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THE BACTERIAL SPOT DISEASE OF THE PEACH AND OTHER STONE FRUITS 1 OF 1

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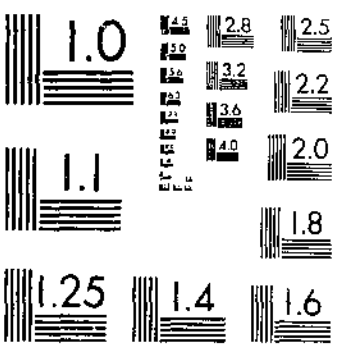

MICROCOPY RESOLUTION TEST CHART
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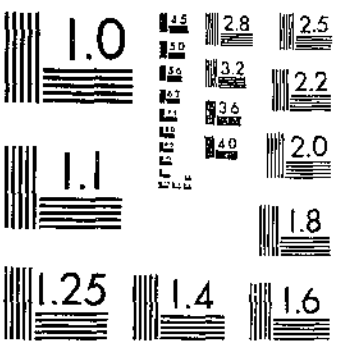

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NATIONAL BURLAU OF STANDARDS-1963-A

TECHNICAL BULLETIN No. 273 JANUARY, 1932

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

THE BACTERIAL SPOT DISEASE OF THE PEACH AND OTHER STONE FRUITS[1]

By JOHN C. DUNEGAN, *Associate Pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry*

CONTENTS

INTRODUCTION[2]

The bacterial spot disease caused by *Bacterium pruni* E. F. Smith was originally described in 1902 by Erwin F. Smith (*40*)[2] as a serious disease of the Japanese plum (*Prunus salicina*)[3] in Michigan. Subsequent investigations revealed that the pathogene was not confined solely to the plum, but that it was capable of producing a serious disease of the peach (*Prunus persica*), apricot (*Prunus armeniaca*), and nectarine (*Prunus persica* var. *nucipersica*).

Although the effect of the disease on the susceptible varieties of the plum has curtailed the production of this fruit in many parts of

[1] The writer wishes to acknowledge the invaluable aid rendered by John W. Roberts through his continued interest and advice during the course of the investigations and his assistance in the preparation of the manuscript. He also wishes to express his thanks to W. F. Turner, formerly horticulturist of the Central of Georgia Railway Co., for assistance in conducting the orchard surveys and harvesting the fruit on the experimental plots; to his former colleague Lee M. Hutchins, of the U. S. Peach Disease Field Laboratory, for the permission to use his experimental nursery plantings; and to the authorities of the College of Agriculture, University of Arkansas, for the use of the laboratories of the department of plant pathology, where certain phases of the investigations were completed during the period 1928 to 1930. In addition he wishes to express his appreciation for permission freely granted by Lynn McKenzie, of Montezuma, and John W. Woolfolk, of Fort Valley, Ga., to make observations and to conduct experiments in their peach orchards.

[2] Italic numbers in parentheses refer to Literature Cited, p. 51.

[3] In this bulletin Bailey's (7) botanical nomenclature has been used.

the country, it is mainly as a malady of the peach that the bacterial spot disease has attracted attention within the last decade.

The rapid expansion of the fruit industry has resulted in the planting of peach trees in many sections of the country in which conditions are particularly favorable to the bacterial spot disease. The expansion of the fruit industry also has increased competition between various production centers, and indirectly it has focused the attention of the fruit growers on any agency, such as this disease, that reduces the quality and marketability of the fruit.

The investigations of the disease from the time of its discovery until 1920 dealt mainly with the study of the pathogene. With the exception of the work of Roberts (*31*) in Arkansas during the years 1913 to 1915, inclusive, little attention was devoted to devising methods of controlling the disease in the orchard. In recent years the investigational work has changed somewhat in its manner of approach, and control experiments have been initiated by a number of investigators in different portions of the country.

Notwithstanding the excellent work that has been done, many questions of importance concerning the behavior of the causal organism have remained unsolved. Possibly the most important of these is the manner in which the causal organism survives the winter season. Mention also may be made of other points, such as the manner in which the pathogene spreads from tree to tree, the accurate identification of the various symptoms, the evaluation of the commercial injury, and even certain of the characters of the pathogene when isolated and grown in pure culture.

The investigations described in the following pages discuss the disease on the peach with but incidental references to the activities of the pathogene on other hosts. Emphasis has been placed particularly on the investigation of those points, enumerated above, upon which detailed information was either lacking or incomplete.

NAMES APPLIED TO THE DISEASE AND TO THE PATHOGENE

The name "black spot" was used by Smith (*42*, *43*, *44*) in his early papers to designate the disease on the plum. This name, while it is particularly descriptive of the symptoms on plum fruit, can not properly be applied to the disease on the peach both because the symptoms produced on the peach are not "black spots," at least in the sense of this term when applied to the plum, and also because the name "black spot" has been used for many years as a synonym of peach scab.[4]

Other names that have been applied to the disease on the peach are bacterial spot, bacteriosis, shot hole, bacterial shot hole, and bacterial crack. With the exception of bacterial spot and bacteriosis, these names are open to the objection that they characterize only certain phases of the disease.

The name bacteriosis has been widely used, but the writer feels that it is an unfortunate term, indicating merely a bacterial attack, and, as such, is too inclusive. Crown gall[5] on peach, for example, could just as properly be called bacteriosis.

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[4] Caused by *Cladosporium carpophilum* Thüm.
[5] Caused by *Bacterium tumefaciens* Sm. and Town.

Since the disease on the various hosts is caused by the same organism, although the symptoms vary, a name is desired that will be descriptive of the disease in all its manifestations. The writer considers that the name "bacterial spot," apparently first mentioned in the literature by Roberts (*31*), is particularly fitting, and it will be used as the name of the disease throughout this bulletin.

The pathogene causing the bacterial spot disease was originally named *Pseudomonas pruni* by Smith (*40*). In 1905 he substituted the generic name Bacterium Cohn emend. for the generic name Pseudomonas Migula as the result of his studies on the classification of the bacteria, and the organism was renamed *Bacterium pruni*.

No further changes were made in the nomenclature of the organism until 1923, when Bergey's Manual of Determinative Bacteriology (*8*) appeared. In this classification all the plant pathogenes were grouped together in the genera Erwinia and Phytomonas of the tribe Erwiniae, family Bacteriaceae. The bacterial spot organism was placed in the genus Phytomonas.

At the present time the organism may be called either *Pseudomonas pruni* E. F. Smith, *Bacterium pruni* E. F. Smith, or *Phytomonas pruni* (E. F. Smith) Bergey et al., depending upon the classification followed. The writer is using the name *Bacterium pruni* as the name of the organism.

HISTORICAL REVIEW

The published studies on the bacterial spot disease represent the work of a number of investigators both in this country and abroad. Only those papers describing the discovery of the organism and the demonstration of its pathogenicity will be discussed here, while those dealing with special phases of the disease will be reviewed in their appropriate places.

The bacterial spot disease was first described by E. F. Smith in a paper delivered at the Washington meetings of the American Association for the Advancement of Science in 1902. A brief résumé of the paper appeared in Science in 1903. In this paper Smith (*40*) discussed a disease of Japanese plum leaves and fruit that had made its appearance in central Michigan. The cause of this disease was a yellow bacterium which he named *Pseudomonas pruni*. This organism, he found, entered the uninjured plant through the stomata. The symptoms of the disease on the foliage and green fruits were described, together with some notes on the growth of the organism on culture media. This discussion apparently constituted the technical description of the newly found organism.

Later, in 1903, Smith (*41*) called attention to the fact that bacterial infection of plants by way of the stomata was not at all infrequent. He cited, among other diseases, the leaf spot of plum and stressed the fact that "sections through very young stages of spots * * * show the epidermis unbroken and the enclosed bacterial masses lying in such relation to the stomata as at once to suggest such infection."

In 1903 Clinton (*10*) noted an unnamed bacterial disease of peach leaves from Pomfret, Conn.

In 1904, at the Philadelphia meetings of the American Association for the Advancement of Science, Smith (*42*) described addi-

tional experiments which substantiated his earlier statements that the bacterium entered the uninjured tissues of the plum through stomata. Numerous infections were secured by spraying suspensions of the organism on leaves and young fruit. Inoculations made two or three weeks prior to the ripening of the plums were not successful, and Smith concluded, for this and other reasons, that the disease was principally one of meristematic tissues.

The bacterial spot organism is mentioned in several places in the first volume of Smith's classic work (*43*), which appeared in 1905. Drawings showing its effects on plum fruit and leaf tissue and one photograph (pl. 18, p. 148) of typical spots produced on the Hale plum in Michigan are included with the discussion. Outside of these rather incidental references the disease is not discussed in the book.

In the 1904–1906 report of the Delaware Agricultural Experiment Station, Jackson (*20*) discussed briefly a plum disease in that State called by the growers "bacteriosis." The disease was described as forming cankers in the young branches. Bacterial cultures were secured from the diseased areas and were used in inoculation experiments. Success was obtained with 4 out of 14 inoculations, and the original organism was reisolated. The disease was not definitely decided to be of bacterial origin, although the preliminary evidence indicated that it was. No description of the organism was appended to the report, but it is quite possible that the disease in question was the one Smith had previously described.

Clinton (*11*) in 1905 identified the organism causing a disease of plums in Connecticut as *Bacterium pruni* and suggested that possibly the bacterial disease of peach leaves he had mentioned in 1903 might be caused by the same organism as the plum disease. In 1909 Clinton (*12*) identified the bacterial disease of peach leaves as that caused by *Bact. pruni*, and he also published an excellent illustration of it.

Another early reference of interest is the report by Heald (*14, p. 32–33*), in 1906, of a twig canker of plums in Nebraska. The symptoms as described by him seem fairly characteristic of the disease, but it is not possible to decide definitely from the brief note that the organism was *Bacterium pruni*.

Smith (*44*) read a paper before the Society of American Bacteriologists in December, 1908, in which he reported the production of spots on the leaves of a peach tree standing in the open following the spraying of the leaves with a suspension of the pathogene isolated from plum. The spots produced in this experiment were identical, according to Smith, with those naturally occurring on the peach, and he felt that "there can be no doubt that the leaf spot of the peach is identical with that of the black spot of the plum, both being due to *Bacterium pruni*."

These experiments were the first cross inoculations of peach leaves with cultures of the organism derived from the plum.

In 1909 Rorer (*34*) discussed a bacterial disease of the peach. He stated that O'Gara had found a disease on peach foliage in Georgia that he thought might be of bacterial origin, and that the same trouble had been observed in 1905 by W. M. Scott. Rorer himself investigated the disease in 1906–07. He isolated a motile yellow organism from young leaf spots in 1906, and in 1907, using this organism,

was able to reproduce the disease on the leaves. As a result of these inoculations he concluded that the leaf-spot disease of peach was bacterial in nature.

Rorer described spots on the young twigs from which he isolated a bacterium apparently identical with that obtained from the leaves. He also observed a spot on the fruit that he considered to be caused by this organism. Attempts to isolate the organism from the fruit spots were not successful, but sections showed masses of bacteria present in the tissues.

Rorer grew the plum and peach organisms side by side in culture and found they had the same cultural reactions. These facts, together with the similar symptoms on the two hosts and the results of the inoculation experiments, indicated strongly that the two organisms were the same species, and he felt it was safe to assume that the organism causing the peach leaf, twig, and fruit spots was *Bacterium pruni*.

Heald and Wolf (*15*) reported the presence of a bacterial disease on plum twigs in the vicinity of San Antonio, Tex., in 1912. From their description of the symptoms it seems likely that they were dealing with the disease caused by *Bacterium pruni*.

Lewis (*25*), also working in Texas, published in 1912 an account of a bacterial canker of plum twigs. The organism was isolated from the cankers, and when suspensions were sprayed upon young plum trees abundant leaf spots developed. Isolations from these leaf spots yielded cultures of an organism similar to the one originally isolated from the twig cankers. Although many leaf spots developed, only a few cankers were produced on the twigs. The cankers that developed resembled those occurring in the field under natural conditions. Further inoculations were made by puncturing the twigs with a sterile needle and then introducing bacteria into the wounds. This procedure resulted in the production of twig cankers on both plum and peach.

The fact that the organism isolated from plum twigs could cause spots on the leaves led Lewis to reexamine the orchard from which the twigs were obtained. Leaf spots were found in abundance and also a few fruit spots. A yellow organism was isolated from both of these sources and grown side by side with the organism originally isolated from the cankers. Their cultural characteristics were similar; and since these agreed in all respects with those of the original description of *Bacterium pruni*, Lewis felt certain that the plum organism he had isolated was none other than *Bact. pruni* E. F. Smith.

Rolfs (*33*) in 1915 presented the first monographic study of the disease. It is in this paper entitled "A Bacterial Disease of Stone Fruits" that we find the first detailed description of the growth of the organism on various media. Likewise, it is in this paper that we have the first attempts to describe in detail the seasonal life history of the organism and the first mention of the cankers as carriers of the disease from one season to another. The apricot and nectarine are added to the peach and plum as susceptible species, and the symptoms on the leaves, twigs, and fruit of all four of these fruits are discussed. The pathological histology, incubation period, effect of environmental factors, and other points are touched. In short, his paper is a true monograph of the disease, although certain

of his conclusions, in the light of recent investigations, need modification, and his discussion of the seasonal life history of the organism can be amplified so that a more exact understanding of the behavior of the organism is gained.

In 1918 McCubbin (*29*) observed an outbreak of the disease in the peach orchards of Ontario, Canada. Severe defoliation occurred on the Elberta variety and to a lesser extent on the J. H. Hale variety. Bacterial lesions were observed on about 20 per cent of the twigs of the current year, and bacterial exudate was observed on the older leaf spots. A microscopic examination of the diseased tissues revealed hollow cavities filled with masses of bacteria.

In 1917 Sackett (*36*) discovered a bacterial disease of the Wragg cherry (*Prunus cerasus*) which he thought was caused by *Bacterium pruni*. He reported upon this disease from time to time (*37*), finally concluding in 1925 (*37*, *Rpt. 38*) that the organism was not *Bact. pruni* but a form which he named *Phytomonas cerasi wraggi*.

Ishiyama (*19*) reported in 1922 that he was unable to secure stomatal infection of the almond (*Prunus communis*) with an organism he determined as *Bacterium pruni*. He stated that negative results were obtained repeatedly and doubted Smith's conclusion that the organism gains entrance to the host through the stomata. He felt that the organism was a wound parasite.

THE DISEASE

SYMPTOMS

The disease occurs on the leaves, twigs, and fruit, and produces characteristic symptoms distinguishing it from other diseases of peaches and plums.

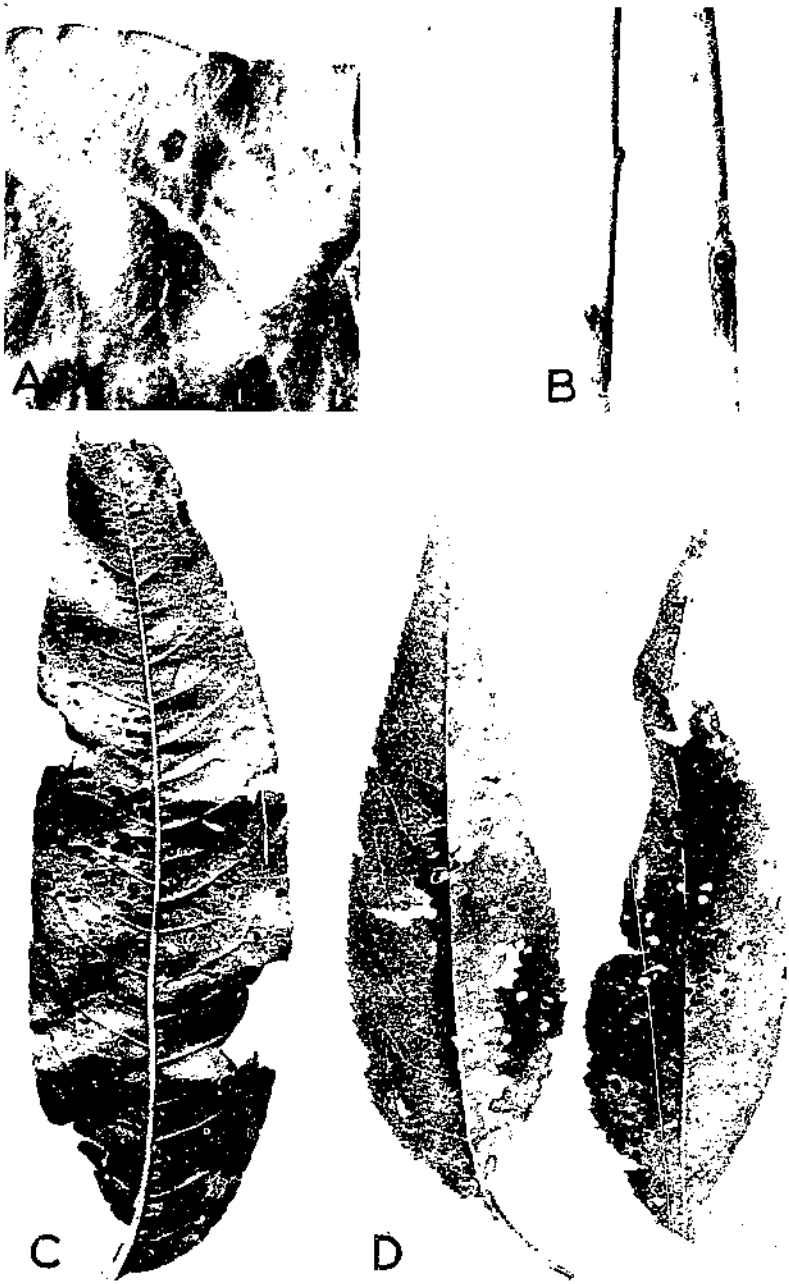
LEAVES

The first indication of the disease on peach leaves is the appearance of circular, pale-green (almost white) spots 0.5 to 1.0 mm. in diameter on the upper surface of the leaves. These spots, which Rolfs (*33*) termed "grey specks," occur singly or in groups, and are frequently very numerous at the tip of the leaf, but may occur scattered over the entire surface of the blade.

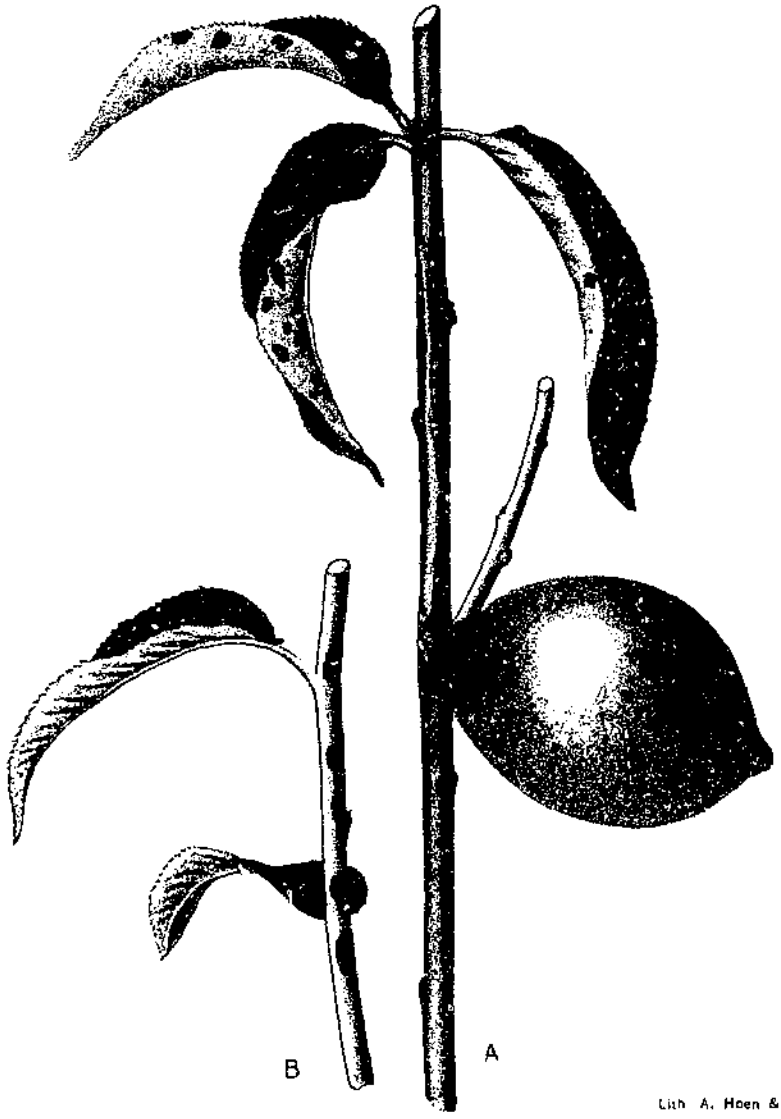
The color of these small circular spots soon changes from the very pale green of the original spot to light brown. The color of the tissue immediately surrounding the brown area fades to a light yellowish green. This band of light yellowish green tissue forms a halo (pl. 1, A) about the brown tissue in the center of the spot.

The next symptom to appear is the development of a "water-soaked" area (pl. 1, C) involving the entire spot, including the light-green halo. The tissue is probably water-soaked in the true sense of the term, as this symptom has been observed only following heavy rains or heavy dews.

The symptoms just described are rather evanescent and give place very soon to those which have been widely observed, namely, small, purple to purplish brown spots from 1 to 5 mm. in diameter. (Pl. 2, A.) These spots are generally angular in shape, and the injury seems to be confined largely to the areas included between the very small veins. A microscopical examination of sections from these



A, Portion of Woodland Cling peach leaf enlarged to show halo about the spot. Collected at Griffin, Ga., June 1, 1927, × 4; B, cankers caused by *Bacterium pruni* on twigs of *Prunus davidiana* collected at Fort Valley, Ga., June 15, 1928, × 1; C, water soaked appearance of leaf spots. Leaf of seedling peach collected at Byron, Ga., May 17, 1927, × 1; D, spots caused by *Bact. pruni* on leaves of *P. davidiana* collected at Fort Valley, Ga., June 15, 1928, × 1



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SYMPTOMS OF BACTERIUM PRUNI INFECTIONS ON THE PEACH

A.—Twig with an overwintered canker and fruit and leaf spots. The canker resulted from infection during the season of 1924, and the fruit and leaf spots were the result of infections in 1925. Elberta variety, collected at Barron's Lane, Ga., May 30, 1925. B.—Seedling twig showing lesions resulting from artificial inoculations made May 8, 1925, at Fort Valley, Ga. The twig was removed from the tree on May 30, 1925

Painted by J. Marion Rhull

spots confirms this observation, as only rarely was the organism observed to spread beyond the vascular units of the small veins.

The individual spots are rather small, but adjacent spots may fuse and involve rather extensive areas of the leaf surface. The tissues killed by the organism become darker in color, dry out, and tear away from the healthy tissues, producing the so-called shot-hole effect, which is a typical symptom of the advanced stages of the disease on the leaf. When the fusion of adjacent spots and the subsequent dropping out of the dead tissues occurs at the tip or along the margins of the leaf a very ragged appearance results.

The spots resulting from the infections of the leaves by *Bacterium pruni* have been confused with leaf injuries resulting from arsenical sprays and with a widespread leaf-spot and shot-hole disease that seems to be physiological in nature.

It is difficult to establish criteria for the rapid and accurate separation of young spots caused by the bacterial pathogene and small spots resulting from arsenical injury. The presence of the halo or light-green area about the bacterial spot is probably its most distinguishing characteristic. In general the arsenical injury spots are rounded to irregular in outline and present a more or less zonate or target-board appearance. They also are accompanied frequently by scorched leaf margins and small cankers about the leaf axils.

The presence of bacterial exudate on the older spots facilitates the identification of the disease in the field. This exudate occurs on the under surface of the leaf in the form of either globular droplets (observed only rarely) or thin whitish membranelike scales, closely appressed to the surface of the leaf. These membranelike scales are the result of the drying out of the mass of bacteria after it has exuded from the necrotic tissues. The presence of these scales can be detected by their slightly upturned margins or by the smooth glassy appearance of the spot. Frequently the membranelike scale is free save a small area in the center. At times the exudate can be detected on the upper surface of the leaves, but the writer has never seen it as pronounced there as on the under surface.

The spot caused by *Bacterium pruni* can be distinguished from the one supposed to be physiological in nature by the fact that the margins of the latter are more or less fimbriate. In the so-called physiological spot there seems to be a production of anthocyan pigment which, instead of becoming concentrated into a solid area, is diffused along the tiny veins. It is this diffused character of the discoloration that produces the fimbriate margins.

The symptoms of the disease on plum leaves are more definite and pronounced than on peach leaves. This is particularly true of the "water-soaked" appearance of the spot on the under surface of the leaf and also of the presence of bacterial exudate. The writer has seen cases on the plum where there were as many as 10 individual membranelike scales on the under surface of a small leaf.

A leaf spot caused by a species of Coccomyces (Cylindrosporium) at times may be confused with the bacterial spot disease, but the presence of the fungus hyphae and usually the conidia on the spot should remove any doubt as to the true nature of the causal organism.

Arsenical burning on plum leaves, although more likely to be marginal, presents the same difficulties that it does with the peach,

and positive identification can be made at times only with the aid of the microscope.

The writer has observed *Bacterium pruni* leaf spots on the nectarine (*Prunus persica* var. *nucipersica*), the apricot (*P. armeniaca*), and *P. davidiana*, and the symptoms are similar to those already described for the peach and plum. However, an opportunity was not available to study these hosts as intensively as the peach and plum, so there is a possibility that certain variations have been overlooked.

FRUIT

The symptoms produced on peach fruit are twofold in nature—primary, those which appear directly after the invasion of the tissue by the organism, and secondary, subsequent changes in the primary lesions produced by the peach itself as the result of growth. Both types are symptoms of the disease, but, strictly speaking, those in the second category are really indirect, as Smith (*45, p. 46*) has pointed out.

The first macroscopic indication of the disease on the fruit is a circular, faint-brown spot on the surface of the skin. The spot is generally 0.5 to 1.0 mm. in diameter and is not readily discernible without the aid of a lens. As the spot enlarges the center becomes slightly darker and sunken, and frequently the margin appears "water-soaked." (Pl. 3, A and B.)

The spots themselves are not limited to any special area on the surface of the fruits nor does any area seem to be particularly favorable for their development. In this respect the disease is decidedly different from peach scab,[6] where the stem end is the favored site for infection. The spots may be isolated or they may occur in groups; in the latter case they very frequently coalesce and involve extensive areas.

As a general rule, primary symptoms appear only while the fruit is small, although in seasons favorable to the spread of the disease primary symptoms may be found on the fruit even at harvest time.

The secondary symptoms, as mentioned before, result from the stresses and strains set up as the fruits increase in size. For the purpose of discussion they may be divided into several different types.

Small-crack type.—This is a very common type (pl. 4, A, and pl. 2, A) found throughout the season. The secondary symptoms seem to be confined mainly to the development of small, shallow cracks. In general, the appearance of the fruit is not seriously marred.

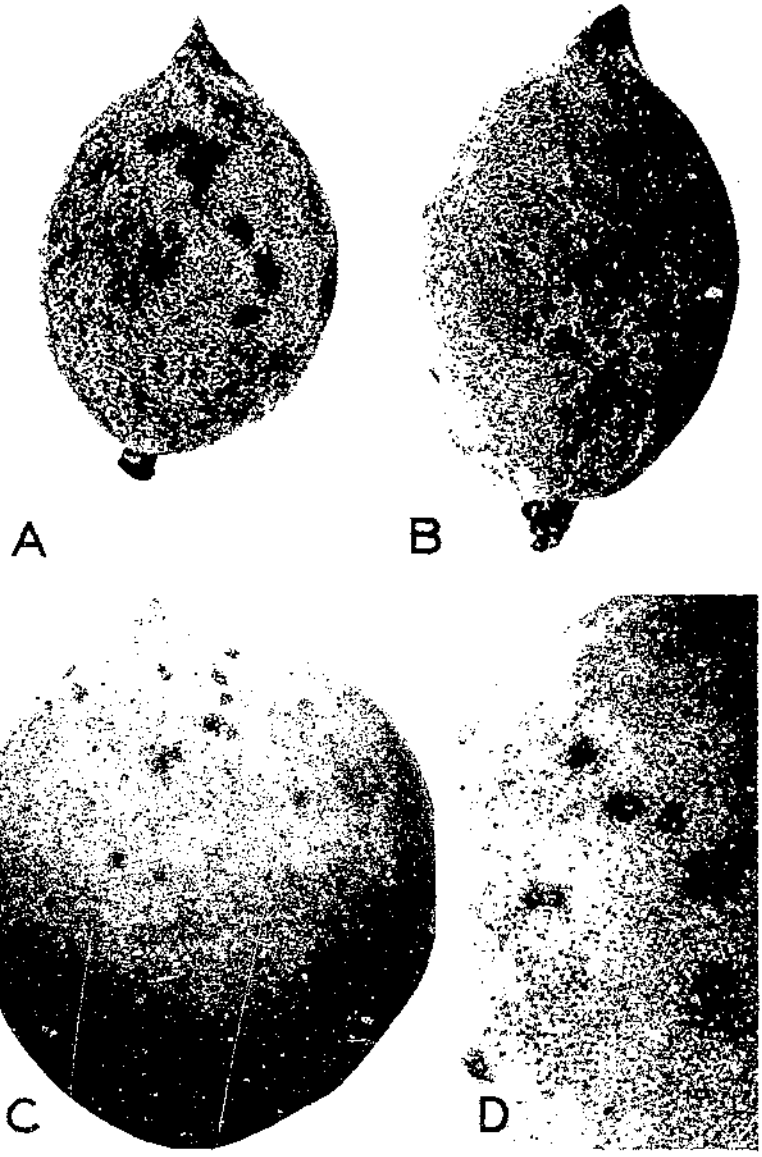
Coalesced-crack type.—This type (pl. 4, B) is very obviously derived from the small-crack type by the coalescing of the individual cracks. In this group there is considerable variation in the severity of the injury, ranging from a few cracks which do not materially injure the appearance of the fruit to cases in which large areas are checked and cracked and the fruit is rendered worthless.

Island type.—The small cracks form circular aggregations (pl. 4, C) with remnants of the original tissue remaining as "islands" in the central portion of the affected areas.

Stellate type.—The cracks in this rather uncommon type (pl. 4, D) coalesce more or less at right angles to form star-shaped lesions.

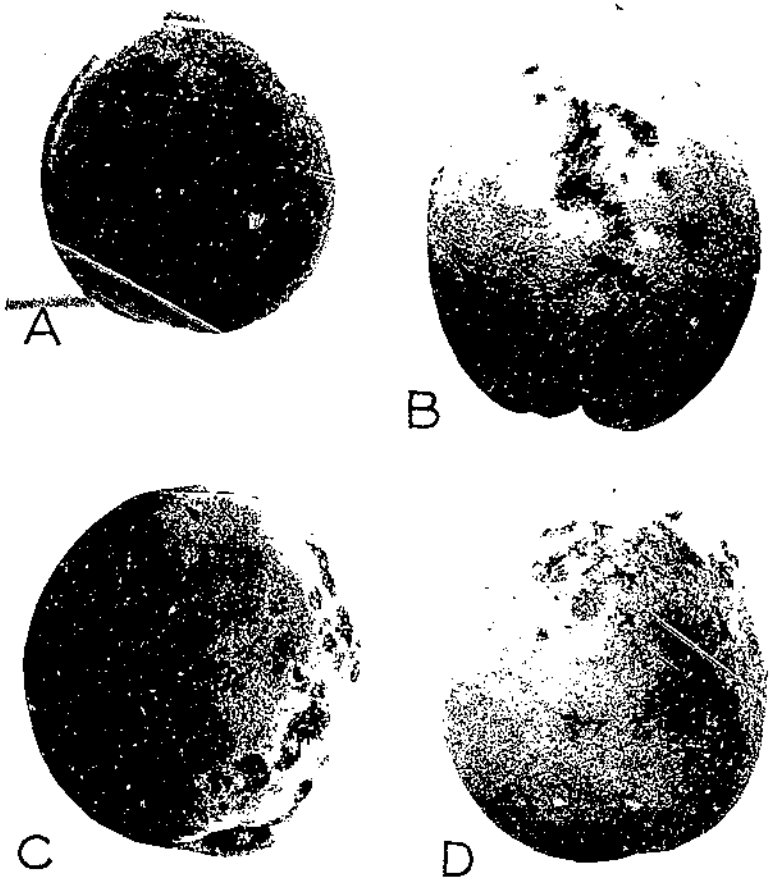
Pin-point type.—In this type (pl. 5, A), which is quite distinct from those just described, the cracks are very small (generally less than 0.5 mm. long). Each crack when present on the blush surface of the fruit is surrounded by a circular light-green halo that gives the surface a mottled appearance. The

[6] Caused by *Cladosporium carpophilum* Thüm.



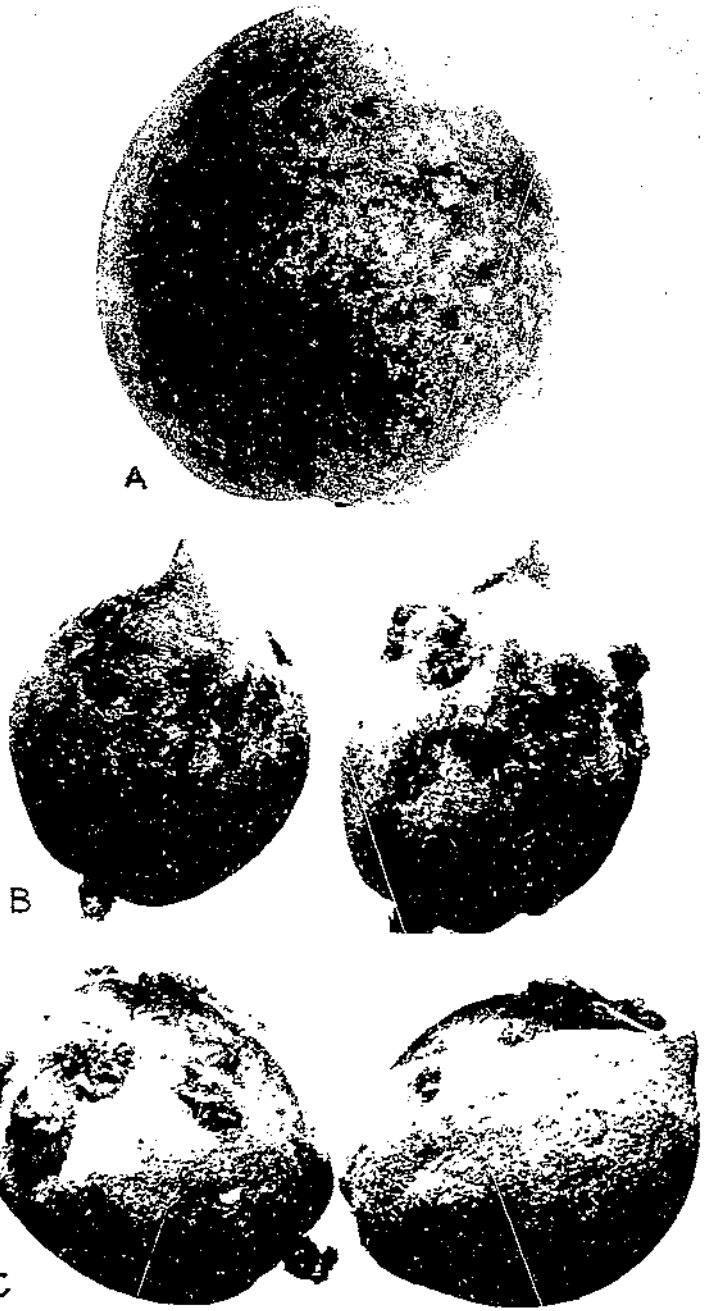
PRIMARY SYMPTOMS OF BACTERIUM PRUNI INFECTION ON PEACH FRUIT

A and B, Young spots on immature Elberta fruit from Barron's Lane, Ga., collected April 7, 1925. The water-soaked appearance of the spots is quite pronounced, but does not show well when photographed, × 1½. C, bacterial exudate on young spots. Elberta peach from Springdale, Ark., collected August 17, 1929, × 1½. D, portion of C enlarged to show more clearly the exudate about the young spots, × 3.



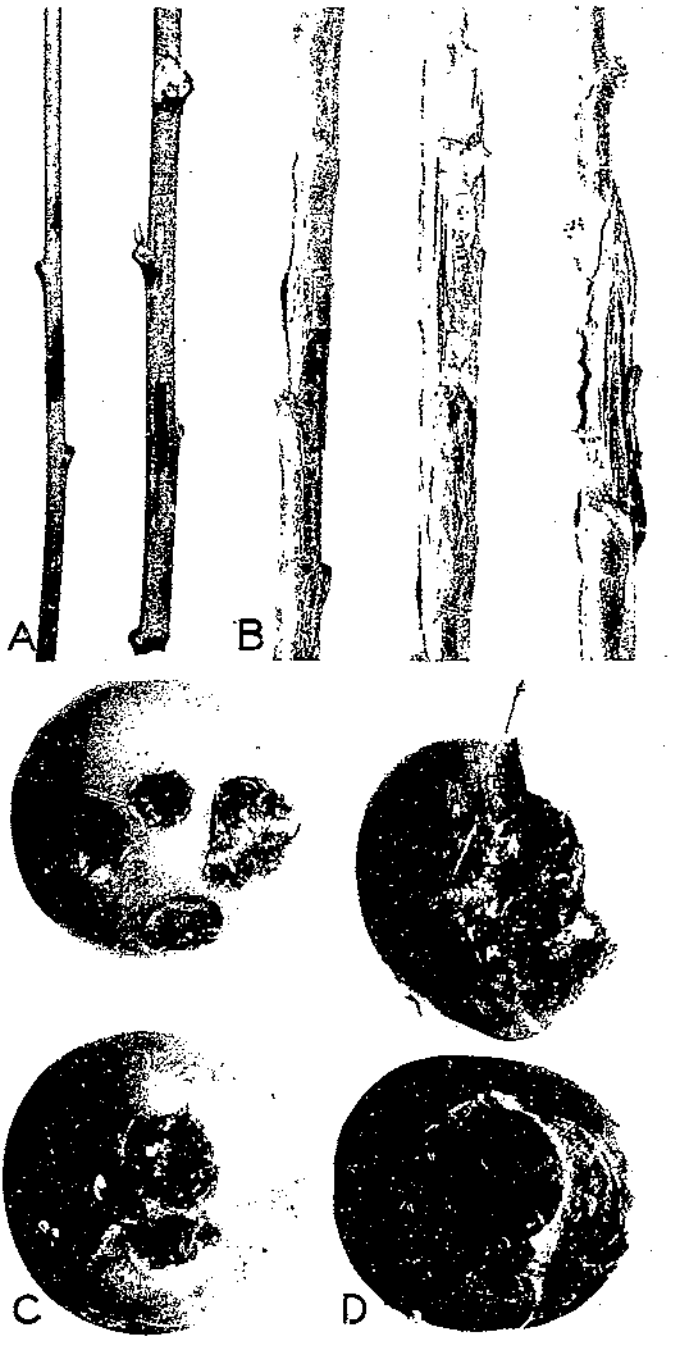
SECONDARY SYMPTOMS OF BACTERIUM PRUNI INFECTIONS ON PEACH FRUIT

A, Small-crack type on Elberta fruit from Barron's Lane, Ga., collected July 16, 1923; B, coalesced-crack type on Elberta fruit from Barron's Lane, Ga., collected July 12, 1927; C, island type on Elberta fruit from Barron's Lane, Ga., collected July 12, 1927; D, stellate type on White English peach from Lamar, Ark., collected August 9, 1929. All × ⅔.



SECONDARY SYMPTOMS OF BACTERIUM PRUNI ON PEACH FRUIT

A, Pin-point type on Early Rose peach from Thomaston, Ga., collected June 21, 1928, × 3½; B, gum-flow type on Woodland Cling peaches from Griffin, Ga., collected June 1, 1927, × 1; C, gum-flow type cut open to show typical necrotic spot in tissue under mass of gum, × 1. (Although B and C might be confused with insect injuries, the writer obtained pathogenic cultures of the causal organism from the diseased tissues.)



A, Young cankers on seedling twigs from Byron, Ga., collected May 3, 1927, ×3½; B, overwintered *Bacterium pruni* cankers on Elberta twigs collected June 14, 1928. Cultures of the organism were isolated from cankers identical with these, × ¾; C and D, spots on mature Shiro plums from De Queen, Ark., resulting from infection by *Bact. pruni*. Note the secondary invasion of the tissue by the brown-rot fungus (*Sclerotinia fructicola*) on the plum at the lower right, × 1

spots may coalesce, but the majority remain distinct. The halo about the spot causes it superficially to resemble young spots caused by the peach-scab fungus. This type is particularly common on the earlier-maturing varieties, such as Carman and Early Rose, but it may occur on all varieties and was observed frequently on the Elberta in Arkansas in 1929.

Gum-flow type.—Occasionally gum exudes from the diseased tissues, causing the spot to be confused with injuries caused by chewing insects. If the gum is lifted away carefully the identity of the spot generally can be determined. The copious production of gum such as is illustrated in Plate 5, B and C, although found on certain varieties of clingstone peaches, is rather exceptional for most other varieties of the peach, the vast majority of the spots remaining free from gum throughout the season.

There are certain other symptoms on the fruit which are of considerable aid in identifying the spots. One is the production of exudate from the young spots. (Pl. 3, C and D.) This phenomenon has been noted occasionally on spots 2.0 mm. in diameter, but is much more common on slightly larger spots. The exudate is slightly viscid under conditions of high humidity, yellowish in color, and dries into small hornlike projections. Occasionally there is a small bit of epidermis resting on top of the mass of exudate which would indicate that the epidermis was torn away by the pressure of the mass of bacteria below it. This exudate serves as inoculum for subsequent infections which occur every season, but are more numerous in rainy seasons when conditions are exceptionally favorable. When these secondary infections occur, spots of varying ages and stages of development are present on the fruit, ranging from the merely discolored areas (primary symptoms) of very recent infections to large cracks (secondary symptoms) where the older spots have coalesced and the tissues have been ruptured by the growth of the fruit.

The fruit spots, particularly those more advanced, are so characteristic that there is little likelihood of the disease being confused with other troubles to which peach fruit is subject. However, as mentioned before, the small spots at the stage when the first cracks begin to appear on the peach may be confused with scab spots. This is particularly true of the pin-point type if the spots occur on the red or blush surface of Early Rose or Georgia Belle fruit. Peach scab, however, is very superficial, and the true identity of the bacterial spots can be determined if the epidermis is scraped away and the necrotic area in the tissues revealed.

Mild cases of arsenical injury to the fruit possibly might be confused with the young spots caused by the pathogene, especially the pin-point type. The symptoms resulting from severe arsenical injury are very distinct and can be distinguished readily from *Bacterium pruni* spots.

The writer has seen one instance in which the injury to fruit resulting from copper dust applied to peach trees might be confused with the pin-point type of infection.

TWIGS

Rorer (*34*) was the first investigator to report the occurrence of *Bacterium pruni* lesions on peach twigs. In the course of his studies he observed cankers on the twigs of the current season's growth and obtained cultures of the organism from them.

Rolfs (*33, p. 393*) stated that the twig lesions usually appear during the month of May or June. According to him, "* * * the first visible indication of a canker is the appearance on the young shoots of a water-soaked spot surrounding a lenticel." He, too, was successful in obtaining cultures of the organism from the diseased tissues.

Roberts (*31*) mentions the occurrence of twig cankers and gives a brief description of their external appearance.

Notwithstanding the work of these investigators, considerable confusion still exists concerning the cankers on peach twigs. This probably is due to the fact that they are much scarcer than the work of Rorer and of Rolfs would indicate, and also to the fact that arsenical injuries and other spots, especially a reddish nonparasitic spot, frequently have been identified erroneously as true bacterial-spot cankers.

The youngest lesions the writer has observed have been water-soaked areas on the twigs. They involved more than one stoma and were not considered the first stage of canker development. Slightly older lesions were brown to purple black in color, only slightly sunken in the center, circular to elliptical in shape, with water-soaked margins. (Pl. 6, A.) They were not confined to any one side of the twig or to a particular location, but occurred at and between the nodes. The true nature of these lesions was established by isolating the organism from the diseased tissue and proving its pathogenicity in subsequent inoculation experiments.

A further stage in their development was observed later in the season when cankers 10 mm. long, elliptical, with their long axes parallel to the long axis of the twig, were noted. The epidermis in the center of the diseased area was ruptured, and gum was oozing from the necrotic tissues. The individual cankers had started to coalesce, forming elongated areas of blackened and sunken tissues. While increase in size mainly was parallel to the long axis, there also was a tendency for the lesion to spread sidewise and encircle the twig. No case was observed in which the twig was girdled completely by a single canker, but practically the same result was achieved by the lateral fusion of adjacent cankers.

Peach twigs are yellowish green to green during the early part of the growing season, and consequently the dark lesions are quite conspicuous on them. Toward the end of the growing season, however, the twigs become dark green and the bacterial cankers are no longer conspicuous.

In the literature there is very little mention of the appearance of twig cankers during their second season. They are not common in Georgia orchards, but a few have been found each year after an intensive search for them. These overwintered cankers are elliptical in shape and black in color. The bark is ruptured, and in some cases considerable gum oozes from the diseased area. Those observed ranged from 8 to 24 mm. long and from 2 to 7 mm. wide. Their general appearance is shown in Plate 6, B, and Plate 2, A.

ECONOMIC IMPORTANCE

The economic losses resulting from outbreaks of *Bacterium pruni* may be divided into three categories:

(1) The devitalization of the tree, with its manifold consequences as the result of defoliation.
(2) Killing of twigs.
(3) Damage to the fruit each season as the result of direct infection.

The average orchardist is inclined to consider the third effect as the one of paramount importance. It must be admitted that this direct injury is more tangible, but in all probability the devitalization of the tree is of equal if not greater importance.

DEVITALIZATION OF TREE

The devitalization of the tree is brought about by the continued dropping of infected leaves throughout the growing season. The extent of this drain on the vitality of the tree is well illustrated in the following observations reported by Gardner, of the Indiana Agricultural Experiment Station, to the Bureau of Plant Industry, United States Department of Agriculture:[7]

Mr. H. E. Newland ascertained the rate of defoliation on 18 representative trees in these plots during the 9-day period between July 26 and August 3, 1923, and found that single trees lost as many as 1,226, 1,340, 1,538, and even 1,848 leaves during the period mentioned. The lowest figure was 314. The average daily rate of leaf fall varied from 52 to 205 per tree, a condition which is really appalling and illustrates well the destructiveness of this disease.

It is very difficult to measure quantitatively the after effects produced by defoliation, but various forms of winter injury and reduction in the size of the crop as the result of improperly nourished buds are frequent aftermaths of severe outbreaks of the disease. It is not to be inferred that these necessarily attend every outbreak, but they have been observed from time to time in widely separated localities.

KILLING OF TWIGS

On the peach the injury to twig growth resulting from the formation of cankers is of minor importance, but on susceptible varieties of plum the organism frequently causes severe damage by girdling the young, developing twigs.

DAMAGE TO FRUIT

The injury to the peach fruit may be as low as 2 per cent on individual trees in seasons unfavorable to the development of the disease, while in an exceptionally favorable season it may reach 100 per cent on weak trees and range from 33 to 76 per cent on average trees. These figures are based on actual counts made in Georgia orchards. Although they can not be applied to more northern orchards, they give some indication of the variability of the disease and how serious it may become in some seasons.

In 1925 the disease was not prevalent in the Fort Valley section of Georgia, and the percentage of infected fruit on the individual trees (Table 1) ranged from 2 to 21. In 1926 the disease was present in an aggravated form, and the figures in Table 2 clearly indicate

[7] ORTON, C. R., and WOOD, J. I. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1923. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Survey Bul. Sup. 33: 35–147. 1924. [Mimeographed.]

the increase in the prevalence of the disease as compared with 1925. The difference in the number of infected fruits on the average trees and the weak trees (Table 2) is also quite pronounced. In 1927 the percentage of infected fruit was about midway between that of the 1925 and 1926 seasons. It varied from 14 to 56 per cent. (Table 3.) These figures do not represent the commercial loss to the growers, because part of the infected fruit was injured only slightly and could be marketed.

TABLE 1.—*Number and percentage of Elberta fruit infected with Bacterium pruni in a peach orchard at Barrons Lane, Ga., in 1925*

Tree No.	Total fruit	Infected		Sound		Tree No.	Total fruit	Infected		Sound	
	Number	*Number*	*Per ct.*	*Number*	*Per ct.*		*Number*	*Number*	*Per ct.*	*Number*	*Per ct.*
1	724	11	2	713	98	6	98	9	9	89	91
2	538	28	5	510	95	7	248	53	21	195	79
3	446	34	8	412	92	8	158	10	6	148	94
4	584	64	11	520	89	9	305	38	12	267	88
5	511	14	3	497	97	10	198	5	3	193	97

TABLE 2.—*Number and percentage of Elberta fruit infected with Bacterium pruni in a peach orchard having average trees at Fort Valley, Ga., and in a peach orchard having weak trees at Byron, Ga., in 1926*

Condition and tree No.	Total fruit	Infected		Sound		Condition and tree No.	Total fruit	Infected		Sound	
Average:	*Number*	*Number*	*Per ct.*	*Number*	*Per ct.*	Weak—Con.	*Number*	*Number*	*Per ct.*	*Number*	*Per ct.*
1	510	386	76	124	24	6	22	22	100	0	0
2	715	300	42	415	58	7	74	70	95	4	5
3	320	107	33	213	67	8	19	18	95	1	5
4	444	177	40	267	60	9	44	41	93	3	7
5	424	286	67	138	33	10	39	32	82	7	18
Weak:						11	50	49	98	1	2
1	138	132	96	6	4	12	60	58	97	2	3
2	31	28	90	3	10	13	70	66	94	4	6
3	26	23	88	3	12	14	229	190	83	39	17
4	121	111	92	10	8	15	49	44	90	5	10
5	48	44	92	4	8						

TABLE 3.—*Number and percentage of Elberta fruit infected with Bacterium pruni in a peach orchard at Barrons Lane, Ga., in 1927 on average trees*

Tree No.	Total fruit	Infected		Sound		Tree No.	Total fruit	Infected		Sound	
	Number	*Number*	*Per ct.*	*Number*	*Per ct.*		*Number*	*Number*	*Per ct.*	*Number*	*Per ct.*
1	576	221	38	355	62	11	812	417	51	395	49
2	564	289	51	275	49	12	868	280	32	588	68
3	1,227	558	45	669	55	13	619	201	32	418	68
4	1,130	596	53	534	47	14	653	295	45	358	55
5	691	385	56	306	44	15	736	270	37	466	63
6	599	335	56	264	44	16	418	93	22	325	78
7	444	190	43	254	57	17	384	70	19	294	81
8	394	200	51	194	49	18	568	78	14	490	86
9	604	231	38	373	62	19	528	114	22	414	78
10	646	211	33	435	67	20	564	266	47	298	53

In estimating the commercial damage many economic factors, such as the grades packed during the season, grades maintained by the individual grower, and the demand for the fruit, must be considered,

but can not be evaluated properly. For example, in 1926 Georgia had a tremendous crop of peaches (17,963 cars). The markets were glutted and sluggish; consequently, the high percentage of infected fruit did not cause the monetary loss that it did in 1929 when the crop was short (5,298 cars) and *Bacterium pruni* again prevalent.

Taking these points into consideration the writer considers it practically impossible to measure in dollars and cents the loss resulting from the infection of the fruit. Orchard counts such as tabulated here give a comparative indication of the extent of the infection from year to year.

Another and neglected phase of the fruit injury which should be pointed out is that peaches severely injured by the pathogene do not make good canning stock. The injury to the fruit by the organism is of such a nature that great difficulty arises when the fruit is peeled by treatment with lye. In this process the skin is readily removed from a normal peach, leaving a smooth surface, but when a severely infected peach is treated similarly a very inferior product results. The skin about the spots does not peel readily, and when it does peel the surface of the peach is left pitted. Such a product is unsatisfactory and this has led canners to reject fruit showing heavy infestation. This results in further loss to the grower, who is thus unable to salvage in some years a considerable portion of the infected fruit.

Plums are frequently more severely damaged than peaches. No counts were made, as only an occasional orchard of the susceptible varieties of plum is to be found in Georgia at the present time. Some indication of the damage to the plum can be gleaned from the fact that in the early years of the present century plums were grown very extensively in central Georgia only to fall prey to the ravages of *Bacterium pruni*. What was at the time an important and extensive industry is now represented by a few scattered plantings.

GEOGRAPHIC DISTRIBUTION

The bacterial spot disease has been reported definitely from the United States, Canada, and Japan. Bacterial leaf spots and twig cankers on plum and cherry have been reported from England and the Continent by various investigators (*5*, *48*, *49*), but the causal organisms are different from *Bacterium pruni*. The same conditions appear to be true of a prune leaf spot in South Africa, according to information received from P. Van Der Bijl, of the University of Stellenbosch. The disease has not been reported from the fruit-growing regions of Australia.

R. H. Porter,[8] of the University of Nanking, China, mentioned the occurrence of a leaf spot of peach in the vicinity of Nanking that resembles the spot caused by *Bacterium pruni*. No isolations were made, however, so the identity of the causal agent is in doubt.

It is not known in which of these sections the disease originated. Either North America or Asia might be the native home of *Bacterium pruni*, but it has become so widely distributed and occurs on so many hosts that it would be difficult or impossible to determine its original home.

[8] PORTER, R. H. A PRELIMINARY REPORT OF SURVEYS FOR PLANT DISEASES IN EAST CHINA. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Survey Bul. Sup. 46: 53–166. 1926. [Mimeographed.]

In the United States the bacterial spot disease has been found on various hosts in an area extending approximately from 30° to 45° north latitude and from 71° to 100° west longitude. Within this area there is great variation in such ecological factors as temperature, precipitation, and topography, and it is only natural that the disease varies from year to year in any one particular locality.

Throughout its entire range the disease has a tendency to become most prevalent in those regions where the soils are lacking in fertility and the trees are in a weakened condition, or in hilly sections where the trees on the hilltops suffer from the lack of food materials.

The sand-hill section of North Carolina and South Carolina is probably the region where the disease attains its greatest importance. This is due to the fact that the Carolina growers, to avoid competition with the Georgia orchards, have had to plant two of the most susceptible commercial varieties, Elberta and J. H. Hale,[9] in a section where conditions are particularly favorable to the development of the disease. A somewhat similar condition exists in southern Indiana and Illinois, where the disease is a serious problem practically every year.

The fact that the disease is most destructive to trees in a low state of vigor has been observed many times and served as a basis for the first control work carried out by Roberts (*31*) in 1913 to 1915.

The disease has never been reported from the large fruit sections west of the Rocky Mountains.

The distribution of the disease in the United States is shown in Figure 1.

HOSTS

The organism causing the bacterial spot disease has been reported as occurring only on species of the genus Prunus. In 1921, however, Kuwatsuka (*23*) demonstrated that *Sorbus japonica* could be infected with the production of galls at the site of the inoculation, but no such disease of *S. japonica* is known to occur naturally. He was unsuccessful in his attempts to infect the following genera of the Rosaceae: Amelanchier (1),[10] Chaenomeles (2), Crataegus (1), Cydonia (2), Eriobotrya (1), Kerria (1), Malus (3), Photinia (2), Pyrus (2), Raphiolepis (1), Rosa (4), Rubus (5), Spiraea (2), and Stephanandra (1).

The disease has been reported in Iowa on *Prunus besseyi*, on cultivated cherries, and on an unidentified species called the Rocky Mountain dwarf cherry; on *Prunus umbellata* in Florida; and on cultivated cherries in New York. It is unfortunate that the reports of the disease on these hosts have not been confirmed by isolations and cross inoculations to peach and plum to establish definitely the identity of the causal organism.

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[9] In normal seasons the comparatively resistant Hiley would ripen in the Carolinas about the time the bulk of the Elberta crop was being shipped from Georgia. The Hiley being a white-fleshed variety generally does not command a satisfactory price when forced to compete in the same market with the yellow-fleshed Elberta.

[10] The numbers in parentheses following the genera indicate the number of species inoculated.

In 1928 the writer had the opportunity of observing the infection, under natural conditions, of a new host. The plant in question, *Prunus davidiana*, an Asiatic species, was grown at Fort Valley, Ga., by Lee M. Hutchins, of the United States Peach Disease Field Laboratory, from seed imported directly from China, and was set out, as an experimental planting, in a block adjoined on two sides by large blocks of Elberta trees in which the bacterial spot disease was known to occur. On June 6 numerous spots, similar to those produced by *Bacterium pruni* on peach leaves, were observed on the leaves of *P. davidiana*. (Pl. 1, D.) Cankers, which were more conspicuous than those produced on the peach, were observed at the same

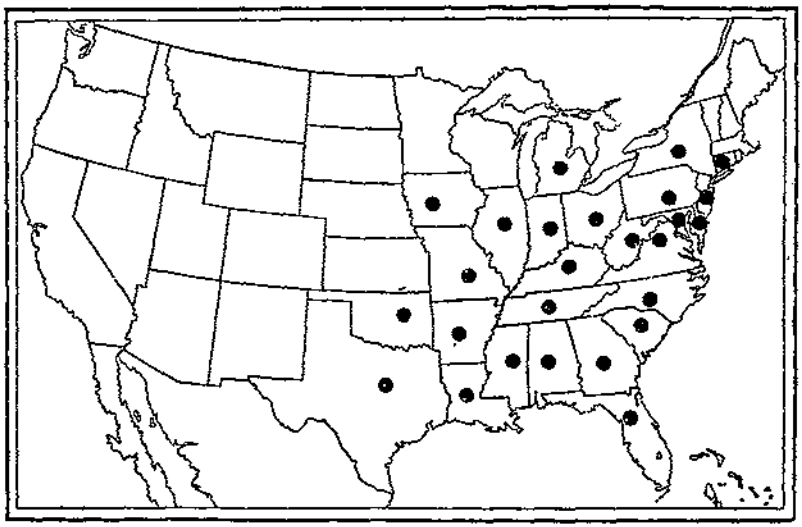

FIGURE 1.—The distribution of *Bacterium pruni* in the United States. The organism and the disease it produces have been reported from every State marked with a dot

time on the twigs. (Pl. 1, B.) A yellow bacterial organism was isolated from these leaf spots and twig cankers, and when inoculated into peach leaves and twigs it produced typical symptoms of the bacterial spot disease. This organism was grown also on various culture media, and its reactions were identical to those of *Bact. pruni* isolated from the peach and plum. It was concluded, as the result of these cross-inoculation experiments and the laboratory studies, that the organism isolated from *P. davidiana* was *Bact. pruni* E. F. Smith. The disease was found again in 1929 on this host and the symptoms were identical with those observed in 1928. The known susceptible species are given in Table 4, with certain other pertinent data.

TABLE 4.—*List of plants susceptible to Bacterium pruni*

Scientific name	Common name	Investigator	Date
Prunus salicina	Japanese plum	E. F. Smith	1903
Prunus persica	Peach	do	1908
Prunus armeniaca	Apricot	F. M. Rolfs	1915
Prunus persica var. nucipersica	Nectarine	do	1915
Prunus avium	Mazzard	K. Kuwatsuka	1921
Prunus buergeriana		do	1921
Prunus crassipes		do	1921
Prunus donarium sub sp. elegans var. glabra		do	1921
Prunus domestica	Plum	do	1921
Prunus japonica	Chinese bush cherry	do	1921
Prunus mume	Japanese apricot	do	1921
Prunus mume var. microcarpa		do	1921
Prunus mume var. bungo		do	1921
Sorbus japonica		do	1921
Prunus communis	Almond	S. Ishiyama	1922
Prunus davidiana	Chinese wild peach	J. C. Dunegan	1928

VARIETAL SUSCEPTIBILITY

The different varieties of peaches, plums, apricots, and nectarines show considerable variation in their susceptibility to the attacks of the pathogene. The plums show the greatest variation, according to Rolfs (*33*), who prepared a comprehensive study of this phase of the disease. The European and American varieties exhibit a marked resistance to the disease, while the Japanese varieties are extremely susceptible.

In Georgia the organism frequently kills many twigs of the Japanese varieties through the formation of girdling cankers. The orchard map in Figure 2 illustrates this condition in a plum orchard at Byron, Ga., where year after year the new growth was severely affected by the organism. The plum was an unidentified variety of the Japanese group, and although the leaves and twigs were very susceptible the disease was never observed on the fruit. The behavior of the disease in this particular orchard was observed for four years and, while the growth of the trees was affected seriously, very few succumbed to the disease.

A plum, also of the Japanese group, observed early in the spring of 1929 near De Queen, Ark., exhibited almost the reverse of the condition in the Georgia orchard. In this tree the fruit was very severely attacked (pl. 6, C and D), while the leaves and twigs were only mildly affected.

The disease has been observed on trees of all the commercial varieties of peaches grown in Georgia. The fruit of early-maturing varieties is harvested before the spots have time to develop into the large cracks and fissures so characteristic of the disease on the Elberta and J. H. Hale varieties, but typical symptoms appear on the leaves and occasionally twig cankers have been observed. The fruit of the varieties Elberta and J. H. Hale is most severely affected by the disease in practically all localities in Georgia. These two varieties are quite susceptible to the disease, and their ripening season is late enough so that there is ample time for the development of advanced symptoms as well as numerous secondary infections.

The writer has never found the twig-canker phase of the disease to be very abundant in the commercial orchards of Georgia, Ten-

nessee, North Carolina, South Carolina, Indiana, and Arkansas. In fact, as will be brought out later, true cankers are exceedingly scarce and difficult to find.

PATHOLOGICAL HISTOLOGY

Smith (*42*), Lewis (*25*), and Rolfs (*33*) have discussed the pathological histology of the disease with particular reference to the

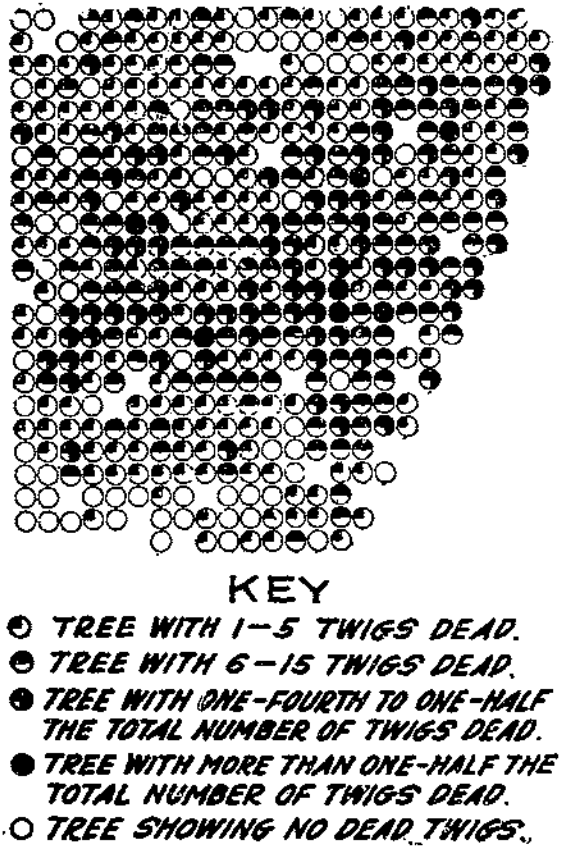

FIGURE 2.—Orchard map showing the extent of twig killing due to the formation of *Bacterium pruni* cankers in a plum orchard at Byron, Ga. The survey was made September 4, 1924

plum. Adams (*3*) has commented on the histology of plum-twig cankers. The discussion which follows will concern the histology of the disease on the peach.

METHODS

Material was killed, embedded, and sectioned in the usual manner. Killing solutions in which the reagents were dissolved or mixed with large quantities of water were avoided, to prevent as much as possible the washing of the bacteria from the tissues. A mixture of

bichloride of mercury (3 gms.) and glacial acetic acid (3 ml.) in 70 per cent alcohol (100 ml.) was found to be very satisfactory, and bacterial ooze on the surface of the fruit remained in situ through the entire process of killing, embedding, and staining. No difficulties were encountered with the peach-leaf material, but the epidermal hairs on the surface of the fruit made it difficult to prepare satisfactory mounts for photomicrographs. Fresh material of the twig cankers was cut on a sliding microtome. Masses of bacteria were observed readily in unstained material mounted directly from the knife, but generally were washed out during the staining process, even though the sections were transferred from the knife to 95 per cent alcohol.

Carbol fuchsin was the most satisfactory stain, particularly if the excess was removed with alcohol and a counter stain of light green or orange G in clove oil applied. The diseased tissues retained the carbol fuchsin and could be distinguished readily from surrounding healthy tissues. Lee and Weller (*24*) have used a very similar technic with satisfactory results in their studies on the red-stripe disease of sugarcane. Carbol fuchsin to which a trace of gentian violet had been added was also used in the study of the leaf tissues.

LEAF SPOTS

A microscopic examination of sections prepared from peach leaves reveals leaf-tissue structure of the normal type. The upper epidermal cells are large and rather irregular in outline. A palisade parenchyma is clearly distinguished, consisting regularly of two or three layers of elongate, more or less cylindrical cells, with their long axes perpendicular to the leaf surface. The columnar arrangement gradually becomes indistinct below the second layer as the cells merge with the cells of the spongy parenchyma. The latter consists of a very loosely arranged network of cells generally slightly longer than broad, extending between the lower epidermis and the cells of the third layer of the palisade parenchyma.

The cells of the lower epidermis as a rule are somewhat smaller than those of the upper epidermis. The important feature of the lower epidermis is, of course, the presence of stomata.

The tissue structure changes abruptly when the veins are reached. If the vein is a large one the palisade layer and the spongy parenchyma are completely obliterated, and their place is occupied by the vascular units surrounded by a sheath of parenchyma cells. Those in the upper portion of the leaf, replacing the palisade cells, are somewhat larger than those which replace the spongy parenchyma tissue. There is a gradation from the condition described for the large veins to one where the vascular element of the very small veins is surrounded with a single sheath of small parenchyma cells, and the normal structure of the palisade and spongy parenchyma is hardly disturbed. A very common condition, however, is that in which the spongy parenchyma is obliterated and its place taken by a compact mass of parenchyma cells while the palisade parenchyma is not disturbed.

In the discussion of the symptoms of this disease on the leaves it was pointed out that the first macroscopic symptom observed is the

presence of a small spot in which the green color of the leaf has faded. Sections prepared from material killed and fixed at this period of development show the substomatal chamber and the cavities in the spongy parenchyma completely filled with bacteria. (Pl. 7, B and D.) There is some indication that the cells of the spongy parenchyma have been pushed apart, but this action is not pronounced. In the palisade parenchyma, however, the cells are clearly being pushed apart by long wedge-shaped masses of bacteria. The cells in both regions are only slightly plasmolyzed and are just beginning to show the effects of the presence of the organism. The chloroplasts in the palisade cells apparently are affected as their reaction to stains differs from that of the chloroplasts in normal cells. This change in the nature of the chloroplasts explains the early macroscopic symptoms (i. e., slightly lighter green areas) mentioned above.

In slightly older spots the cells, in both the spongy parenchyma and the palisade parenchyma, show clearly the effects of the presence of the organism. The contents of the cells have become disorganized, the chloroplasts have practically disappeared, and the protoplasm has pulled away from the cell walls.

At this stage in the development of the spot the cell walls are intact, and the bacteria are entirely intercellular. The pushing apart of the cells by the bacteria causes an increase in thickness of the leaf, as indicated in Table 5, which is a series of measurements of the distance between the upper and lower epidermis in healthy and diseased leaves.

TABLE 5.—*Increase in the thickness of peach leaves as the result of the presence of Bacterium pruni in the tissues*

Thickness of normal leaf	Thickness of invaded area	Thickness of normal leaf	Thickness of invaded area	Thickness of normal leaf	Thickness of invaded area
Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*
133.0	150.0	133.0	148.2	129.2	153.9
138.7	153.9	138.7	135.8	133.0	150.1
136.8	152.0	136.8	148.2		
131.1	150.1	131.1	152.0	Av. 134.1	Av. 151.4

It is interesting to consider at this point the effect of the bacteria on the tissues and how they bring about the death of the cells. The most obvious effect is the splitting apart of the cells through the dissolution of the middle lamella. This action postulates the isolation of single cells and prevents the interchange of food materials—a thing in itself that a priori would be sufficient cause for the subsequent disintegration and death of the cells. However, before this splitting and isolation of the cells takes place there is another effect which the writer feels is perhaps the primary reason for the death of the tissue, particularly in the spongy parenchyma.

In the healthy tissues these cells are afforded an abundant supply of oxygen and water vapor through the connection with the stomata. When the bacteria invade the tissues and fill the large intercellular cavities the supply of oxygen is cut off, or at least very materially reduced, and the hypothesis is advanced that the cells are actually killed by asphyxiation. Rosen (*35*) working independently, although

at the same laboratory as the writer, recently has advanced this hypothesis in connection with his studies of the fire-blight organism.[11]

Complete disorganization of the leaf tissues in the infected region ensues very soon after the bacteria have separated the individual cells. The cell contents become completely plasmolyzed, the cell walls collapse, and some of the walls becoming ruptured, the bacteria enter the cells. Because of the complete collapse of the tissues (pl. 7, E), the infected area, instead of being thicker than the adjacent healthy part of the leaf, now becomes thinner. It is difficult to prepare sections of the advanced stages, as the dead tissues crumple and tear, producing very unsatisfactory results. The necrotic areas, unless they fall out (shot-hole effect) are invaded by saprophytic fungi, and the pathological picture is further complicated by their presence.

During the spring and early summer the shot-hole effect in the leaves seems to be due to a passive breaking away of the dead tissue without the formation of a special separation tissue about the area killed by the causal organism. It has been pointed out that the spread of the organism within the leaf is delimited by the vascular units of the small veins, and the points of separation between the dead and living tissues occur adjacent to these vascular units.

Duggar (*13, p. 69*), in his study of the shot-hole effect, observed the same passive breaking away of the dead tissues. He described the process as follows:

> I anticipated finding some unusual cellular development along the line of abscission, but free-hand sections of various stages indicate that advantage is taken of the normal leaf development. The cells which make up the minutest ramifications of the veinlets seem to afford a place through which a break most readily occurs with least injury to delicate parts adjacent.

Toward the end of the growing season the pathogene occasionally does not invade all the tissues between the veins. In these cases the intercellular spaces in the uninvaded spongy parenchyma are completely occluded by the resumption of cell division, and a layer of cork cells forms a barrier between the dead tissues on the one side and the healthy tissues on the other. The break, which frees the dead tissue, occurs at the cork layer which is left as a protective layer covering the healthy tissues. The meristimatic activities, which produce the occlusion of the intercellular spaces, stop abruptly when the vascular unit of a vein is reached, so that even in the late summer the activities of the pathogene still are delimited by the small veins.

Samuel (*39*), in a study of the infection of almond leaves by *Clasterosporium carpophilum* (Lev.) Aderh. in South Australia, observed that in the spring and early summer the infections were abscissed practically always, but that in the latter part of the summer abscission may not occur. Even in the latter case he found that certain cellular changes occurred, involving the suberization and lignification of the cell walls. Apparently he did not encounter, to any extent at least, the passive breaking away of the tissue with the utilization of the vascular units and the cells immediately surrounding them as barriers to protect the healthy tissues.

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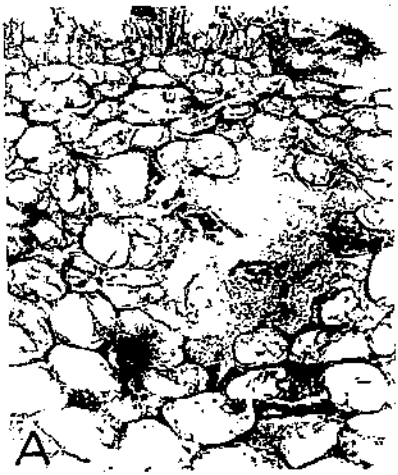
[11] *Bacillus amylovorus* (Burr.) Trev.



A, Cavity formed in fruit tissue as the result of the activities of *Bacterium pruni*, × 225; B, bacterial masses present in the substomatic chamber, × 500; C, bacterial masses in the intercellular spaces of peach fruit tissue, × 500; D, bacterial masses visible in the palisade layer and the cells beginning to collapse, × 500; E, advance stage of leaf invasion revealing the complete disorganization of the tissue, × 225

FRUIT SPOTS

A radial section of the outer portion of a peach fruit reveals a very irregular epidermis, consisting of single horizontally elongated cells and cushions of several small cells separated at unequal intervals by the basal portions of the epidermal hairs. The outer margin of the epidermis is covered with a moderately thick cuticle which dips up and down following the irregular contour of the cells. The inner margin is rather uniform, the bases of the hairs, the bases of the elongated cells, and the lower cells of the cushions in the main being at one general level.

The epidermal structure is broken at irregular intervals by the stomata which, in the varieties studied (Elberta, J. H. Hale, Carman, and Early Rose), occur in depressions between the horizontally elongated cells or between the cushions of small cells.

The hypodermis is not very clearly differentiated, but may be said to extend downward 6 to 10 cell layers. The cells directly under the epidermis are much flattened, but the succeeding layers become more and more isodiametric until they merge with the large parenchyma cells of the pulp tissue. This fusion of the two tissue systems is very gradual, and it is difficult to indicate in a given section where one ends and the other begins.

Vascular bundles are scattered through the pulp tissue, but they never have been observed in that portion invaded by the organism and need not be considered in this discussion.

The writer has been unable to produce infections by spraying or otherwise inoculating the peach fruit with pure cultures of the pathogene, and, therefore, has been unable to observe the entrance of the bacteria through the stomata and the initial stages of infection. The earliest stage observed is that in which masses of bacteria are collected in the intercellular spaces below the epidermis in the vicinity of the stomata. (Pl. 7, A, C.) The hypodermal cells in this early stage of the disease are not visibly affected; however, by the time the bacteria have completely surrounded the cells the protoplastic contents may be seen as a plasmolyzed mass at one side of the cell. The walls soon collapse and a distinct cavity is formed. (Pl. 7, A.) This cavity is confined to the hypodermis and may extend from just beneath the epidermis to a depth of 8 or 10 cell layers, or it may be shallow and involve only 3 or 4 cell layers.

The cells of the epidermis while apparently affected, as indicated by a change in staining reaction, do not collapse but remain as a thin membrane over the bacterial cavity. Eventually the epidermis is ruptured either by the stretching of the tissue in growth or by the upward pressure of the mass of bacteria which then exudes through the rupture. The writer is inclined to believe the latter interpretation is the correct one, as small bits of epidermal tissue have been observed at the top of the "hornlike" mass of dried bacteria which has oozed from the cavity.

The epidermis before it is ruptured probably protects the bacteria from desiccation and the toxic action of sunlight; after it is ruptured the period of active cell destruction comes to an end. The bacteria almost completely disappear from the cavity proper, but can be detected between the cells forming the sides of the cavity.

A periderm is initiated in the vicinity of the cavity, and a layer of wound cork, starting at the epidermis on one side and passing diagonally through the hypodermis and under the cavity and up to the epidermis on the other side, surrounds the affected region. This layer of wound cork effectively prevents the desiccation of the healthy cells below the cavity. A number of noninfected cells are present in the occluded region, but they are not subsequently attacked by the bacteria. In view of the rapid and total cell destruction brought about in forming the initial cavity, it is remarkable that secondary cavities are not formed in the noninfected tissue remaining in the occluded region.

When the bacteria emerge and the period of rapid cell destruction is ended, the macroscopic appearance of the spot is merely that of a slight depression in the surface of the fruit with a small rupture in the epidermis. The roughened surface of the spots and the extensive cracking that frequently develops are both secondary effects of the disease. The roughened surface occurs in part because the remnants of the collapsed hypodermal cells are forced to the surface with the increase in diameter of the fruit, and the cracking because of the failure of the cells of the wound cork to expand at a rate commensurate with that of the surrounding tissue. It is these later stages which make the disease of such economic importance, since the appearance of the fruit is so strikingly marred. The bacteria, of course, initiate the whole train of events by killing the small pocket of tissue, but the cracking and the irregular surface are aftermaths brought about by the subsequent growth of the host.

TWIG CANKERS

The study of the histology of twig cankers has been hampered by the difficulties involved in obtaining authentic material.

A cross section of a young peach twig reveals nothing unusual in the type of stem structure. The epidermis, covered by the cuticle, is one cell thick and is broken at intervals by the stomata which are placed below the general level of the epidermis. The tissue beneath the epidermis consists of a variable number of layers of parenchyma cells, loosely arranged, and with definite intercellular spaces connected to the substomatal chambers. Embedded in the parenchyma at irregular intervals are cells containing masses presumably of calcium oxalate crystals. The cortical parenchyma is separated from the vascular tissue by a band of contiguous groups of thick-walled bast fibers, which very effectively confines the activities of the pathogene to the cortical parenchyma; and only rarely does the pathogene gain entrance to the phloem region by destroying the parenchyma cells between the groups of fibers.

Although actual penetration of the stomata of the young twigs has not been observed, masses of bacteria have been seen just under the stomata in such a position that their path of entrance is clearly indicated.

The organism, after it gains entrance to the twig tissues, occupies the intercellular spaces in the parenchyma tissue just below the stomata. The walls of the cells in the invaded region become thickened and brown in color and many of the cells are filled with a

brown, gummy substance. Those cells collapse in which the walls are not actually ruptured, and the walls are pressed together as though they were under a pressure radially. Bacteria can be observed in those cells where the walls have been ruptured, and eventually distinct cavities are formed in the tissues by the breaking down of the cells. Whether the cavity results from the actual destruction and decomposition of the cellular material by the bacteria or is merely the result of the pressure exerted by the mass of multiplying bacteria is not clear.

The bacterial cavities may be just beneath the epidermis, in which case the structure of the stem is not seriously disrupted. Often, however, the cavities are formed more deeply in the tissues, at times being adjacent to the ring of bast fibers. The invaded region is soon separated from the healthy tissue of the stem by the initiation of a wound periderm which extends from the epidermis on one side of the region diagonally through the cortical parenchyma to the epidermis on the other side.

Since the pathogene is confined almost without exception to the cortical parenchyma and the vascular system is scarcely affected, it is easy to understand why wilting and killing of peach twigs rarely accompanies the formation of these cankers. In the plum the situation is quite different, in that the bacterial cankers occur not only in the cortical parenchyma but also in the region of the phloem, thus seriously interfering with the translocation of food materials. A possible explanation of this difference in behavior may lie in the fact that the groups of bast fibers in the peach twig practically are in contact with each other, while in the plum they are separated from each other by three or four cells parenchymatous in nature, affording, through the increased number of intercellular spaces, a means of entrance to the vascular cylinder.

THE PATHOGENE

ORIGINAL DESCRIPTION OF THE ORGANISM

The original description of the organism was published by Smith (*40*) in 1903 as a species of Pseudomonas, and is reproduced here in full:

> The organism is distinctly yellow and grows readily in ordinary culture media, bouillon, milk, potato, agar, etc. It was easily obtained in pure culture from small spots. In agar-plate cultures it looks much like *P. campestris*, but is readily distinguished by its feebler growth on potato and by its behavior in Uschinsky's solution, which is converted by it from a limpid fluid to one as viscid as egg albumen. The bacteria are small to medium size and occur singly, in pairs, or short chains. They are motile by means of one to several polar flagella. The thermal death point is approximately 51° C. Gelatin is not liquefied rapidly. Litmus in milk is reduced, but finally returns to its former color. Casein is slowly precipitated and finally redissolved. No gas is produced from any medium.

The essential characteristics of the organism are mentioned in the above description, but it is to be regretted that Smith did not amplify this description at a later date. However, two of the most important characteristics, i. e., growth on Uschinsky's solution and failure to produce gas on any medium, are given in this description.

Rolfs (*33*), in his monograph on the disease, discussed the morphological and cultural characters in much greater detail. With

the exception of the discussion of acid production on carbohydrate media, the writer agrees with Rolfs's observations on the behavior of the organism in culture. Accordingly, the bacteriological studies presented in the following pages are not to be construed as criticisms of the earlier work, but rather as confirmatory evidence.

SOURCES OF THE CULTURES

During the period 1925–1928 cultures were obtained from 23 different sources and grown in the laboratory on various media. Seven of these cultures were obtained from Delaware through the courtesy of J. F. Adams, and one culture was obtained from Erwin F. Smith. The others were isolated from different sources by the writer. (Table 6.)

TABLE 6.—*Sources of Bacterium pruni cultures used in the bacteriological studies*

Isolation	Date	Sources of cultures	Locality
A–1	Apr. 30, 1925	Small spot on Elberta peach	Barrons Lane, Ga.
A–2	May 4, 1926	do	Do.
A–3	May 19, 1926	Necrotic spot on peach twig (1925 wood)	Do.
A–4	Apr. 16, 1927	Small spot on Elberta leaves	Do.
G	June 4, 1926	Small spot on Elberta peach	*Gay, Ga.*
H	May 26, 1926	Spot on fruit of cultivated plum	Hillsboro, Ga.
L	June 20, 1928	Spot on Elberta peach	*Lugoff, S. C.*
M	May 3, 1926	do	Americus, Ga.
P	May 4, 1927	Exudate on plum leaf spot	Byron, Ga.
T	May 14, 1926	Spot on Elberta peach	Talbotton, Ga.
W	May 21, 1927	Spot on Woodland Cling peach	*Griffin, Ga.*
ADL	June 20, 1928	Leaf spot on Prunus davidiana	Fort Valley, Ga.
TC–1	June 9, 1928	Overwintered twig canker on Elberta	Do.
TC–2	June 18, 1928	do	Do.
TC–3	do	do	Do.

MORPHOLOGY

The organism is a short rod, with rounded ends, usually occurring singly, although occasionally in pairs. Smears from young infections on peaches yielded bacteria measuring 0.8μ to 1.7μ long and from 0.2μ to 0.8μ wide. The majority of the organisms were 1.4μ to 1.7μ long and 0.6μ to 0.8μ wide. Smears from dextrose agar cultures yielded bacteria which measured 0.8μ to 1.0μ long and 0.2μ to 0.4μ wide, although a few were seen that were as large as those present in the smears made from the fruit.

Smith (*40*) and Rolfs (*33*) found the organism to be motile by means of one to several polar flagella. Motility in the writer's cultures has been observed very clearly a number of times with a dark field objective. Flagella have been demonstrated by the Casares-Gil method and by the method devised by Rosen.[12] The writer never has observed more than one polar flagellum attached to an organism in any of his preparations.

No spores have been observed on any of the smears. Attempts to demonstrate the presence of spores by the chromic-acid-staining method gave negative results.

[12] This method, although unpublished, was used through the courtesy of H. R. Rosen, who demonstrated the procedure to the writer.

Capsules were observed in cultures 9 days old, stained according to Moore's technic.

The organism is stained readily with aqueous methylene blue, Ziehl's carbol fuchsin, and saturated alcoholic gentian violet.

Rolfs (*33*) considered the organism to be Gram-negative although the results in many of his tests were not sharp and decisive. The writer tested five different isolations with Hucker's ammonium oxalate modification and in all cases the crystal violet was completely lost when the smears were flooded with 95 per cent alcohol or acetone. According to these results the organism is Gram-negative.

CULTURAL CHARACTERS

DEXTROSE AGAR SLANTS

On dextrose agar (pH 6.4) growth was visible after 24 hours as a colorless filiform line following the path of the needle. At the end of three days the bacterial mass had started to spread over the surface of the agar. After eight days the growth was abundant, smooth, light cadmium [13] in color, broadly filiform, glistening, translucent, and convex to capitate. No odor was present and the agar was not discolored. When viewed through transmitted light the center of the culture was darker than the margins. The bacterial mass had started to flow to the base of the slant and the mass was streaked with "currents" or bands of light and dark color. Growth completely covered the slant in 28 days.

DEXTROSE AGAR STABS

In stab cultures on dextrose agar (pH 6.4) growth was slower in appearing than on the slants of the same agar. After three days it was distinctly visible as a finely beaded line following the path of the needle halfway to the base of the tube. A small circular colony was present on the surface at the point of inoculation. After eight days the growth was distinctly echinulate but had not extended to the base of the tube along the path of the needle. At the end of 28 days the growth was still echinulate, the agar was not discolored, and there were no signs of liquefaction of the medium. The agar at the surface was completely covered, and the organism had reached the bottom of the tube along the path of the needle.

BEEF-EXTRACT AGAR SLANTS

Growth appeared after 24 hours on beef-extract agar (pH 7.4) as a faint colorless line following the path of the needle. After four days it was clearly discernible as a filiform line following the path of the needle and widening at the base of the slant where the bacterial mass was slowly collecting. The growth was rather scanty, smooth, empire yellow in color, slimy to viscid, and translucent. No odor was present, and the agar was not discolored. The center of the bacterial mass was darker, giving the mass a mottled appearance when viewed by transmitted light.

[13] The following publication was used as the basis of color determinations in these studies: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

At the end of 28 days the character of the growth had changed very little. It was decidedly viscid and light cadmium in color but had not spread over the agar to any extent. The bacterial mass did not cover the surface of the slant at the end of 40 days. This was markedly different from the growth on dextrose agar, where the bacteria had covered the entire surface of the slant in 28 days.

BEEF-EXTRACT AGAR STABS

In stab cultures of beef-extract agar (pH 7.4) growth was slow in appearing. At the end of four days a filiform growth followed the path of the needle halfway down the stab. A small circular colony was present on the surface about the point of inoculation. Bacterial growth reached the bottom of the tube after 11 days, and as the culture aged the growth became echinulate in character. No discoloration or liquefaction of the agar was observed.

BEEF-EXTRACT AGAR PLATES

Colonies on plates of beef-extract agar (pH 7.4) were just visible after 48 hours at laboratory temperatures (24° to 26° C.). The colonies were white in color with a faint tinge of yellow. When examined under a binocular microscope the surface colonies were slightly raised, circular to oval in shape with an entire margin and finely granular internal contents. A clear, almost colorless, band surrounded the outer margin of the larger colonies. Buried colonies were spindle or discus shaped.

The colonies assumed a definite yellow color at the end of three days. At the end of 14 days the colonies on the surface measured 1.5 to 2 mm. in diameter. They were apricot yellow in color, circular to oval in shape, convex in elevation, with a smooth surface, and the internal structure was finely granular. The edges of the colonies were entire. Buried colonies were generally less than 1 mm. in diameter, apricot yellow in color, spindle to oval in shape, and generally both buried and surface colonies were translucent. There was no discoloration of the medium.

BEEF-EXTRACT BROTH

On beef-extract broth (pH 6.9) a faint clouding of the medium was visible after inoculation. In 48 hours the clouding was marked throughout the medium, and there was a faint trace of surface growth in the form of a membrane. By the end of the ninth day there was a marked surface growth which settled to the base of the tube as white flocculent sediment when the tubes were agitated. The broth was translucent but not transparent, and no odor had developed. The broth was changed to a viscous fluid with a consistency much like egg albumen in periods varying from 28 to 40 days after inoculation.

DEXTROSE BROTH

The organism produced very similar reactions in dextrose broth (beef-extract broth plus 1 per cent Bacto dextrose). This medium was also converted into a viscous fluid.

POTATO CYLINDERS

Growth was fairly abundant after two days. It followed the path of the needle over the slanted surface of the cylinder and was wet, raised, smooth, glistening, and between lemon chrome and lemon yellow in color. The tissue of the inoculated potato cylinders was slightly discolored at the end of three days. At the end of five days the bacterial mass had flowed down the slanted portion of the cylinder and was spreading over the rest of it. At the end of 13 days the growth had practically covered the entire cylinder. At the end of 32 days the bacteria had spread over the surface of the cylinders forming a thin film, with a slight metallic luster. The iodine test for starch was as strong in the inoculated as in the noninoculated cylinders, indicating the absence of any diastatic action.

SODIUM TAUROCHOLATE AGAR

Sodium taurocholate agar was prepared according to the directions of Patel (*30*) with the exception that Bacto peptone was substituted for Witte's peptone. Growth was visible after 48 hours at 25° C. and in three days had developed almost as rapidly as on dextrose agar inoculated at the same time. At the end of 30 days there was little difference between the growth of the pathogene on sodium taurocholate agar and on dextrose agar. The crystal violet in the medium was decomposed almost completely. Growth on sodium taurocholate agar was more vigorous than on beef-extract agar. The growth of *Bacterium pruni*, a gram-negative organism, was not inhibited by the crystal violet while the growth of a gram-positive organism (*Staphylococcus* sp.) used for comparison was completely inhibited.

USCHINSKY'S SOLUTION

A faint clouding of Uschinsky's solution was present after four days. No ring or pellicle formed in young cultures, but as the culture increased in age these began to appear. The medium became translucent, and a yellowish precipitate collected on the bottom of the tubes. The solution was not viscous at the end of 25 days, but on older cultures (2½ months) it became viscous like the white of egg.

The solution used in these experiments had the following formula:

	Grams
Sodium chloride, $NaCl$	5.0
Calcium chloride, $CaCl_2$	0.10
Magnesium sulphate, $MgSO_4$	.30
Dipotassium phosphate, K_2HPO_4	2.0
Ammonium lactate, $NH_4C_3H_5O_3$	6.0
Sodium asparaginate, $NaC_4H_7O_3N_2$	3.0
Glycerin, $C_3H_5(OH)_3$	30.0

All the ingredients except the glycerin were dissolved in a liter of warm distilled water and then the glycerin was added. The solution was steamed for 20 minutes at 100° C., then filtered through paper, tubed, and sterilized 10 minutes at 110° C.

Variations in the procedure of preparing Uschinsky's solution have considerable effect on the resultant solution, and the above procedure

was strictly adhered to after first trials. In preliminary tests a different procedure was followed, and the solution would not support the growth of the organism.

COHN'S SOLUTION

Growth on Cohn's solution was very weak, being confined to a very faint clouding of the liquid. There was no other development.

PHYSIOLOGY

LIQUEFACTION OF GELATIN

Growth was vigorous on slants of Bacto gelatin (pH 6.5). Liquefaction was evident along the path of the needle after 42 hours, and by the end of 5 days a narrow trough-shaped depression had developed. Bacteria were carried to the base of the slant with the products of liquefaction, and as the culture grew older the slant was completely separated from the gelatin in the lower portion of the tube. Finally all the material originally in the slanted area was liquefied, and the bacteria rested as a yellowish mass (color between light cadmium and lemon chrome) on the unslanted portion of the medium with the liquid above it. The process of liquefaction continued, although the rate diminished as the volume of liquid above the bacterial mass increased.

All the gelatin was liquefied after 35 days in most of the tubes. At this time a bacterial mass, apricot yellow in color, had collected at the bottom of the tube. The liquid produced by the action of the bacteria on the gelatin was clear.

In stab cultures of Bacto gelatin (pH 6.5) growth was just visible at the end of 48 hours and was very evident at the end of five days. Liquefaction had commenced in the upper portion but was not evident in the lower half of the tubes. The growth was first napiform in character, although no liquefaction was evident along the path of the needle in the lower portion of the tube. From napiform the growth slowly changed to infundibuliform, and finally a stratiform character was reached in all tubes. At this time a bacterial mass, light cadmium to lemon chrome in color, rested on the still solid gelatin with a layer of clear liquid above it. The process of liquefaction proceeded slowly with the rate decreasing as the layer of liquid increased. At the end of 35 days one-half of the gelatin had been liquefied, and the bacterial mass, now apricot yellow, rested on the solid gelatin. The liquid above this was still clear. Growth stopped at this stage, due probably to lack of sufficient oxygen, and no further liquefaction was observed after an additional 12 days.

REACTIONS IN MILK

Bacto dehydrated milk was used in these tests. The medium was sterilized on three successive days for 15 minutes at 100° C. There was slight trace of clearing (whey formation) in the tubes 48 hours after inoculation. In four days there was a cleared zone at the tops of the tubes 1 to 2 mm. deep in all the tubes, and at the end of seven days the cleared zone was 5 to 10 mm. deep. A curd was not formed.

Jodidi (*22*) has demonstrated that *Bacterium pruni* elaborated proteolytic enzymes when grown in milk, and these enzymes probably produce an immediate dissolution (peptonization) of the casein and prevent the formation of a curd. Jodidi (*21*) also found that tyrosine, leucine, and a mixture of the higher fatty acids (myristic, palmitic, and stearic) were some of the products formed by the growth of *Bact. pruni* in skim milk. These materials occur as crystals and globular aggregates in the culture media. Reduction of litmus began within 3 days and in the writer's cultures was complete in 12 days. In some of Rolfs's (*33*) cultures the litmus was not completely reduced at the end of 45 days.

AMMONIA PRODUCTION

Strips of filter paper saturated with freshly prepared Nessler's solution and hung over 9-day-old beef-extract broth cultures became slightly brown within a few minutes and were completely browned in 18 hours, denoting the production of ammonia.

HYDROGEN SULPHIDE PRODUCTION

Strips of filter paper saturated with lead acetate solution did not develop black color when hung over beef extract broth cultures, indicating that hydrogen sulphide was not being produced. The tests were continued for 18 hours with negative results.

REDUCTION OF NITRATES

Cultures on Bacto nitrate agar were tested on the first, second, and fourth days for nitrate reduction with sulphanilic acid and alpha-naphthylamine. The results were negative. There was no indication of gas formation in any of the tubes. Noninoculated tubes also were tested and gave negative reactions.

INDOL PRODUCTION FROM TRYPTOPHANE BROTH

Cultures on Bacto tryptophane broth (pH 6.6) were tested at the end of 14 and 28 days with both the vanillin and the Ehrlich test. The vanillin tests gave a positive reaction in practically all cases, but the results from the Ehrlich test generally were inconclusive. Noninoculated tubes of broth when tested by the vanillin method gave negative reaction for indol but positive for tryptophane.

HYDROLYSIS OF STARCH

Streak cultures on plates of soluble potato-starch agar (pH 6.4) were flooded with a saturated solution of iodine in 50 per cent alcohol. One isolation was found to have no action on the starch, while another isolation showed a very faint halo, indicating that the starch was being acted upon. The cultures were 14 days old when tested.

RELATION TO FREE OXYGEN

Dextrose-agar shake cultures developed a yellow layer of bacterial growth at the top of the agar. No colonies were visible below the

surface, but it was felt that the turbidity of the agar might mask any small colonies present. However, if such colonies were present below the surface of the agar they failed to develop to any appreciable size, as none could be detected in the tube at the end of 70 days.

The experiment was repeated, using nutrient gelatin to avoid the turbidity of agar, and the results were the same. Growth was present only at the surface. Since the organism liquefies gelatin, the surface layer of growth was soon covered with the products of this liquefaction. The liquefaction continued very slowly after the bacterial layer was covered with liquid, indicating that the organism flourishes best when it has access to free oxygen.

PRODUCTION OF HYDROXYL IONS FROM PEPTONE AND BEEF EXTRACT

This phenomenon was observed in the course of some experiments to test Rolfs's (*33*) statement that the organism breaks down carbohydrates and produces acid.

The experiments involved the use of dehydrated Bacto Andrade indicator agar, a commercial product of the following composition:

Bacto beef extract	3.0 parts.
Bacto peptone	5.0 parts.
Sugar	10.0 parts.
Bacto agar	12.0 parts.
Andrade indicator	0.0275 part.
Sodium hydroxide, NaOH	q. s.

Thirty grams of the agar powder was dissolved in 1 liter of distilled water.

The following sugars were tested: Sucrose, dextrose, lactose, maltose, and mannite.

The fact that sugars themselves may be broken down by hydrolysis during sterilization, as pointed out by Wolf (*47*), was disregarded in these preliminary experiments, since any pronounced hydrolysis and acid formation would be revealed by the change in color of the indicator in the medium and could be taken into consideration at the beginning of the experiment. The various media were faintly pink in color after sterilization, indicating that they were slightly acid in reaction. Noninoculated tubes were included as checks with each type of media.

Growth on the various sugar media was visible 24 hours after inoculation. In the case of the sucrose, dextrose, and maltose media the bacterial mass flowed down the slant and collected in a yellow viscid mass at the base. Growth was not so abundant on the lactose and mannite media; the bacterial mass was confined to the slant throughout the entire experiment and did not collect at the base.

There was no evidence of gas production in any of the media, and the color of the indicator did not change from the faint pink to red as would have occurred if there had been production of acid. In fact, at the end of the experiment the inoculated tubes were less pink than the check tubes, indicating that instead of acid production there was the production of alkali from some source.

The experiments were repeated several times, always with the same results. The amount of carbohydrate was increased from 1 per cent to 2 per cent and finally to 3 per cent, but even with the increased

amount of carbohydrate available there was no evidence of acid production.

Different isolations of the organism were used in the tests, including one obtained from Erwin F. Smith. The pathogenicity of all the isolations had been established by inoculation experiments, eliminating any doubt that the true organism was being used.

The experiments just discussed were carried out in soda-lime glass test tubes. It has been demonstrated that this type of glass is a fruitful source of alkali ions, and a portion of the next supply of medium prepared was tubed in pyrex glass test tubes.

The initial reaction of the medium in the pyrex tubes was pH 6.6 and in the soda lime tubes pH 6.8. At the end of 20 days the hydrogen-ion concentration of the inoculated medium and the noninoculated medium in both types of glass was determined colorimetrically.

It is evident from these results (Table 7) that the glassware used was responsible for very little of the pronounced swing to the alkaline range of the scale. This experiment also definitely confirmed the previous experiments and demonstrated why the red color of the acid range of the indicator did not appear.

TABLE 7.—*The effect of type of glassware on the hydrogen-ion concentration of lactose medium not inoculated and inoculated with Bacterium pruni*

Type of glass	Treatment of medium	Initial reaction	Reaction at end of 20 days
		pH	*pH*
Pyrex	Not inoculated	6.6	7.1
	Inoculated with Bact. pruni	6.6	[1] 7.4–8.0
Soda lime	Not inoculated	6.8	6.9
	Inoculated with Bact. pruni	6.8	[1] 7.6–8.0

[1] The upper and lower limits are given. In both cases the majority of cultures were pH 7.9 or 8.0.

The next experiment demonstrated clearly that the peptone and beef extract were the source of the hydroxyl ions. In this experiment the organism was grown in solutions of these two materials. Growth was vigorous on the peptone solution but was scanty on the beef extract solution. The swing of the reaction to the alkaline side is clearly demonstrated in Table 8.

TABLE 8.—*The production of hydroxyl ions from solutions inoculated with Bacterium pruni*

Solution and culture No.	Isolation	Hydrogen-ion concentration			Solution and culture No.	Isolation	Hydrogen-ion concentration		
		At start	After 2 days	After 9 days			At start	After 2 days	After 9 days
From peptone:					From beef extract:				
3975	A-1	6.2	6.6	7.8	3967	A-1	6.1	6.1	7.6
3976	A-1	6.3	6.5	7.5	3968	A-1	6.1	6.1	7.3
3977	A-1	6.6	6.6	7.6	3969	D-15	6.0	------	(1)
3978	S-1	6.2	6.2	[1] 6.2	3970	D-15	5.9	5.9	7.1
3979	S-1	6.1	6.3	7.8	3971	S-1	6.1	------	(1)
3980	S-1	5.8	6.3	7.7	3972	S-1	6.0	------	(1)
					3973	Griffin	5.9	5.9	7.3
					3974	do	6.0	------	(1)

[1] No growth in these tubes.

The results of these experiments and others of like nature demonstrate that the organism attacks peptone and beef extract with the production of hydroxyl ions. Likewise, the experiments demonstrate the fallacy of using solutions containing these two materials as a basal solution for carbohydrate fermentation tests with *Bacterium pruni*.

FERMENTATION OF CARBOHYDRATES

Rolfs (*33*), as mentioned before, stated that the organism broke down certain carbohydrates with the production of acid.

St. John-Brooks, Nain, and Rhodes (*38*) did not observe acid production by *Bacterium pruni* on carbohydrate medium in their experiments.

The writer has shown that the copious production of hydroxyl ions from peptone and beef extract masks the acid production, if it is present at all, in the ordinary media.

The ammonium phosphate medium recommended in the Manual of Methods of the Society of American Bacteriologists (*46*) is very satisfactory for the demonstration of the utilization of various carbohydrates by *Bacterium pruni* and the resultant production of acid. To prevent as much as possible the hydrolysis of the carbohydrates, the material to be tested was sterilized separately in a 20 per cent solution at 10 pounds pressure for 10 minutes. Wolf (*47*) has demonstrated that this procedure largely eliminates the hydrolysis of the carbohydrates.

The reaction of the ammonium phosphate medium with agar added (1.5 per cent) was brought to the neutral point and the solution sterilized at 15 pounds pressure for 15 minutes. A sufficient quantity of the 20 per cent carbohydrate solution was added to the cooled but still liquid ammonium phosphate medium to make a 2 per cent carbohydrate solution, and the resultant mixture was tubed and slanted under aseptic conditions into previously sterilized pyrex test tubes. One milliliter of a 1.6 per cent alcoholic solution of brom cresol purple was added to each liter of medium. All the tubes were incubated for a period of 48 hours at 23° to 26° C. before inoculation, as an added precaution against contamination.

The following carbohydrates were tested:

- Monosaccharides
  - Pentoses
    - Arabinose
    - Xylose
    - Rhamnose
  - Hexoses
    - Dextrose
    - Levulose
    - Galactose
    - Mannose
- Disaccharides
  - Sucrose
  - Lactose
  - Maltose
- Trisaccharides
  - Raffinose
  - Melezitose

In testing each carbohydrate, except sucrose, at least 15 different isolations of the organism were used, and noninoculated tubes of the media were held as checks.

Bacterium pruni grew vigorously on the monosaccharide media but made very poor growth on the disaccharide and trisaccharide media. With the monosaccharide media best growth was obtained on the four hexoses, and a somewhat less vigorous growth was obtained on the three pentoses. The cultures on all these carbohydrate media were kept under observation for 21 days. The first indication of acid production was noted in three to five days after inoculation. At the end of the experiment (after 21 days) the color of the indicator was yellow, indicating a change in hydrogen-ion concentration from 7.0[14] to pH 5.4 or below. Acid production was observed with all the materials tested. The reaction in the case of rhamnose, sucrose, maltose, lactose, raffinose, and melezitose was not so pronounced as with arabinose, xylose, dextrose, levulose, galactose, and mannose.

No signs of gas production were detected in any of the tubes throughout the experiment.

ENZYME PRODUCTION

Rolfs (*33*) found that the organism attacks pure sucrose and produces invert sugar. The writer has observed the same phenomenon in his carbohydrate studies. Cultures of ammonium phosphate medium with 2 per cent Difco sucrose were inoculated with various isolations of the organism. At the end of 21 day 5 ml. of the solution were withdrawn from the flasks and tested with freshly prepared Fehling's solution. Every culture gave a positive reaction for invert sugar, while the noninoculated medium held as a check gave negative results. The inversion of sucrose indicates the presence of the enzyme invertase.

Jodidi (*21*, *22*) has studied the chemistry of the changes occurring in skim milk inoculated with *Bacterium pruni* and has demonstrated that proteolytic and lipolytic enzymes are present among the products of the growth of the organism on milk.

EFFECT OF SUNLIGHT

The effect of sunlight on the organism was determined by the standard method of exposing Petri dishes resting on cakes of ice to sunlight for varying periods. Heavily seeded dextrose agar was used, and one-half of each Petri dish was shielded from the sun's rays by covering it with heavy black paper. The toxic action of the sun's rays was not apparent on the plates exposed for five minutes in any of the tests. The results on the plates exposed for 10 minutes were somewhat variable, but positive effects were noted in the 15, 20, 30, and 45 minute exposures. The number of colonies that developed in the exposed portion of the Petri dish gradually diminished until on the 30-minute exposure growth was inhibited almost completely in all the tests. No colonies ever developed on the exposed portion of the plates subjected to action of the sun for 45 minutes.

[14] This is approximately the concentration at the beginning of the tests, but it varied between 7.2 and 6.6 with the individual batches of media.

RESISTANCE TO DESICCATION

Drops of an aqueous suspension of the organism were placed, under aseptic conditions, on sterile cover slips in sterile Petri dishes. The Petri dishes containing the inoculated cover slips were placed in a light-tight container. At fixed intervals one cover slip was removed with a sterile forceps and dropped in a tube of sterile broth and the tube placed in an incubator. If the broth became cloudy, denoting bacterial growth, dilution plates were poured from it to make certain that the cloudiness was due to the growth of *Bacterium pruni* and not some contaminating organism. Using this technic, the organism was recovered from cover slips that had been dried for 2, 4, 5, 6, 7, 8, 9, 10, and 13 days, but no growth occurred from cover slips held for 15, 18, or 20 days. The dilution plates which were poured in all these tests made possible the positive identification of *Bact. pruni* as the cause of the clouding of the broth.

LONGEVITY

Cultures on beef-extract agar kept at laboratory temperature (24° to 26° C.) were still viable after 90 days. In one case growth was obtained from a culture 146 days old, but cultures older than this failed to develop when placed on beef-extract agar slants.

THERMAL DEATH POINT AND GENERAL TEMPERATURE RELATIONS

The thermal death point of the organism was found by Smith (*40*) to be approximately 51° C. Rolfs (*33*) found in his preliminary experiments that it ranged between 49° and 54° C. He finally concluded that 51° C. was very close to the exact figure, since growth occurred several times in cultures held at that temperature for 10 minutes while at 52° C. no growth was obtained.

The organism grows most vigorously between 24° and 28° C., the range which Rolfs considered the optimum temperature.

The color of the colonies, normally yellow, becomes paler as the temperature at which they are grown is increased. The room temperatures in the Fort Valley, Ga., laboratory were regularly high enough during the summer to produce this effect, which also could be produced at will during the winter by incubating the cultures at temperatures between 30° to 35° C.

The maximum temperature for growth was found by Rolfs (*33*) to be 37° C. A definite determination of the minimum temperature has not been made.

INDEX NUMBER

The index number of *Bacterium pruni* is 5020–31105–x222. The reactions indicated in this number are grouped according to the descriptive chart of the Society of American Bacteriologists of December 29, 1924.

RELATION TO OTHER ORGANISMS CAUSING ANALOGOUS DISEASES

The mode of entrance into the host tissue (i. e., through the stomata) and the resultant symptoms, particularly on the leaves, would

indicate that *Bacterium pruni* might show relationship with certain other phytopathogenic organisms.

Smith (*45*) in 1920 considered that the members of the "yellow group," *Bacterium campestre*, *Bact. citri*, *Bact. phaseoli*, *Bact. juglandis*, *Bact. malvacearum*, and *Bact. translucens*, were more or less related to each other. In fact, he felt that possibly some of them were identical, for he stated: "Indeed, some of these names are perhaps synonymous, but this can be settled only by many cross inoculations and much further study."

The problem of the interrelationship of these organisms has been attacked in recent years from the serological standpoint. St. John-Brooks, Nain, and Rhodes (*38*) in England and Link (*26*, *27*, *28*) and his coworkers in this country have subjected many of these organisms, including *Bacterium pruni*, to serological tests.

Briefly stated, the serological test consists in treating homogeneous suspensions of an organism with its specific serum. The bacteria develop flakelike clumps, i. e., agglutinate when treated with the sera. The results, to be reliable, should be positive even when the specific serum is diluted as much as 1 to 10,000.

Cultures of the organism are grown on standard agar for varying periods (48 hours to 4 days). Suspensions are made of the organism in saline solution (0.85 per cent) and are either tested for a known bacterial count or brought to an approximate density by comparing with one another. This suspension known as the antigen treated with phenol to kill the organism or not treated, according to the desires of the investigator, is then injected into a laboratory animal, generally a rabbit, at varying intervals and in varying amounts. Ten days after the last injection the animal is bled from the heart and the serum is prepared by removing the corpuscles from the blood. This serum, which is stored under aseptic conditions, is termed the antiserum, and it is diluted with varying amounts of saline solution by a series of progressive dilutions resulting in a final series 1 to 5, 1 to 10, 1 to 20, and so on up to 1 to 10,204 or higher.

Five milliliters of the diluted antiserum and a similar quantity of the antigen are thoroughly mixed together, incubated for 1 hour at 37° C., and then placed in a refrigerator for 12 hours. At the end of this period readings are taken. A complete clearing of the tube means strong agglutination. It should be understood that there are minor variations in the method, but this is a general outline of the procedure.

Closely related organisms are frequently agglutinated in the low dilutions by antisera other than their own. Link and Sharp (*27*), for example, found that *Bacterium phaseoli* and *Bact. flaccumfaciens* were agglutinated by the antiserum of *Bact. campestre* in dilution of 1 to 5, 1 to 10, 1 to 20, but *Bact. campestre* was agglutinated by its own antiserum dilution of 1 to 7,680.

Bacterium pruni when investigated in England was not agglutinated by the antisera of other members of the yellow group. Similar results are reported by Link and Link (*26*) in which there was no agglutination of *Bact. pruni* antigen even in dilutions as low as 1 to 10 when tested against the antisera of *Bact. campestre*, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. phaseoli sojense*, smooth and rough strains, *Bact. flaccumfaciens*, *Bact. medicaginis* var. *phaseo-*

licola, and *Bact. tumefaciens*. These organisms were not tested against the antiserum of *Bact. pruni* by Link and Link, but such tests were made by St. John-Brooks, Nain, and Rhodes (*38*) and *Bact. pruni* was agglutinated only by its own antiserum, and the other organisms investigated were not agglutinated by the antiserum of *Bact. pruni*. These results in the light of present-day interpretations of serological tests lead them to conclude that *Bact. pruni* is not serologically related to the other members of the yellow group. Link, Edgecombe, and Godkin (*28*) have modified this conclusion as the result of further studies which indicated that *Bact. pruni* is very slightly related serologically to *Bact. cucurbitae* and *Bact. translucens* var. *undulosum*.

PATHOGENICITY

The pathogenicity of *Bacterium pruni* has been demonstrated conclusively by Smith (*42*, *44*), Lewis (*25*), Rorer (*34*), and Rolfs (*33*). Smith inoculated an isolation from plum into plum and peach; Rorer used an isolation from peach to reinoculate peach; Lewis used an isolation from plum to inoculate peach and plum; and Rolfs used an isolation from plum to inoculate plum, peach, apricot, and nectarine. Reisolations from these four hosts were used the following year in cross-inoculation experiments, which were successful.

Smith obtained infections on the leaves and young plum fruit simply by spraying the organism suspended in water on the leaves and fruit of the host plant. These experiments were most successful when the inoculation was made during a rainstorm, or when the inoculated plants were kept moist for 24 to 48 hours after inoculation. He observed that under field conditions the number of leaf spots produced by spraying the leaves with aqueous suspensions of the organism was much smaller than one would expect considering the number of organisms used. If, however, the host was kept in a saturated atmosphere after the inoculation, a greater number of spots would appear. Rorer, who used the same technic in his studies of the disease on the peach, observed the same phenomenon, i. e., disparity of number of leaf spots produced as compared with the number of organisms used.

Lewis also produced spots on plum leaves simply by spraying them with a suspension of the organism, and also found that a few cankers developed on the twigs. He produced cankers on plum and peach twigs very readily by puncturing the twigs with a sterile needle and then introducing the organism into the puncture.

Rolfs inoculated his experimental plants by spraying, by rubbing the parts to be inoculated between the fingers moistened with a suspension of the organism, and by injecting an aqueous suspension of the organism into the tissues with a hypodermic syringe. Successful results were obtained with all three procedures.

In Georgia the writer experienced difficulty in obtaining infections by spraying aqueous suspensions of the pathogene on the leaves and twigs. The "iceless refrigerator" described by Hunt (*17*) was used in the early experiments, but even this device under Georgia conditions did not keep the leaves in a saturated atmosphere long enough for numerous infections to develop. A few leaf spots developed when suspensions of the organism were sprayed on peach trees during light

rains. Infections of peach leaves were obtained under similar conditions when the leaves were rubbed between the fingers previously moistened with a suspension of the organism.

Most of the inoculations were performed by injecting an aqueous suspension of the organism into the twigs (pl. 2, B) and leaves with a hypodermic syringe. This method is not strictly comparable with the method in which natural infections take place (i. e., through the stomata), but by actually placing the bacteria within the tissues, the effect of environmental conditions upon the entrance of the bacteria is more or less nullified. This is a feature not to be overlooked when facilities for producing favorable environmental conditions are wanting.

If the hypodermic needle is drawn gently along the under surface of the leaf, it is possible to produce a series of punctures which do not rupture the upper epidermis. Leaf inoculations of this type give rise to a series of coalesced spots following the path of the needle. These spots are similar, except for their lack of isolation, to those produced by spraying the organism on the leaf.

The writer has tried to produce infections on the peach fruit a number of times without success. The failure of these experiments, which were limited in number, probably is due to the hirsute character of the fruit, which makes it extremely difficult to duplicate experimentally the conditions under which natural infections take place.

The pathogene was reisolated from the leaf spots and twig cankers, compared in culture with the original isolation of the organism, and subsequently reinoculated and reisolated, thus fulfilling Koch's postulates. In all cases the symptoms on the host and the characters in culture were the same as those produced by the original culture used in the primary inoculations.

OVERWINTERING

The survival of the pathogene from one season to another is generally believed to be connected intimately with the presence or absence of twig cankers.

Early workers, Heald (*14*), Jackson (*20*), Lewis (*25*), and Rorer (*34*), all reported the occurrence of twig cankers on plum and peach, presumably caused by the activities of *Bacterium pruni*. Apparently they did not investigate the rôle these cankers play in the seasonal life history of the organism, and the first reference to the manner in which the organism overwinters is to be found in Rolfs's monograph (*33*).

Rolfs considered that the cankers were of paramount importance in the problem of overwintering of the organism, for he stated (*33, p. 413*):

> *Bacterium pruni*, so far as is known, passes its entire life cycle in nature within the tissue of the host. * * * Cankers on the twigs and limbs are the principal sources of infection in the spring. Trees bearing twigs on which inoculations result in the development of well-formed cankers, are invariably the first to develop leaf spot abundantly the following year. Young trees which suffer severely from repeated outbreaks of the shot-hole condition usually show many twig cankers. Twig cankers are also constantly associated with early spring outbreaks on older trees. * * *

These statements by Rolfs have been accepted widely by plant pathologists, although, as pointed out by Anderson (*6*), he does not state in his monograph that he isolated the bacteria from overwintered twig cankers resulting from natural infections.

Kuwatsuka (*23*) in 1919 was successful in isolating the organism from an overwintered plum canker, and Adams (*3*) reported in 1926 that he had isolated the organism from overwintered plum cankers. Later, Adams (*4*) reported that he had isolated the organism from peach-twig cankers during October and November, 1928, and January, March, and May, 1929.

Anderson (*6*), after failing in several hundred attempts to obtain the organism from cankers on dormant peach twigs, searched for other sources of overwintered inoculum. He succeeded in isolating the organism from overwintered leaf spots during 1924–25 and in subsequent inoculation experiments demonstrated that the isolations were pathogenic. As a result of these studies he concluded that in Illinois the pathogene passes the winter in the fallen leaves rather than in twig cankers.

Adams (*2, p. 51*) has commented on Anderson's theory in so far as it applied to the behavior of the disease in Delaware orchards as follows:

> Anderson * * * has shown that bacteria may overwinter in the dead leaves of the peach under Illinois conditions but advances no proof as to the method of distribution for infection in the spring. The rapid disintegration along with our early and frequent cultivation would lend little support to this contention under our conditions but must be entertained as a possibility until proved otherwise. While Anderson has failed to isolate bacteria from the cankers no proof is advanced that he was actually working with typical cankers.

In justice to Anderson it should be stated that isolation of the organism from overwintered leaves is a distinct contribution to our knowledge of the seasonal life history of the organism, but at present there is no proof that bacteria from these leaves initiate infections the following spring.

Rolfs (*33*) observed that the bacteria may enter the tissues of the buds, and he stated that infected buds are often responsible for outbreaks the following spring. He isolated the causal organism from the center of 11 buds of a total of 50 buds collected in August from peach twigs on which the leaves were seriously infected by the organism.

Anderson (*6*) and Adams (*1*) were not successful in their attempts to isolate the organism from overwintered buds, although Adams felt that some of the early infections in Delaware in 1925 were associated with diseased buds.

Working in Georgia, the writer first attempted to overwinter the organism in cankers produced by artificial inoculations. Rolfs reported that the disease invariably appeared on the leaves about such cankers earlier the following spring than it did elsewhere in his experimental plots. The writer produced cankers on the twigs by artificial inoculations early in the spring, during the summer, and in the fall over a period of several years, but no infections appeared on the leaves about these cankers the following spring.

While this work was in progress a series of observations starting in 1925 were made in an orchard near Montezuma, Ga., for the

purpose of obtaining further information on the overwintering of the pathogene. This orchard had suffered severe losses from *Bacterium pruni* every year, and it was thought that a detailed examination of a limited number of trees early each spring might shed some light on the problem. Selection was made at random of a block of Elberta trees consisting of seven adjacent rows, four being 20 trees deep and three 10 trees deep. The off varieties and missing trees brought the theoretical total of 110 trees down to 103.

OBSERVATIONS IN 1925

The trees were examined from time to time as the new growth began to develop, and on April 29, 1925, the presence of the disease on the leaves and fruit was detected in distinct regions in the trees. By examining carefully all the limbs in the region where the infection appeared, a twig canker generally could be located in this part of the tree. The most striking feature was that the canker, when once located, was seen to be at the apex of a conical region of infection in the tree. The bacteria had been washed to the limbs below and produced the primary infections on the young leaves and fruit. No infected fruit or leaves were found above the canker. These cone-shaped regions below cankers have been reported in connection with fire-blight disease of apple by A. N. Brooks (*9*) and the bitter-rot disease of apples by a number of investigators.

Each tree in the block was examined carefully for the presence or absence of cankers associated with outbreaks of the disease, and the results of this survey are presented in Table 9.

TABLE 9.—*Results of a survey made April 29, 1925, showing an apparent correlation of outbreaks of the bacterial-spot disease with overwintered twig cankers*

Items of comparison	Total number	Percentage
Trees examined	103	
Bacterial-spot infections observed	56	
Bacterial-spot infections associated with twig cankers observed	39	
Bacterial-spot infections apparently not associated with twig cankers observed	17	
Bacterial-spot infections associated with twig cankers		72

These regions of infection were found on various sides of the trees. In general there was only one region in each tree.

Unfortunately, attempts to isolate the organism from these cankers were unsuccessful, and this much-needed evidence to complete the proof of their identity could not be obtained. Nevertheless, the subsequent behavior of the disease during the season, which was a dry one, clearly indicated that the original inoculum had come from the cankers, and they were considered overwintered cankers even though definite proof through isolations of the pathogene was lacking.

Observations made at harvest time showed that the infected leaves and fruit were still in definitely delimited regions. The dry weather had practically checked further spread of the pathogene. This localization of the diseased region was so marked that unskilled laborers, who were assisting in the harvesting of the fruit and who

had no conception of the disease whatsoever, remarked upon the fact. The trees accordingly presented throughout the season excellent examples of drip infection from overwintered sources of inoculum.

It should be pointed out that these cankers were not located until the infections appeared on the leaves and fruit. These infections, if found early enough—i. e., before there is any secondary spread of the disease—indicate the portion of the tree that should be examined intensively for the original source of infection. Without this the task of finding the cankers resolves itself into a discouraging and time-consuming examination of every twig and branch on the tree, and their scarcity makes it seem very improbable that they could be located in the dormant season.

In addition it is quite possible that the cankers which survive the winter are not conspicuous until the bacteria emerge in the spring. At any rate the writer has spent days searching for them in the dormant season without success, only to find them with comparative ease after the first leaf and fruit infections appeared.

OBSERVATIONS IN 1926

The early spring temperatures were lower in 1926 than in 1925, and the first survey of the selected block of trees was not made until May 3, four days later than the date of the observations made in 1925, because it was thought the development of the disease would be retarded by the lower temperatures. This assumption was found to be incorrect. The disease had progressed beyond the localized drip-infection stage and had spread to all sides of the trees. Twig cankers could not be found, but this was not surprising because without the localized drip infections to act as guides it is practically a hopeless task, as explained before. There had been a number of rainy days with high winds, and the bacteria, as they oozed from the cankers, apparently were blown throughout the trees.

As a result of searches made during the balance of the 1926 season a few cankers on the 1926 wood were found early in the season and the organism isolated from them. Subsequent inoculation experiments demonstrated the pathogenicity of these isolations and their identity with *Bacterium pruni*.

Since no cankers were found at the end of the season after the leaves had fallen, no information was gained concerning the macroscopic appearance of the cankers at the beginning of winter.

OBSERVATIONS IN 1927

The block of Elberta trees that had been under observation for the two preceding years was examined carefully in February and 52 necrotic areas which were thought to be bacterial-spot cankers were marked with tags in an attempt to see if cankers could be located in advance of the appearance of the disease on the leaves and fruit. The results were negative, since it was impossible to correlate the outbreak of the disease with the presence of the alleged cankers.

The block was examined on April 12 and the development of the disease was found to be at a stage midway between that reached on the

date of the first observation in 1925 and that of the first observation in 1926. In most trees the disease had spread through the tree and no cankers could be found, but occasionally a tree would be observed with a cone-shaped region of infected leaves and fruit with a canker at the apex. These cases were not so clear-cut as the 1925 observations. Similar drip infections associated with twig cankers were observed in several other orchards at this time. Attempts to culture the organism from these cankers again resulted in failure.

Cankers on peach and plum twigs, which resembled closely those produced in the inoculation experiments, were collected late in the fall (November), and efforts were made to isolate the organism from them. No cultures of *Bacterium pruni* were secured from any of the cankers, but in the case of the peach the negative results may indicate only that the cankers used were not true bacterial-spot cankers. The cankers on the plum twigs, however, were considered true bacterial-spot cankers.

OBSERVATIONS IN 1928

In 1928 the work was transferred from the orchard near Montezuma, Ga., to orchards in the vicinity of Fort Valley, Ga., as many trees in the original block under observation near Montezuma had died from winter injury, or were removed because of the phony disease.[15] This high mortality made the block no longer suitable for other studies of the disease which were being made concurrently with the search for cankers in which the pathogene passed the winter.

The observations in 1925 and 1927 favored the theory that the organism overwintered in the twig cankers, but definite proof in the form of cultures from these cankers was lacking. The work in 1928 was, therefore, centered about the isolation of the organism from overwintering cankers. Early in May young spots were observed on the leaves, and by searching carefully in the immediate vicinity of those leaves a canker generally was located on an adjacent twig. All suspicious looking cankers were brought to the laboratory, and attempts were made to isolate the organism from them, using the following technic:

Previously cleaned glass slides were dipped in 95 per cent alcohol and the adhering alcohol ignited. While still hot a previously flamed glass cell was placed on the slide. When the slide was cool the cell was lifted for a moment, and a drop of sterile tap water was placed in the center of the area covered by the cell, which was then replaced.

The epidermis of the canker was cut away with a sharp, sterile scalpel, and small fragments of the cortical tissue were removed aseptically and placed in the drop of sterile water. The tissue fragments were left in the drop of water for a few minutes, and then the cell was removed for an instant and the drop examined under a binocular microscope. If masses of bacteria had oozed out into the water, the fragment was removed from the drop with a sterile needle and the cell again placed over the drop. Plates of dextrose agar prepared in advance and allowed to solidify were placed on the stage of the binocular microscope. The cell was lifted up and portions of the drop removed with small sterile platinum loops were streaked across the surface of the solid agar. Bac-

[15] An infectious disease of unknown cause, in which the affected tree develops shortened internodes, many lateral twigs, and large, flattened, dark-green leaves, giving the appearance of compact, dense growth with very healthy foliage. Each year there is a notable and progressive decrease in the average size and yield of fruit, which is likely to be distinctly poorer in flavor than normal fruit, though slightly better in color. Eventually the infected trees fail to produce any commercial crop.—Hutchins (*18*).

terial growth generally appeared within 24 to 48 hours, and the pathogenicity of those colonies which resembled *Bacterium pruni* was tested by inoculation experiments.

The writer holds no particular brief for this technic other than that it makes possible the elimination at the start of all those cankers which do not yield bacteria of some sort, whether *Bacterium pruni* or merely secondary organisms in the dead tissues. In experiment No. 7 the organism was isolated from two cankers using the usual technic of poured plates.

Thirty cankers were examined during the period May 11 to June 14, and the organism, as demonstrated by subsequent inoculation experiments, was secured from six of them.

A summary of these experiments is presented in Table 10, and the following notes briefly describe those cankers from which the organism was isolated:

Experiment No. 1.—This was not a definite canker but merely a necrotic spot about a bud on 1927 wood. The organism was isolated from the tissues just beneath the epidermis.

Experiment No. 2.—(*a*) In this case the twig was swollen and cracked by a canker extending from the third to the fourth node below the apex of the 1927 wood. The organism was isolated from the inner tissues.

(*b*) The terminal of the 1927 growth in this twig was blackened and dead. Bacteria were not found just beneath the epidermis, but were present deeper in the cortical tissue.

Experiment No. 6.—The canker on this twig began just below the apex of the 1927 growth and extended downward a distance of 10 cm., involving five nodes. Longitudinal slits were present on all sides of the twig and there was some evidence of callus formation just below the epidermis. The affected area was sunken, and was brown to purplish brown in color. Bacteria were not found just under the epidermis but in several pockets deeper in the cortex.

Experiment No. 7.—(*a*) The outer surface of the twig was fissured and cracked by a canker 4.5 cm. long, beginning at the fifth node below the apex of the 1927 growth. The organism was secured from the inner cortical tissues.

(*b*) The canker was 4.5 cm. long and extended downward from the ninth node below the apex of the 1927 growth. Fissures or cracks were absent in this canker, and the bacteria were found just beneath the epidermis. Fragments from both cankers of experiment No. 7, as mentioned before, were dropped in tubes of sterile dextrose broth and dilution plates poured.

TABLE 10.—*Summary of the bacterial-spot twig canker isolation experiments in 1928*

Experiment No.	Date	Number of cankers used	Number of cankers from which Bacterium pruni was obtained	Experiment No.	Date	Number of cankers used	Number of cankers from which Bacterium pruni was obtained
1	May 11	1	1	5	May 31	1	0
2	May 14	5	2	6	June 1	7	1
3	May 22	4	0	7	June 14	10	2
4	May 28	2	0				
				Total		30	6

These studies conducted during the years 1925 to 1928 indicate that twig cankers are the source of the overwintered inoculum in Georgia. Higgins (*16*) also believes that the twig cankers are the most important source of spring infections in Georgia.

Although no infection cones were observed in 1926 and the pathogene might have been distributed by the wind, the writer is of the

opinion that the initial observations were not made early enough to observe the infection cones. The disease appeared earlier than normal, with conditions, including several rainstorms accompanied by high winds, favoring a rapid spread. Under these conditions the infection cones are soon obliterated and the disease is distributed generally throughout the tree.

These studies also indicate that it is not necessary for many cankers to survive the winter to insure the perpetuation of the disease from year to year. In the spring of 1925 there was generally but one canker to a tree, and a number of trees were without cankers. The same condition was observed in 1927, while in the spring of 1928 as many as five cankers were observed in a tree.

The cankers on peach twigs seem to be more prevalent in Delaware than in Georgia, according to the recent observations of Adams (4). In September, 1928, he examined three Elberta orchards in Delaware for twig cankers—"* * * that were conspicuous to the eye on the new growth up to a height of five feet from the ground." On trees that had been severely affected by the disease for three years he found an average of 35 cankers in the area examined on each tree. On trees that were affected seriously for the first time in 1928 he found an average of 10 cankers to each tree.

Adams did not state how many of these cankers survived the winter and initiated infection the following spring, so his observations, unfortunately, can not be compared with those made by the writer.

DISSEMINATION OF BACTERIUM PRUNI

The area included in the infection cones generally is too extensive to be accounted for by a mere passive oozing and dripping of the bacteria from the overwintered sources. Field observations indicate that the inoculum causing the primary infections is spread by raindrops striking the bacterial ooze and rebounding from the twigs in scattered bacteria-charged droplets.

Secondary infections can be established by the splash from raindrops rebounding from fruit and possibly leaf spots oozing bacteria. The exudate is generally on the lower side of the leaf, however, and the chances here for this type of dissemination do not seem to be so great as in the case of the fruit spots.

The writer feels that dew is of primary importance in the dissemination of the bacteria which exude from the leaf spots. Frequently a leaf is observed on which there is a series of spots almost in a straight line down the blade. This condition is probably the result of a drop of dew charged with bacteria running down the leaf.

Wind as well as rain must be considered an important agent in the spread of the pathogene. Drops of rain and dew charged with bacteria can be blown from one portion of the tree to another or even to an adjacent tree. The effectiveness of the wind is in direct proportion to its velocity, since the higher the velocity the greater distance the drop may be carried. The drop will also break into a greater number of droplets when it is being carried by a wind of high velocity. During dry weather the wind is probably a minor agent

in the spread of the disease, as the other conditions are not favorable for infection.

Rolfs (*33*) felt that insects played a rôle in the dissemination of the organism. The possibility of this is not denied, but the writer has seen no evidence of insect transmission in his studies of the disease.

Turning now from this rather speculative discussion of the agencies concerned in the dissemination of the disease, some observations on the actual spread of the disease will be presented.

Mention has been made of the detailed surveys that were made for a number of years in a block of Elberta trees at Montezuma, Ga. The results obtained in 1926, a season very favorable to the spread of the disease, are presented in Table 11, and a graphic demonstration of the spread is shown in Figure 3.

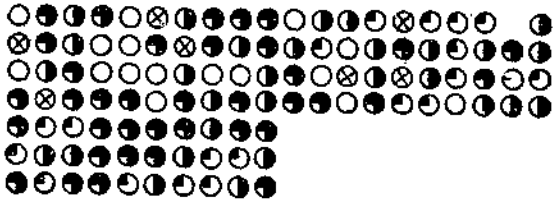


FIGURE 3.—Orchard diagram showing the spread of *Bacterium pruni* during the growing season. The data are based on surveys made May 18, June 30, and July 13, 1926

TABLE 11.—*Increase in number of trees infected with Bacterium pruni*

Date	Total trees	Trees infected		Increase in—	
				Number infected	Percentage of number infected
1926	*Number*	*Number*	*Per cent*		
May 18	102	20	19		
June 30	102	49	48	29	29
July 13	102	86	84	37	36

Table 11 shows the number of trees bearing infected fruit on different dates. Fruit spots were used, as they can be identified rapidly and positively, while young leaf spots may be confused with other troubles. The increase in the number of trees showing fruit infections is interesting in itself, but in making a survey a record was kept of the location of each tree showing the disease.

From this record it is possible to ascertain to a certain degree the relationship of the earlier infections and the new cases. On June 30, 29 new cases were observed, and of these, 17 trees were adjacent to those that were observed infected on May 18. On July 13, 37 additional cases were observed, and of these, 29 trees were adjacent to those previously observed to be infected. The importance of the original infections is shown clearly, since more than one-half of the new cases in both observations were adjacent to trees previously infected. In the observations of July 13 over three-fourths of the new infections were adjacent to the previously infected trees.

These results were obtained in a year (1926) when conditions favorable to the spread of the disease prevailed throughout the growing season. In the previous year the growing season was abnormally dry except a brief period in April when the initial infections were established. The disease did not spread from tree to tree to any extent as the result of the unfavorable conditions. In 1927 the spread of the disease was very rapid the early part of the season, and 64 per cent of the trees were found infected on April 25, as contrasted with 19 per cent on May 18 in 1926, and 54 per cent for the entire season of 1925.

The detailed discussion of the spread of the disease in 1926 is merely to explain how the disease behaved under a certain set of environmental conditions. These conditions vary from year to year, and the time of the initial appearance and subsequent spread of the disease necessarily varies in accordance with them.

EFFECT OF ENVIRONMENTAL CONDITIONS

The environmental conditions play important rôles in the dissemination and development of bacterial plant diseases. Smith (*45*) in a general discussion of the subject has pointed out that "excessive rainfall, shading, high winds, wet earth, and heavy dews" furnish conditions that are ideal for the rapid dissemination of the pathogenes causing these diseases, especially when the air temperature is high.

An abundance of moisture is probably the most important factor affecting the dissemination of *Bacterium pruni*. Field observations by investigators in different portions of the country have led to the conclusion that the yearly fluctuations of the severity of the disease can be attributed largely to fluctuations in the amount of precipitation. Periods of excessive rainfall are accompanied by cloudy skies which shut-off to a certain extent the sun's rays, and the films of water on the parts to be infected do not evaporate so rapidly as they would under conditions of bright sunlight. Shading produced by the foliage of the trees themselves has much the same effect. This is very clearly demonstrated on a clear day following a heavy dew. The writer frequently has observed that the leaves on the west side of the tree were damp with dew at 10 o'clock in the morning, while those on the east side under a bright sun were completely dry. This condition frequently brings about a localization of the disease on the west side of the tree. It is quite possible in view of the toxic effect of the sun's rays on the pathogene on culture media that a similar effect exists in nature, and shading

reduces the number of bacteria that are killed by the toxic action of the sun's rays.

The effect of high winds has been mentioned previously, and it is sufficient to say here that a high wind accompanied by rain can blow the bacteria through the trees and completely overcome the effect of shading in contributing to the prevalence of the disease on the west side of the trees.

Temperature is also an important factor and clearly influences the time of the initial appearance of the disease in the spring and its subsequent development. Unfortunately, accurate records are not available, and the initial appearance of the disease can not be correlated so clearly with temperature as is possible with apple scab,[16] peach-leaf curl,[17] and the apple bitter-rot disease.[18] In Georgia the disease appears during the period from April 10 to April 30, and it is evident that the temperatures normally reach a point favorable to the organism every year during this period and eliminate any wide variation in the time of appearance. The conditions are different in other portions of the country, and Rolfs (*33*) observed a variation in the time of the appearance of the disease from April to August. In Arkansas, during the year 1929, the low temperatures during April and May had a marked influence on the disease. Rainfall was excessive, and it was accompanied by many cloudy days, yet the disease did not become common in the orchards until June.

Kuwatsuka (*23*) made the interesting observation in 1921 that his inoculation experiments using 2-year-old potted plants were more successful when the pots were standing in pans of water, so that the soil was nearly saturated, than when the plants were wilting or held at the lowest water supply consistent with keeping the plants alive. This effect was probably due to the fact that the stomata were closed in the wilted or dry plants and the pathogene was unable to enter through them.

INCUBATION PERIOD

Smith (*42*) observed that small spots were visible on plum leaves seven days after spraying the leaves with a suspension of the organism. In his experiments with peach leaves he (*44*) did not give any definite data, but stated: "The result of this experiment was the appearance after a number of days of several thousands of typical leaf spots."

Rolfs (*33*) found that in his experiments the incubation period varied from 7 to 15 days in warm weather, but in cold weather the period was prolonged to between 20 and 25 days. These results were obtained when leaves and fruit were moistened with a suspension of the bacteria. In the case of the twigs where the organism was actually introduced into the tissue, he found that the first symptoms appeared in from 4 to 10 days.

The incubation period varied from 5 to 12 days in the writer's experiments, reported in Table 12. The inoculation experiments from which these data were derived were all of the type in which the suspensions of the organism were introduced into the tissue with a

[16] Caused by *Venturia inaequalis* (Cke.) Wint.
[17] Caused by *Exoascus deformans* (Berk.) Fekl.
[18] Caused by *Glomerella cingulata* (Ston.) Spauld. and Schrenk.

hypodermic needle. The results obtained were comparable with those reported by Rolfs when he used a similar technic.

TABLE 12.—*Duration of the incubation period in inoculation experiments with Bacterium pruni*

Date	Tissue involved	Method of inoculation	First symptoms appeared	Incubation period
				Days
May 8, 1925	Twigs	Puncture	May 13, 1925	5
May 18, 1925	do	do	May 25, 1925	7
June 5, 1925	do	do	June 12, 1925	7
June 12, 1925	do	do	June 18, 1925	6
Apr. 15, 1926	do	do	Apr. 23, 1926	8
Apr. 23, 1926	do	do	May 1, 1926	8
May 20, 1926	do	do	May 28, 1926	8
Do	Leaves	Scraped	do	8
May 29, 1926	do	do	June 7, 1926	10
June 1, 1926	do	do	do	6
Aug. 6, 1926	do	do	Aug. 13, 1926	7
Mar. 19, 1927	Twigs	Puncture	Mar. 31, 1927	12
May 28, 1927	do	do	June 3, 1927	6

The experiments in which the leaves were either sprayed or rubbed between the fingers moistened with a suspension of the organism were not particularly successful under Georgia conditions. In one experiment, peach leaves were sprayed with a suspension of the organism on March 25, 1926, and the first symptoms were not observed until April 16, indicating an incubation period of 22 days.

To summarize the results of the various investigations, the incubation period appears to be rather prolonged in cool weather and may be as much as 25 days in duration. The period is shortened in warm weather and varies from 7 to 15 days. In those experiments in which the organism was injected into the tissue the incubation period in some cases was as short as 4 or 5 days.

DURATION OF INFECTION PERIOD

Observations have shown that the fruits of susceptible varieties are liable to infection at practically any time with the possible exception of a brief period early in their development (i. e., before the calyxes have split off the young fruit).

The writer can not agree with Higgins (*16*) who stated that the fruit probably was immune to infection after it was half grown. A careful examination of the fruit over a series of years has shown that infection is possible throughout the period of fruit development. In fact, very young spots exuding bacteria have been observed on mature fruit of the Elberta variety in Georgia in July, one week before it was harvested. In Indiana the writer has observed bacteria oozing from young spots on mature Elberta fruits in August. In Arkansas, bacterial ooze has been observed on mature Elberta peaches which had been harvested.

Infection is clearly correlated with proper temperature and moisture conditions, and negative departure (i. e., low temperatures or the absence of precipitation) from the optimum conditions often produces periods of considerable duration in which no infection takes place. The 1925 season in Georgia, which was abnormally dry, was

an excellent illustration of this. Conditions such as these can easily lead one to think that the fruit acquires an immunity after a certain period in its development, when in reality the absence of infections is due to unfavorable environmental conditions.

Leaf spots in the early stages of their development have been observed throughout the growing season, but as there is a continual dropping of old leaves and a continual production of new ones, it may be quite possible that the leaves can be infected only during certain stages of their development. It is not known whether or not a leaf formed in April could be infected in August or October if it remained on the tree.

The evidence from inoculation experiments indicates that the twigs are most susceptible when the tissues are quite young and succulent. The organism makes very little progress when introduced into current-year twigs in October during periods when the temperature and precipitation are favorable to the organism as shown by its ready development on leaves inoculated at the same time. There is no evidence that twigs are capable of being infected after their first year.

The infection period persists, in the case of the fruit, until maturity is reached. In the case of the leaves it may or may not be confined to certain periods, while in the case of the twigs the evidence at hand indicates that it is of relatively short duration.

CONTROL MEASURES

It is hoped that the discussion in the previous pages of some of the more obscure symptoms of the bacterial spot disease will aid in its control by facilitating its early recognition in the orchards. Mention may be made of the relation between the development of successful control measures and such features in the seasonal life history of the pathogene as the production of inoculum throughout the growing season and the relative scarcity of twig cankers. The almost continuous production of inoculum indicates very clearly that a prolonged schedule of control measures is in order, while the relative scarcity of the twig cankers largely precludes the possibility of controlling the disease through a program based on their elimination.

The first extensive control experiments were conducted by Roberts (*31*) in Arkansas during the years 1913 to 1915. In these experiments due cognizance was taken of the fact, established by numerous field observations, that the disease was more severe on weak trees and in orchards that had not been properly pruned, fertilized, or cultivated. Roberts obtained satisfactory commercial control of the disease by improving the vigor of the trees, through the use of nitrate of soda, and through pruning and cultivation. As a result of these experiments he made the following statement concerning the control of the disease:

> Experiments carried on by the writer and others indicate that the disease may be kept in check in southern peach orchards by proper pruning, cultivation, and especially fertilization. Nitrate of soda was by far the most efficient fertilizer used. Trees in which a high state of vigor and health is maintained are commercially resistant to the disease.

These recommendations have been satisfactory in sections where the disease was not severe and where the character of the soil was

such that improvement can be rapidly accomplished. In many sections (particularly the newer peach sections) soil improvement was slow and expensive, and the disease had gained such a foothold that it seemed desirable to develop, if possible, additional control measures, particularly some form of spray solution that would control the disease.

Various investigators have tested a large number of compounds in the past five years, but practically all have been eliminated either because they showed little or no signs of control or because they caused severe injury to the peach. The extreme susceptibility of the peach leaves, fruit, and twigs to spray injury has made the development of a satisfactory spray an extremely difficult task.

One of the most promising sprays for the control of this disease is the zinc-lime spray developed by Roberts and Pierce (*32*). Various combinations of this spray (which in its latest formula consists of 4 pounds of zinc sulphate and 4 pounds of hydrated lime to 50 gallons of water) have been used by Roberts and Pierce in southern Indiana every year since 1925 and by the writer in northwestern Arkansas in 1929 and 1930. The spray does not eliminate the disease completely, but the number and size of the spots (particularly on the fruit) are reduced and a larger percentage of the fruit is merchantable on the trees sprayed with it. The amount of defoliation is reduced, and the general vigor of the trees is improved through this reduction in defoliation and through a stimulation produced by the sprays. Measurements have shown that the leaves on the trees sprayed with the zinc-lime spray are larger than on the unsprayed trees and they are deeper green in color.

While this spray does not eliminate the disease completely the results obtained with it are promising and it is hoped that further experimentation will produce a combination that will reduce the ravages of the disease to a minimum.

SUMMARY

Bacterium pruni, the cause of the bacterial spot disease of stone fruits, was first described in 1902.

The organism has been reported from the United States, Canada, and Japan, and possibly may occur in China.

In the United States the disease has been observed over a wide area east of the Rocky Mountains, but has never been reported from the fruit-growing sections of the far West and Northwest.

The organism causes the disease only on members of the genus Prunus. *Sorbus japonica* has been infected artificially by Kuwatsuka in Japan, but the resulting symptoms are not typical.

The disease is of economic importance because it (1) brings about a devitalization of the tree through defoliation, (2) kills the twigs, and (3) lowers the marketability of the fruit by injuring its appearance.

The symptoms of the disease on peach leaves, fruit, and twigs have been described in detail. On the fruit the symptoms of economic importance are secondary in nature, resulting from the growth of the tissues after injury by the pathogene has ceased. These secondary symptoms are grouped in an arbitrary descriptive classification.

The pathological histology of the disease on the peach is discussed. The organism is intercellular in the early stages of infection, but finally the cell walls are ruptured and bacteria can be found within the remnants of the cells. In the leaf and fruit spots only parenchymatous tissue is attacked, while in the twigs occasionally the phloem is invaded. The ultimate result of the invasion of the tissue by the bacteria is the formation of a cavity in which all semblance of the normal tissue structure is lost. The necrotic area is isolated from the healthy fruit and twig tissues by a layer of wound cork.

The discussion of the morphology and physiology of the organism agrees in the main with the results of previous investigators. The production of hydroxyl ions from media containing peptone or beef extract is demonstrated, and media containing these ingredients are not satisfactory for the study of acid production by the organism. Twelve carbohydrates were tested, using a standard synthetic medium. The organism was able to ferment the carbohydrates without the production of gas, and produced an acid reaction in the media. The organism grew vigorously on monosaccharides, but made very poor growth on disaccharides and trisaccharides.

The recent serological work with phytopathogenic bacteria is reviewed. According to these tests the organism is related only slightly to other members of the so-called "yellow group," in spite of the analogies in mode of entrance and resultant symptoms.

The occurrence of twig cankers and their relation to the overwintering of the organism is discussed in detail. Evidence is advanced to show that in three seasons the initial outbreaks of the disease in Georgia peach orchards could be correlated with the presence of overwintered twig cankers. The organism was isolated from a limited number of these cankers. No evidence was obtained to show that the organism overwinters in the fallen leaves.

Field observations indicate that the organism is spread through the agency of wind, rain, and dew. The actual spread of the disease based on the results of several orchard surveys is discussed. In a block of 102 trees the number of infected trees increased from 20 on May 18 to 49 on June 30 and 86 on July 13, 1926. On each of the last two dates more than 50 per cent of the trees infected were adjacent to trees previously infected. The rate of spread of the disease depends largely upon the environmental conditions and will necessarily vary from year to year.

Fruit of the susceptible varieties of peach is liable to infection any time during its development after the calyxes have dropped off. Unfavorable environmental conditions will produce periods of considerable duration in which no infection takes place and may lead to the belief, considered erroneous, that the fruit acquires a degree of immunity at a certain stage in its development. The twigs are susceptible when young and succulent, but the evidence indicates they can not be infected after the tissues mature.

In portions of the United States where the disease is not severe and the soil is relatively fertile the disease can be kept under commercial control through the use of fertilizers, particularly nitrate of soda, and proper pruning and cultivation. There are many sections, however, where these measures have failed to give satisfactory control, and various investigators have devised sprays to be used in combating the

disease. The zinc-lime combination is such a spray and seems to be one of the most promising that has been developed for this purpose. While the disease is not eliminated through the use of this spray, the amount of defoliation is reduced, and a larger percentage of the fruit is merchantable on the trees sprayed with it.

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