BACTERIAL LEAF SPOT OF BEGONIA

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INTRODUCTION

For some years begonias affected with a bacterial leaf spot have been sent to the United States Department of Agriculture for diagnosis and for advice regarding control or prevention of the disease. The earliest record is a report (without specimens), in 1907 from Nebraska, to the Division of Mycology and Disease Survey, Bureau of Plant Industry, of the occurrence of bacterial trouble on begonia leaves. This was followed by a number of other reports of its occurrence in various States. During the past 4 or 5 years specimens and reports have come in more frequently and from widely separated localities. Considering the length of time the disease has been known, the records contain very little information regarding the causal organism. Losses of from 1 to 50 percent are reported, mostly in young plants. The considerable variation in its occurrence and in the degree of infection is perhaps closely connected with cultural conditions.

REVIEW OF LITERATURE

In 1894 Prillieux and Delacroix (8) noted a bacterial stem disease of begonias in France and stated that cultures of the organism resembled *Bacillus pyocyaneus*.

In 1909 Heald and Wolf (6) collected diseased begonia leaves in Texas and reported later (6, pp. 82-83):

Begonia. Bacterial leaf-spot (*Bacillus pyocyaneus* [B. pyocyaneus] P. and D. (?).—This is probably the same disease that occurs in France, although it was not observed to attack the stems but only the leaves. * * * No cultural work with the organism was attempted.

Begonia leaf specimens of the Heald and Wolf collection of 1909 are now in the mycological collections of the Bureau of Plant Industry (no. 1411, bacterial leaf spot, *Bacillus pyocyaneus* [B. pyocyaneus] on begonia). These specimens and a photograph of an infected leaf in Heald and Wolf’s publication (6) show lesions entirely similar to the leaf spot described in the present paper.

O. C. Boyd reported this bacterial leaf spot of begonia from Georgia in 1928 and sent typical specimens to the Division of Mycology and Disease Survey. R. P. White reported in 1928 that the variety Melior is very susceptible and the variety Peerless is resistant to the bacterial leaf spot of begonias. In 1929 he reported that there was a yellow halo about each spot. The writer in 1930 reported that the colonies of the causal bacteria are yellow. Numerous reports of its

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1 Received for publication Aug. 1, 1936; issued May 1937.
2 Reference is made by number (italic) to Literature Cited, p. 589.
3 The reports in this paragraph relate to card records on file in the Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.
occurrence came in before 1930, but unfortunately specimens were seldom sent.

In Europe, Buchwald in 1929 and again in 1933 reported a bacterial disease of begonias. Fotsch in 1933 mentions the disease. In each report the European disease is described as a vascular infection. Leaf veins are blackened, leaves wilt, stems rot and fall over. Buchwald states that the symptoms are similar to those caused by *Bacterium campestre* in cabbage. He named the causal organism *Bact. begoniae*.

In 1935 Wieringa reported more fully a wilt and stem rot disease of begonias. His descriptions of the symptoms and the causal bacterium suggest *Bacterium campestre*. Wieringa named the organism *Phytoponas flavas begoniae*. It is an interesting fact that the records of bacterial diseases of begonias in Europe from 1894 to 1935 are of vascular infections with resulting leaf wilt, vein blackening, and stem rot, whereas in the United States all the records are of leaf spots with no indication of vascular disease. Allowing for possible errors in description or technique, the bacterium of the European vascular disease is very similar in character to the bacterium causing leaf spot disease in the United States. It is possible but not likely that the same or a very similar organism affects vascular tissue under some conditions and only leaf mesophyll under other conditions.

The bacteria on which the present study is based were isolated from begonia leaves (var. Lloydii) collected in Oregon in June 1934 by F. P. McWhorter, plant pathologist, of the Oregon State College, and from leaves inoculated and infected with this Oregon strain.

**DESCRIPTION OF THE DISEASE**

The spots are usually well isolated and well distributed over the leaves (fig. 1). Sometimes they are more numerous along the leaf margin, but are never observed on the petioles or veins. They are occasionally found on the leaf bracts.

Infections are first visible as tiny, clear specks on the lower leaf surface. These enlarge into more or less circular, translucent, pale-green or colorless spots that later have brown, opaque centers with translucent, yellow halos. The size varies from 1 to 8 mm in diameter, averaging 2 to 5 mm. The bacteria probably enter through the stomata, which are fairly numerous on the lower surface of the leaf, fewer on the leaf bracts, very rare on the flower parts, and absent from the upper leaf surfaces, veins, petioles, peduncles, and stems. Sections through infected areas show that the destruction of tissue and the multiplication of the bacteria begin in the lower layers of cells, later spreading to the palisade cells and upper epidermis. With conditions favorable for the parasite the spots enlarge and coalesce, sometimes forming rather large areas of affected tissue. If considerable moisture is present these areas undergo soft rot. In no case has there been any evidence of vascular infection, of wilt or stem rot, or of entire destruction of the plants. However, the attacked plants become unsightly and worthless for sale, because even the moderately infected leaves fall and the less infected ones are imperfect. The plants continue to grow, but parts of the stem remain bare and the new leaves are often small. The new leaf growth is apparently healthy, but in many cases a trace of infection is present in tiny, inconspicuous brown
Figure 1.—A. *Begonia semperflorens*, 10 days after inoculation; B. natural infection on *B. semperflorens*; C. *Begonia*, hort. var. Jessie, 16 days after inoculation. All X about 2.
spots from which a general infection spreads whenever conditions are again favorable for the parasite.

Warm, moist, poorly ventilated, and crowded conditions favor the development of the disease. Adequate spacing, with regulated temperature and moisture, is usually effective in preventing or in curing the disease. Rapid forcing should be avoided, and spotted leaves should be removed or destroyed.

THE CAUSAL ORGANISM

ISOLATION AND INOCULATION

Pure cultures of the bacteria are easily isolated from recent lesions. Other types of bacteria are sometimes found in old lesions and in the soft rot encouraged by moisture. The organism has been isolated repeatedly from natural infections and from those resulting from inoculations. All of the available species and varieties of begonia—*rex*, *lucerna*, *semperflorens*, *Chatelaine*, *maculata*, *ricinifolia*, "Lorraine", *Jessie*, and one unknown variety—have always developed the typical leaf spots after the plants have been inoculated by spraying them with water containing the bacteria.

The stems, petioles, leaf veins, and flowers appear to be immune. Repeated and heavy inoculations by various methods failed to give even a trace of infection in these tissues.

Because the organism causing this leaf spot of begonia so closely resembles in its morphological and cultural characters the group of yellow bacteria of which *Bacterium campestre* is perhaps the best known representative, it was necessary to test its pathogenicity on various hosts. Cabbage, rutabaga, bean, radish, and horseradish plants were inoculated in parallel tests with begonias. The tests were repeated a number of times under different conditions of moisture and temperature. No infection developed on any of the plants except the begonias, which invariably became typically infected.

Geraniums (*Pelargonium* spp.) are very slightly susceptible to the begonia pathogen. In one of several inoculation tests, small, circular, translucent spots appeared on the leaves. Common garden varieties of geranium (*P. hortorum* Bailey) and the Lady Washington (*P. domesticum* Bailey) were used in these tests.

*Bacterium pelargonii* Brown (1) and *Bact. erodii* Lewis (7) cause definite leaf spotting of geraniums. These organisms are unlike the begonia organism.

MORPHOLOGY

The bacteria are slender rods with rounded ends. Stained with carbol-fuchsin, single rods are 0.9μ to 1.8μ long and 0.3μ to 0.4μ wide. Paired rods are frequent in most culture media. Chains occur in beef broth and in beef broth plus 2-percent sodium chloride. No definite capsules were demonstrated. There is a slimy substance in practically all cultures, and this sometimes suggests a capsule, as it stains less deeply than the rod. No spores or involution forms have been observed. The bacteria are motile. There is a single, polar flagellum three to four times the length of the rod and distinctly wavy. The bacteria are Gram-negative and are not acidfast. They stain readily with the usual aniline stains.
CULTURAL CHARACTERS

On beef-peptone agar plates inoculated directly from a leaf spot or from a culture, colonies appear in 2 days, and in 4 days well-isolated colonies are 4 mm in diameter. The colonies are circular, smooth, flat, with margins entire and translucent. The centers are nearly or quite opaque, but with age the whole colony becomes transparent. The color is "massicot yellow." Submerged colonies are opaque, oval to broad spindle-shaped.

Beef-agar slants have a smooth, thin, translucent, later transparent growth. Sometimes there is a small opaque area of growth at the base of the slant.

In beef-agar stabs the growth is thicker but slow in covering the surface, and after several weeks small secondary centers of growth appear. There is no growth in the stab.

Growth on beef-extract agar is similar to but considerably less than that on the beef-infusion agar.

On all the solid beef media the growth is usually slightly viscid.

In beef-peptone broth growth begins at the surface as a moderate clouding which increases to form irregular rims and fairly heavy, discontinuous, yellow pellicles. This surface growth breaks into irregular pieces and falls if even slightly disturbed. Pseudozoogloae are often present. There is a slight translucent yellow sediment. The clouding persists for 3 to 4 months and the medium becomes viscid, extremely so in the lower half of the culture. No green pigment was formed in any culture.

Thickly sown beef-gelatin plates at 20°C. are completely liquefied in 3 days; thinly sown plates liquefy in 8 days. The opaque, granular, yellow colonies remain intact and floating in the clear liquid. Gelatin tubes at 10° to 12° are entirely liquefied in 8 to 10 weeks; the medium is clear, without surface growth, but with yellow sediment at the base of the tube.

On potato-dextrose agar of pH 5.6 to 5.8 the growth is rapid and abundant. The surface becomes covered with thick, soft, opaque growth. The color is at first "honey yellow," later "tawny olive" or "isabella color."

Growth on potato cylinders is luxuriant, thick, smooth, shining, and deeper yellow ("old gold" or "buffy citrine") than on beef media. Cultures 8 weeks old are "olive brown."

In Cohn's solution there is no growth. Uschinsky's solution clouds slowly. In 2 weeks there is a definite surface growth that slowly increases to form yellow ("Naples" to "mustard") rims and pellicles. There is a slight, pale-yellow sediment. The medium between the surface growth and the sediment is a clear, pale yellow.

Growth is good in nitrate broth, but there is no reduction of nitrates to nitrites in 7-, 10-, or 16-day cultures. Bacillus phytophthorus Appel grown in the same medium and tested by the same methods gave a positive test for nitrate reduction (tests were made with the a-naphthylamine sulphanilic acid method).

In beef broth plus 2-percent sodium chloride, growth is considerably retarded and limited to a thin surface clouding and thin rims (the

4 Unless otherwise stated, all beef media were made with beef infusion and had a pH value of 6.8 to 7.0.

5 The color readings given in quotations are based on Ridgway's Color Standards (9).
yellow rim growth is very viscid). In 3-percent sodium chloride the growth is even less, and in 4-percent the growth is entirely inhibited.

Very good growth developed in a 1-percent tryptophan solution, but tests made on the fourth and on the tenth day gave no evidence of indol (tests were made with paradimethylaminobenzaldehyde and potassium persulphate).

There is definite evidence of hydrogen sulphide in potato-dextrose agar, potato cylinders, milk, and beef cultures.

Ammonia in small amounts is produced in most culture media.

Milk cultures quickly show a layer of clear whey at the surface. In 8 to 10 days the milk is colorless and transparent except for the yellow surface film and the scanty translucent yellow sediment. The liquid becomes pale amber or pale orange and the translucent sediment increases until it fills the lower half of the tube. Tyrosine crystals were abundant in some cultures and rather scanty in others.

Litmus in milk is completely reduced in 8 to 10 days. The color begins to return about the fifteenth day. Old cultures are pale wine red.

Fermentation and acid reaction are prompt and strong in saccharose, slightly less in dextrose, slow and weak in lactose, still slower in mannite and glycerin. Some strains failed to grow in the glycerin medium. The medium used for these tests was a peptone-free, synthetic agar with brom-cresol purple as indicator and 1 percent of the carbohydrate to be tested. No gas was produced and no clouding occurred in the closed arm of fermentation tubes containing the same medium without agar.

The optimum pH value for growth in beef media is 6.8 to 7.0, and 5.0 and 8.8 are the limits for growth.

The diastatic action was tested in plate cultures of beef-extract agar plus 0.2 percent starch. Surface streak inoculations produced vigorous and abundant growth. Tests with an iodine solution on the third day showed entire starch reduction in a band 10 to 11 mm wide on either side of the bacterial growth. On the ninth day this reduction was 30 to 35 mm wide. Parallel plates, with brom-cresol purple as an indicator, showed that no acid production accompanied the starch reduction.

The optimum temperature for growth is about 28° C. Cultures in liquid and on solid media produced under the same conditions equal growth at 27°, 28°, and 29°. The maximum temperature is 37° or slightly higher. Liquid media at 37° produced no growth, but potato-dextrose agar slants showed visible growth in 3 days at 36° and in 6 days at 37°. The minimum is 8° or lower. The thermal death point is about 50°. Cultures on solid media produced visible growth more quickly and also endured greater extremes of temperature than liquid cultures. Well-developed cultures that had been frozen solid for 10 days were only slightly reduced in vitality. Freshly inoculated cultures were frozen (—3°) for 25 days, then removed to room temperature. Growth was slow in starting, but in 10 days after thawing it was definite and typical. Beef-bouillon and potato-dextrose agar cultures were alive and only slightly reduced in vigor after being frozen for 5 months.
Cultures at room temperature and at 15° C. remained alive for 12 months or more but were reduced in vigor after 8 to 10 months. When dried on cover glasses, the vitality of the bacteria was good for 40 days, but afterwards many tests failed; the last positive test was after 56 days of desiccation. Exposure of freshly inoculated plates to direct sunlight for 20 minutes killed all the bacteria. Exposure for 15 minutes caused a reduction of 98 to 99 percent; 10 minutes, 50 percent; and exposure even for 5 minutes caused a noticeable reduction in numbers.

**TECHNICAL DESCRIPTION**

**Bacterium flavozonatum** n. sp.

Slender rods, rounded at ends, single and in pairs. Average size 0.9μ to 1.8μ by 0.3μ to 0.4μ. Motile; single, wavy polar flagellum, three to four times the length of the rod. Capsules absent, or very indefinite. No spores nor involution forms. Aerobic. Growth yellow on beef agar. Gelatin liquefied. Ammonia and hydrogen sulphide produced. Nitrates not reduced. Good growth in Uschinsky's solution. No growth in Cohn's solution. Milk rapidly cleared. Starch hydrolyzed. Maximum temperature 37° C., minimum below 8°, optimum about 28°; thermal death point about 50°. Resists drying 56 or more days. Produces acid but no gas from dextrose, saccharose, lactose, mannite, and glycerin. Gram-negative. Not acidfast. Stains readily with aniline dyes. Pathogenic to begonias, producing leaf spots and causing defoliation.

Specimens of this disease on begonia leaves have been deposited in the mycological collections of the Bureau of Plant Industry.

**SUMMARY**

A bacterial leaf spot of cultivated begonia that causes defoliation in severe attacks and unsightly plants and leaves in lighter attacks occurs widely in the United States.

The causal organism has been isolated and its pathogenicity for begonias proved. Because the organism resembled *Bacterium campestris*, inoculations were made but no infections were secured on cabbage, rutabaga, radish, and horseradish. On geranium leaves a very slight infection was sometimes produced.

Methods of control and prevention are suggested.

The name *Bacterium flavozonatum* is proposed for the organism causing this begonia disease.

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