PSEUDOMONAS CITRI, THE CAUSE OF CITRUS CANKER

[A PRELIMINARY REPORT]

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During the summer of 1914 reports of the rapid spread of Citrus canker and the severe injury caused by this new Citrus disease were received by the Bureau of Plant Industry from orange and grapefruit growers in Florida, Texas, and Mississippi. It soon became evident that this disease was one of unusual virulence, which made the investigation of its cause a matter of urgent importance. From the reports of various investigators it appears that Citrus canker was known and recognized as a new disease before any specimens were received by this Bureau.[1] The first specimens received by the Bureau consisted of fruits, leaves, and twigs of grapefruit and showed cankers in every stage of development, from the youngest infections, which were scarcely more than a millimeter in diameter, to the large corky forms, as much as 5 mm. in diameter. A careful microscopic study was made of some of the youngest cankers, and the presence of bacteria was immediately detected. Bacteria were found in fresh sections and have been demonstrated in a large number of stained sections, as represented in the accompanying illustration (Pl. IX, fig. 1).

Numerous plate cultures were made from fresh specimens of cankers received at different times, and an organism was isolated which has been proved to be pathogenic to grapefruit seedlings.

Due attention has been given to all the rules governing bacteriological technique, and every precaution has been observed in making the inoculations. The inoculations were made on young, healthy, vigorously growing grapefruit seedlings, which were kept in the laboratory because the highly infectious nature of the disease made it impossible to carry on the experiments in the Department greenhouses. Pure cultures of the organism were mixed with sterile distilled water, and the suspension thus obtained was placed upon the upper and the under leaf surfaces by means of a sterile pipette in such a manner that the leaves were, for a short time at least, covered with a film of the inoculating fluid. The main stem and branches were treated in the same way. In some cases the leaves and stems were punctured with a sterile needle, but this is not

[1] Stevens, H. E. Citrus canker. A preliminary bulletin. Fla. Agr. Exp. Sta. Bul. 122, p. 113–118, fig. 44–46. Mar., 1914.

Berger, E. W., Stevens, H. E., and Stirling, Frank. Citrus canker. II. Fla. Agr. Exp. Sta. Bul. 124, p. 27–53, fig. 7–14. Oct., 1914.

Edgerton, C. W. Citrus canker. La. Agr. Exp. Sta. Bul. 150, 10 p. Oct., 1914.

Wolf, F. A., and Massey, A. B. Citrus canker. Ala. Agr. Exp. Sta. Circ. 27, p. 97–101, illus. May, 1914.

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necessary, as infections may be obtained without this procedure. As soon as the plants were inoculated they were placed under bell jars and kept at a temperature of about 86° F. Under these conditions the organism takes a vigorous hold on its host, and in three or four days evidences of infection can be noted. At the end of a week definite, well-defined cankers which penetrate the tissue of the leaf have been formed. Owing to the stimulating influence which the organism has upon the infected leaf tissue, there is a rapid development of cells, and the tension resulting from the abnormal growth quickly ruptures the epidermis and exposes the soft, spongy, underlying canker tissue, which is distinctly visible on both sides of the leaf. The cankers produced by artificial inoculation present a characteristic appearance and closely resemble natural cankers in macroscopic as well as in microscopic features. They penetrate the tissue of the leaf and are more or less raised on both the upper and the lower surface. The outline is circular, and there is a sharp, distinct demarkation between the canker and the surrounding normal leaf tissue. Young cankers have a soft, spongy structure and at first show a light-green color, which later turns red-brown. The cells in the canker tissue become suberized and produce a corky growth, which is a symptom of the disease. This open, spongy type of canker is the result of rapid growth due to favorable conditions of temperature and moisture.

The identity of natural and artificial cankers is shown in Plate X. Sections of cankers about 2 weeks old show the pathological and histological features observed in young natural infections. (See Pl. IX, fig. 1.) The cells are found to be filled with short rod bacteria, and the stimulus exerted by the organism on the infected tissue is distinctly visible. The natural differentiation of palisade and parenchyma tissue has been obliterated, and all the cells exhibit more or less enlargement and distortion, which is due to the activity of the invading organism. As a result the diseased tissue of the canker is raised above the normal leaf surface. In later stages in the development of the canker some of the cells disintegrate, and lesions are formed. The organism appears to act more vigorously on the cell contents than on the cell walls, and in due time the cell contents are exhausted. The cell walls which remain become suberized and constitute the corky cankerous growth which is a characteristic symptom of this disease. Numerous cankers obtained from pure-culture inoculations upon grapefruit seedlings are shown in Plate IX, figures 3, 4, 5, 6.

While the canker is still soft and young, the organism is in a very active condition and can be isolated very readily. Upon teasing out a small piece of canker tissue in a drop of sterile water, motile bacteria in great numbers ooze out and give the water a milky, turbid appearance. The motility of the organism can be most satisfactorily observed by means of dark-field illumination. The organism was reisolated from

these cankers by plating out on beef agar and was found to be identical with the original organism. Inoculations on grapefruit plants with the organism obtained from this reisolation produced characteristic cankers.

The open surface of the canker and the spongy character of its structure afford an excellent lodging place for spores of all sorts, and it is not surprising to find fungi, some of which may perhaps play a minor part in the later stages of the disease. A number of fungi have been isolated from old Citrus cankers, and a study of their relation to the canker problem shows that the fungous flora of the Citrus canker perhaps may be an interesting problem in itself.

The organism appears to be a new species and is briefly described as follows:

Pseudomonas citri, n. sp.

This organism is a short, motile rod with rounded ends and a polar flagellum. It occurs singly or in pairs and varies in shape from a short, ellipsoidal form to the typical rod. Its dimensions show corresponding differences, but rod forms usually are 1.5 to 2 by 0.5 to 0.75μ.

When plated out on beef agar at room temperature, the organism appears at the end of 36 to 48 hours, the colonies showing up as fine, glistening points just visible to the naked eye. The surface colonies increase quite rapidly in size and in three or four days show very distinctly. They are circular in outline, with entire margins and a slightly raised, smooth surface. By reflected light the colonies show a dull yellowish color, while a bluish translucent color is observed by transmitted light. The internal structure is finely granular and the motility of the organism can sometimes be noted in the outer border of the colony by examining the culture under the low power of the microscope.

In needle-stroke cultures on beef agar a moderate filiform growth is produced which does not penetrate the agar. The streak widens slowly and spreads more at the base of the slant surface. The bacterial mass is slightly raised, smooth, shining, and dull yellow in color.

A very characteristic growth is obtained on potato cylinders. In young cultures the organism follows the line of the streak and produces a somewhat raised, shining growth which has a bright-yellow color. A narrow, white zone is noted on the uninfected surface of the potato, following the margin of the bacterial mass. This feature does not persist very long, as the organism grows vigorously on this medium and soon the entire surface of the cylinder is covered with a thick, yellow, shining, viscid mass.

Beef bouillon shows a visible growth in 24 hours. In older cultures a yellow ring is formed at the surface.

Litmus milk shows a deeper blue color, the casein is precipitated, and the clear supernatant liquid appears a deep reddish color when viewed by transmitted light.

Gelatin is liquefied, the line of puncture is filiform, and the growth of the organism takes place at the surface of the culture.

Dunham's solution shows more or less clouding, the heaviest growth taking place in the open end of the tube, where a flocculent growth is noted at the surface. No traces of indol were noted.

This organism produces no gas in the presence of Dunham's solution in combination with dextrose, lactose, or mannit. The organism grows well in all these combinations, especially at the open end of the tube, where a flocculent growth is produced. Dextrose appears to favor the development of this organism particularly, as a heavy, flocculent growth is formed throughout the entire tube. It grows but sparingly in Ushinsky's solution, and in starch-nitrate solution does not reduce the nitrate. The organism grows best under aerobic conditions.

The organism stains readily with carbol fuchsin, and flagella have been demonstrated by means of the methods of Van Ermengem and Dr. Hugh Williams. (See Pl. IX, fig. 2.)

Much confusion and uncertainty seem to exist in the minds of Citrus growers and others in regard to the identification of the true Citrus canker. Many specimens supposed to be infected with canker which have been sent for identification have been found to be injured by fungi or some other cause. A most careful and detailed comparative study of Citrus canker and other diseases resembling it must be made in order to clear up the canker problem and reduce the necessity of frequent bacteriological diagnoses.

Although this paper gives only a very brief account of the etiology of the Citrus canker and many important facts in the life history of the causal organism remain to be determined, the immediate publication of this preliminary report is considered necessary on account of the great economic significance of this disease, which up to the present has been supposed to be due to a fungous parasite. Because the methods of control for bacterial diseases differ quite radically from those employed for fungous diseases it is hoped that the presentation of this report at this early stage in the investigation will lead to a more adequate understanding of the precautions which may be essential in an effective campaign of eradication.

PLATE IX

Pseudomonas citri

Fig. 1.—Drawing of a stained section of a portion of a grapefruit leaf bearing a young canker resulting from inoculation with a pure culture of *P. citri*. × 250.

Fig. 2.—Photomicrograph of *P. citri* stained by the Williams method for flagella. × 1,000.

Fig. 3.—Top view of a grapefruit seedling showing the results of artificial inoculation with *P. citri* isolated from Texas specimens.

Fig. 4.—View of the lower side of the leaves shown in figure 3.

Fig. 5.—Top view of a grapefruit seedling showing the results of inoculation with *P. citri* obtained from Florida specimens.

Fig. 6.—View of the lower side of the leaves shown in figure 5.


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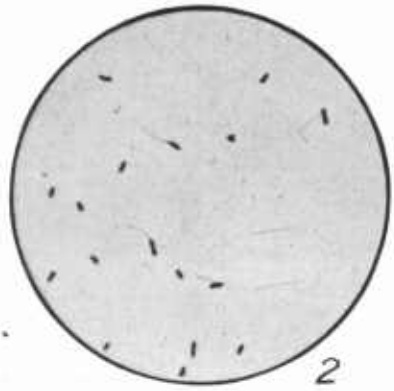
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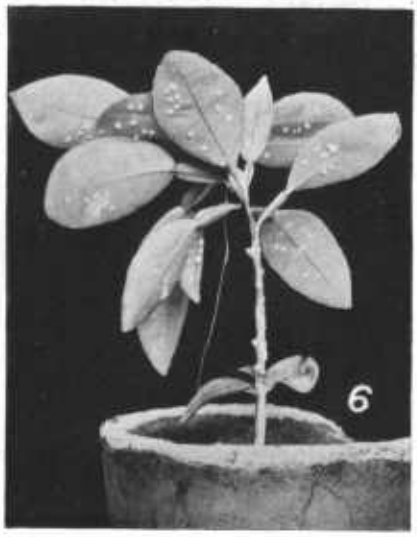
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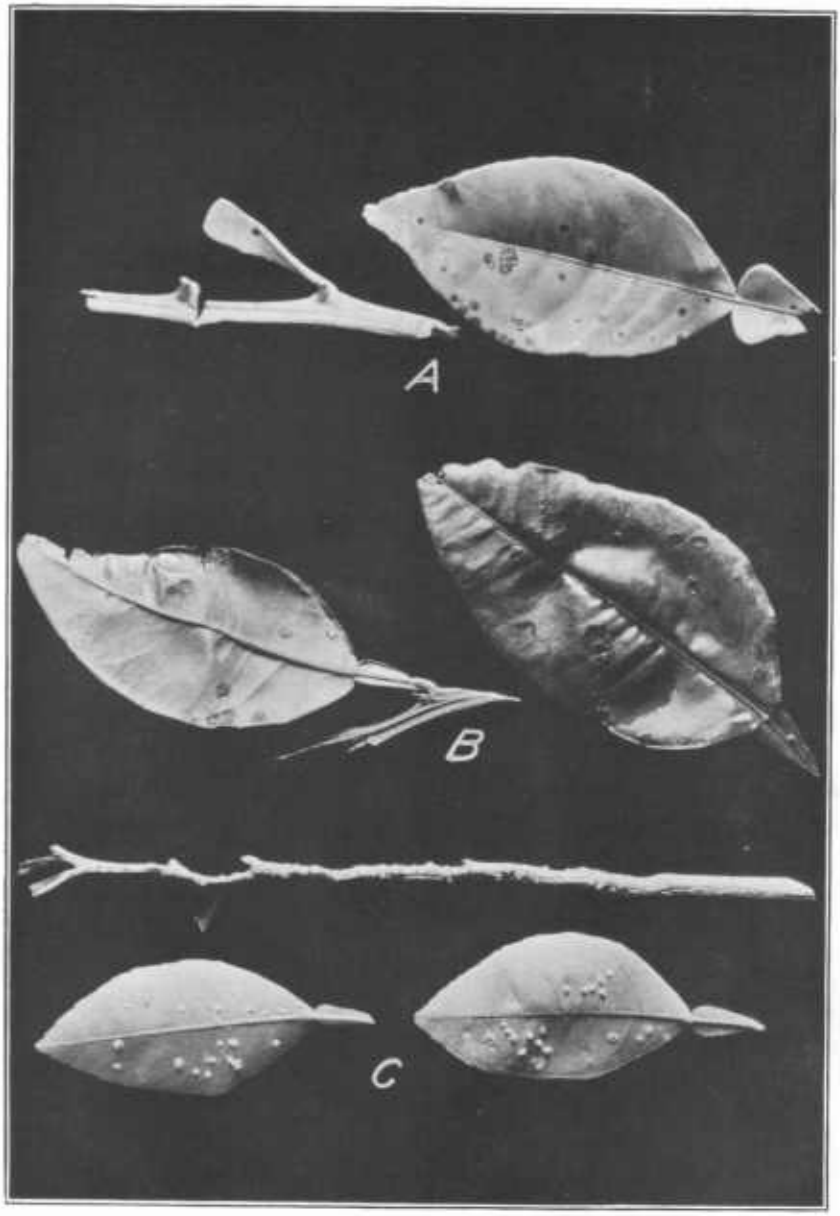
A
B
C

PLATE X

Pseudomonas citri: Small lesions on Citrus twigs and more obvious cankers on Citrus leaves. *A*, Cankers on twig and leaves from Florida produced by natural infection; *B*, natural infections on leaves from Texas; *C*, cankers on twig and leaves produced by artificial inoculation.

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