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BACTERIAL CANKER OF STONE-FRUIT TREES IN CALIFORNIA^{1,2}

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INTRODUCTION

The gummosis phenomenon in trees of the genus *Prunus* has been much investigated since the early nineteenth century. Although many studies were primarily concerned with the origin and composition of the gum itself, some attention was paid to cause. In general, most investigators regarded climatic and soil factors as the cause of the gummosis. Apparently Brzezinski,⁽¹⁰⁾ working at Krakow in 1902, first attributed to bacteria a certain gumming cankerous disease of apricots, plums, and cherries. He claimed to have demonstrated the pathogenicity of these bacteria to their respective hosts, but neither named nor described them.

In 1905, Aderhold and Ruhland^(1,2,8) found a bacterial canker disease, caused by *Pseudomonas spongiosa*, producing severe damage to cherries in Germany. Their work, which received wider recognition than that of Brzezinski, served to focus the attention of plant pathologists on the role of bacteria in producing gummosis of limbs and trunks of the stone-fruit trees; all types of this gummosis had heretofore been thought to result from purely physiological causes.

Griffin's work,⁽¹³⁾ in 1911, definitely established the bacterial origin of a gummosis disease of cherry limbs in Oregon and also showed that the bacterium (*Pseudomonas cerasus*)⁴ produced a blighting of dormant buds. Barss,^(5, 6, 7) who followed Griffin in this work, demonstrated

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² The writer wishes to express his appreciation to Professors R. E. Smith and M. W. Gardner for advice during the investigations and for aid in preparation of the manuscript.

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⁴ The possessive, *cerasi*, is preferable to *cerasus*.

that this same bacterium produced cankers on limbs of practically all the stone fruits. These two workers introduced the terms "bacterial gummosis" or "bacterial canker" to distinguish their disease from gummosis resulting from other causes.

INVESTIGATIONS OF A GUMMOSIS AND A "SOUR-SAP" DISEASE IN CALIFORNIA

In California, in 1916, Barrett⁽⁴⁾ discovered a severe cankerous condition of apricot trees, with all the symptoms described by Griffin in Oregon. After this work the disease in California was regarded as the "bacterial gummosis."

For a number of years, a severe cankerous condition of plum trees has occurred annually in the foothills of the Sierra Nevada Mountains, causing especially great damage in the fresh-fruit district of Placer County. Goldsworthy and Smith⁽¹⁵⁾ have reported studies on the disease under the name of "sour-sap." Although they found symptoms differing somewhat from those of "bacterial gummosis," no one species of bacteria appeared to be constantly associated with the trouble; any of several types could produce the "sour-sap" symptoms. The data presented herein are based upon a comparison of "sour-sap," as described by Goldsworthy and Smith, with the more common gummosis disease.

INJURIES FROM OTHER CAUSES SOMETIMES CONFUSED WITH THE BACTERIAL DISEASE

Before the symptoms of "sour-sap" and gummosis are detailed, it should be emphasized that the term *sour-sap* here designates the symptoms described by Goldsworthy and Smith.⁽¹⁵⁾ The term as used by some other workers refers to troubles unrelated to the disease now under consideration. Thus Cockayne⁽¹²⁾ and Waters^(24, 25) mentioned a sour-sap of pears in New Zealand as caused by excessive soil moisture; Miss Willis⁽²⁶⁾ applied the term to a root trouble of prunes in Washington, which she attributed to drought injury; Miss Phillips,⁽¹⁷⁾ in California, mentioned a sour-sap disease of apricots that was later shown to result from *Verticillium albo-atrum*;⁽¹⁸⁾ and Birmingham⁽⁸⁾ has recently reported a sour-sap of cherry tree in New South Wales, which he attributes to extreme variations in soil moisture.

With the exception of Phillips, all these workers had reference to a browned and fermented condition, particularly within the bark of trunks and within the cortex of roots and crowns of the trees. The term *sour-sap* has therefore been applied to certain symptoms but not, in general, to any particular disease.

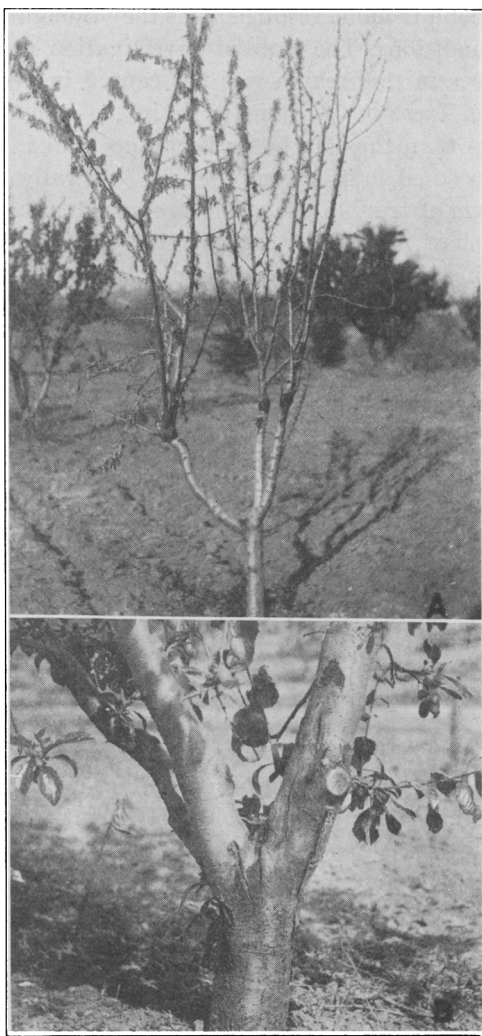


Fig. 1.—Upper, young plum tree which died from the sour-sap type of disease. Limbs on right side of tree died at the time they began to leaf-out; those on left side died after a good many leaves had been produced. Lower, Grand Duke plum with large diseased area on trunk and first scaffold branches. Note the absence of gum.

Although Goldsworthy and Smith⁽¹⁵⁾ attributed most of the trouble in Placer County to the bacterial form of sour-sap, they recognized the presence of a second trouble, resulting—as they thought—from adverse soil moisture conditions. The present investigation soon showed that the death of trees in this region was not caused in every case by the bacterial disease. Trees in low-lying areas in the orchard often died in greater numbers than those in better locations, and in these cases the roots and crowns died first, a symptom not generally associated with the bacterial form of trouble. Isolations from such affected crowns and roots failed to show any consistent bacterial or fungal flora. Studies were therefore instituted for the purpose of answering two questions: (1) Does soil moisture play a part in producing this type of trouble; and (2) does soil moisture influence the bacterial disease? This work has been done in coöperation with Dr. A. H. Hendrickson of the Pomology Division, who takes full responsibility for the soil moisture phase of the problem. The results of the studies are reserved for a future publication.

SYMPTOMS OF GUMMOSIS AND SOUR-SAP COMPARED

The disease known as sour-sap in the Placer County fruit district is characterized by the failure of entire trees or portions of trees to produce leaves in the spring (fig. 1*A*). Some may put out foliage, only to die shortly thereafter. The bark of limbs or trunks is girdled by necrotic areas, the tissue of which is dull brown, moist, and sour-smelling. After a tree is dead one can seldom determine whether death was preceded by a girdling of the trunk or by a general involvement of the entire above-ground system, inasmuch as the bark of even the smallest limbs turns brown. Studies of numerous cases, however, have revealed that death of the entire tree follows only if the trunk has been previously girdled by the diseased area (fig. 1*B*).

Cankers start as small, brown to reddish-brown spots in the outer bark (fig. 2*A*, *B*). When conditions are favorable, these spots enlarge by means of small, water-soaked streaks, which during the fall and winter extend up and down the branch. Sometime during early spring a brownish discoloration begins to appear in the tissue between the streaks. This dying of the bark proceeds rapidly at about the time the buds begin to open; and by the time the leaves appear, the area that earlier manifested the disease only by the presence of the streaks now becomes uniformly brown and moist (fig. 2*C*). As a rule little, if any, gum is exuded from the affected tissues; but a watery material may

flow from cracks in the bark and cover the limbs. The absence of gum is particularly noticeable in the case of plums. A more detailed description of the cankers will follow later.

One rather striking and unique feature of the disease is its remaining

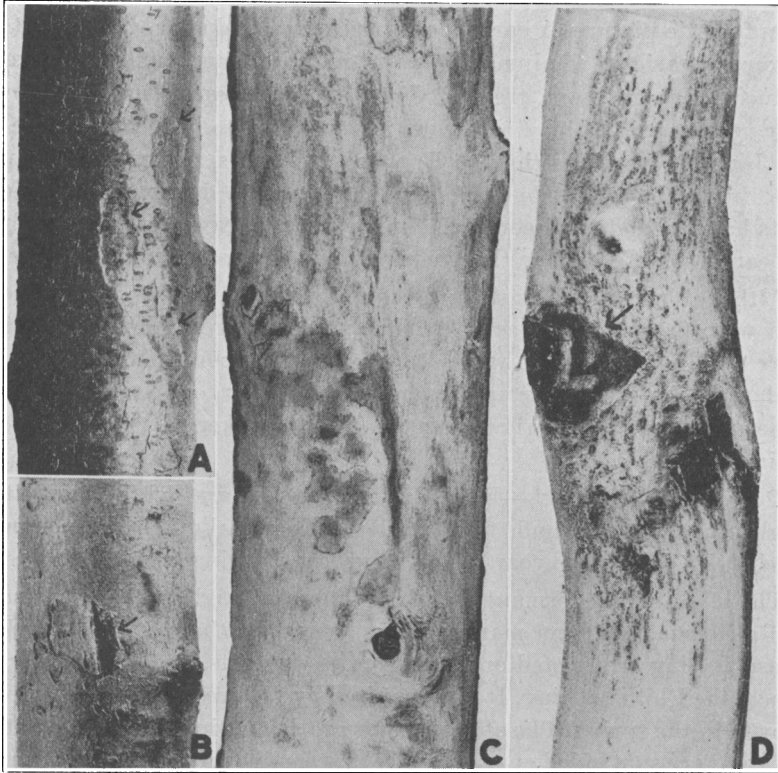


Fig. 2.—Sour-sap cankers. *A*, *B*, young cankers (indicated by arrows), visible from the exterior only by slight sinking or cracking of the periderm. *C*, periderm removed from an area beyond the necrotic center of a canker (visible in the upper right-hand corner). This area is beginning to turn uniformly brown. *D*, a diffuse canker, composed of a small necrotic center (indicated by arrow) surrounded by a wide zone of loosely connected, reddish brown streaks. The only exterior signs of this canker were a slight sinking and cracking of the periderm.

aboveground. The writer has never found a proved case in the roots. It generally stops at the ground level or slightly below.

The more common gummosis type of disease has been generally regarded as differing from the sour-sap in the presence of abundant gum and in better defined, deeper cankers. A detailed comparison of symptoms, however, has failed to show any differences that remained constant throughout the year. Such differences as appear at certain times

are those of degree. Thus, though the cankers of the sour-sap type do not usually exude gum, they have been found to do so at certain times and under certain conditions. Strong evidence, related both to the feature of gum formation and to the character of the cankers, indicates that factors other than the causal organism may influence the entire symptomatic picture. A careful comparison of cankers suggests that in European varieties of plums (*Prunus domestica*) most cankers are well-defined areas, while on trees of Japanese varieties (*Prunus salicina*) they are generally ill-defined. Gum flow is likely to be more profuse in the Japanese varieties than in European. Peaches, cherries, and apricots, furthermore, exude gum more readily than do plums. All these facts have tended to discount the importance of symptoms that at first appeared to differentiate the gummosis and sour-sap types of cankers.

Although these studies showed the great similarity between cankers that were first regarded as of two symptomatic types, dependence for more conclusive evidence was naturally placed upon isolations and inoculations. Before taking up these results, however, one should consider some other symptoms whose relation to the canker phase will be explained later.

Dormant-Bud Blight.—Both Griffin⁽¹³⁾ and Barss^(5, 6, 7) showed the blighting of dormant buds to be a phase of bacterial gummosis, common on cherries during the years when they were working with the problem.

The blighting of dormant buds by bacteria, though by no means rare in California, has been markedly less prevalent than the limb-canker phase, having been noted only on cherries, apricots, and one variety of peach, the Phillips Cling. It is recognized by failure of the buds to start growth in the spring. The affected buds are darker in color than healthy ones and are subtended on the branch by a small canker (fig. 3A), which generally exudes gum—sometimes in such abundance as to cover the bud. In California the fungus *Coryneum beijerinckii* Oud. produces on apricot a very similar trouble, which may be identified by the characteristic spores of the fungus appearing between the bud scales.

Blossom Blight.—The only case of blossom blight observed was found on apricot twigs sent from the Santa Clara Valley. Although the disease was thought at first to be brown rot, caused by *Sclerotinia fructicola*, subsequent isolations proved it to be a bacterial trouble. Close study indicated that the bacteria had entered through the base of the bud rather than through the blossoms. Probably, therefore, this was not strictly a blossom blight, but merely a girdling of the pedicels, after the blossoms had separated in the bud, by a lesion that had been established before the blossoms opened.

Green-Shoot Blight.—Up to the time the writer published his comparison⁽²⁷⁾ of *Pseudomonas prunicola* with the common gummosis bacterium of California, no case of the disease on green shoots of *Prunus* had been found. This publication stated: "Even though natural infec-

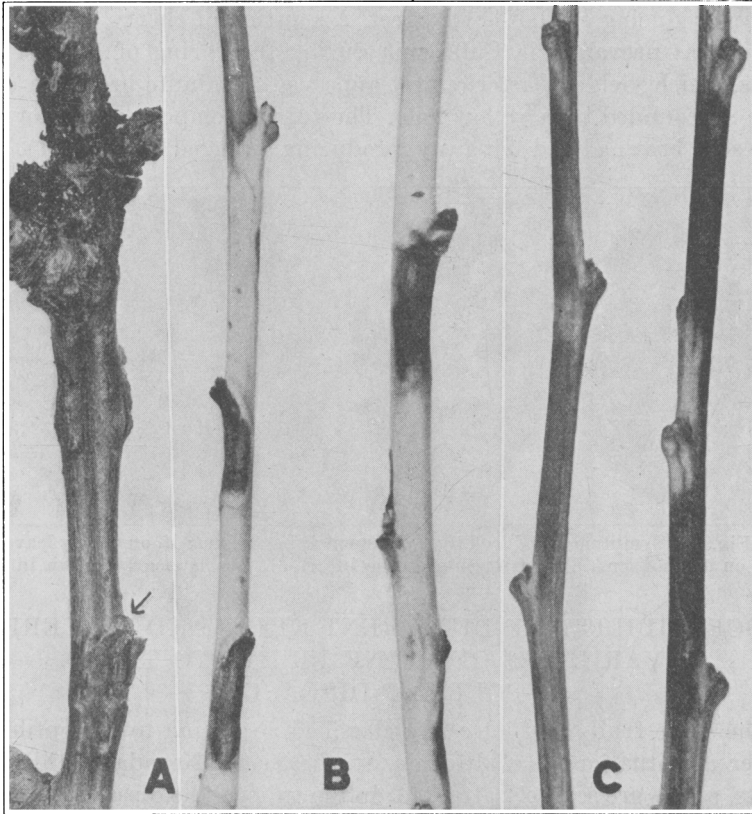


Fig. 3.—Bacterial gummosis. *A*, infection of a two-year-old Bing cherry branch through dormant buds. The remains of a bud and the accompanying canker are indicated by the arrow. *B*, the disease on current-year terminal growth of J. H. Hale peach. *C*, the disease on current-year terminal growth of apricot.

tion of green shoots was possible, the low humidity conditions of the Pacific Coast during the growing season would probably militate against an abundance of this type of injury." Within the last year and a half, however, two cases of blighting of green shoots by a bacterial organism have been found—one on apricot and one on peach (fig. 3*B, C*). On the apricot the disease was present as ill-defined, superficial, black streaks extending for some distance along the shoot. On the peach, the disease

had started at the axils of leaves and had extended downward as black, elliptical lesions bordered by a narrow band of water-soaked tissue.

Leaf-Spot.—Barss⁽⁶⁾ mentioned a leaf-spotting of cherries produced by *Pseudomonas cerasi*. The spots were at first roughly circular and slightly water-soaked; but later the affected tissue browned and dropped away, producing a "shot-hole" effect. A spotting of cherry and apricot leaves was prevalent in California during the spring of 1932. These spots, which yielded bacteria, first appeared as minute brownish dots, each surrounded by a yellow halo. The tissues composing the dot and halo soon browned and fell away, producing a ragged hole (fig. 4).

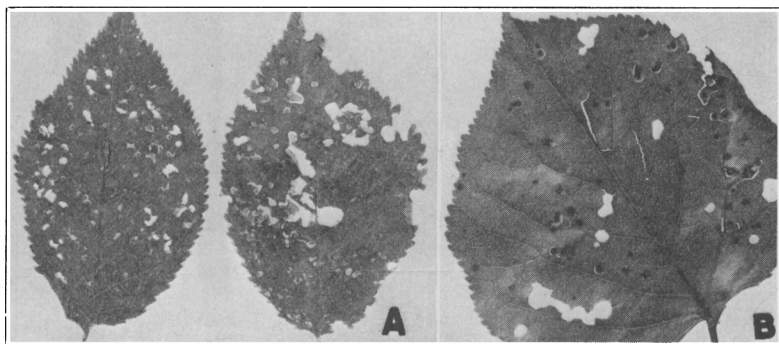


Fig. 4.—Symptoms produced by the gummosis bacterium: *A*, on cherry leaves; *B*, on apricot leaf. The abscission of the infected areas is clearly shown in *B*.

SUSCEPTIBILITY OF DIFFERENT KINDS AND DIFFERENT VARIETIES OF STONE FRUITS TO THE SOUR-SAP DISEASE

The stone fruits are not easily classified according to susceptibility under all situations in California. In the Sierra Nevada foothill districts, plums are severely attacked; in the valleys, the disease is usually not serious on either plum or prune varieties. By and large, the apricots, nectarines, cherries, and plums (including certain varieties used both as plums and as prunes) are more susceptible than the peaches or almonds. Little is known regarding the comparative susceptibility of different varieties of almonds; the disease has been found only on Nonpareil and Ne Plus Ultra.

The following discussion of susceptibility among varieties of cherries, plums, and peaches is based on observations in different parts of California and on records supplied by the Penryn Fruit Company, which owns or controls about a thousand acres of orchards in Placer County. The company's data consisted in a comparison of the percentage of trees lost during the serious outbreak of the disease in the spring of

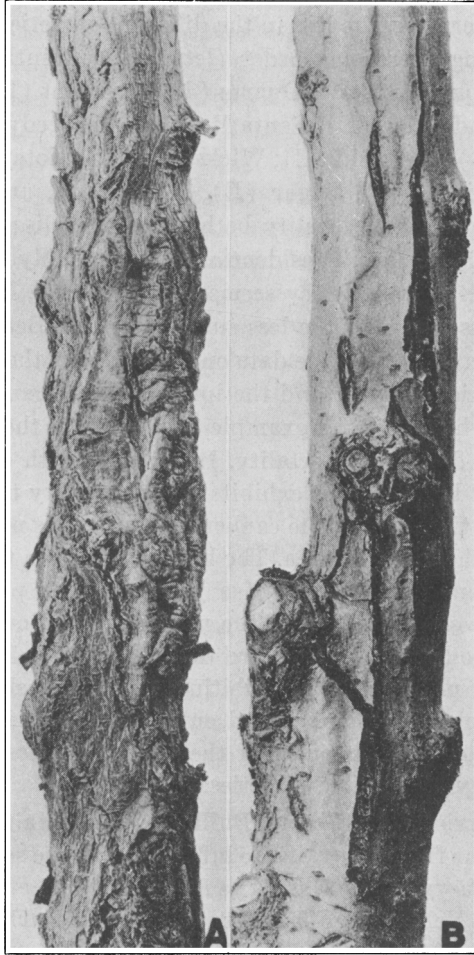


Fig. 5.—Reaction of two plum varieties to the disease. *A*, branch of Climax plum that has become gnarled through a vigorous healing along the later margins of cankers. These ridges of new tissue enable the distal branches to remain alive. *B*, badly diseased branch of President variety exhibiting comparatively little healing.

1930. The loss in the more important cherry varieties falls into the following decreasing order: Lambert, Napoleon (Royal Ann), Bing, Chapman, Republican, Black Tartarian. According to the Penryn Fruit Company, furthermore, the loss in the different varieties of plums falls into the following decreasing order (letters in parenthesis indicating European or Japanese stock): Duarte (J), President (E), Climax (J), Giant (J), Grand Duke (E), Santa Rosa (J), Tragedy (E), Burbank (J), Formosa (J), Diamond (E), Wickson (J), Gaviota (J), California Blue (E), Earliana (E), Sugar (E), Kelsey (J), and Beauty (J). Observations in the same locality both agree and disagree with these data. Whereas Duarte and President are unmistakably two of the most susceptible varieties and Kelsey seems most resistant, Burbank should probably be classed among the less susceptible and Beauty among the more susceptible. Of course, the data on losses reflect the ability of trees of certain varieties to withstand the inroads of the cankers even after infection. Though Beauty, for example, readily takes the disease, it does not show a high degree of mortality. In common with others (Climax, Santa Rosa, and Burbank), it exhibits marked ability to produce large amounts of new tissue along the canker margins, thus maintaining connections across the diseased area. The branch shown in figure 5A represents the healing in many trees of a young Climax plum orchard at Penryn. Despite severe infection, the mortality was low, and the trees were making good growth. Compare this with figure 5B, a badly diseased President plum branch exhibiting comparatively little healing ability, even though the canker had entirely girdled it. Such diverse responses may partially account for the different losses experienced in otherwise equally susceptible varieties.

Such peach varieties as Elberta, Phillips Cling, "Halford No. 2," and J. H. Hale appear from all available information to be severely affected; Alexander and Levy can be classed as moderately affected; Lovell, Tusken (Tuscan), Yellow St. John (St. John), and Early Crawford are slightly affected.

Apriots are not planted on a commercial scale in Placer County. The few that are grown exhibit great susceptibility. Of the two most important commercial varieties, Blenheim was at first considered more subject to the disease than Tilton; but data collected in other districts have not substantiated this belief. In an orchard of two-year-old trees, where the disease appeared throughout the planting in the spring of 1932, 20 per cent of the Blenheim trees and 18 per cent of the Tiltons were affected. Whether loss of trees will be greater for one variety than for the other cannot be predicted at present.

SIMILAR BACTERIA ISOLATED FROM DIFFERENT TYPES OF INFECTION

Materials representing the gummosis type of limb canker were collected from widely separated parts of the state. This type of canker was found on apricot, cherry, peach, nectarine, almond, plum, and prune. In isolations made from the actively extending margins of such cankers, the bacteria appeared in most cases very similar when grown on potato-dextrose and nutrient agars. In isolations made from cankers representing the sour-sap type, the bacteria resembled those from gummosis cankers. Outside the Sierra Nevada foothill districts, the sour-sap type of cankers was found in only two places; on peach at Merced and on apricot at Madera, both in the San Joaquin Valley.

Isolations from several cases of blighted dormant buds, from the one case of blossom blight mentioned earlier, from green-shoot blight, and from leaf-spot, all yielded bacteria like those from gummosis and sour-sap cankers of limbs.

TWO TYPES OF BACTERIA DIFFERING IN PIGMENT PRODUCTION

Had the isolation work been discontinued after the first six months, the results would have strongly suggested that only one type of bacteria was constantly associated with both the sour-sap and gummosis types of cankers. In the spring of 1930, however, a collection of the gummosis type of canker on apricot, obtained from Orland, Glenn County, yielded a bacterium which, when grown on potato-dextrose and nutrient agars, resembled those obtained earlier in all but one respect—pigment production. The bacteria that had been obtained so consistently up to this time produced no pigment on potato-dextrose agar and only a slight lemon-yellow⁵ discoloration of nutrient agar. The organism obtained from the Orland material, on the other hand, produced a brilliant green (lumiere green to apple green) discoloration of both media. In further collections from different orchards in the Orland district, the green organism was found exclusively. Since this time other samplings have been made in this locality with the same results. Shortly after the first isolation of the green organism, peach and cherry limbs bearing typical sour-sap cankers were collected in Placer County. Isolations yielded the green organism exclusively. That this organism was not constantly associated with the sour-sap cankers in Placer County was shown when many subsequent isolations yielded organisms of the first group only.

⁵ All accurate color descriptions in this article are based on: Ridgeway, R. Color standards and color nomenclature. Published by the author, Washington, D. C. 1912.

Up to the present time isolations from all types of infections, including those of limb cankers, buds, blossoms, green shoots, and leaves, have revealed the green organism in but 10 to 15 per cent of the cases. Only once have the two types of organisms been found in the same canker. Needless to say, had materials been collected more frequently from the Orland district, the percentage for the green organism would probably have been much higher.

TABLE 1

SUMMARY OF THE NUMBER OF POSITIVE RESULTS OBTAINED FROM INOCULATING
YOUNG PLUM TREES ON JANUARY 15, 1931, AT PENRYN, CALIFORNIA

Source of bacteria	Variety inoculated	Group* of bacteria inoculated	Total number of inoculations	Number of successful inoculations	Per cent of inoculations successful
Sour-sap cankers.....	Wickson.....	White.....	63	63	100
	President.....	White.....	63	32	51
	Wickson.....	Green.....	24	20	83
	President.....	Green.....	24	18	75
	Wickson.....	Control.....	50	2	4
	President.....	Control.....	50	0	0
Gummosis cankers.....	Wickson.....	White.....	33	32	97
	President.....	White.....	33	27	82
	Wickson.....	Green.....	25	20	80
	President.....	Green.....	25	15	60

* White=bacteria that produced no pigment on potato-dextrose agar; green=bacteria that produced on potato-dextrose agar a green pigment, which diffused through the medium.

To facilitate references, these two groups of organisms will be designated in the table and the text as "white" and "green"—terms referring to pigment production on potato-dextrose agar only, since the more common "white" organism produced a lemon-yellow pigment in nutrient media. To designate the source of the culture, bacteria obtained from the sour-sap type of cankers will be assigned the letter "S"; those from the gummosis type, the letter "G."

BACTERIA FROM DIFFERENT SOURCES SIMILAR IN PATHOGENICITY

After some experimentation, a very successful method of inoculating branches and trunks of the host was devised. When a teasing needle had been passed tangentially through the bark, the bacteria in water suspension could be injected, by means of a hypodermic needle, into the hole thus made. Vaseline was then used to seal the holes, although this procedure was not necessary during the moist periods of winter and spring.

Pathogenicity of Bacteria from Gummosis and Sour-Sap Cankers.—
In studying the different isolations from the standpoint of pathogen-
icity, comparisons were made between bacteria of the two groups (white

TABLE 2
RESULTS OF INOCULATING THE TWO GROUPS OF BACTERIA FROM THE TWO TYPES OF
CANKERS INTO YOUNG PLUM TREES AT PENRYN, CALIFORNIA; 1931

Original source of bacteria	Group* of bacteria	Inoculation No.	Variety inoculated	Length† of cankers (inches)
Sour-sap cankers...	White.....	1	Wickson.....	1-4
		2	Wickson.....	1-10
		3	Wickson.....	10-12
		4	Wickson.....	1-4
		5	Wickson.....	2-12
		6	Wickson.....	6-8
		7	Wickson.....	5
		8	Wickson.....	1-2
		9	Wickson.....	3-5
		10	President.....	3-4
		11	President.....	1-4
		12	Grand Duke.....	1-4
		13	Grand Duke.....	4-8
		14	Grand Duke.....	1-2
		15	Grand Duke.....	1-7
		16	Grand Duke.....	2-5
Gummosis cankers	Green.....	17	Wickson.....	1-2
		18	Wickson.....	1-5
		19	President.....	3-5
		20	Grand Duke.....	1-3
	White.....	21	Wickson.....	1-4
		22	Wickson.....	4
		23	Wickson.....	1-2
		24	Wickson.....	6
		25	Wickson.....	1-4
		26	President.....	2-4
		27	Grand Duke.....	1-2
		28	Grand Duke.....	2-7
		29	Grand Duke.....	4-5
	Green.....	30	Wickson.....	1
		31	Wickson.....	2-4
		32	President.....	3-5
		33	Grand Duke.....	1-4
	Control.....	34	Wickson.....	0
		35	Wickson.....	$\frac{1}{2}$
		36	President.....	0
		37	Grand Duke.....	0

* White=bacteria that produced no pigment on potato-dextrose agar; green=bacteria that produced on potato-dextrose agar a green pigment, which diffused through the medium.
† Measurements of cankers were made 66-78 days after inoculations.

and green) and bacteria from the two types of cankers (sour-sap and
gummosis). During January, 1931, a total of 510 inoculations were
made into 120 trees of the President, Wickson, and Grand Duke vari-

eties of plums. Table 1 summarizes the number of positive results secured by March 22, the time of examining the inoculations. Table 2 gives the measurements of the cankers produced by the various cultures. The first table shows little difference in the percentage of successful inoculations between similar bacteria from the two types of cankers or between the two groups of bacteria. As to length of cankers (table 2), no significant difference appeared between bacteria of the same group



Fig. 6.—Sour-sap in young President plum trees. The tree on the left was inoculated with a white-S bacterium; the two trees on the right were not inoculated. No gum was exuded from the diseased tree.

even when obtained from the different types of cankers. There is a slight suggestion, however, that the green bacteria did not produce such extensive cankers as the white. Though a detailed description of the cankers will follow under a later heading, it may be said here that bacteria from gummosis and sour-sap cankers produced similar symptoms; nor was there evidence that bacteria of the two groups (white and green) produced different types of cankers. Figure 6 shows the results of inoculating a young plum tree with a white-S bacterium in February, 1932. While a large portion of the trunk was involved within a month, the tree survived the following summer, but died in the spring of 1933.

On January 14, 1932, twenty-five inoculations were made with each of the four cultures (white-S, white-G, green-S, and green-G) into six-year-old Bing cherry trees at Davis. By March 3, definite sunken areas

4 to 5 inches long and 2 to 3 inches wide had formed around the inoculation points. Gum was being exuded profusely from cankers produced by each culture. In several cases the cankers had, by extending in a spiral manner, completely encircled the branch. Here, again, bacteria of the two groups (white and green) and those from the two types of cankers (S and G) produced identical symptoms.

Next, inoculations were made into both peaches and apricots, and typical gummosis cankers were produced by each of the different cultures. On February 11, 1932, an exceedingly successful series of inoculations was made into trees of the Phillips Cling variety of peach and the Blenheim variety of apricot. Within two weeks definite sunken areas from 1 to 2 inches long had formed around the inoculation points, gum cavities had appeared along the margins of the cankers, and large globules of gum had been exuded through the inoculation wound. Within a month many cankers had become 4 inches long; a few had girdled branches 2 inches in diameter; and secondary infections, in every respect similar to natural infections, had appeared on branches below the inoculation points.

During the fall and winter of 1932-33, inoculations were made with single-cell cultures⁶ of both the white and green organisms; these showed conclusively that unmixed cultures of each of the two types produced cankers on cherry and plum trees.

Inoculations of Dormant Buds.—Although most of the work has been concerned with the production of limb cankers, a few inoculations of dormant buds were made. Positive results have been obtained only with the white bacteria. This work, however, is probably not extensive enough to prove that the green bacteria are incapable of blighting dormant buds.

Inoculations of Green Shoots.—After the publication⁽²⁷⁾ of the comparison of *Pseudomonas prunicola* with a white-G bacterium designated as 357, an attempt was made at Berkeley to produce a blighting of green shoots with a number of the isolations, including *Ps. prunicola*. In a few cases, blighting of the growing tip for a distance of $\frac{1}{2}$ to 1 inch was obtained; but there was no extensive blighting of the twig like that secured by Wormald⁽³⁰⁾ with *Ps. prunicola*. More severe blighting would probably result if other varieties of plums and trees in more succulent conditions were used, since Wormald⁽³⁰⁾ found extensive symptoms only on vigorously growing green shoots.

Pathogenicity of Bacteria Obtained from Dormant-Bud Blight and Green-Shoot Blight.—Inoculations and reisolations have positively dem-

⁶ The writer wishes to extend his thanks to Dr. A. J. Riker and associates of the University of Wisconsin for their coöperation in obtaining these single-cell cultures.

onstrated that bacteria obtained from blighted dormant buds and green shoots can produce cankers as extensive as do bacteria from limb cankers. Two cultures, both of the white group, isolated from blighted dormant buds of apricot in the fall of 1929, have repeatedly produced cankers on limbs of plums and cherries.

GUMMOSIS AND SOUR-SAP TYPES OF CANKERS PRODUCED BY INOCULATION WITH THE SAME BACTERIUM

Descriptions of the cankers produced by the inoculations reported in tables 1 and 2 are presented here for comparison with results obtained at Davis. There was a marked contrast between cankers on the Wickson variety and those on the President. Whereas the majority of the former were advancing along the cambium, the latter were confined almost entirely to the outer phloem and cortex. Those on Wickson were gumming freely, while those on President were not producing gum. The cankers on Grand Duke trees were of an intermediate nature: some were advancing along the cambium, others through the phloem; some were gumming, while others were not. A comparison of these cankers with those from natural infection gave convincing evidence that the sour-sap type of symptom was being produced by the inoculations in President and Grand Duke. Figure 7*C* shows the diffuse nature of the canker produced on President by a bacterium of the white-S group; figure 2*D*, the same condition resulting from natural infection. The cankers on Wickson, on the other hand, in the exudation of gum and in the formation of well-defined necrotic areas, exhibited the gummosis type of symptoms (fig. 7*A, B*).

Symptoms of the diffuse, at first superficial, type were repeatedly obtained by inoculations of plums during the winter and spring of 1931-32. Inoculations made during January and February produced, on the whole, cankers that did not gum; those made during March and April produced cankers that gummed rather profusely in certain cases. The important point to remember here is that the same bacteria produced at different times symptoms varying as widely as those produced by natural infection. These variations were not surprising after a study was made of canker development throughout the winter and spring. During the winter (December and January) the cankers progressed, in the main, as water-soaked streaks. At this time there was very little uniform browning of the tissues between the streaks. Later (March and April), however, the tissues between the streaks browned more rapidly, producing a well-defined necrotic area—one closely similar to the gummosis types of cankers. Substantially the same thing has been found in

the study of cankers from natural infection. The difference in the amount of gum exuded by cankers produced in January and in April may result partially from the difference in the rapidity of necrosis and partially from the difference in the condition of the host.

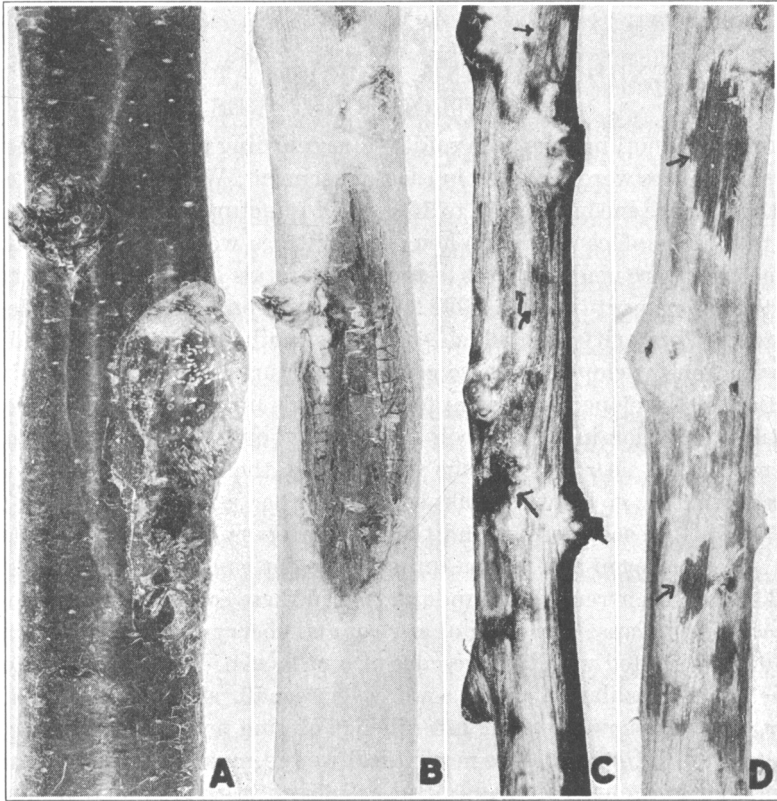


Fig. 7.—Various symptoms produced by the same organism (white-S). *A*, profuse exudation of gum from a canker on a Blenheim apricot branch. *B*, the same canker, somewhat reduced—a well-defined, elliptical, necrotic area representative of the gummosis type of symptom. *C*, a very diffuse diseased area representative of the sour-sap type of symptom. The small necrotic zone surrounding the inoculation point is indicated by the lower arrow. The watersoaked, slightly brown streaks extending above and below the inoculation point are indicated by small arrows. *D*, two midwinter inoculations resulting in slight necrosis even though the bacteria had invaded a large area. Bands of pigmented cortical tissue indicate the invaded zone.

In several instances, the same isolation produced different symptoms upon being inoculated into plums at Davis (on the floor of the Sacramento Valley) and into plums at Penryn (about 40 miles from Davis in the foothills of the Sierra Nevada mountains). One case in point was a series of inoculations made with a white-S culture into President

plums at Davis on October 23, 1931, followed by a series into the same variety at Penryn on October 24. Cankers that gummed freely, forming well defined, elliptical, necrotic areas, developed at Davis, while those produced at Penryn were small necrotic areas bordered by wide zones of loosely connected, only slightly brown, water-soaked streaks that did not exude gum.

GUM EXUDATION AND NECROSIS AT A MINIMUM IN CONTROL PUNCTURES

Although nothing has been said so far regarding control inoculations, large numbers were included in each experiment. When five or six-year-old trees were inoculated, 10 to 15 control punctures were made on one or two limbs of each tree. When smaller trees were used, the control punctures were made on trees located at intervals in the inoculated row. In the experiment listed in table 2, 150 control punctures were made on 30 trees. The rarity with which symptoms similar to those produced by inoculation developed in these control punctures is important. Only 2 of the 150 developed such symptoms, and this infrequent occurrence of natural infection in the controls held throughout the inoculation work. In most cases, necrosis of tissue surrounding the needle punctures was at a minimum: no brown streaks occurred either in the bark or along the surface of the sapwood, a condition likewise true of inoculations made with the pathogen at a season unfavorable for canker development.

The rare occurrence of abundant gum in these control punctures was interesting because gumming of apricot and cherry inoculations seemed closely correlated with the presence of a pathogen. It should be remembered that inoculation wounds were very small, a fact that probably contributed somewhat to the infrequency of gum production in the control punctures, inasmuch as mechanical wounding alone is said to cause gumming of stone-fruit trees (Butler⁽¹¹⁾ and others). Small beads of gum were sometimes exuded from the control punctures in apricot, peach, and cherry trees; but they were never so large nor so frequent as from trees inoculated with the pathogen. On these hosts the pathogen almost always caused profuse gumming. On plum, control punctures rarely produced gum.

CONSTANCY OF THE TWO TYPES OF BACTERIA IN REISOLATIONS

In 1931, reisolutions were made from representative cankers produced by the series of inoculations presented in table 2. These were compared with the original cultures, and a number of each were used in inoculations the following winter. Where the green group of organisms

was inoculated, the same type of bacterium was obtained in the reisolutions. Similarly, bacteria of the white group were obtained from cankers that had been produced from inoculation with this group. In this respect, the results were so consistent that during the winter of 1931-32 the following experiment was devised to study further the constancy of the two strains. Young President plum trees that had been growing for a year in pots at Berkeley, well isolated from any known source of the disease, were divided into six lots of ten trees each. Each lot was inoculated on October 7 with one of the following isolations: white-S-1, white-S-2, white-G-1, white-G-2, green-S-1, and green-G-1. The groups of trees were then placed out-of-doors 10 feet from each other, in order to minimize spread of the bacteria by splashing rain. Cankers from 2 to 4 inches long were produced by the majority of inoculations. After two months, five isolations were made from each canker. The bacteria were then carefully studied by plating in potato-dextrose agar. The results agreed closely with those obtained earlier: cankers produced by the green organisms yielded similar organisms in reisolutions, while those produced by the white organisms yielded bacteria resembling those used for inoculation. Although this experiment points to a high degree of stability in the character of pigment production, much more work should be done along this line. Trees of different kinds, and different varieties of stone fruits growing in different localities, should be inoculated with the two groups of organisms; reisolutions should be studied carefully.

MORPHOLOGY AND STAINING REACTIONS OF THE BACTERIA

In an earlier publication⁽²⁷⁾ the writer reported that culture 357 from gummosis cankers and *Pseudomonas prunicola*, as well, appeared to be Gram negative when a modification of the staining technique recommended in the S. A. B. Manual⁽²³⁾ was employed. When this procedure was followed in staining bacteria of the green group from both types of cankers and the white isolation from sour-sap cankers, these also were found to be Gram negative, as was further evidenced by their ability to grow well on a medium containing 1 part in 10,000 of gentian violet. The discussion of these tests will follow under an appropriate heading.

In the same publication the measurements of the bacterium 357 (white-G) and *Pseudomonas prunicola* (culture received from Wormald) were considered to exhibit no constant differences. In the present work organisms from these two isolations were again measured in comparison with isolations of the green group and with the white isolation

from sour-sap cankers. Table 3 summarizes the results of measuring 400 bacteria of each isolation. The fifth column of the table shows the probable error for each mean length. A statistical treatment of these results by calculating, for any two sets of measurements, the ratio of the mean difference to its probable error, reveals significant differences in length between *Ps. prunicola* on the one hand and white-G, white-S, and green-S on the other. That is to say, such a statistical treatment shows significant differences between these particular sets of measurements. One should not infer that these differences definitely separate the bacteria of the different isolations on the basis of length, particularly in the

TABLE 3
MEASUREMENTS OF BACTERIA

Group of bacteria*	Extremes of width	Extremes of length	Mean width	Mean length
	<i>microns</i>	<i>microns</i>	<i>microns</i>	<i>microns</i>
White-S.....	0.35-0.79	0.83-2.76	0.62	1.63±0.0121
White-G.....	0.35-0.79	0.83-3.03	0.55	1.67±0.0144
Green-S.....	0.35-0.79	0.83-2.76	0.57	1.62±0.0129
Green-G.....	0.35-0.79	0.83-3.03	0.62	1.72±0.0134
<i>Pseudomonas prunicola</i>	0.35-0.79	0.83-3.03	0.68	1.80±0.0143

* White=bacteria that produced no pigment on potato-dextrose agar; green=bacteria that produced on potato-dextrose agar a green pigment, which diffused through the medium. S=bacteria from sour-sap cankers; G=bacteria from gummosis cankers.

face of results presented in the earlier publication,⁽²⁷⁾ which showed equally great differences between measurements made of the same bacterium taken from different-aged cultures. For instance, the length of *Ps. prunicola* from a 48-hour-old culture averaged 2.1 μ ; that from a 12-hour-old culture, 1.8 μ . With the results of this earlier work as a background, the data in table 3 can be interpreted as indicating that the bacteria are fairly similar in length, averaging from 1.6 to 1.8 μ .

In the earlier publication⁽²⁷⁾ the writer states that both the bacterium designated as 357 and *Pseudomonas prunicola* commonly occurred in pairs. At times the former had been seen to produce long chains of cells similar to those reported by Wormald⁽³⁰⁾ for *Ps. prunicola*. This feature has since been exhibited by all the isolations in table 3 and by a single-cell culture of green-G. No attempt has been made to study the cause of the phenomenon.

Motility of the organisms in all the cultures in table 3 has been observed a number of times by means of dark-field illumination. Young, vigorously growing bacteria stained by Leifson's method were seen to bear from one to three polar flagella. The same staining technique demonstrated that many of the organisms in smears of each of the cultures were capsulated.

CULTURAL STUDIES

Methods.—Although certain methods employed in the cultural work will be described under the headings to which they pertain, a few may be mentioned here. Beef extract broth was prepared according to the formula in the S. A. B. Manual.⁽²³⁾ In addition to peptone and beef-extract, dextrose at the rate of 10 grams per liter was added. Beef-extract agar was made by adding 15 grams of agar per liter to this broth.

The synthetic media used in the carbon source studies were as follows:

Basal medium No. 1: ammonium dihydrogen phosphate, 0.5 gram; potassium chloride, 0.2 gram; and calcium chloride, 0.01 gram per liter.

Basal medium No. 2: potassium dihydrogen phosphate, 1.0 gram; magnesium sulfate, 0.5 gram; potassium chloride, 0.5 gram; sodium nitrate, 2.0 grams; and ferrous sulfate, 0.01 gram per liter.

These media were adjusted to pH 6.6 to 7.0 by addition of sodium hydroxide; then the desired carbon compound was added at the rate of 10 grams per liter. After tubing, the media were sterilized at 8 pounds pressure for 20 minutes.

Although basal medium No. 2 supported growth of all organisms better than did No. 1, it was less satisfactory in obtaining pH readings by the colorimetric method, inasmuch as the bacteria produced in the medium a pigment that hindered close matching with the standards.

Three different cultures of each of the two groups of organisms from the gummosis cankers and also from the sour-sap cankers were used in most of the culture work. *Pseudomonas prunicola*, sent to the writer by Dr. Wormald in the early part of 1931, was included in all the tests. Unless otherwise stated, all culture work was carried out at a temperature of 25° C.

Beef-Extract Agar.—It was on beef-extract and potato dextrose agars that the difference in pigment production between bacteria of the white and green groups was first seen. Although the growth characteristics of the bacteria on these media have been mentioned earlier, they will be summarized here.

Bacteria of the white group and *Pseudomonas prunicola*, when streaked on slants of this medium, produced a flat, slightly grey to colorless growth, the margin of which was lobed and the lobes in turn finely toothed. The surface of the growth was often marked by minute pits. The consistency was butyrous. After the bacteria had been growing for several days, the medium was discolored a lemon chrome to lemon yellow. No noticeable difference was observed between bacteria from the gummosis type of canker and those from the sour-sap type.

Bacteria of the green group produced growth similar in physical characteristics to that of the white group. The distinguishing feature, however, was the production by the green group of a lumiere green discoloration of the medium. On the whole, bacteria of the green group from the two types of cankers were indistinguishable, although one isolation from sour-sap cankers produced a lumiere green pigment that changed to a brown after several days, while the pigment of the other isolations remained green.

Beef-Extract Broth.—Bacteria of both groups and from both types of cankers produced in this medium a uniform cloudiness, followed by the slow accumulation of a granular sediment. Those of the green group tended to produce a fairly distinct surface film; those of the white, a slight film that easily fragmented when the tube was disturbed. The same difference in pigment production was observed in this medium as on the agar. Bacteria of the white group produced the lemon-yellow discoloration; those of the green, the distinctly green pigment. *Pseudomonas prunicola* was indistinguishable from bacteria of the white group.

Potato-Dextrose Agar.—Here, again, the physical characteristics of all bacteria were similar. The growth was flat, grey, glistening or (at times) glazed; and the margins were unevenly lobed. A zonated condition that often appeared near the margins apparently resulted from the uneven thickness of the growth. The consistency was butyrous. In pigment production, however, bacteria of the two groups separated themselves very sharply, those of the green group producing an intense yellowish green (lumiere green to apple green), those of the white no pigment. Bacteria from the two types of cankers were similar in all respects. As was mentioned before, certain of those from sour-sap produced the green pigment and were placed in the green group, while others produced no pigment and were placed in the white group.

Czapek's Agar.—All isolations of both groups of bacteria grew very vigorously on this agar. The bacterial mass possessed a more liquid consistency than on any of the agars mentioned above. It was slightly raised and glassy, with almost entire margins. Bacteria of both groups produced a distinct yellowish-green discoloration of the medium, although that produced by the green group was more intense.

Uchinsky's Medium.—Only fair growth was made by the bacteria in this medium. A uniform cloudiness of the medium was followed by a slow accumulation of a flocculent sediment, but no pellicle. Here, again, bacteria of both groups produced the distinct yellowish-green pigment, that of the green group being the more intense.

Malic Acid Agar.—This medium was prepared by adding 10 grams of malic acid and 15 grams of agar to 1,000 cc of basal medium 1. In this

case the physical characteristics of the bacterial growth were similar in all isolations from both types of canker. *Pseudomonas prunicola* differed only in making a less luxuriant growth than did the others. The growth along the streak was flat, glistening, glassy, with a slight buff tinge. The margins were entire or were only slightly lobed. In one experiment *Pseudomonas prunicola* and bacteria of the white group had not produced pigment by the end of 7 days, while those of the green group had produced the characteristic yellowish-green discoloration of the medium. In two later tests, however, bacteria of the white group produced within 8 days a greenish-yellow discoloration of the medium, though never so intense as that produced by bacteria of the green group.

Salicin Agar with Sodium Nitrate.—This was basal medium 2 with 10 grams of salicin per liter. All cultures made similar growth on slanted tubes of this medium. The growth was colorless, flat, filiform, and butyrous in consistency, with margins slightly lobed and crinkled. Only a slight decrease in pH of the medium was noted. All organisms produced a greenish pigment that diffused through the medium, those of the green group causing the most intense color.

Salicin Agar with Sodium Asparaginate.—Basal medium 2 was used, with sodium asparaginate substituted for sodium nitrate and with 10 grams of salicin as the carbon source. Somewhat poorer growth occurred on this than on the medium last discussed. The physical characters of the growth of all the cultures was the same; there was a difference, however, in pigment production, in that bacteria of the green group produced the characteristic green pigment, while those of the white group imparted a slightly brownish tinge to the medium.

Gentian Violet Bile Agar.—This medium was made by adding gentian violet (0.1 gram per liter) and Bacto-Oxgall (10 grams per liter) to beef-extract agar. When this medium was inoculated with a water suspension of the organisms and plates were poured, colonies appeared at 25° C within two or three days. The colonies of *Pseudomonas prunicola* and those of the white-G and white-S attained a size of 2 to 3 mm. The colonies were flat near the margin but were slightly raised in the centers. The gentian violet was present in the center of the colony as a well-defined, violet-colored disk surrounded at the periphery by a zone of unstained growth. The colonies of green-G and green-S were similar except that in the former the accumulation of stain was practically uniform throughout the colonies. The vigorous growth which the organisms made on this medium is considered good evidence that they are Gram negative.

Basic Fuchsin Sodium Sulfite.—This medium was made by adding 5 cc of a 5 per cent alcoholic solution of basic fuchsin and 35 cc of a 10 per

cent aqueous solution of sodium sulfite to basal medium 2 containing sucrose or dextrose and agar. Plates were poured, and the organisms in water suspensions were placed at six different points in each plate by means of a platinum loop.

On the plates containing dextrose as the carbon source all the organisms produced pink, circular, concentrically ridged growths. The agar surrounding the bacterial growth was partly decolorized. No distinction could be made between bacteria of the two groups. On plates with sucrose as the carbon source, all the cultures produced similar types of red colonies, but those of the white group from both sour-sap and gummosis cankers were a more intense red. In other words, the white bacteria produced a more pronounced Endo reaction.

Liquefaction of Gelatin.—These experiments were carried out at 19° C. All cultures under test liquefied the gelatin. The growth of the bacteria was, from the first, confined to the upper portion of the stab near the surface. Liquefaction proceeded in a stratiform manner at a rate that differed somewhat among the different isolations. The green group from gummosis cankers produced a more rapid liquefaction than the others, completely liquefying a column of gelatin 47 mm long in 30 days. This culture further differed from the others, including *Pseudomonas prunicola*, in producing a light yellow-green pigment in the medium; none of the other cultures produced pigment in these experiments.

Reaction in Milk.—Fresh skimmed milk was divided into two lots; to one was added 50 cc per liter of a saturated aqueous solution of litmus; to the second, 2 cc per liter of a saturated alcoholic solution of methylene blue. The material was then tubed and sterilized at 100° C for 15 minutes on each of 3 successive days.

Within a few days all the cultures produced in the litmus milk a definite alkaline condition, followed by a gradual peptonization. In these processes, cultures of the green organism from gummosis cankers were more active than any of the rest. By the end of 16 days, however, all cultures had completely peptonized the milk and had considerably decolorized the litmus, the green-G bacteria being more active than the others in the latter process. Throughout the experiment a white granular sediment gradually collected in all tubes except the checks. As no acid was formed in the milk, this sediment could not have been acid curd; nor did it have the characteristics of a rennet curd. It was thought to consist of materials thrown out of suspension by the change in pH.

The methylene blue was reduced by all the bacteria. In this respect, *Pseudomonas prunicola* was the slowest, and green-G the fastest. During the reduction the methylene blue changed from a beryl blue to a

Venice green. The green-G bacteria differed from the rest in producing a slight greenish cast in the cleared zone at the surface of the liquid.

Starch Hydrolysis.—Two types of starch agar were prepared as follows: (1) beef-extract broth (without dextrose) plus soluble starch at the rate of 10 grams per liter, as directed in the Manual of the Society of American Bacteriologists;⁽²³⁾ and (2) basal medium 2 plus 10 grams per liter of soluble starch. The bacteria in water suspension were streaked on poured plates of these agars. After 24, 48, and 120 hours of incubation, three plates of each isolation were treated with a saturated solution of iodine in 50 per cent alcohol.

By the end of 24 hours, all cultures of both groups of bacteria from both types of cankers had made visible growth on the beef-extract medium. Iodine tests, however, up to the end of 120 hours failed to show hydrolysis of starch by any of the cultures. In the case of the synthetic medium, no culture had made any visible growth by the end of 120 hours, and consequently no hydrolysis of starch was found. Apparently, therefore, these bacteria cannot hydrolyze starch to a degree measurable by the method employed. The growth of the organisms observed in the beef-extract-starch agar probably resulted from the utilization of a carbon source other than the starch—for example, the peptone.

Utilization of Various Carbon Sources.—Earlier in the work the fermentation of various carbohydrates was studied in a beef-extract broth. In the presence of peptone, however, the bacteria so greatly increased the alkalinity as to counteract the acid produced from certain of the sugars. The use of this medium was thereafter discontinued in favor of either basal medium 1 or 2. The latter was preferred because it supported a more vigorous growth. The results with the two types of basal media agree in all respects. Those reported herein are from the use of basal medium 2.

The data in table 4 show that the organisms from the two types of cankers and from the two groups exhibited no differences in their ability to ferment the various carbon compounds. They all produced acid, but no gas, from xylose, arabinose, mannose, dextrose, levulose, galactose, sucrose, mannitol, and glycerin. Certain inconsistencies on the levulose medium led to experiments in which the sugar was sterilized by filtration. A comparison of this medium with one in which the levulose was sterilized by heating at 8 pounds' pressure for 20 minutes demonstrated that the inconsistencies were caused by the heating. All bacteria consistently produced acid from the filtered sugar. In the experiment reported in table 4 the bacteria had made no visible growth on rhamnose by the end of 10 days; other experiments have shown that such growth

appears only after a somewhat longer period and results in acid production. When lactose, maltose, trehalose, or raffinose was used as a carbon source, the bacteria made rather sparse growth. The data indicate that the pH of the media containing these four sugars was shifted into the higher ranges. After 10 days, none of the isolations had grown on media

TABLE 4

UTILIZATION OF VARIOUS CARBON SOURCES BY ORGANISMS FROM THE TWO TYPES OF CANKERS

Carbon source	Change* in pH after 10 days at 25° C				
	White-S	White-G	Green-S	Green-G	<i>Pseudomonas prunicola</i>
Xylose.....	+	+	+	+	+
Arabinose.....	+	+	+	+	+
Rhamnose.....	0	0	0	0	0
Mannose.....	+	+	+	+	+
Dextrose.....	+	+	+	+	+
Levulose.....	+	+	+	+	+
Galactose.....	+	+	+	+	+
Sucrose.....	+	+	+	+	+
Lactose.....	-	-	-	-	-
Maltose.....	-	-	-	-	-
Trehalose.....	-	-	-	-	-
Raffinose.....	-	-	-	-	-
Mannitol.....	+	+	+	+	+
Glycerin.....	+	+	+	+	+
Peptone.....	-	-	-	-	-
Sodium asparaginate.....	-	-	-	-	-
Sodium acetate.....	0	0	0	0	0
Sodium tartrate.....	0	0	0	0	0
Sodium succinate.....	-	-	-	-	-
Sodium citrate.....	-	-	-	-	-
Sodium malate.....	-	-	-	-	-
Sodium lactate.....	-	-	-	-	-
Sodium benzoate.....	0	0	0	0	0

* Plus sign=production of acid; minus sign=production of alkali; zero=no growth.

containing sodium acetate, sodium tartrate, or sodium benzoate as a carbon source, while abundant growth accompanied the use of sodium asparaginate, sodium succinate, sodium malate, sodium citrate, or sodium lactate.

Utilization of Various Nitrogen Compounds.—In this work the following nitrogen compounds were substituted for sodium nitrate in basal medium 2: ammonium sulfate, sodium nitrate, sodium nitrite, and sodium asparaginate. The carbon source was dextrose.

All the cultures, including *Pseudomonas prunicola*, readily utilized all the nitrogen compounds except sodium nitrite. Likewise all isolations of both groups and from both types of cankers, as well as *Pseudo-*

monas prunicola, produced similar changes in the pH of the media. On the ammonium sulfate medium they progressively increased the hydrogen ion concentration during the first week of growth and maintained this acid condition throughout the 18 days of the experiment. On media in which the nitrogen source was either sodium nitrate or sodium asparaginate, the hydrogen ion concentration likewise increased for the first week of growth; but reversion followed, until by the end of the 18 days the sodium nitrate medium was only slightly more acid than the controls, while the sodium asparaginate medium was distinctly more alkaline.

Influence of Culture Media on Pigment Production by the Bacteria.—As has been emphasized throughout this paper, the only basis on which the bacteria were originally separated into the two groups, white and green, was the differences in pigment production on potato-dextrose and beef-extract media. Bacteria of the green group produced a definitely green pigment on both media, while those of the white group produced a pigment (lemon yellow) only on beef extract. The experiments reported under the inoculation studies show that bacteria of the green group retained the ability to produce the green pigment in a passage through the host, while the white bacteria showed no tendency towards assuming this ability. Isolations made over a period of three years have only once revealed bacteria of both groups in the same canker. All these indications therefore point to a stability of the chromogenic characters that differentiate these two groups.

In all culture tests the production of pigment by bacteria of the two groups was carefully compared. The bacteria of the green group produced pigment in the greatest number of cases. For instance, in an experiment where various carbon sources were added to medium 1, the green bacteria developed pigment in the xylose, arabinose, and mannose media, while the white bacteria did not. On the other hand, in the sodium succinate medium, bacteria of both groups produced pigment very similar in quality if not in intensity. Although the two types of bacteria are dissimilar on beef-extract and potato-dextrose media, they apparently tend to be less so on other media.

TEMPERATURE RELATIONS OF THE BACTERIA

To study the effect of temperature on growth of the bacteria, two methods were employed. By the first, the turbidity produced in basal medium 1 at the different temperatures was compared with a set of standards made from varying amounts of barium hydroxide and sulfuric acid. The second method consisted in placing the bacteria on the

surface of potato-dextrose agar by means of a platinum loop, incubating the plates at the desired temperatures, and measuring the diameter of the colonies at intervals.

By the first method the greatest turbidity was obtained in tubes incubated at 25° C, although only slightly less appeared in tubes kept at 20° and 30° C. All cultures were similar in this respect. By the second method the increase in diameter of colonies of the green organisms was greatest at 30° C, while increase in diameter of the white organisms and *Pseudomonas prunicola* was greatest at 25° C. Here, again, growth was only slightly less rapid at 20° and 30° C in certain experiments. Apparently, although these experiments indicate that bacteria of the green group possess a slightly higher optimum temperature than those of the white group, both groups grow vigorously between 20° and 30° C.

IDENTITY OF THE CAUSAL BACTERIA

Before discussing the previously described canker-producing pathogens of *Prunus* species, we shall briefly summarize the results of comparing the green and white organisms. First, as to the comparison of the bacteria from the two types of cankers (gummosis and sour-sap), neither the pathogenicity nor the cultural studies seem to indicate that the two types of cankers are produced by different organisms. On the contrary, all evidence supports the conclusion that they are produced by the same bacteria. Second, as to the comparison of the green and white types of bacteria, certain features besides pigment production apparently differentiate these two types. One possible difference is that the green organism required a slightly higher temperature for optimum growth, although the evidence is by no means conclusive. Certain slight differences appeared in the production of surface films in liquid media and in the rapidity of gelatin liquefaction and peptonization of milk. Attention, however, is called to the fact that both types of organisms did liquefy gelatin and did show similar reactions in milk (for example, reduction of methylene blue). A difference hard to evaluate was found by growing the two types of organisms on a modified Endo medium: the white bacteria gave more pronounced Endo test than did the green. Contrasted with these differences are the numerous similarities existing between the bacteria of the two types. Their carbohydrate metabolism was strikingly similar, as was their utilization of nitrogen compounds. Both types of bacteria possessed polar flagella and capsules. Although bacteria of the several isolations differed somewhat in length, the green organisms were not consistently differentiated from the white. According to a study of pigment production on various media, though the two

types of organisms differ in pigment production on potato-dextrose agar, on other media they produced pigment differing only in intensity.

Probably the most convincing evidence of similarity between the two types of bacteria was afforded by the pathogenicity studies. On plums, cherries, apricots, and peaches, both types produced cankers in every way similar. Inoculations made during the winter of 1932-33, with single-cell cultures of both types of bacteria, proved conclusively that the similarity of symptoms obtained in earlier studies did not result from mixed cultures.

In short, the evidence seems to warrant the conclusion that the two types do not differ widely enough to be placed in different species. Such differences as were observed may have resulted from strains within the same species.

We shall now consider the literature on bacteria pathogenic to species of *Prunus*. Of these, *Bacterium pruni*, described by E. F. Smith⁽²²⁾ in 1903, and *Pseudomonas cerasi wraggi*, described by Sackett⁽¹⁹⁾ in 1925, differ widely from the organism found in this work. Both these species of organisms are yellow on culture media and possess other characters not encountered in the canker organisms. Having obtained a culture of *Pseudomonas cerasi wraggi* from Sackett in 1930, the writer found that it not only had distinctive characters in culture media, but produced no cankers in several trials on either apricot, cherry, or plum trees.

In 1905 Aderhold and Ruhland⁽¹⁾ named *Bacillus spongiosus* n. sp. as the organism producing a serious cankerous disease of cherry trees in Germany. In later publications^(2, 3) they showed that this organism possessed polar flagella, a fact that would place it in the genus *Pseudomonas* (*Bacterium*). Although their description of this bacterium is complete in certain respects, many important details are lacking. The organism does not appear to have been studied by other European workers. Braun⁽⁹⁾ and Pape,⁽¹⁶⁾ who mention it in connection with a cankerous condition of cherries in Germany, have apparently never studied it apart from the host.⁷ It remains in the literature, therefore, as a putative species; its pathogenicity appears to have been established, but it has not been fully enough described to permit comparison with other organisms.

Griffin,⁽¹³⁾ in his publication on bacterial gummosis, names *Pseudomonas cerasus* as a new species, resembling *Ps. spongiosa* closely in many respects, but differing from this organism in its failure to produce vacuolate or spongy colonies on certain media and in its production of a

⁷ Neither in publications nor in correspondence with the author has Braun or Pape mentioned studies of *Pseudomonas spongiosa*. Neither was able to furnish a culture of the organism.

green pigment; the latter characteristic is not attributed to *Ps. spongiosa*. Although Griffin did not mention any difference in the length of *Ps. spongiosa* and *Ps. cerasi*, according to Aderhold and Ruhland's⁽³⁾ measurements the former ranges from 1.6 to 4.0 μ in length, while Griffin's⁽¹³⁾ measurements for *Ps. cerasi* ranged from 1.5 to 2.5 μ (majority 1.8 \times 0.6). Still another difference exists in the descriptions of the two organisms: *Ps. spongiosa* is reported to produce ammonia; *Ps. cerasi* is not. There seems to be a reasonable doubt that the two organisms are identical, despite their similarity in many respects.

Wormald⁽³⁰⁾ named the organism that he found to be the cause of the green-shoot wilt, *Pseudomonas prunicola*. He states that his bacterium did not appear to be identical with *Ps. spongiosa*, from descriptions of which it differed as to colony characteristics, action on milk, vigor of growth in Uschinsky's solution, and reaction to stains. He did not mention the difference between the length of *Ps. spongiosa*, as reported by Aderhold and Ruhland, and that of his organism. As stated in the last paragraph, the measurements of *Ps. spongiosa* ranged from 1.6 to 4.0 μ . Wormald⁽³⁰⁾ reports *Ps. prunicola* as ranging from 0.9 to 2.5 μ in length. He does not mention whether or not *Ps. prunicola* produces ammonia in culture media; but tests made by the writer⁽²⁷⁾ indicated that it does not. In this feature, therefore, it further differs from *Ps. spongiosa*.

Since *Pseudomonas cerasi* is reported to produce a distinct green pigment in common culture media, while *Ps. prunicola* produced only a lemon-yellow, Wormald⁽³⁰⁾ considered the two as distinct. The writer⁽²⁷⁾ questioned the advisability of accepting Wormald's organism as a new species because, in a comparison, *Ps. prunicola* appeared very similar to the common gummosis-canker-producing organism of California, both in cultural characters and in pathogenicity to limbs of plum and cherry. Furthermore, the writer hesitated to accept *Ps. prunicola* as a new species because a bacterium had been found resembling both *Ps. prunicola* and the common gummosis strain in all tests tried up to that time, with the exception of pigment production. This second bacterium produced in potato-dextrose and nutrient media a distinctly green pigment (fluorescence) similar to that reported for *Ps. cerasi*.^(5, 13) A further reason for suspecting that the common white organism resembled the green was Goldsworthy's⁽¹⁴⁾ earlier work in California with two organisms, which were said to be similar in most cultural features but different in pigment production and in serological tests. Goldsworthy asserted that both organisms produced gummosis in stone-fruit trees. In view of this situation the writer deemed further work necessary to establish the relationship of *Ps. prunicola* and the two types of gummosis organisms to one another and to *Ps. cerasi*. The present investigation

further indicates that *Ps. prunicola* and the common (white) canker organism of California are identical.

Asserting that *Pseudomonas prunicola* alone was responsible for the green-shoot wilt, Wormald^(30, 31, 32, 33) names a second organism, *Ps. mors-prunorum*, causing a canker disease prevalent in England. Although both organisms were capable of producing limb cankers, fruit-spot, and leaf-spot,^(30, 33) and were similar in certain cultural features, he considered them separate species, inasmuch as *Ps. mors-prunorum* failed to produce pigment in nutrient broth and Uschinsky's solution, it increased the hydrogen-ion concentration in lactose media, and it remained alive only four to six days in a 5 per cent sucrose medium. Wormald's results, however, show that separate cultures of *Ps. mors-prunorum* differed among themselves almost as widely as did the two species. A careful comparison of the English canker organism and the bacteria described herein is obviously desirable.

Admitting the limitations imposed on a discussion of the relationships of two organisms that have not first been tested comparatively⁸ in the same laboratory, the writer wishes to submit the results here presented as evidence that the green strain described is identical with *Pseudomonas cerasi*. The white organism, accordingly, would be considered a strain or variety of *Ps. cerasi* and might be designated as the variety *prunicola*.

Pseudomonas cerasi var. *prunicola* n. var.—A rod-shaped, aerobic, capsulated, Gram negative, motile (one to three polar flagella) organism, commonly occurring in pairs, occasionally forming long chains. Individual cells are 0.83 to 3.03 μ long and 0.35 to 0.79 μ wide, averaging 1.7 by 0.6 μ . On nutrient agar it produces flat, butyrous, colorless to white colonies, the margins of which are finely lobed. A lemon-chrome to lemon-yellow discoloration of the medium occurs after the bacteria have been growing for several days. On potato-dextrose agar the colonies are whiter than on nutrient-agar; otherwise, the growth characteristics are about the same. No pigment is formed on this medium. The organism produces acid but no gas from arabinose, xylose, mannose, dextrose, levulose, galactose, sucrose, mannitol, and glycerin; it grows poorly on rhamnose. It decreases the hydrogen-ion concentration when growing on lactose, maltose, trehalose, raffinose, or peptone. Other carbon compounds utilized are sodium asparaginate, sodium succinate, sodium citrate, sodium malate, and sodium lactate; but apparently not sodium acetate, sodium tartrate, or sodium benzoate. Starch is not hydrolyzed. No ammonia is produced, and nitrates are not reduced to nitrites. Ammonium sulfate, sodium asparaginate, and sodium nitrate are satisfactory nitrogen sources. The bacterium produces a moderately rapid stratiform liquefaction of gelatin stabs. In milk, there is first an increase in alkalinity, then a peptonization, but without the formation of curd. In this medium litmus is partially decolorized, while methylene blue is reduced. The optimum temperature for growth

⁸ The author was unable to obtain either the original or a more recent isolation of *Pseudomonas cerasi* from Oregon.

of the bacterium is about 25° C. The organism is pathogenic to species of *Prunus* causing dormant-bud blight, green-shoot blight, leaf-spot, and limb cankers.

The varietal distinction is based largely on differences in chromogenesis. *Pseudomonas cerasi* Griffin produces on potato-dextrose agar a lumiere-green to apple-green pigment with fluorescent properties; *Ps. cerasi* var. *prunicola* forms no pigment on this medium. Differences in intensity of pigment are observable on other media, *Ps. cerasi* producing a more distinctly green color than *Ps. cerasi* var. *prunicola*.

According to some indications in the literature, it may become desirable to reclassify a number of closely related organisms that have heretofore been considered distinct species. Attention is called to Smith and Fawcett's⁽²⁰⁾ comparison of *Bacterium syringae*, *B. citriputeale*, and an isolation from stone fruits which they called *B. cerasi*.⁹ These three organisms showed marked similarities in cultural characters and in their reaction on various hosts. Although Smith and Fawcett did not propose to group these organisms under one species, they stated that if this is ever done, *B. syringae* should be the species name.¹⁰ Smith,⁽²¹⁾ who has more recently compared Wormald's *Ps. prunicola* with *B. citriputeale*, finds the two very similar and probably identical. On the basis of his and Fawcett's earlier work he avers that the same relations exist between *Ps. prunicola* and *B. cerasi*.

Should the reclassification be undertaken, the gummosis-canker bacterium of stone-fruit trees would probably be included; it would then not retain the species name *B. cerasi*, since the species *B. syringae* antedates it by nine years.

NEW CANKERS APPEAR DURING WINTER AND EARLY SPRING

As the gummosis and sour-sap types of symptoms are evidently caused by the same bacterium, the two can be discussed as one disease. Although most of the following observations were made on plums in Placer County, supplementary inspections of peach, apricot, and cherry trees were carried out in other localities.

New cankers, appearing first as small, brownish flecks in the cortex of the bark, are extremely hard to detect. In winter, they are slow to develop such external symptoms as gummosis or cracking and sinking of the periderm; in spring, they exhibit these signs almost immediately.

⁹ Smith and Fawcett's culture of *B. cerasi* was obtained in California. After corresponding with Smith, the writer is convinced that it belongs to the white group described herein.

¹⁰ Since the publication of Smith and Fawcett's work, Miss Charlotte Elliott, in her "Manual of Bacterial Plant Pathogens," Williams and Wilkins Co., page 217, has made *Bacterium citriputeale*, but not *B. cerasi*, synonymous with *B. syringae*.

Consequently, the time of their first appearance can be only approximately established.

During the season of 1929-30 an outbreak of disease was discovered in late January. A month later, the cankers were exuding gum or a watery material; by March many of them had girdled limbs and directly caused serious losses of peach, nectarine, and plum trees. During the season of 1930-31, two major outbreaks occurred. The first was discovered on January 9, just as numerous cankers began to appear a few inches below old diseased areas. Apparently they arose from infection by bacteria washed down in rain water from the old cankers. The second outbreak was found on April 1, 1931, when the diseased areas were producing large amounts of gum. Worse infection occurred during the season of 1931-32 than in either of the preceding seasons. The first cankers were noted on December 31; and from this time to February 1 numerous cases developed. Beginning again about the first week in March and continuing for approximately two weeks, new cankers appeared on apricot, peach, and plum trees, both in Placer County and in the Sacramento-San Joaquin valley area. Although, as a rule, not large enough to cause much damage during the spring, they constituted a danger the following season. No new disease was observed during the season of 1932-33 until February 15, when a moderate amount developed in pruning wounds.

As there is only fragmentary evidence regarding the length of incubation periods under various climatic conditions, the time when the different outbreaks were initiated cannot be determined. Since the disease appeared in midwinter and spring rather than autumn, a good deal of rain was apparently necessary for infection; or at least, infection occurred with greatest frequency during long rains.

EXTENSION OF ESTABLISHED, OR HOLD-OVER, CANKERS CONFINED TO WINTER AND EARLY SPRING

The diseased areas remaining in the tree from one season to another, in addition to serving as sources of abundant and readily available inoculum, are centers from which new tissue is invaded. Such cankers pass the summer in a quiescent state. Resuming activity in October or November, they continue to extend throughout the winter by means of small, slightly brown streaks. Sometime in early spring—generally late February—the tissue between the streaks begins to die; by March it turns uniformly brown and sour. If the streaks are numerous, necrosis continues up to the limits of the zone they occupy, forming well-defined cankers (fig. 7*B*); if they are few, only the center of the diseased area is

killed (fig. 2*D*), and the result is diffuse cankers. In April, canker extension begins to wane; and by May or June it stops or becomes too slow to be detected.

TABLE 5

PRODUCTION OF CANKERS BY *PSEUDOMONAS CERASI* VAR. *PRUNICOLA* AT DIFFERENT TIMES OF THE YEAR

Date of inoculation	1931-32: On plum at Penryn			1932-33: On cherry at Davis		
	Inoculations producing symptoms	Average length 20 days after inoculation	Average temperature between inoculations	Inoculations producing symptoms	Average length 20 days after inoculation	Average temperature between inoculations
	<i>per cent</i>	<i>millimeters</i>	<i>degrees Fahr.</i>	<i>per cent</i>	<i>millimeters</i>	<i>degrees Fahr.</i>
April 7.....	0	0	64
June 5.....	0	0	72
August 18.....	22	3	92
September 4.....	29	2	69
October 1.....	25	4	68
October 11.....	40	3	61
October 20.....	82	76	59
October 31.....	75	32	70	83	14	56
November 15.....	87	8	58
November 18.....	80	7	46
December 1.....	83	4	40
December 2.....	81	16	43
December 17.....	54	6	44
December 29.....	89	4	41
December 31.....	69	12	47
January 13.....	79	6	41
February 2.....	84	9	45	93	10	44
February 17.....	100	19	47
March 8.....	92	50	57
March 22.....	87	30	60	100	5	55
April 12.....	63	14	56
May 10.....	0	0	68

THE SEASONAL ASPECT OF CANKER ACTIVITY IN RELATION TO TEMPERATURE AND RAINFALL

Barss⁽⁶⁾ in Oregon and Goldsworthy and Smith⁽¹⁵⁾ in California found that inoculations produced the disease only during winter and spring, thus establishing its seasonal character. The present investigation revealed a similar situation in the autumnal transition of cankers from a quiescent to an active state and in the springtime reversal of the process. In a search for causes of this phenomenon, such seasonal variables as temperature and rainfall were considered, particularly as the latter affects soil moisture. Certain facts appear to eliminate soil moisture as a possible cause. First, in two of the four years, canker activity began before the rainy season, at a time when the soil moisture was low. Second, although late summer irrigation increased soil moisture in certain orchards, no effect on canker activity was noticed.

For the purpose of collating canker development and temperatures, inoculations were made at intervals throughout the autumns, winters, and springs of two seasons. Table 5 contains data on average canker length, percentage of inoculations developing symptoms, and mean temperature between inoculations. The results of the 1931-32 experiment were as follows: no symptoms were produced in April and June, 1931; only a few small necrotic areas developed during August, September, and early October; but definite cankers resulted from late October and November inoculations. Then followed a period during December (1931), January, and February (1932), when a high percentage of the inoculations developed small but definite lesions. March and April, 1932, were favorable for rapid canker extension. No symptoms whatever attended the inoculations of May 10. The 1932-33 experiment gave essentially the same results, although, on the whole, the symptoms were less extensive than those of the year before. Larger cankers developed in inoculations of October 31 and November 15 than in those made either immediately before (October 1) or immediately after (December 1) these dates. Although a second December series produced only small lesions, a high percentage of the trials were successful. February and March, on the other hand, favored more rapid progress of the disease.

These experiments justify the conception, gained earlier by observation, that activity which begins each autumn in certain established cankers continues until spring. They go beyond the observations, in that the extension rate is shown to vary, decreasing in winter and increasing in spring. An examination of table 5 shows that these variations coincide with fluctuations of temperature. In 1931, for example, large cankers developed during late October and November at mean temperatures from 57° to 70° F; during December (1931) and January (1932) small ones developed at temperatures from 41° to 47° F. In 1932, likewise, larger diseased areas were produced at 56° to 58° F (October 31 and November 15) than at 40° to 41° F (December 1 and December 29).

The resumption of canker activity in autumn, however, cannot be explained as a direct response to temperature. To regard it as such, one must assume that a drop of 9° F in 1931 (from 68° in early October to 59° F in late October) and of 5° F in 1932 (from 61° in October to 56° F in November) caused the differences in size of cankers shown in table 5. On the contrary, two experiments, in which inoculated trees were held at 36°, 50°, and 65°-70° F, indicated that the greatest canker extension occurred at this last temperature and that 50° was definitely more favorable than 36° F.

The failure to obtain cankers during the higher temperatures of early

autumn may possibly be explained by the host's reactions to the diseased areas. The few small lesions that formed in September and early October were promptly and effectively buried between new tissue; thereafter, they made no noticeable progress. Even the smallest necrotic streaks were surrounded by a meristematic layer of cells (fig. 8). In late Octo-

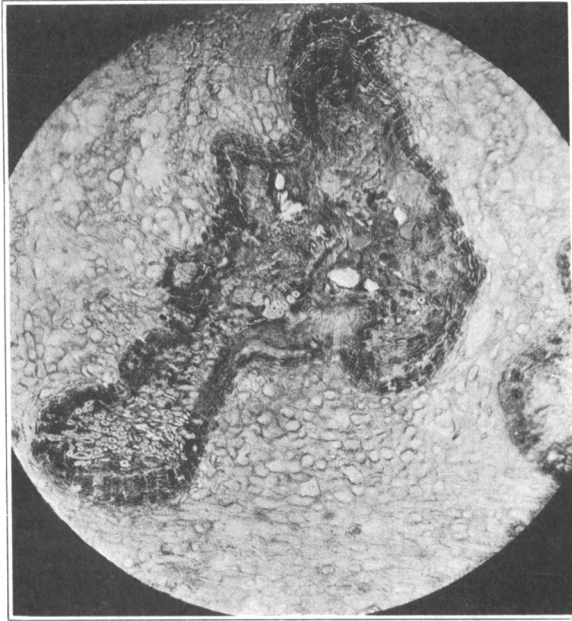


Fig. 8.—Cross section through a necrotic streak surrounded by layers of meristematic tissue—a characteristic of quiescent cankers.

ber or early November, the trees apparently lost this ability to respond to the presence of cankers; they did not regain it until spring. In both 1931–32 and 1932–33, large cankers developed if inoculations were made in late October or November, a time when the trees were incapable of responding to wound stimuli.

If temperature influences the start of canker activity in autumn, it must do so through its effect on the host. The literature dealing with the relation of temperature to the rest period in orchard trees is scattered; to review it would require more time and space than this brief discussion affords.

SUMMARY AND CONCLUSIONS

Investigations, beginning with that of Brzezinski in Poland in 1902, followed by that of Aderhold and Ruhland in Germany in 1905, have shown that bacteria produce on *Prunus* spp. certain canker diseases, characterized by copious gum exudation. Whether the disease described in Poland is identical with that found in Germany cannot be determined from the literature.

Griffin in Oregon (1911) was the first American investigator to establish the bacterial nature of a cherry gummosis. Thus far, this disease has been reported only from states on the Pacific Coast, having been found in California by Barrett in 1916. In 1930, Goldsworthy and Smith described a cankerous disease attacking stone-fruit trees in the Sierra Nevada foothills. This malady, differing somewhat in symptoms from gummosis, was shown to result from bacterial infection, although the pathogen was not described.

In the present investigations, bacterial gummosis was compared with the disease described as "sour-sap" by Goldsworthy and Smith. The sour-sap disease is characterized by the failure of entire trees or portions of trees to produce leaves in the spring. Others start to grow, but the foliage suddenly wilts after the first warm days. The bark of limbs and trunks is girdled by ill-defined, brown, moist, sour-smelling necrotic areas.

The disease generally remains in the aboveground portion of the tree; no proved case has been found in the roots. A second striking feature is the lack of gum formation except on infrequent occasions.

The gummosis disease has been regarded as differing from the sour-sap in the presence of abundant gum and in better defined, deeper cankers. These characteristics differentiate the two troubles at certain periods but not at others. Such factors as time and kind of stone fruit appear to influence greatly the expression of the disease. Bacterial infection of leaves, green shoots, blossoms, and dormant buds is described; and its connection with the limb canker phase is discussed.

Apricots, cherries, and plums are apparently more susceptible to the sour-sap type of trouble than are peaches and almonds. Differences in varietal susceptibility are found, the Lambert cherry, the President plum, and the Phillips Cling peach being among the most severely affected; the Black Tartarian cherry, the Kelsey plum, and the Early Crawford peach, less affected. Certain plum varieties heal vigorously along canker margins, thus maintaining, across diseased areas, ridges of healthy tissue that enable the distal portion of branches to remain alive. Other varieties exhibit little healing ability.

Isolations from sour-sap and gummosis types of cankers yielded similar organisms. Two types of bacteria differing in chromogenesis were found: one group produced a distinct lumiere-green pigment on potato-dextrose agar; the other, no pigment. The latter type was found in from 85 to 90 per cent of the isolations. The chromogenic character of the bacteria apparently remained fairly constant, no noticeable change taking place in a passage through the host.

Inoculations, using single-cell isolations in certain cases, proved that both groups of bacteria were able to produce identical symptoms on plum, peach, apricot, and cherry limbs. Both groups of bacteria produced in certain instances the sour-sap type of cankers, in other cases the gummosis type. In 1931, parallel inoculations of President and Wickson plum trees produced, in the former, indefinite cankers that did not form gum, and, in the latter, well-defined cankers exuding abundant gum. This experiment and others in conformity with earlier observational results, proved that the sour-sap and gummosis symptoms are manifestations of the same disease. Factors other than cause appear to determine the type of symptom.

Cultural studies failed to reveal marked distinctions between bacteria of the two types. They were similar in size, in number and arrangement of flagella, in growth characteristics on various media, and in utilization of carbon and nitrogen compounds. They showed only slight differences in liquefaction of gelatin and in reactions on milk. The most consistent difference was intensity of pigment production, one group causing a lumiere-green discoloration of most media, the other forming a lemon-yellow pigment in certain media but not in others. On a medium containing basic fuchsin decolorized with sodium sulfite, both types of organisms accumulated the fuchsin in the colonies, but with different degrees of intensity. Some evidence, by no means conclusive, indicated that the green organism might require a slightly higher temperature for optimum growth than the white. In view of the pathogenicity studies, however, the two groups of organisms are not considered separate species, but probably represent strains of the same species.

The cultural studies showed, furthermore, that similar bacteria were obtained from the sour-sap and gummosis types of cankers. *Pseudomonas prunicola* Wormald was found to be identical with the white bacteria obtained from both types of cankers.

A review of the literature on other pathogens shows *Bacterium pruni* E. F. S. and *Pseudomonas cerasi wraggi* Sackett to differ widely from the organism herein described, and raises legitimate doubts that the bacterium mentioned in this paper is identical with *Ps. spongiosa* Aderhold and Ruhland, despite its similarity. The green strain, on the other

hand, appears identical with *Ps. cerasi* Griffin. Earlier studies had shown the marked similarity between the white strain and *Ps. prunicola* Wormald; the present investigation confirms this view. Evidently, therefore, the green strain is *Ps. cerasi*. The white strain should be called *Ps. cerasi* var. *prunicola* n. var., a description of which is included.

No opinion is expressed as to the relation between *Pseudomonas mors-prunorum* Wormald and the organism described herein. Wormald asserts that *Ps. mors-prunorum* differs from *Ps. prunicola*, which, as already shown, is probably identical with *Ps. cerasi* var. *prunicola*, the white strain mentioned in this paper.

If certain allied organisms such as *Bacterium syringae* van Hall, *Bacterium citriputeale* C. O. Smith, and *Pseudomonas cerasi* are reclassified, the status of the bacterium last mentioned will be affected.

New disease appeared during the winter and spring. As young cankers are extremely hard to detect, very little is known concerning the length of the incubation period under various climatic conditions.

After beginning activity in the autumn, established (hold-over) cankers continue to extend during winter and early spring; becoming quiescent in late spring, they remain so throughout the summer. Inoculations in early autumn and late spring produced only small lesions; those in late autumn and early spring, extensive cankers—further evidence that the disease has a marked seasonal character. Although temperature affects the rate of canker extension, it is apparently not directly responsible for failures to obtain marked symptoms in early autumn and late spring. On the other hand, according to certain evidence, the host itself may influence the disease, inasmuch as the lesions developed during these periods were effectively buried between new host tissue, while those initiated in late autumn or early spring were not.

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