galactose, levulose, xylose, and mannitol, but is inert to maltose, lactose, dextrin, glycogen, inulin, and starch. Slight acid but no gas produced from utilized carbohydrates; starch not hydrolyzed. Does not oxidize lipoids or sodium tartrate.

SUMMARY

A bacterial spot found in 1939 causes severe defoliation of *Ribes aureum* in experimental, ornamental, and windbreak plantings and in plants growing naturally in the mountains in the vicinity of Cheyenne, Wyo. Necrotic spots are produced on leaves, tender shoots, and young fruits; severe attacks cause defoliation. Koch's rules of proof demonstrated that the disease is caused by a green-fluorescent, white bacterium. The morphology, staining reactions, and cultural characteristics of the parasite indicate that it is related to the green-fluorescent, white bacteria now included in the genus *Pseu-*

[11] WEISS, F., and WOOD, J. I. A LIST OF NAMES AND SYNONYMS OF PHYTOPATHOGENIC BACTERIA OCCURRING IN THE UNITED STATES (AND ITS POSSESSIONS AND A FEW OTHERS) EMBODYING RECENT CHANGES IN NOMENCLATURE. U. S. Bur. Plant Indus., Plant Dis. Rptr. 27: 42–62. 1943. [Processed.]

domonas by most students of the bacteria. The name *Pseudomonas ribicola* n. sp. is proposed and a technical description is included.

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