BACTERIAL LEAFSPOT OF DELPHINIUM[1]

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INTRODUCTION

The leafspot of Delphinium (larkspur), commonly known as the "Black spot," or "Black disease," is a very destructive disease, widespread throughout the northern United States, more particularly in the North Atlantic States. Although this decorative plant has been eliminated from the gardens of some large estates because of its ravages, the disease has received scant attention from pathologists.

The only references in pathological literature are brief notes by Dr. Erwin F. Smith. In "Science," for March, 1904,[2] he gave a very brief description of the causal organism which he had isolated, proved pathogenic by inoculations and named *Bacillus delphinii*. Cultures were allowed to die before adequate cultural work was done, and although the organism was again isolated in 1907, pressure of other work crowded out its further study. The spots are figured by him in "Bacteria in Relation to Plant Diseases,"[3] and in the same volume, page 92, it is stated to be one of the diseases transmitted through stomata and water-pores. He later[4] refers again briefly to the subject, as follows:

"The spot disease of Delphinium (Vol. I, fig. 127) is another malady in which infection takes place readily through the unbroken leaf-surface and stem-surface, i. e., through stomata. The disease has been obtained a number of times during the last seven years by placing the bacteria in water and spraying this upon the plants. The leaf-serratures also blacken in this disease, and here infection probably occurs through the groups of water pores situated on their apex."

In 1920 characteristically spotted leaves were received from Woodstock, N. Y., from which isolations were made of a white bacterial organism. These and subsequent isolations from various sources have been used for the study here presented.

HOST PLANTS

This disease has not been reported on any plant except larkspur, being most destructive on the choice hybrid delphiniums. A few small but definite infections have been produced on Aconite by rubbing the inoculum on the lower surface of leaves, but no natural infections have been reported on this plant. No infections were obtained by repeated inoculations on cultivated varieties of Ranunculus and Aquilegia.

GEOGRAPHICAL DISTRIBUTION

Specimens have been received from points in Maine, New Hampshire, Massachusetts, Connecticut, New York, Long Island, Pennsylvania, Illinois, and in one case from Portland, Oreg. One correspondent wrote in 1922, "I have just

[1] Received for publication Jan. 23, 1924.

[2] SMITH, E. F. BACTERIAL LEAF SPOT DISEASES. Science 19: 417. 1904.

[3] SMITH, E. F. BACTERIA IN RELATION TO PLANT DISEASES. v. 1, fig. 127. 1905.

[4] SMITH, E. F. BACTERIA IN RELATION TO PLANT DISEASES. 2: 61–62. 1911.

returned from a trip and find that the delphinium all the way from Ridgefield, Conn. out to Chicago, Ill., is badly affected by the 'black disease.'" A delphinium grower and breeder in California reported in 1923 that the "Black disease" which gives so much trouble in the East had not as yet appeared or seemed not to be known on the West Coast.

Leaves from Castine, Me., received early in July, 1922, were 23 cm. across and came from plants 3 meters high. They were thickly covered with the largest spots observed, i. e., 1 to 2 cm. in diameter (Pl. 1, B). Probably the cool, moist climate in this location which was a large factor in the vigor of these plants also increased the virulence of the disease. On less vigorous plants the spots are usually smaller (Pl. 1, A). No specimens have been received from farther south than Pennsylvania, and inoculations in Washington, D. C., where the climate does not favor this plant, while giving numerous typical infections, have never produced spots more than a few mm. wide (Pl. 1, C). During the heated summer term it has been found impossible to produce artificial infections in Washington, D. C., except in the event of a cool, rainy spell.

APPEARANCE OF DISEASED PLANTS

The leafspots are very striking in appearance. On the upper surface they form tarry black areas of irregular shape and size that may reach a diameter of 2 cm. on large vigorous leaves (Pl. 1, B). On the lower surface they are brown and the smaller ones do not yet show through to the upper side. Old spots lack the water-soaked edges that so frequently accompany other bacterial spots, although in very young stages water-soaking is present and is the first indication of infection observable under a hand lens. Although the lower surface of spots may appear sunken, the upper surface usually appears to be raised slightly and, in many spots, there is a tendency to concentric rings strongly suggesting fungus infection (Pl. 2, D and E). Such spots were repeatedly sectioned to make sure that no mistake was being made, but in every case bacteria oozed out in vast quantities, while no fungus was observed and cultures of *Bacterium delphinii* were obtained when poured plates were made. Spots resulting from pure culture inoculations also often showed this zoning, which has since been noted in bacterial spots on other plants, notably tobacco.

Spots may occur on any part of the leaf blade, the result of stomatal infection, and are also common on the leaf tips where they make their entrance through the large water-pores (Pl. 2, A, B, and C). When infection takes place on young leaves, and especially in the deep sinuses, distortion usually results from the failure of the diseased areas to keep pace with the growing healthy tissue. Flower buds are also occasionally attacked, becoming black-spotted and distorted, but this should not be confused with the common distortion of buds and young leaves caused by mite infestation where a blackening of the very young tissues often occurs in severe cases. This is a continuous blackening instead of a spotting and no bacteria are present. The bacterial spot occurs also on petioles and stems. In later stages of the disease the spots may coalesce, forming large black areas, involving in some cases almost the entire blade of the leaf.

HISTOLOGY OF DISEASED LEAVES

The bacteria enter the leaf through the stomata of the lower epidermis or through the water pores at the tips of the serratures. As the spongy parenchyma just beneath this epidermis is very loose, it is difficult to demonstrate stomatal infection in its earliest stages, since the bacteria scatter so readily on entering. Also, unless sections are made early enough the infection spreads so rapidly as

to obscure any evidence of the point of entry. Material fixed 24 hours after spraying gave no sections suitable for photographic demonstration although the spots were water-soaked and scattered bacteria were found under the stomata in microtome sections. After 48 hours, however, very good sections were obtained showing bacteria in the intercellular spaces under the stomata chiefly lying on the cell walls, singly or in oval masses (Pl. 3, A and B). At this stage the spot shows as a minute water-soaked point on the lower surface, to be seen only under a lens. In microtome sections the bacteria are confined to the loose parenchyma. Cells in the vicinity of the invaders often show on their walls what appear to be extrusions of cell sap. These are hemispherical bodies which stain very deeply (Pl. 4, A). Later the palisade tissue is invaded and killed and the lesion penetrates to the upper surface as a black spot involving the whole thickness of the leaf. At this stage of infection the bacteria lie on the walls of the cells and crowd the intercellular spaces.

ISOLATIONS

Poured plates were made in this laboratory in 1903 from spotted leaves from New York and Massachusetts and again in 1907 from Massachusetts. In each case a white organism was obtained with which successful infections were secured on delphiniums. On account of the small amount of cultural work done at that time no adequate comparison can be made with isolations of 1920–1923. Descriptions and photographs of agar plate colonies agree with those of later date but in other cultural characters there is disagreement, not only between these early isolations and those of 1920–1923 but also among the several early isolations themselves, and it is apparent that some of the early cultural work was not fully checked up, since striking inoculations were obtained in 1903 proceeding from subcultures of single poured-plate colonies of what was undoubtedly this organism.

Isolations have now been made from typical infected plants from all of the States mentioned under "Geographical distribution." All gave colonies apparently of the same white organism. Comparative cultures have shown these to be alike in all important points.

In several cases where material was not in good fresh condition when received a yellow organism appeared on the plates in numbers about equaling the white colonies and, in one case, only a yellow organism was obtained although three separate platings were made from typical spots. This was of a saprophytic type frequently appearing on plates poured from many species of plants when not in perfectly fresh condition, and it failed to produce any infections when the inoculum was either sprayed or rubbed on the lower surface of delphinium leaves.

INOCULATIONS

Successful inoculations have been made in the hothouse and out of doors, on young and on mature plants, both in cages where moisture could be maintained and also without cover. Infections obtained on young plants in the hothouse never increased much in size (1 or 2 mm. wide) and few secondary infections were found. Reisolations were made from hothouse inoculations of 1920 and used for inoculations in the hothouse in 1921 and 1922 with similar results. Outdoor inoculations in the summer of 1921 failed, that is only a very few slow-growing infections were secured, probably because of the late start made and supervening dry, hot weather.

On May 31, 1922, vigorous young seedling plants of hybrid delphiniums, which were set out of doors May 1 and were just beginning to send up blossom stalks, were sprayed with subcultures from an isolation of March 29, 1922, from a hot-

house infection. It was a cool cloudy day with threat of rain and the plants were left uncovered. They were sprayed with the organism on two successive cloudy days, and then a three days' continuous rain set in. Nine days after the first spraying the leaves were thickly speckled with infections, especially those inoculated from one colony, individual spots being as much as 2 mm. wide and some confluent areas as large as 1 cm. across. These spots were black, surrounded by yellow areas on some leaves while on others (older mature stem leaves) no yellowing occurred. Poured plates from such spots gave pure cultures of the white organism used for inoculation. The weather then turned very warm, the spots did not increase materially in size and later attempts at inoculation failed, both with reisolations from these leafspots and with copious colonies used to produce them. It seems evident that climatic conditions, after 3 cold and moisture, play a large part in the success of inoculations, while temperatures inhibit, and moist cool conditions greatly favor the disease.

During April and May, 1923, several sets of inoculations were made indoors on delphinium plants that had wintered outdoors and increases of white precipitate in the bottom of the tube within 4 or 5 days and were moderately clouded. After 3 months' with numerous small crystals. 1921 which had produced the infections of 1922 were used. Although

USCHINSKY'S SOLUTION.—Within 24 hours a faint clouding may be seen in the upper part of the culture. By the second day a delicate pellicle has formed with blue-fluorescence just below it in the upper 2 or 3 mm. and by the fifth day clouding is heavy with a heavy pellicle and green fluorescence throughout. A heavy white precipitate is formed.

FERMI'S SOLUTION.—There is prompt growth in Fermi's solution with a beautiful blue fluorescence sometimes becoming green but more often remaining blue though held for several weeks. A heavy wrinkled pellicle is formed which does not fall readily on shaking, but clouding usually remains weak. Thumm[7] states that all of the colors produced by fluorescent bacteria are due to the same pigment, the blue becoming green with the production of alkali by the organism.

The addition of dilute ammonia to blue cultures of *Bact. delphinii* in Fermi's solution turned them a vivid green. Blue and green cultures were tested for relative acidity with the following results:

Blue P_H 6.5 or +41.

Green P_H 6.6 or +34.

NITRATE REDUCTION.—Nitrate is not reduced. Tests were made with nitrate bouillon cultures 5 days old and 10 days old in which moderate clouding had taken place, using the starch-iodin-sulphuric acid test.

NITROGEN COMPOUNDS.—The ability of the organism to obtain its nitrogen from various nitrogen compounds was tested in 1 per cent water solutions of the following: peptone, asparagin, asparagin plus dextrose, ammonium citrate, ammonium tartrate and ammonium succinate. The results are shown in Table I.

[6] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. pl. 15. Washington, D. C. 1912.

[7] THUMM, K. BEITRÄGE ZUR BIOLOGIE DER FLUORESCIERENDEN BAKTERIEN. Arb. Bakt. Inst. Tech. Hochschule Karlsruhe 1: 291-377. 1897. Abstract in Smith, E. F. Bacteria in relation to plant diseases. 1: 238. 1905.

CULTURAL CHARACTERS

BEEF AGAR PLATES.[5]—Colonies are visible on the second day and by the third day in thin sown plates are 3 to 5 mm. wide, round, white, transparent, opalescent, slightly convex, sometimes slightly umbonate, smooth shining, finely granular. Young colonies show coarse cross-hatching by oblique transmitted light (Pl. 4, F and G) and sometimes these internal cross-hatchings persist in the older colonies at summer temperatures but usually they give place to internal concentric markings (Pl. 4, E). Buried colonies are lenticular. When older, the colonies may reach a diameter of 8 mm. The consistency is sometimes slightly viscid. Occasionally a ring of irregular opaque areas or radiating lines may appear in the colonies (Pl. 4, D) and old colonies may become slightly lobed. Plates from old litmus milk and from old bouillon cultures have given very convex colonies with depressed centers which, as they enlarge, have a wrinkled surface. After return to favorable cultural conditions, transfers from these colonies again gave normal colonies on agar plates.

BEEF AGAR SLANTS.—Growth is thin, white, smooth shining, transparent, opalescent, with internal wavy markings and entire margins. Growth does not cover the surface but tapers upward from a width of 3 to 6 mm. There is considerable white precipitate in the V. Numerous small crystals form beneath the growth and the agar becomes green.

POTATO CYLINDERS.—The moderate, thin, spreading growth on potato cylinders is dirty white becoming pale tan colored, with a slimy consistency; the potato begins to gray in 24 hours and in a few days is gray throughout. There is no distinct odor. Starch is only partially digested, i. e., a purple color is produced by adding iodin to the crushed potato cylinders on which the organism has been growing.

STARCH AGAR PLATES.—Smears were made on starch agar poured plates from young agar cultures. When tested after 7 days by drenching the surface of the plate with iodin solution an area 5 to 8 mm. wide surrounding the growth was purple in color but there was no area of complete starch destruction.

WHEY AGAR.—On whey agar titrating +8 or P_H 7.4 colonies are very convex, creamy white, opaque, round with entire margins, not opalescent, with internal concentric markings in the margins and in thin sown plates are 5 to 7 mm. wide when 10 days old.

BEEF BOUILLON.—Clouding occurs near the surface in 24 hours with a delicate pellicle which falls readily in fragments on being disturbed. Undisturbed cultures 2 days old are well clouded throughout and have a heavy pellicle, borne down in the center with a mass of white growth, giving the appearance of gelatin liquefaction (Pl. 4, H) when viewed from the side, but from the top showing a nail-head of white in the center of the membranous pellicle. This pellicle falls readily as a whole on shaking. Disturbed cultures re-form their pellicle quickly when young but not after the cultures are 6 or 8 days old. Blue-green fluorescence begins at the top in young cultures. Old cultures are green throughout.

BEEF GELATIN PLATES.—In +10 (P_H 7.2) beef gelatin, kept at 20 to 22° C., colonies on the second day are minute white spots lying in shallow pits of liquefaction several times their width. By the fourth to the sixth day colonies may reach a diameter of 1 cm., clouding the liquefied gelatin uniformly except for a heavier mass of growth at the center.

BEEF GELATIN STABS.—Liquefaction begins on the second day (20 to 24° C.) and is napiform, becoming stratiform. Liquefaction is complete in two to three

[5] All beef media used were made with beef infusion plus 1 per cent Difco peptone. Titrations expressed by + and − are according to Fuller's scale.

weeks with deep green fluorescence. Cultures kept one year on culture media liquefy gelatin very slowly.

MILK.—Milk clears uniformly without coagulation, beginning about the seventh day. Cultures one month old are translucent and honey yellow. No crystals are formed at this time but an occasional culture held for three months has a few small masses of tyrosin crystals. The milk at this age is tawny yellow and translucent (Ridgway XV)[6] and the precipitate has become yellow.

LITMUS MILK.—Litmus milk begins to blue on the third day from the top downward, then clears without coagulation. The cream rim when present is reddened. Reduction of the litmus begins in 10 days and is complete in 20 days. A copious white precipitate is formed. The blue color returns in 2 to 4 weeks. After 3 months the milk is still blue and translucent but the precipitate has become yellow.

METHYLENE BLUE MILK.—Reduction begins on the second day and is complete in 3 to 5 days. No coagulation occurs.

COHN'S SOLUTION.—Weak clouding is visible within 4 or 5 days and increases slowly for 5 or 6 weeks when the cultures are moderately clouded. After 3 months' growth there is a small amount of white precipitate in the bottom of the tube with numerous small crystals.

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TABLE I.—*Sources of nitrogen*

	1 day old	4 days old	10 days old	20 days old
Peptone	Very weak clouding.	Good clouding, heavy pellicle.	Same as 4 days, some precipitate.	No further growth.
Asparagin + dextrose.	.....do.....	Weak clouding, delicate pellicle.	Moderate clouding, good pellicle.	Do.
Asparagin	.....do.....	.....do.....	Weak clouding, delicate pellicle.	Do.
Ammonium citrate.	.....do.....	Weak clouding, no pellicle.	Weak clouding, no pellicle.	Do.
Ammonium succinate.	.....do.....	.....do.....	.....do.....	Do.
Ammonium tartrate.	No clouding.....	No growth.....	Very weak clouding....	Do.

LITMUS SUGAR AGARS.—Litmus agar containing 1 per cent peptone and 1 per cent of saccharose, maltose, lactose, dextrose, galactose, levulose, l. arabinose, glycerin or mannit was used for stroke cultures. The litmus is reddened decidedly on the second day and is red throughout in 6 days in the presence of dextrose, levulose, l. arabinose or galactose. Saccharose causes no color change except sometimes a very feeble reddening after 2 to 3 weeks. Cultures with maltose, lactose, glycerin and mannit become decidedly blue, beginning on the second day.

When the same carbohydrates are added to beef infusion peptone litmus agar the organism produces a blue reaction with all of them.

FERMENTATION TUBES.—In fermentation tubes containing 1 per cent peptone water and 1 per cent of saccharose, lactose, maltose, dextrose, galactose, glycerin or mannit, there is no growth in the closed end and no gas. In dextrose and galactose the reaction is acid to litmus while the others are alkaline to litmus. They were tested after 1 week, 2 weeks, and 4 weeks. Saccharose gives a neutral or slightly acid reaction.

BLOOD SERUM.—Only a very moderate growth takes place on Loeffler's blood serum and there is no liquefaction.

TOLERATION OF SODIUM CHLORID.—The organism grows promptly and well in 1 per cent NaCl in +10 or +12 beef bouillon and fairly well in 2 per cent, weakly in 3 per cent, and some isolations make very weak growth in presence of 4 per cent NaCl.

OPTIMUM P_H IN BEEF BULLION.—The best growth was made in +16 (P_H 6.7) to +12 (P_H 7.1). There was gradual but finally very good clouding in +25 (P_H 5.6), but none in +30 (P_H 5.2). Moderate clouding occurs in 0 (P_H 8.2), weak clouding in −2 (P_H 8.4) and in −4 (P_H 8.6), but none in −9 (P_H 9.2).

TOLERATION OF ORGANIC ACIDS.—Tests made with citric, malic, and tartaric acids are summarized in Table II. It was found that the P_H value is the controlling element here, the organism being able to grow in the same P_H of all acids while varying in its tolerance of the different acids judged by their Fuller's scale values.

AMMONIA PRODUCTION.—There is strong ammonia production. Cultures in beef bouillon 2 to 17 days old were tested by suspending over them strips of filter paper wet with Nessler's solution. Browning of the paper began immediately and became intense on heating the cultures in a water bath.

HYDROGEN SULPHID PRODUCTION.—No hydrogen sulphid is given off from bouillon, Uschinsky's solution, Fermi's solution, or potato cultures. Tests were made by suspending filter paper wet with lead acetate water in the tubes. No discoloration of the paper occurred.

TABLE II.—*Toleration of organic acids*

Citric acid			Malic acid			Tartaric acid		
Fuller's scale	P_H	Growth	Fuller's scale	P_H	Growth	Fuller's scale	P_H	Growth
+17	.....	Prompt heavy clouding.	+17	.....	Prompt heavy clouding.	+17	.....	Prompt heavy clouding.
+28	5.7	Delayed heavy clouding.	+25	5.8	Delayed heavy clouding.	+24	5.7	Delayed heavy clouding.
+33	5.4	No growth.....	+33	5.2	No growth.....	+29	5.2	No growth.

INDOL PRODUCTION.—No indol is produced in 10 days' growth either in Dunham's solution or in Uschinsky's solution to which 2 per cent Difco peptone was added. Tests were made with sodium nitrite and sulphuric acid. Cultures of *Bacillus coli* tested at the same time gave a good pink reaction.

EFFECT OF DESSICATION.—The organism is rather resistant to drying. Small drops from 24-hour beef bouillon cultures were spread on sterile cover slips in sterile Petri dishes and kept in the dark. Most of the covers when dropped into tubes of bouillon after 9 days' drying caused clouding, many clouded the bouillon after 12 days' drying, and some were able to cloud the bouillon after 20 days' drying. Plates were poured from these last which gave pure cultures of the right organism.

THERMAL RELATIONS.—The organism grows at temperatures from 1° to 30° C. in beef bouillon +12, P_H 7.1, with an optimum growth at 25°. At 1° clouding began on the sixth day and gradually (within three weeks) became heavy and green fluorescent with a pellicle. At 30° growth was very weak, even after three weeks, and without either pellicle or green color. Fresh transfers to bouillon kept at 34°, 35°, and 37° for eight days, when removed to room temperatures (23° to 25°) failed to cloud although held for two weeks.

The thermal death point is 50° C. when test-tube cultures in beef bouillon (P_H 6.8 to 7.2) are exposed in a water bath for 10 minutes (tube diameter 16 mm.)

EFFECT OF FREEZING.—Transfers were made to beef bouillon (+12, P_H 6.7) from 24-hour-old bouillon cultures. These were allowed to stand 10 minutes, shaking at intervals; then beef agar plates were poured. The freshly transferred cultures were then frozen solid in a salt and ice mixture, kept frozen for 15 minutes and thawed in cool water. Plates were then again poured as before. From 40 per cent to 60 per cent of the bacteria were killed by this treatment.

EFFECT OF SUNLIGHT.—Tests were made in Washington, D. C., in August. There were no clouds but the sky was not brilliantly clear. Plates poured from 24-hour bouillon were exposed bottom side up on ice to the direct rays of the sun. One-half of each plate was protected from the sun by several folds of black paper. Exposures were made for 5, 10, 20, 30, 45, and 60 minutes. A colony count gave the following results: One-third were killed by a 5-minute exposure, one-half by 10-minute, three-fourths by 20-minute and all by 45-minute and 60-minute exposures.

LONGEVITY.—The organism lives at room temperatures for six months in beef bouillon and in milk, or until the culture is actually almost completely evaporated.

GROUP NUMBER.—The group number is 211.2322123 according to the descriptive chart of the Society of American Bacteriologists.

TECHNICAL DESCRIPTION

Bacterium delphinii (EFS).

A short nonsporiferous rod with rounded ends; usually single or in pairs, sometimes in short chains; flagella 1 to 6 bipolar: capsules, aërobic, white, but producing a blue-green fluorescence in cultures; diastasic action weak; liquefies gelatin; does not reduce nitrates; clears milk without coagulation; blues, then reduces litmus milk; produces ammonia but no hydrogen sulphid or indol; forms acid from dextrose, galactose, levulose, and with more difficulty from saccharose, but not from lactose, maltose, glycerin or mannit; does not produce gas from any of these carbon compounds; grows well in Uschinsky's, Fermi's and Cohn's solutions; optimum temperature 25° C., maximum 30°, minimum 1°, or less; thermal death point 50°; Gram-negative; not acid-fast; stains readily with carbol fuchsin and gentian violet; is pathogenic to delphiniums, producing dead black spots on the leaves, stems and flower buds.

CONTROL METHODS

It has been found impractical to do any control work in Washington. The delphinium plant does not reach its best development here, preferring the cooler northern States. The organism also is very sensitive to high temperatures. Moreover, isolations kept on culture media over winter have lost much of their virulence and by the time fresh isolations can be obtained from northern material the weather here has become unfavorable to both host and parasite. Control studies should therefore be made in a region where the disease prevails.

A few suggestions may be made, however. It seems probable that the organism winters-over in the soil since it is rather resistant to cold and drying, and since infection generally appears first on the lower leaves, suggesting the spattering of infected soil by rain. On this theory all diseased material should be gathered and burned. Drenching the soil surrounding the plants with alkaline Bordeaux mixture before growth begins in the spring and later spraying both the soil and the lower surface of early leaves with Bordeaux mixture should prevent infection from this source.

SUMMARY

The black spot of delphinium is a bacterial disease widespread in the northern part of the United States and is very destructive to choice hybrid varieties.

The bacteria gain entrance to the plant through the water pores and stomata, causing irregular tarry black spots on the leaves and sometimes on stems and flower buds.

The causal bacterium has been isolated and successful infections obtained by spraying with water suspensions of young subcultures from single colonies.

For control of the disease it is suggested that all diseased material be collected and burned and that the surrounding soil and early leaves be sprayed with Bordeaux mixture.

Since the flagella have been demonstrated to be polar, the name of the organism becomes *Bacterium delphinii* (EFS).

PLATE 1[a]

A.—Delphinium leaf from Maine, 1923. Small spots.

B.—Delphinium leaf from Castine, Me., 1922. Large spots caused by *Bact. delphinii*. Reduced slightly.

C.—Spots on delphinium leaf produced by spraying with water suspension of a subculture of a single colony freshly isolated from a leaf from Maine, in July 1923, 32 days after inoculation.

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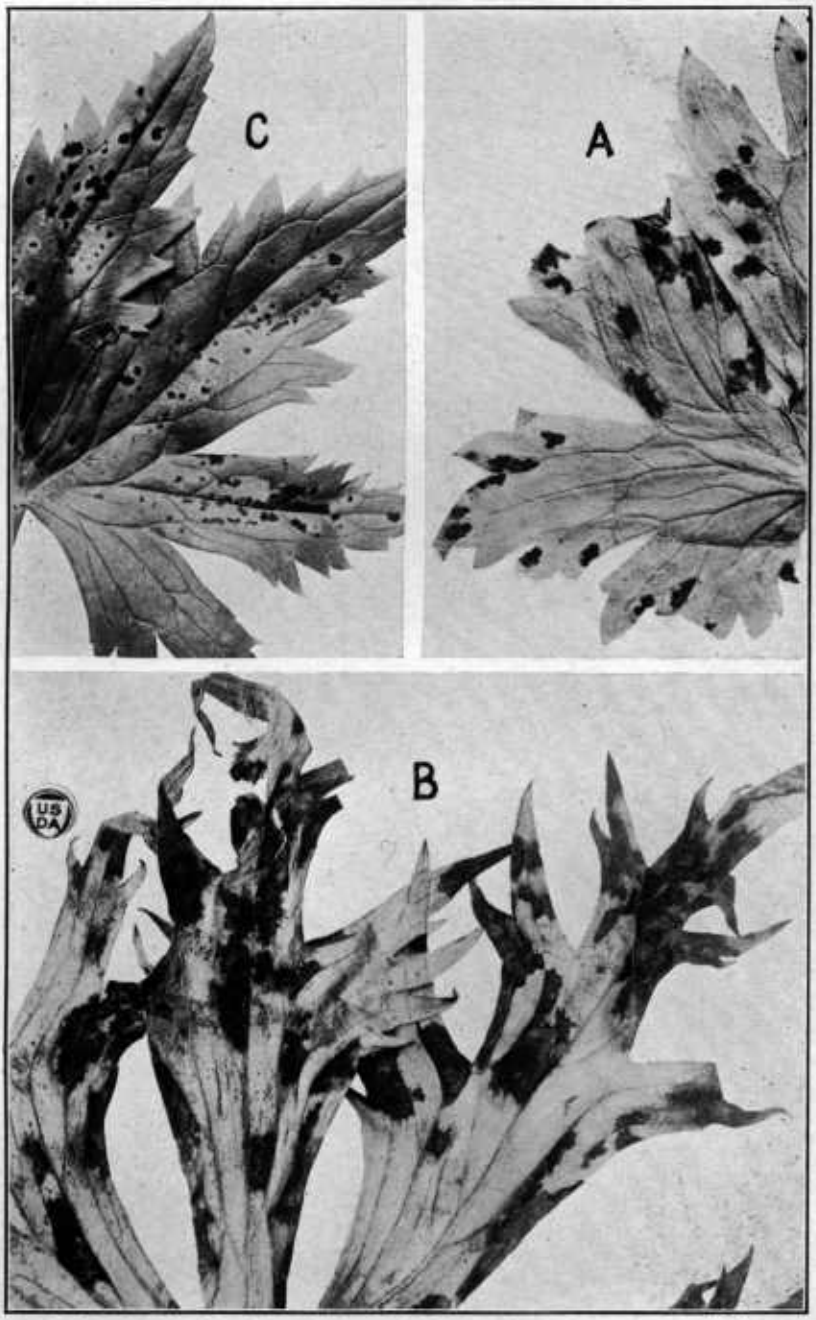
[a] All photographs were made by James F. Brewer.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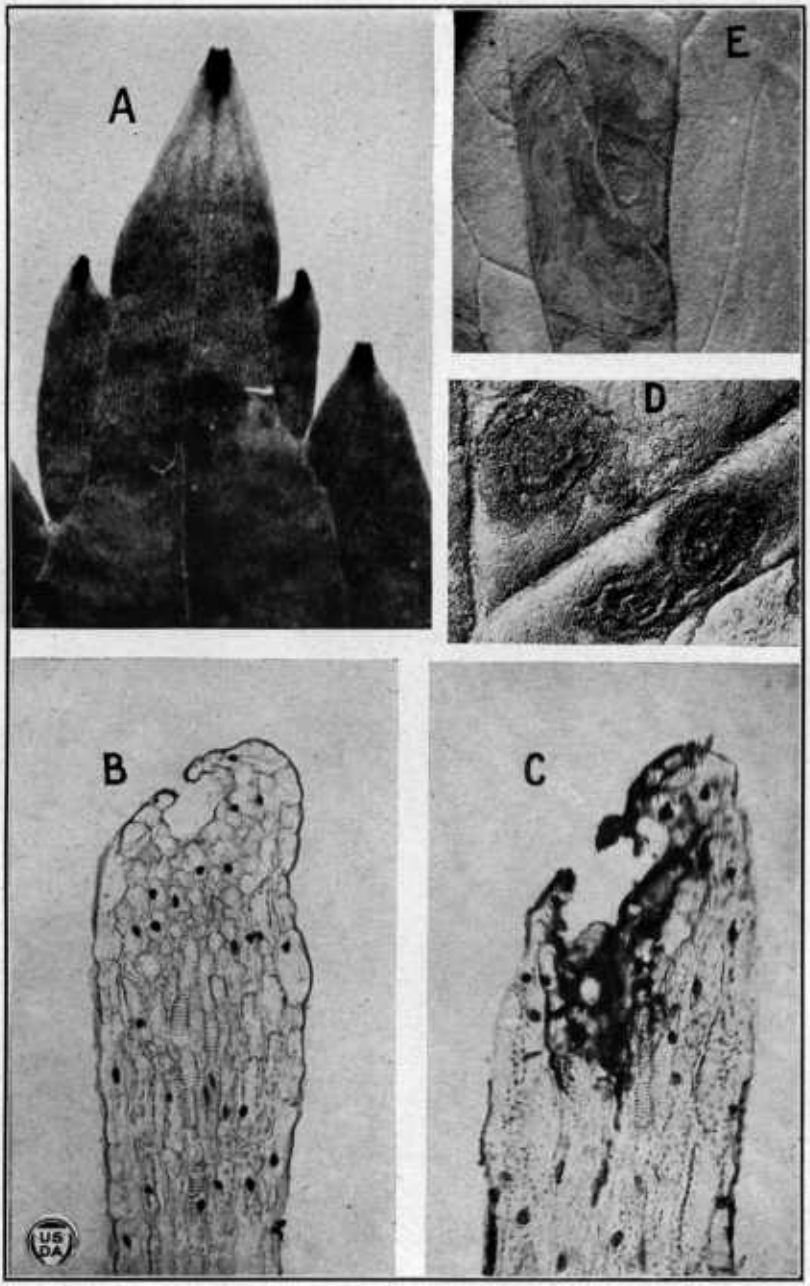
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PLATE 2

A.—Tip of a delphinium leaf showing infected (blackened) water pores. Time, 20 days after inoculation in the hothouse with the organism from Oregon. × 10.

B.—Microtome section of a normal water pore. The black spots are nuclei.

C.—Microtome section of an infected water pore.

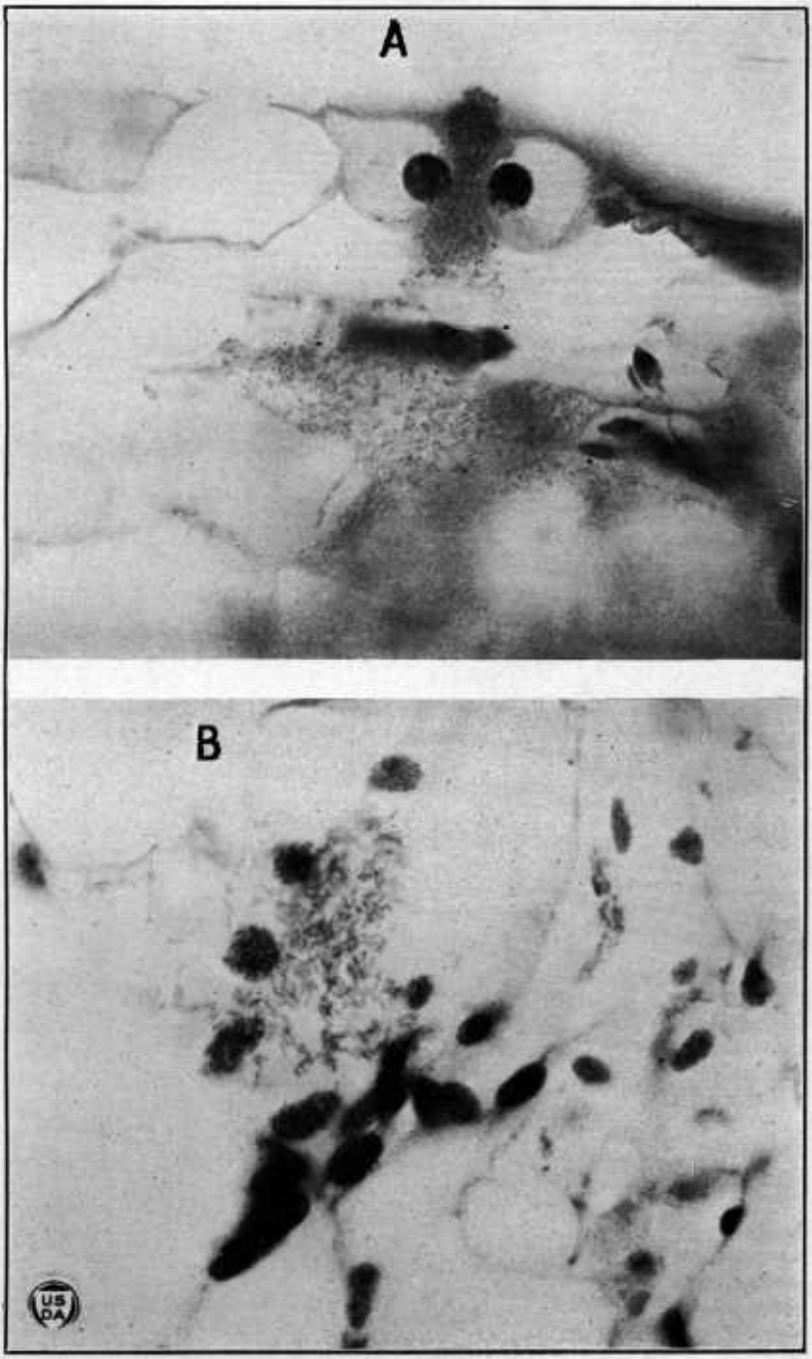
D.—Small leaf spots showing zoning. Photographed from a fresh leaf received from Connecticut. × 4.

E.—Large leaf spot showing zoning. Photographed from a pressed leaf from Castine, Me. × 2½.

PLATE 3

A.—Stomatal infection. Microtome section of a leaf spot 48 hours after inoculation by spraying. Bacteria lie on the walls of the cells nearest the stoma, and in and under the stoma. × 1400.

B.—Bacteria in the tissues. The large oval bodies are masses of bacteria. × 1400.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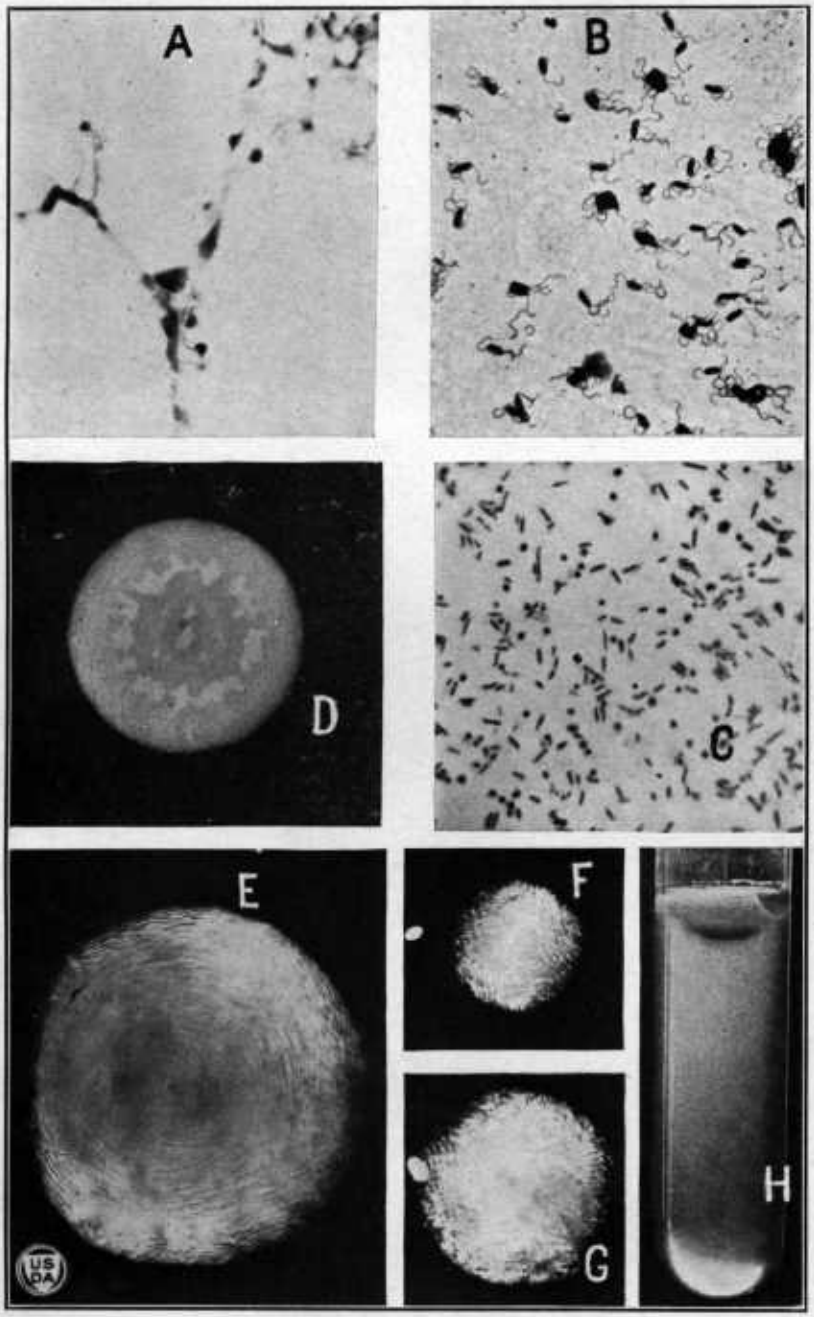
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PLATE 4

A.—Hemispherical extrusions on cell walls in the vicinity of the invading bacteria. × 500.

B.—*Bacterium delphinii* showing flagella. Stained by Casares-Gil's method. × 940.

C.—Rods and spherical bodies from the sediment in the pellicle of bouillon cultures.

D.—Agar plate, colony 4 days old showing ring of opaque areas. × 10.

E.—Agar plate, colony 9 days old, by oblique transmitted light. × 10.

F.—Agar plate, colony 2 days old, by oblique transmitted light. × 10.

G.—Agar plate, colony 3 days old, by oblique transmitted light. × 10.

H.—Culture in beef bouillon 4 days old, showing pellicle weighted down in center by a mass of sediment.