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Citrus Canker

By
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CITRUS CANKER

INTRODUCTION.

During the past few years considerable attention has been directed to the study of a citrus disease, commonly known as citrus canker. A number of publications dealing with these observations and investigations have been prepared so that a considerable amount of information has been secured relative to the distribution and the appearance of the disease, to the nature of the organisms which cause it, to the agencies concerned in its dissemination and to the difficulties experienced in its control and eradication. Many problems connected with this disease have not yet been investigated, but it is deemed advisable to present at this time the results of the studies thus far conducted.

A report embodying the investigations made by the writer upon citrus canker has been published in the Journal of Agricultural Research, (Vol. VI, No. 2.) (1). The present publication contains a rather more brief account of the most important results presented in this report, and in addition contains such information as could be drawn from other papers dealing with citrus canker.

HISTORICAL.

Citrus canker was introduced into the Gulf States from Japan on nursery stock. It has been found to occur in Japan, the Philippine Islands and parts of Eastern Asia. Whether or not it is indigenous to these countries is not known with certainty, but it is known that it is not of American origin. Specimens were first brought to the writer's attention in February, 1914. The Office of Nursery Inspection of Florida had collected specimens in September, 1912, but did not realize the presence of a new citrus disease within the State until July of the following year. The disease has been collected in all of the Gulf States in the sections adapted to the growth of citrus trees. Within Alabama it is confined principally to Baldwin and Mobile coun-

(1) Wolf, F. A. Citrus Canker. Jour. Agr. Research 6, No. 2, 69-100, Fig. 8, plates VIII-XI, 1916.

ties. A very small percentage of the trees within the state are affected with citrus canker, but when the infectious and virulent nature of the disease is realized it is seen that these few trees are a serious menace to the citrus industry in Alabama. It is for this reason that such energetic efforts are being made to prevent its further introduction and spread and to eradicate the disease already present.

Hosts.

Citrus canker has been found to occur on many of the species and varieties of citrus. Among them are grape-fruits, King oranges, trifoliate oranges, sweet oranges, naval oranges, mandarines, Satsumas, tangerines, lemons and limes. It has been observed in Louisiana on species of Fortunella, to which genus the kumquat oranges belong. Swingle (2) has recorded the presence of canker on kumquats in Japan. It is not equally severe on all of these hosts but grapefruits and trifoliate oranges seem to be much more susceptible than the other varieties. Satsuma oranges appear to be quite resistant. Trees growing in rows adjacent to badly diseased grapefruits have been observed to remain free from disease during an entire season. In other cases Satsuma trees in nursery rows have been observed, all of whose leaves were diseased, some of which leaves had several hundred cankerous areas.

Symptoms of the Disease.

Citrus canker manifests itself by the presence of characteristic spots on foliage, twigs, larger branches, and fruits. The diseased areas are usually light brown in color and are raised more or less above the surrounding tissue. The cankers are circular in outline when they occur singly and irregular lesions are formed when several spots fuse. The cankerous tissue consists of a corky mass of cells covered by the lacerated grayish outer membrane of the host. The disease was at first mistaken for scab, but it cannot be confused with scab or other leaf troubles when once one has seen citrus canker in its several stages of development.

Infections on the leaves first appear as small, oily

(2) U. S. Department of Agriculture, Citrus Canker in the Philippines. U. S. Department of Agriculture Cir. 1, No. 1, plate 8, 1915.

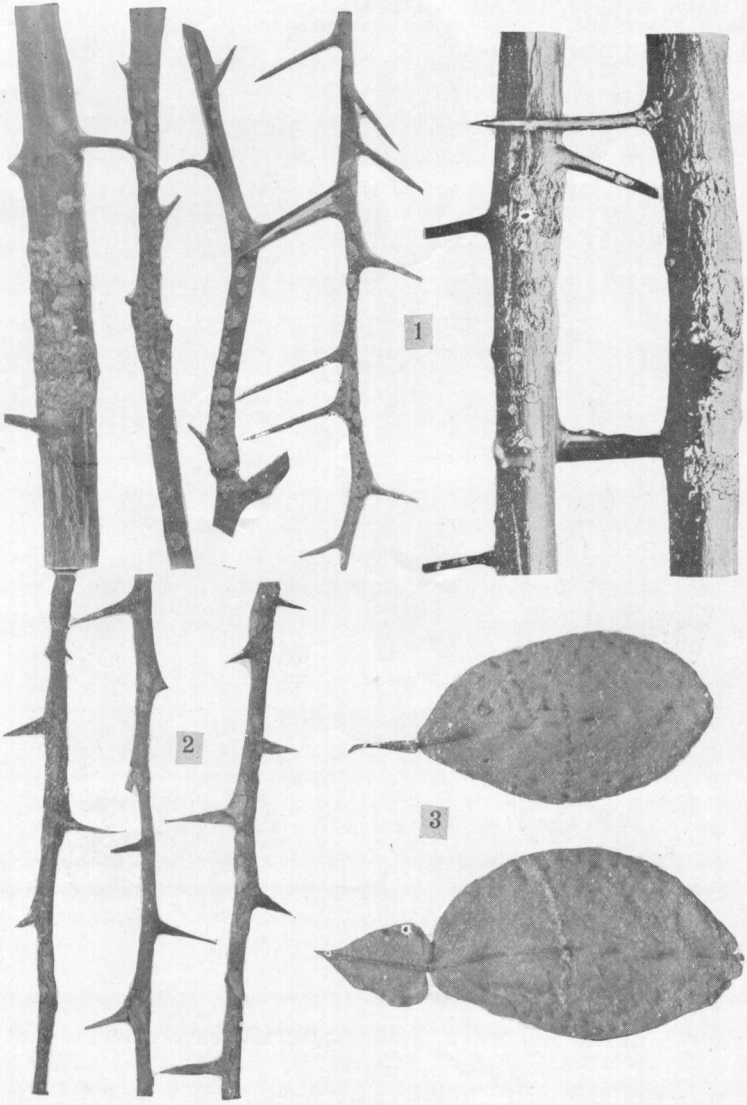

Fig. 1. Citrus canker on branches of grapefruit.

Fig. 2. Citrus trifoliata twigs affected with citrus canker.

Fig. 3. Infection through wounds made by thorn scratches on seedling grapefruit leaves.

PLATE II.

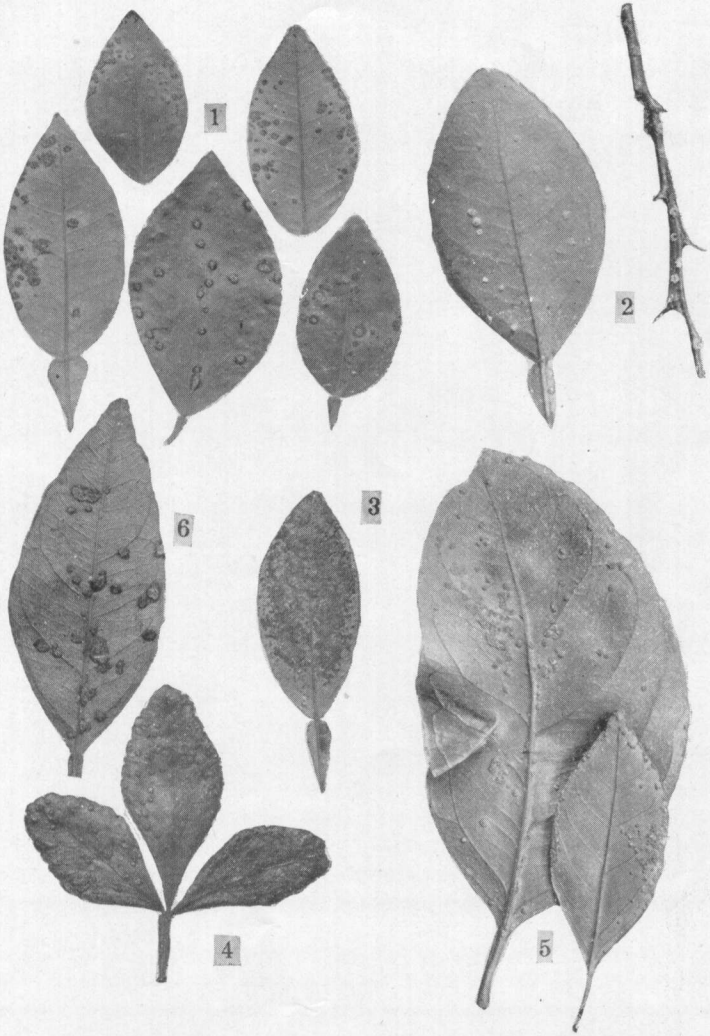

Fig. 1. Citrus canker spots on grapefruit leaves, natural infection.

Fig. 2. Spongy, white, prominently projecting cankers on leaf and twig of grapefruit. Plant inoculated with pure culture and continuously kept in a moist atmosphere.

Fig. 3. Infection resulting from immersion of leaf in suspension containing Pseudomonas citri. Cankers occupy nearly the whole of the lower leaf surface.

Fig. 4. Natural infection on Citrus trifoliata leaf.

Fig. 5. Suspension of Pseudomonas citri applied with an atomizer to Satsuma orange leaves, with resultant canker.

Fig. 6. Old cankers on Satsuma leaf.

or watery dots, most commonly on the lower leaf surface. They are slightly convex and within a few days will have extended entirely through the leaf. The spots gradually increase in size, the convex surface comes to be more and more elevated on one or both leaf surfaces until the outer membranes of the leaf are broken, whereupon the exposed canker tissues becomes light brown in color (Plate 2, figs. 1, 4 and 6.) An oily border marks the margin of the cankers which come to be surrounded by a yellowish zone. All of the tissues not occupied by the lesions may become yellow, in which case the leaves fall, especially when grapefruits and trifoliate oranges are affected.

Cankers on twigs and larger limbs do not differ materially in appearance from lesions on the leaves. (Plate 1, figs. 1 and 2.) They are larger, project more or less prominently and become variously cracked or fissured. Their presence on the twigs may result in the stunted growth or death of the distal parts. The cankerous areas on the fruits are also similar in appearance to the leaf cankers. Scurfy, elevated spots surrounded by a yellowish zone are formed. When large areas are involved the fruits crack open, thus permitting organisms which cause decay to enter. Affected fruits usually fall, or if they remain on the tree they are rendered very unattractive.

Cause of Citrus Canker.

The primary cause of citrus canker is a bacterial parasite, Pseudomonas citri. Announcement of this fact was first made by Miss Hasse (3) as a result of isolation of the organism from grapefruit and reinoculation with pure cultures upon grapefruit seedlings. The disease had previously been regarded as of fungous origin but the successful artificial inoculations from which this conclusion was drawn were produced by the use of mixed cultures containing Pseudomonas citri. The bacterial organism has repeatedly been isolated during the past summer from cankers on grapefruits, Satsumas, trifoliate oranges and lemons. No difficulty has been experienced in effecting cross inoculations from any of these hosts upon either grapefruit,

(3) Hasse, Clara H. Pseudomonas citri, the cause of citrus canker, Jour, Agr. Research 4. No. 1, pp. 97-101, Pls. IV and X, 1915.

pine apple, oranges, Satsumas or seedling trifoliate oranges. Successful inoculations were made by spraying the trees with a bacterial suspension obtained from pure cultures, plate 2, fig. 5, by immersion of the leaves in such suspensions, plate 2, fig. 3, by transferring the bacteria into the tissues through needle punctures, or by rubbing the leaves between the thumb and fingers after having dipped them in a suspension containing bacteria.

The period of incubation appears to vary, depending on temperature, moisture, and age of the plant tissues. The disease may be evident to the unaided eye three days after inoculation in some cases and ten days may be required in others. The most rapid development of the disease occurs under humid conditions, on young tissues. Mature parts, however, may become diseased. The illustrations, representing natural and artificial inoculations in Miss Hasse's report, differed so materially and the latter were so unlike anything which had ever come under the writer's observations that no explanation of the differences could be made at first. When, however, inoculated plants were kept under bell jars in a saturated atmosphere cankers represented in plate 2, fig. 2. were formed which are regarded as similar to the artifical infections produced by Miss Hasse, plates IX and X.

Pseudomonas citri is a yellow, rod-like organism about three or four times as long as broad. It occurs singly or in chains of six or more elements. The organism possesses a lash-like process which enables it to move about in liquids. It grows readily in culture on a variety of artificial media. It is capable of withstanding somewhat higher temperatures than many other bacteria which cause plant diseases. Stevens (4) found that the bacteria can be killed at temperatures ranging from 55-60 degrees C. Tests conducted under other conditions by the writer led to the conclusion that the thermal death point of Pseudomonas citri is about 65 degrees C. It is believed to be rather resistant to drying since it retained its vitality for about two months on microscopic slides placed in moist chambers. Stevens (4) found that it did not survive exposure on glass slides for two weeks under laboratory conditions.

(4) Stevens, H. E. Citrus canker, III, Fla. Agr. Exp. Sta. Bull. 128: p. 3-20; figs. 4, 1915.

He found it to be alive, however, after five weeks on cheese cloth, wetted in a bacterial suspension.

Fig. 1. (a) Pseudomonas citri stained with carbol fuchsin, (b) stained with Williams' flagella stain (adapted from Hasse); (c) stained with analine gentian violet.

It has been found that fungi belonging to the genera Phoma, Gloeosporium, and Fusarium are associated with citrus canker. The former alone is notably active in the disintegration of host tissues. This was determined by specific tests for the production of certain enzymes. It was found to be capable of dissolving cellulose, starch, cane sugar and maltose. It is also able to utilize the organic acids of the host as evidenced by a decrease in acid content of tissues on which it is growing. Because of its activity in the disintegration of citrus tissues, of its common association with citrus canker, which fact would help in its identification, its probable introduction to the Gulf States with Pseudomonas citri, and the impossibility of assigning it to species already described, it is regarded as an undescribed species and given the name Phoma socia.

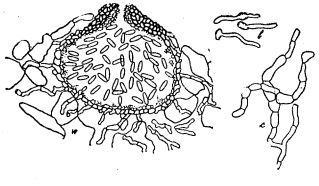

Fig. 2. (a) Pycnidium of Phoma socia, (b) Germination of conidia of Phoma socia, (c) mycelium in old culture.

Life History.

So far as is known Pseudomonas citri passes its entire life cycle under natural conditions within the host tissues. New infections appear in spring shortly after the new growth has begun. The first appearance in Alabama was on May 11th in 1914 and on May 27th in 1915. Old cankers on leaves and twigs are undoubtedly the source of infection in spring since new leaves formed near such old cankers are especially liable to first become diseased. Infections occur not only on new growth but on old leaves and twigs. Old diseased areas may enlarge by the renewed growth of the organism at the margin of the old cankers. New infections may appear at any time throughout the growing season and have been observed to occur as late as in the month of November. It is not known how long the organism can remain viable under natural conditions on fallen leaves, but it is believed that it can survive the winter. It appears to have perished in the laboratory in packets of leaves kept from September until May in one case, and from March until October in another.

Stevens (4) found that the canker organism was not only alive but also actively growing in inoculated test tubes of soil kept in the laboratory for six months. That it remains alive in the soil is indicated by the appearance of diseased sprouts from the roots of diseased trees which are burned.

Infections occur through natural openings, breathing pores on the leaves and twigs, and through wounds. It was observed in the inoculation experiments that infection appears first on the lower leaf surface, upon which side the stomata occur. From this it was inferred that the bacteria enter the leaf through the stomata. That such is the case was established by a study of leaf sections which were fixed 72 hours after inoculation, infiltrated with paraffin, cut and properly stained. This observation is contrary to that of Stevens (3) in which he states without giving evidence that the organisms are capable of penetrating either surface of the leaf. Infections through natural openings are possible only in the presence of a film of moisture on the host parts. Wounds inflicted on leaves and branches by thorn scratches have been observed to have afforded entrance to the canker organism, plate 1, fig. 3.

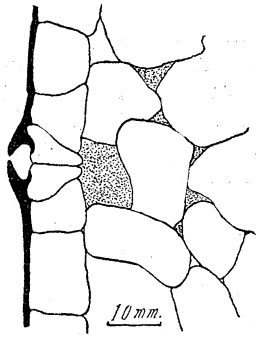

Fig. 3. Infection of Pseudomonas citri through a stoma or breathing pore with bacteria in substomatal cavity, and adjacent intercellular spaces, seventy two hours after inoculation.

When once the bacteria are within the host they multiply rapidly, effect a passage between the host cells and come to occupy the intercellular spaces. Their presence within the tissues is evidenced in three to five days by oily or watery dots which within another week will have developed into open cankers. At this stage before the exposed cankerous cells have become dry, the greatest danger of spreading the disease exists.

Effect Upon The Host.

The most manifest effect of canker upon the host, as determined by microscopical examination, is the en-

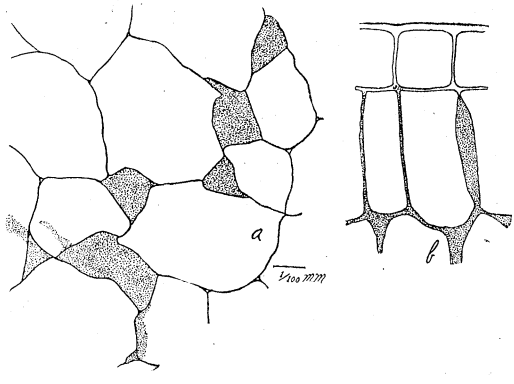

Fig. 4. (a) Pseudomonas citri occurring between the cells of the mesophyll tissue and (b) of the palisade parenchyma.

largement of affected cells. Little if any cell division in cankerous tissue is believed to occur. The tension re-

sulting from the enlargement of cells causes the rupture of the epidermis and the exposure to desiccation of the cankerous cells. Such cells are only lightly attached to each other as shown in fig. 5, and will separate intact in a drop of water on a slide when spongy cankers, plate 2, fig. 2, are examined. The bacteria are normally found to occur between the cells and not within them, as stated by Hasse.

Several causes operate in bringing about the enlargement and separation of cankerous cells. Among them are (1) the presence of the bacteria between the cells with the consequent passage of materials which are used in the growth of the bacteria, through the host cell walls, (2) the bacteria dissolve the middle lamellae; (3) they dissolve starch and otherwise affect the cell contents so that diseased cells have a greater affinity for water.

Death of diseased cells results in part from drying after the rupture of the epidermis and the cell walls gradually become suberized.

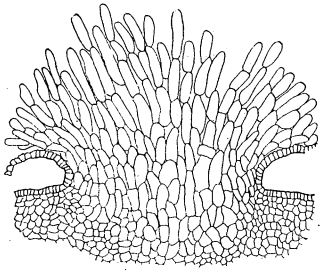

Fig. 5. Spongy canker in outline on rind of grapefruit showing enlargement of cells and indicating the ease with which they may be separated.

A chemical analysis of diseased and of healthy grapefruit leaves by the employment of a refined method of analysis shows that there have been in cankerous tissues profound changes, especially in the carbohydrate and nitrogenous substances. It was also found that there is a decrease in acid content in diseased tissues. An attempt was made to correlate the difference in acidity of grapefruit leaves and Satsuma leaves with the difference in susceptibility of these two species to canker. Satsuma leaves are consistently higher in acid content than grapefruit leaves, but the difference

in total acid content of the two is not regarded as sufficient to account for the difference in susceptibility.

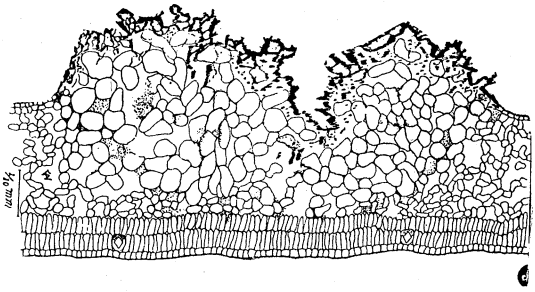

Fig. 6. Cross section of citrus canker on grapefruit leaf showing enlargement of cells of mesophyll and collapse of exposed cells.

Spread of Citrus Canker.

Rain and dew are probably to be regarded as very important factors in carrying the disease to unaffected parts of trees in which the disease is already present. Man himself is a very important agent in effecting the spread of canker from diseased trees to nearby healthy ones. The bacteria may be present in drops of water or in a film of moisture on the affected trees, especially if newly formed cankers occur on these diseased trees. In the cultivation and care of the groves man may come in contact with these infected trees and carry the bacteria to healthy ones. The spread of canker to two groves which have come under observation was very probably effected by the human agency. Sterling (5) reports transmission of the disease through handling diseased leaves prior to touching healthy ones. Certain birds and insects may also transfer the organism from diseased to healthy parts.

Control.

Efforts toward control have been directed along three lines: exclusion, protection and eradication.

The further introduction of the disease into the United States from foreign countries and localities has

(5) Berger, E. W., Stevens, H. E., and Sterling, Frank. Citrus canker II. Fla. Agr. Exp. Sta. Bull 124, 27-53, figs. 6-14, 1914.

been prevented by Federal quarantine. The several states themselves have passed regulatory measures to prevent the further spread of canker within the states and from any one of them to any other of them.

The use of fungicides and disinfectants indicates that there is little to be hoped for in their use for protection against citrus canker. Field tests have been made with Bordeaux mixture, ammoniacal copper carbonate, soluble sulfur, Bordeaux to which bichloride of mercury, 12 tablets to three gallons, had been added, Bordeaux containing formaldehyde, 1-100, and Pyrox. Even when all visible signs of the disease are removed from the trees prior to the application of the fungicides their use does not prevent the reappearence of the disease on these trees.

The only method known of checking citrus canker is the complete destruction of all infected trees. Eradication by this procedure seems possible, but only when the work has been thoroughly done, with the observance of the strictest sanitary precautions.

The early efforts toward the eradication of citrus canker were confined to the removal of diseased parts in case the trees were only slightly diseased. When they were badly diseased the trees were severely pruned even though this necessitated the removal of all or nearly all of the branches. Trees thus treated were then sprayed thoroughly with Bordeaux mixture. After a few months trial it was found that the trees were still diseased. Further than this, the adjacent trees had become diseased, although they were apparently healthy when the pruning was done. As a result of this it was decided that only the complete destruction of affected trees as they stand in groves or nurseries would be effective. The eradication campaign with its concerted, heroic effort to stamp out citrus canker from the Gulf States is the outgrowth of this decision.