

HILGARDIA

*A Journal of Agricultural Science Published by
the California Agricultural Experiment Station*

VOLUME 10

NOVEMBER, 1936

NUMBER 8

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SYMPTOMATIC AND ETIOLOGIC RELATIONS OF THE CANKER AND THE BLOSSOM BLAST OF PYRUS AND THE BACTERIAL CANKER OF PRUNUS¹

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INTRODUCTION

IN 1931 a heretofore undescribed bacterial canker of pear trees was found in Sierra Nevada foothill orchards. A brief discussion of symptoms, giving results of inoculations and comparing the disease with fire blight, *Erwinia amylovora* (Burrill) Bergey *et al.*, was published in 1934.⁽¹⁸⁾ ³ The causal organism was not described except as it differed from *Erwinia amylovora* in producing a greenish pigment on many media, thus allying itself with *Phytomonas cerasi* (Griffin) Bergey *et al.*, cause of the stone-fruit bacterial canker.⁽¹⁷⁾

A blossom blast of pear in California differing from that caused by fire blight was briefly described by Thomas and Ark,⁽¹⁵⁾ ⁴ who report the causal organism as similar to those of citrus blast and stone-fruit canker.

The orchards in which the writer first found the limb-canker disease have remained free of blossom blast, though planted with Beurre Bosc, a variety elsewhere susceptible to blossom infection. Limb and blossom symptoms in the trees growing in other districts indicate that all are phases of the same disease. One purpose of this work, therefore, was to compare the bacteria obtained from these parts of the host.

Reports from New York^(8, 4) and Arkansas^(9, 10) regarding infection of pear leaves, fruit, and blossoms by bacteria possessing cultural char-

¹ Received for publication July 13, 1936.

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³ Superscript numbers in parentheses refer to Literature Cited at end of this paper.

⁴ Thomas and Ark designated the blossom disease "blast" to distinguish it from that caused by *Erwinia amylovora*. Rosen⁽¹⁰⁾ in Arkansas had used the term earlier to designate a pear-blossom blight caused by an organism similar to *Phytomonas citriputeale* (C. O. Smith) Bergey *et al.*, the cause of citrus blast.

acteristics similar to these organisms were additional reasons for the study.

Evidence obtained earlier⁽¹⁸⁾ pointed to similarities between the California pear-canker organism and *Phytomonas cerasi*, cause of the stone-fruit canker. This study, therefore, also includes symptomatic and etiologic comparisons of the two diseases.

COMPARISON OF THE PEAR AND STONE-FRUIT DISEASES

The bacterial canker of stone-fruit trees is described in detail in an earlier publication.⁽¹⁷⁾ Pear blossom blasts and limb cankers are briefly described by Thomas and Ark⁽¹⁵⁾ and by the present writer.⁽¹⁸⁾ Certain features of the pear disease not previously discussed are included herein.

Twig Infection.—In certain years an infection of small branches and twigs causes a considerable loss (fig. 1, *A* and *B*). Sloughing away of the periderm and a spongy condition of the cortex and outer phloem are characteristic symptoms present in all bark cankers whether in large or in small branches. Although differing in external appearance, the twig cankers of the pear and stone-fruit diseases have a similar manner of involving the tissues of the bark. This point will be discussed later.

Dormant-Bud Blast.—Figure 1, *C* and *D*, shows small lesions surrounding dormant buds. Both blossom and leaf buds are susceptible to infection and are points from which the disease enters and kills small branches. *Phytomonas cerasi* causes a similar infection of the dormant buds of stone-fruit trees. Twigs as well as small branches are killed by the extension of these infections.

Infection of the Fruit-Cluster Base.—A phase of the pear disease that has no counterpart in the stone-fruit disease is the infection of the fruit-cluster bases. Although the exact time of infection is not known, presumably the bacteria enter the fruit-stem scars after the fruit is picked.

Limb Cankers.—Branch cankers, adequately pictured in the earlier article,⁽¹⁸⁾ were the most common symptoms in the pear orchards where the disease was first found. New cankers and the active margins of old ones present the same appearance as the twig infection shown in figure 1, *A* and *B*. The centers of old cankers are characterized by a longitudinal and transverse cracking of the periderm and by a gradual sloughing away of affected cortex. In many cases where the disease does not at once extend to the cambium, the underlying healthy tissue, in forming a new periderm, forces the diseased tissue outward. Branches diseased for a few years will, therefore, become roughened before natural longitudinal suturing begins. Figure 2 shows a tree in which the bark of certain limbs is rough whereas that of others is smooth.

Branch cankers of the pear and stone-fruit diseases have very similar internal characteristics. In both cases the margins of the affected area are made up of numerous, loosely knit streaks, the paths along which

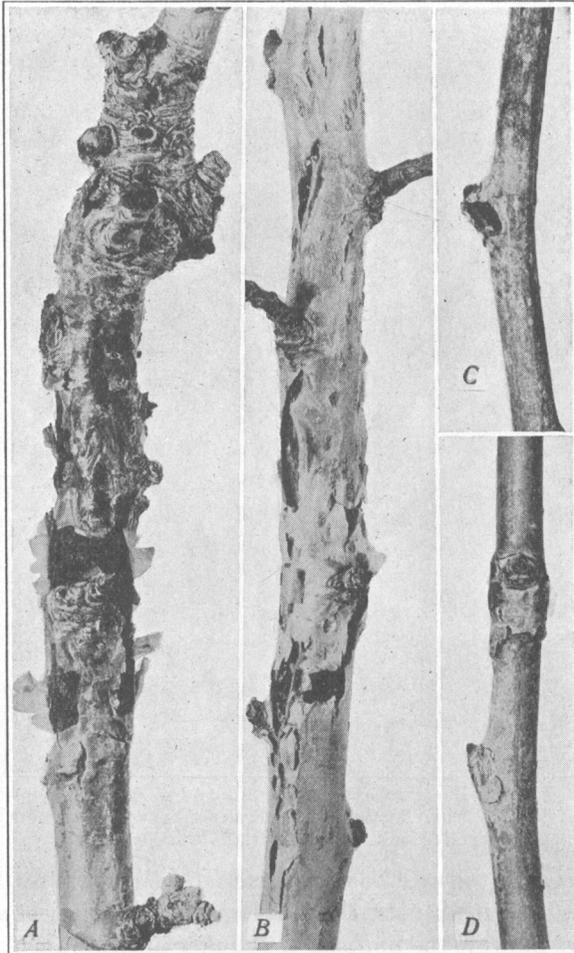


Fig. 1.—Bacterial canker on small branches of pear: *A* and *B*, young terminal shoots with characteristic sloughing away of periderm; *C* and *D*, infection of dormant buds and accompanying lesions in the twig.

bacterial invasion has progressed. On both hosts the streaks of active cankers are light brown and water-soaked. When inactive the streaks are dark brown to black on pear and brown to reddish brown on stone fruits.

Blossom Blast.—Thomas and Ark⁽¹⁵⁾ found that the pear blossom blast closely resembled blight, caused by *Erwinia amylovora*, but is distinguishable because blast seldom extends more than 1 to 2 inches into the

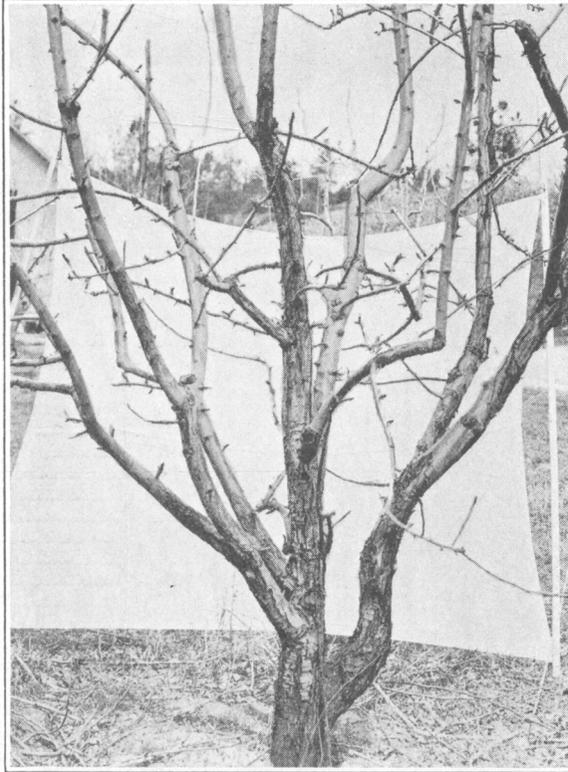


Fig. 2.—Bacterial canker on trunk and scaffold branches of Wilder pear. The longitudinal and transverse cracking of periderm does not occur on all the branches.

spur and never involves a bacterial exudate. Figure 3 of the present article shows the withered, blackened blossoms and leaves of an infected spur. Arrows indicate the limits of the canker in the branch.

Cherry and apricot blossoms are sometimes blighted by *Phytomonas cerasi*,⁽¹⁷⁾ the general symptoms being the same as those of pear blast.

Leaf and Fruit Infection.—The disease has not been observed on pear leaves in California, but apricot and cherry leaves are frequently attacked by *Phytomonas cerasi*.

No natural infection of pear fruit has been noted in California. Fruit infection by inoculation has, however, been obtained (fig. 6). In the only case of fruit infection by *Phytomonas cerasi* definitely established, small,

superficial, black pits appeared on the surface of green apricots. Infection has, however, been secured on apricot and plum fruit by needle-puncture inoculations with *P. cerasi*, the resulting symptoms being small, black, sunken pits like those from natural infection.

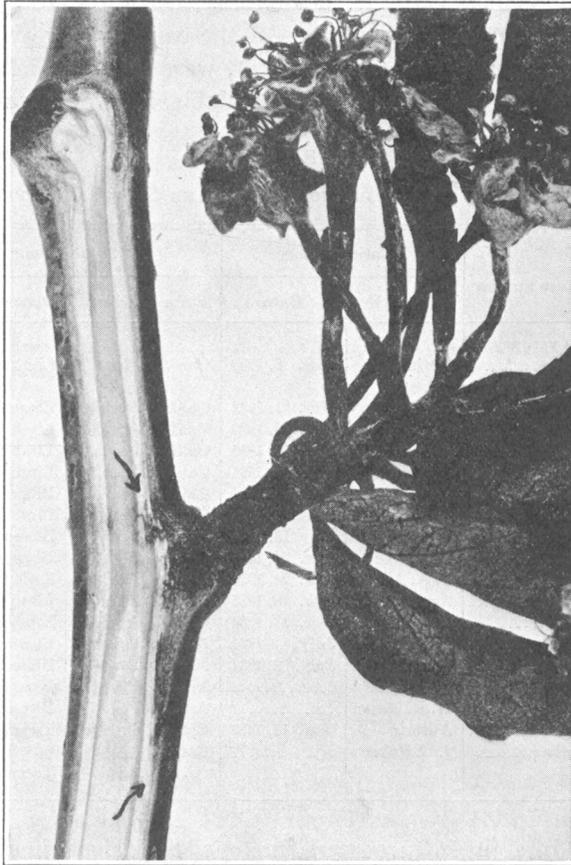


Fig. 3.—Blossom blast of pear. Arrows point to the margins of the diseased area in the twig. This is usually as far as blossom blast progresses the first year.

Other Points of Similarity Between the Pear and Stone-Fruit Diseases.—The pear canker disease differs from fire blight in being active during fall, winter, and early spring, when blight is inactive.⁽¹⁸⁾ A similar seasonal nature of stone-fruit bacterial canker has been observed.

To summarize: the pear and stone-fruit diseases possess marked similarities in the parts of trees they attack, in appearance of the invaded tissue, and in their activity during the same seasons of the year.

PATHOGENICITY STUDIES

The primary object of the inoculation work was to compare bacteria from the pear canker and blossom blast with the organism of stone-fruit canker (*Phytomonas cerasi*). Incidentally, certain similar organisms attacking the pear in other sections of the country, together with the citrus-blast bacterium (*P. citriputeale*), were included in these tests. The history of the cultures, so far as available, is given in table 1.

TABLE 1
HISTORY OF CULTURES USED IN INOCULATION AND CULTURAL EXPERIMENTS

Organism or culture number	Isolation history		Source of culture		
	Isolated by	Date	State	Host	Diseased part of host
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	Author	Aug. 8, 1930	Calif.	Plum	Limb canker
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II.....	Author	Mar. 21, 1932	Calif.	Apricot	Blossom blight
<i>Phytomonas cerasi</i> I.....	Author	Feb. 22, 1930	Calif.	Apricot	Limb canker
<i>Phytomonas cerasi</i> II.....	Author	Apr. 10, 1933	Calif.	Peach	Limb canker
Wilder I.....	Author	Jan. 1, 1931	Calif.	Pear	Limb canker
Wilder II.....	Author	Mar. 7, 1933	Calif.	Pear	Blight of blossom base
Wilder III.....	Author	Aug. 12, 1932	Calif.	Pear	Limb canker
Bartlett I.....	P. A. Ark	May 15, 1932	Calif.	Pear	Blossom blight
Winter Nelis I.....	P. A. Ark	Apr. 24, 1933	Calif.	Pear	Blossom blight
Winter Nelis II.....	P. A. Ark	Apr. 24, 1933	Calif.	Pear	Twig blight
Apple I.....	Author	Apr. 28, 1933	Calif.	Apple	Limb canker
Apple II.....	Author	Apr. 28, 1933	Calif.	Apple	Limb canker
<i>Phytomonas utiformica</i> "r".....	F. Clara	July, 1931	N. Y.	Pear	Blossom blight
<i>Phytomonas utiformica</i> "f".....	F. Clara	July, 1931	N. Y.	Pear	Blossom blight
Arkansas I.....	H. R. Rosen	Ark.	Pear	Blossom blight
Arkansas II.....	H. R. Rosen	Ark.	Pear	Blossom blight
<i>Phytomonas citriputeale</i>	Author	Mar. 14, 1932	Calif.	Orange	Twig blast
<i>Phytomonas papulans</i>	J. W. Roberts	East. U. S.	Apple	Target canker

Eighteen different cultures were employed at various times. Two were cultures of *Phytomonas cerasi* var. *prunicola*: two were *P. cerasi*: and three were cultures the author had isolated from limb cankers of Wilder pear from an orchard in Placer County (Wilder I, II, III). Three (Bartlett I, Winter Nelis I and II) were furnished by P. A. Ark, who had obtained them from blossom and twig blight of pears from El Dorado, San Benito, and Santa Cruz counties, respectively. Two cultures from apple were obtained from Mendocino and Sonoma counties. The two of *Phytomonas utiformica* Clara (isolates "r" and "f") were sent by Clara⁶ to Harvey E. Thomas of this Station. Unfortunately, isolate "r" was lost after the first two series of inoculations in 1933. H. R.

Rosen⁽¹⁰⁾ furnished two cultures designated "receptacle" and "petal" (Arkansas I and II, respectively). The culture *P. citriputeale* was obtained from twig blast of orange in Placer County. Inoculations of this organism into lemon and orange fruits produced the sunken lesions typical of the black-pit disease in nature. John W. Roberts furnished the culture that he provisionally called *P. papulans*,⁽¹¹⁾ the organism originally described by Rose⁽⁶⁾ as the cause of apple blister spot. Roberts obtained this culture from the target canker of apples.

Inoculations were made by first piercing the bark tangentially, then injecting into the holes a drop of the organism in water suspension.

TABLE 2
RESULTS OF INOCULATING WILDER PEAR TREES JANUARY 20, 1933

Organism	Inoculations producing symptoms, per cent	Length of cankers, mm*
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	77	15- 25†
Wilder I.....	92	25-102
<i>Phytomonas utiformica</i> "r".....	88	25- 64
<i>Phytomonas utiformica</i> "f".....	100	25-127
Controls.....	5‡	5- 10

* Measurements made 62 days after inoculation.

† Later observations showed that these cankers continued to extend until they involved considerably more area.

‡ Three infections of control wounds on one tree were clearly cases of secondary infections. The remaining 61 wounds were not infected.

From 50 to 75 inoculations were made from each culture used in each experiment into three to five different trees. Control punctures were made in different limbs of the same trees.

Although most of these organisms are similar in certain respects, some difference of opinion exists as to the closeness of the relation. The literature on this phase will be reviewed in a later section.

Results of Inoculations.—Table 2 shows the results of inoculations into pear during late winter of 1933. Cultures Wilder I and *Phytomonas utiformica* ("r" and "f") produced extensive cankers (fig. 4) in every way typical of those in nature. *P. cerasi* var. *prunicola* had produced small but definite cankers (fig. 5, C and D). As later observations showed, these cankers continued to extend and eventually became 6 or more inches long. A second series of inoculations made with Wilder I, *P. cerasi*, and *P. cerasi* var. *prunicola* into Wilder pear resulted in cankers from 3 to 6 inches long in all cases. No differences could be found between cankers produced by *P. cerasi* and Wilder I.

In January, 1933, Blenheim apricot limbs and Phillips Cling peach limbs, inoculated with the stone-fruit organisms, Wilder I culture, and

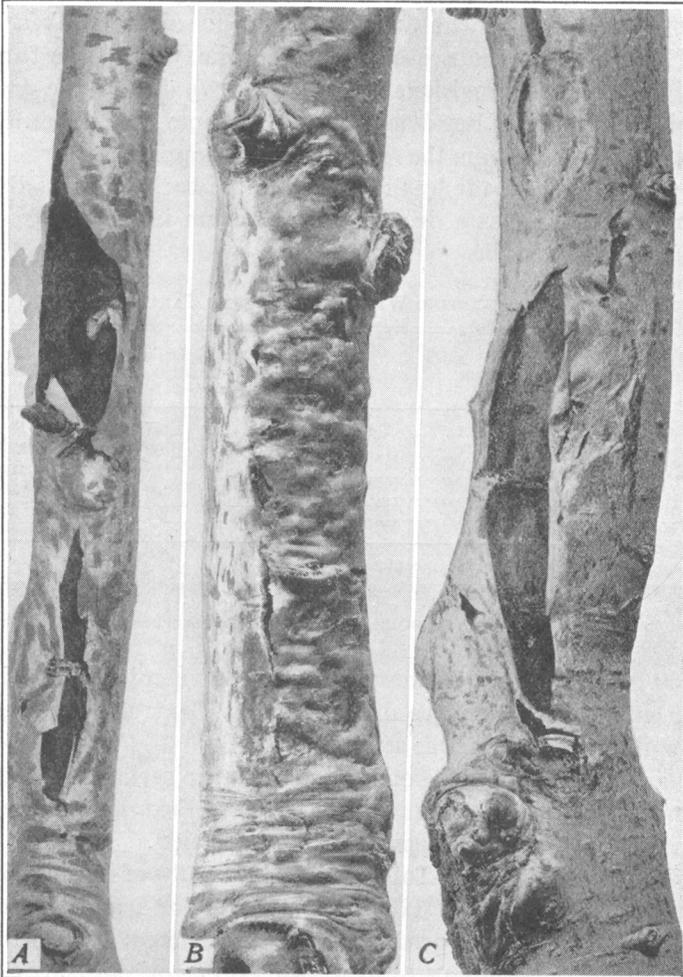


Fig. 4.—Symptoms produced by *Phytomonas utiformica* "r" (A), by *P. utiformica* "f" (B), and by Wilder I (C), on Wilder pear branches. Compare these symptoms with those from natural infections shown in figure 1.

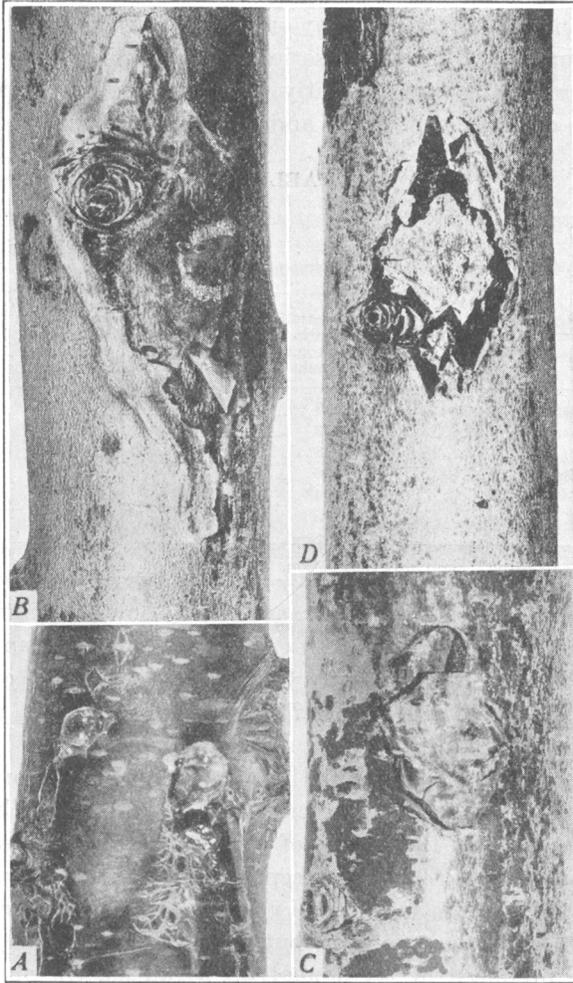


Fig. 5.—Cankers produced by culture Wilder I on Wilder pear (B) and Blenheim apricot (A). On the apricot, the bark canker was $1\frac{1}{2}$ inches long. C and D are cankers produced by *Phytomonas cerasi* var. *prunicola* on Wilder pear.

the two cultures of *Phytomonas utiformica*, developed definite cankers in every way typical of the bacterial canker of these hosts in nature (table 3). The fact that Wilder I produced somewhat smaller cankers than the stone-fruit organism may or may not be significant.

In February, 1934, when a series of inoculations were made into Duarte plum, conditions apparently did not favor rapid canker development, since cultures of *P. cerasi* and *P. cerasi* var. *prunicola* produced

TABLE 3

RESULTS OF INOCULATING APRICOT AND PEACH TREES JANUARY 7, 1933

Organism	Blenheim apricot		Phillips Cling peach	
	Inoculations producing symptoms, per cent	Length of cankers,* mm	Inoculations producing symptoms, per cent	Length of cankers,* mm
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	82	20-64	60	20-40
<i>Phytomonas cerasi</i> I.....	93	20-30	56	20-50
Wilder I.....	48	20-40	42	20-30
<i>Phytomonas utiformica</i> "r".....	90	20-70	78	10-50
<i>Phytomonas utiformica</i> "f".....	54	20-30
Controls.....	0	0	0	0

* Measurements made 26 days after inoculation.

rather small lesions. Nevertheless, as table 4 shows, distinct symptoms were produced by the six cultures obtained from blossom blast, twig blight, and limb canker of pear in California. At least one culture from apple (Apple I), the culture of *Phytomonas utiformica*, and culture Arkansas I also produced distinct symptoms.

On January 21, 1935, inoculations were made into apricot, peach, sweet cherry, and plum. Eleven cultures were used, each being inoculated at 60 places on three trees of each species. As table 5 shows, on apricot and peach all cultures except *Phytomonas papulans* produced diseased areas as large as *P. cerasi* var. *prunicola* or larger. On cherry the diseased areas were smaller, but were otherwise indistinguishable from those produced by the stone-fruit organism. On plum, *P. citri-puteale* and culture Winter Nelis I produced cankers somewhat larger than did *P. cerasi* var. *prunicola*, whereas the rest of the cultures, except *P. papulans*, produced cankers somewhat smaller. Thus, *P. papulans* was the only culture that was distinctive on these four hosts. Secondary infections, which occurred on apricot and which will be discussed later, gave additional evidence of the pathogenic abilities of certain pear cultures.

On December 19, 1935, *Phytomonas cerasi* var. *prunicola*, *P. cerasi*,

TABLE 4
RESULTS OF INOCULATING DUARTE PLUM TREES FEBRUARY 13, 1934

Organism	Inoculations producing symptoms, per cent	Length of cankers,* mm	Organism	Inoculations producing symptoms, per cent	Length of cankers,* mm
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	86	5-25	Bartlett I.....	86	5-44
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II.....	86	10-29	Winter Nelis I.....	79	10-34
<i>Phytomonas cerasi</i> I.....	92	5-30	Winter Nelis II.....	83	5-25
<i>Phytomonas cerasi</i> II.....	100	20-30	Apple I.....	79	5-25
Wilder I.....	86	5-16	Apple II.....	39	5-10
Wilder II.....	79	5-20	<i>Phytomonas utiformica</i> "f".....	66	5-25
Wilder III.....	79	10-25	Arkansas I.....	73	5-22
			Arkansas II.....	79	5-20
			Controls.....	0	0

* Measurements made 35 days after inoculation.

TABLE 5
RESULTS OF INOCULATING BLENHEIM APRICOT, PHILLIPS CLING PEACH, LAMBERT CHERRY, AND GRAND DUKE PLUM TREES ON JANUARY 21, 1935

Culture	Apricot		Peach		Cherry		Plum	
	Inoculations producing symptoms, per cent	Average length of cankers,* mm	Inoculations producing symptoms, per cent	Average length of cankers,* mm	Inoculations producing symptoms, per cent	Average length of cankers,* mm	Inoculations producing symptoms, per cent	Average length of cankers,* mm
<i>Phytomonas cerasi</i> var. <i>prunicola</i>	70	15	53	15	84	53	75	35
<i>Phytomonas utiformica</i>	91	24	83	23	60	32	70	14
<i>Phytomonas citriputeale</i>	74	17	60	30	60	26	60	43
<i>Phytomonas papulans</i>	0	0	0	0	0	0	0	0
Wilder I.....	70	14	67	17	48	24	30	15
Wilder II.....	64	20	38	25	47	23	45	13
Winter Nelis I.....	80	18	100	27	80	30	90	40
Winter Nelis II.....	66	19	50	15	50	16	20	13
Apple I.....	56	18	43	19	72	29	30	22
Arkansas I.....	87	25	74	21	72	32	65	25
Arkansas II.....	90	31	53	24	75	35	50	18
Controls.....	0	0	0	0	0	0	0	0

* Twenty inoculations into each of three trees. The measurements represent the average length of diseased areas along cambium and in bark 40 days after inoculation.

and *P. prunicola* Wor. were each inoculated at 100 places in five Duarte plum trees. Since the temperature did not favor rapid disease development during December and January the cankers produced averaged only 13 millimeters by January 19. Observations on March 4, however, showed that *P. prunicola* had produced cankers as extensive as had the other two organisms, some being 65 millimeters long and 30–35 millimeters wide.

On March 4, 1936, Wilder I and Winter Nelis I were inoculated into young Wilder pear trees. On the same date *Phytomonas cerasi* var. *prunicola*, culture Wilder I and culture Winter Nelis I, were inoculated into Bing sweet cherry. On March 9 examinations showed that in all cases the organisms were invading the bark tissues of both pear and cherry above and below the inoculation points. By March 14 the invaded zones in the pear trees were from 15 to 20 millimeters long and 10 to 15 millimeters wide. No difference existed in symptoms produced by cultures Wilder I and Winter Nelis I. On cherry the three cultures (*P. cerasi* var. *prunicola*, Wilder I, and Winter Nelis I) had produced diseased areas somewhat larger than on pear. Culture Winter Nelis I and *P. cerasi* var. *prunicola* produced somewhat larger cankers than did culture Wilder I, the average lengths being 31, 27, and 21 millimeters respectively.

Table 6 condenses the inoculation data to permit comparisons between results of different years. Whenever a particular culture was listed as pathogenic to a particular host, the character of the symptoms produced was considered in addition to the data on measurements. If on apricot, for example, the symptoms produced by a pear culture were not comparable with those caused by *Phytomonas cerasi* and *P. cerasi* var. *prunicola*, the results were listed as doubtful. This point is stressed because some cultures from pear produced on stone-fruit trees typical bacterial canker symptoms,⁽¹⁷⁾ although the diseased areas were not always so large as those produced by *P. cerasi*.

The California cultures from pear limbs and culture Winter Nelis I from pear blossoms were pathogenic to the five species of stone fruits and to pear. The California cultures from pear blossoms were pathogenic to the stone fruits with but one exception, that of culture Winter Nelis II on European plum and on peach. A culture from apple (Apple I) was pathogenic to Japanese plum and apricot; but inoculations into European plum, peach, and sweet cherry were doubtful. A second culture (Apple II) was doubtful on Japanese plum, the only host into which it was inoculated. The Arkansas cultures were pathogenic to the five stone fruits. *Phytomonas utiformica*, in addition to pear, was pathogenic to

Japanese plum, peach, apricot, and sweet cherry; but doubtful results were obtained on European plum. *Phytomonas citriputeale* produced positive results on European plum, apricot, peach, and sweet cherry. No trials were made on pear. *Phytomonas cerasi* and *P. cerasi* var. *prunicola* were pathogenic to the stone fruits and also produced cankers in pear. *Phytomonas papulans*, on the other hand, produced no symptoms on any of the stone fruits.

TABLE 6
SUMMARY OF PATHOGENICITY STUDIES*

Culture	Source of culture		Japa- nese plum†	Euro- pean plum	Apricot	Peach	Sweet cherry	Pear
	State	Host						
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	Calif.	Plum	+	+	+	+	+	+
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II.....	Calif.	Apricot	+	+‡	+‡	+‡	+‡	..
<i>Phytomonas cerasi</i> I.....	Calif.	Apricot	+	+‡	+‡	+‡	+‡	+
<i>Phytomonas cerasi</i> II.....	Calif.	Peach	+	+‡	+‡	+‡	+‡	..
<i>Phytomonas utiformica</i> "f".....	N. Y.	Pear	+	±	+	+	+	+
<i>Phytomonas utiformica</i> "r".....	N. Y.	Pear	+	+
<i>Phytomonas citriputeale</i>	Calif.	Orange	..	+	+	+	+	..
<i>Phytomonas papulans</i>	East. U.S.	Apple	..	-	-	-	-	..
Wilder I.....	Calif.	Pear	+	+	+	+	+	+
Wilder II.....	Calif.	Pear	+	+	+	+	+	+
Wilder III.....	Calif.	Pear	+	+
Bartlett I.....	Calif.	Pear	+
Winter Nelis I.....	Calif.	Pear	+	+	+	+	+	+
Winter Nelis II.....	Calif.	Pear	+	±	±	±	±	..
Apple I.....	Calif.	Apple	+	+	+	±	±	..
Apple II.....	Calif.	Apple	±
Arkansas I.....	Ark.	Pear	+	+	+	+	+	..
Arkansas II.....	Ark.	Pear	+	+	+	+	+	..

* Key to symbols: +=pathogenic; -=nonpathogenic; ±=doubtful.

† This variety of plum (Duarte) is said to be *Prunus (salicina × munsoniana) × salicina*.

‡ These cultures had proved pathogenic to European plum, apricot, peach, and cherry in earlier tests.

Evidence of Natural Spread of Disease from One Host to Another.—A host that will develop symptoms when inoculated with bacteria pathogenic to another host will not necessarily contract the disease under field conditions. Evidence, however, points toward the spread of bacteria from stone-fruit trees to pear trees. In one pear orchard, for example, blossom blast occurred only adjacent to three badly diseased apricot trees. The blast was most abundant near the apricots, but was absent a few rows away. In another case, two-year-old pear trees adjacent to old diseased peach trees developed limb cankers, while those farther away remained healthy. One of the worst cases of pear blast observed occurred in a pear orchard interplanted with plums. The plums had apparently suffered from bacterial canker for some years. Although few limb

cankers were present in the pears, for several years blossom blast was prevalent, a fact indicating that the holdover source might have been the plums.

In connection with the inoculation results presented in table 5, attention was called to the development of secondary infections in apricot limbs inoculated with the following pear cultures: Wilder I and II, Winter Nelis I, and Arkansas II. The secondary infection probably did not result from bacteria coming from other trees, since the lesions occurred only below the inoculation points and since control punctures made at one side and above these limbs remained healthy. Healthy trees, furthermore, occurred to windward of those inoculated.

The following observation regarding possible spread of bacteria from pear to citrus should be recorded. In 1932, within two weeks after numerous new cankers appeared in an orchard of Wilder pears, typical citrus blast appeared in a row of orange trees bordering the orchard. No blast had occurred in these orange trees within the preceding three or four years; and close examination after the outbreak in 1932 failed to show any recognizable blast symptoms of previous years.

CULTURAL TESTS

Unless otherwise stated, the following tests were made at 25° C. All organisms made good growth at this temperature.

The synthetic medium used in most of the carbohydrate tests was the same as that designated basal medium 2 in an earlier article.⁽⁷⁾ Its constitution was as follows: 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. The pH was adjusted to 6.8 to 7.0 with sodium hydroxide.

Degree of Fluorescigenesis as a Distinguishing Feature.—The relations between the pathogenic bacteria that produce a green fluorescence were studied by Burkholder,⁽⁸⁾ who attempted to adduce from his own and others' studies the degree of cultural homogeneity exhibited by species included in Bergey's genus *Phytomonas*. In many respects the fluorescent species constituted a closely related group, having common characteristics other than fluorescence. Burkholder's work encouraged Clara⁽⁹⁾ to bring together and to study under the same conditions the cultural and pathogenic attributes of many of these species. Clara's conclusions will be reviewed later; he, as well as others, considers fluorescigenesis a cardinal diagnostic character.

The present author's study⁽⁷⁾ of the stone-fruit canker indicated that the bacteria involved fell into two types or, as was finally concluded,

two strains or varieties. The type more commonly found on plum, cherry, and apricot differed from the less common type in not producing pigment on potato-dextrose agar. Other slight cultural differences were evident, the consistency of which will be considered later. The less common type was regarded as *Phytomonas cerasi* Griffin, while the more common was designated *P. cerasi* var. *prunicola* n. var.

While information was being secured on the presence of the pear bacterial canker in various localities, the large number of cultures obtained were seen to vary in their ability to produce pigment on potato-dextrose agar. Representative cultures, consequently, were compared with those from stone fruits in the following manner: The cultures were first grown for 24 hours in beef-extract broth and were then transferred to tubes of potato-dextrose agar. After a period extending to 27 days in some cases transfers were again made to beef-extract broth, and after 24 hours to potato-dextrose agar. In all, six such transfers to potato-dextrose agar were made. As the results showed, the organisms from pear separated themselves in the same manner as did the stone-fruit cultures. They consistently did or did not produce a green pigment on this medium. Of the 12 cultures from pear, 4 produced pigment and 8 did not. Of the 13 cultures from stone fruits, 5 produced pigment and 8 did not. The two cultures from apple (Apple I and Apple II) did not produce pigment, nor did those of *Phytomonas utiformica*, *P. citriputeale*, *P. prunicola* (Wormald) Bergey *et al.*, and *P. papulans*. Among those that produced pigment there was some variation; culture Wilder I, for example, produced a clear yellowish-green fluorescence in the medium, similar in every respect to that of *P. cerasi* I, whereas culture Wilder II produced at first a clear yellowish-green fluorescence, but after a few days a brownish discoloration of the agar. The same type of variation existed between *P. cerasi* I and *P. cerasi* II.

In order that later reference may be made to the ability of the individual cultures used in the inoculation experiments to produce pigment in potato-dextrose agar, the following list is given:

<i>Fluorescent</i>	————— <i>Nonfluorescent</i> —————	
Wilder I	Bartlett I	<i>Phytomonas citriputeale</i>
Wilder II	Winter Nelis I	<i>Phytomonas utiformica</i>
Wilder III	Winter Nelis II	<i>Phytomonas prunicola</i> Wor.
	Apple I	<i>Phytomonas papulans</i>
	Apple II	Arkansas I
		Arkansas II

The stone-fruit cultures designated *Phytomonas cerasi* are, of course, fluorescent, and those designated *P. cerasi* var. *prunicola* are nonfluorescent on potato-dextrose agar.

The difference in pigment production that distinguished *Phytomonas cerasi* and *P. cerasi* var. *prunicola* on potato-dextrose agar was not so clear-cut on certain other media,⁽¹⁷⁾ being more a difference of intensity than of quality. Thus, in a synthetic liquid medium containing manitol, glycerol, or sodium succinate as the energy source, a greenish-yellow pigment was produced by both, although that produced by *P. cerasi* was more intense. When cultures Wilder I, *P. cerasi*, *P. cerasi* var. *prunicola*, and *P. utiformica* were grown comparatively in the presence of various carbon sources (23 in all), culture Wilder I resembled *P. cerasi*, while *P. utiformica* resembled *P. cerasi* var. *prunicola* in fluorescogenesis.

Carbohydrate Utilization.—The medium used in these tests was basal medium 2, described earlier in this section. It soon proved ill-adapted to studies of increase in hydrogen-ion concentration when the carbon source used was not readily utilized by the bacteria. Trehalose and raffinose, for example, are less readily utilized than dextrose; yet growth is moderately luxuriant after about one week. If basal medium 2 is used, the pH is unchanged or is slightly increased. If, on the other hand, the basal medium is that proposed by the Society of American Bacteriologists *Manual*,⁽¹⁸⁾ in which the nitrogen source is monobasic ammonium phosphate, the bacteria produce a definite decrease in pH when utilizing trehalose and raffinose. As will be mentioned later, a substitution of ammonium sulfate for sodium nitrate in basal medium 2 permits the hydrogen-ion concentration to increase more rapidly in the presence of dextrose as an energy source. Since the pH change is determined not only by the hydrogen or hydroxyl ions derived from the carbon source, but by the ions derived from all other constituents of the medium and by the buffering effects of the constituents, the conditions of the test must be specified. The basal medium used for the carbon-utilization tests reported in table 7 was basal medium 2. Despite the objectionable features of this medium in the presence of a poorly utilized carbon source, it supported a luxuriant growth and favored development of the fluorescent pigment. For those reasons it was used extensively.

As shown in table 7, the pear-canker organism (Wilder I) was grown comparatively with *Phytomonas cerasi* var. *prunicola*, *P. cerasi*, *P. prunicola* Wor., and *P. utiformica* on basal medium 2 in the presence of twenty-three carbon sources. *Phytomonas utiformica* was grown on all but three of these carbon compounds. The plus and minus signs represent greater acidity and greater alkalinity than the control tube after the bacteria had been growing for 10 days at 25° C. With but one exception the organisms produced the same type of reactions on all carbon

sources. The exception was in the case of rhamnose, where culture Wilder I had made visible growth although the others had not. This difference is not significant, since in further tests the other cultures produced visible growth after 14 days or so.

In later tests all the cultures used in the pathogenicity studies were grown comparatively on basal medium 2 with xylose, dextrose, and

TABLE 7
CARBOHYDRATE UTILIZATION*

Culture	Carbon source																								
	Xylose	Arabinose	Mannose	Dextrose	Levulose	Galactose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Rhamnose	Mannitol	Glycerol	Peptone	Sodium asparaginate	Sodium succinate	Sodium citrate	Sodium malate	Sodium lactate	Sodium tartrate	Sodium acetate	Sodium oxalate	Sodium formate	Starch
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I...	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	0	0	0	0	0
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II...	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
<i>Phytomonas cerasi</i> I.....	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
<i>Phytomonas cerasi</i> II.....	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
<i>Phytomonas prunicola</i> Wor.....	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
Wilder I.....	+	+	+	+	+	+	+	-	t	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
Wilder II.....	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
Wilder III.....	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
Bartlett I.....	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
Winter Nelis I.....	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
Winter Nelis II.....	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
Apple I.....	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
Apple II.....	t	+	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	0	0	0	0
<i>Phytomonas utiformica</i> I.....	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
Arkansas I.....	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
Arkansas II.....	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
<i>Phytomonas citriputeale</i>	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
<i>Phytomonas papulans</i>	+	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	-	-	0	0	0	0
<i>Erwinia amylovora</i>	0	+	+	+	+	+	+	+	0	+	+	0	0	0	-	-	0	0	0	0	0	0	0	0	0

* Key to symbols: +=change towards acid side; -=change towards alkaline side; t=growth but no change in pH; 0=no growth.

sucrose as energy sources (10 grams per liter). With but one exception, that of culture Apple II in the presence of sucrose, all the organisms produced acid from the sugars (table 7). Although culture Apple II made a good growth on sucrose, no change in pH was visible after 10 days. In the degree to which the pH was changed, an apparent consistent difference was noticed on sucrose. Thus *Phytomonas cerasi* and the pear cultures that produced pigment on potato-dextrose agar caused less increase in hydrogen-ion concentration that did *P. cerasi* var. *prunicola* and those cultures (except Apple II) that did not produce pigment on potato-dextrose agar. In three experiments, for example, after 5 days the

latter group of cultures had reduced the pH to 3.8–4.1, whereas the former had reduced it only to 6.4–6.6. Under the conditions of these experiments at least, the cultures separated along the same lines as on potato-dextrose agar.

All the organisms were inoculated into tubes of basal medium 2 with formic acid (0.15 per cent, pH 6.8) as the only carbon source. None of

TABLE 8
MISCELLANEOUS CULTURAL FEATURES*

Culture	Gelatin liquefaction	Hydrogen sulfide production	Milk		Growth on nitrogen compounds			
			pH change	Peptonization	Ammonium sulfate	Sodium asparaginate	Asparagine	Glycine
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II.....	+	-	Ak	-	+	+	+	+
<i>Phytomonas cerasi</i> I.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas cerasi</i> II.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas prunicola</i> Wor.....	+	-	Ak	+	+	+	+	+
Wilder I.....	+	-	Ak	+	+	+	+	+
Wilder II.....	+	-	Ak	+	+	+	+	+
Wilder III.....	+	-	Ak	+	+	+	+	+
Bartlett I.....	+	-	Ak	-	+	+	+	+
Winter Nelis I.....	+	-	Ak	+	+	+	+	+
Winter Nelis II.....	+	-	Ak	+	+	+	+	+
Apple I.....	+	-	Ak	+	+	+	+	+
Apple II.....	-	-	Ak	-	+	+	+	-
<i>Phytomonas utiformica</i> I.....	+	-	Ak	+	+	+	+	+
Arkansas I.....	+	-	Ak	+	+	+	+	+
Arkansas II.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas citriputeale</i>	+	-	Ak	+	+	+	+	+
<i>Phytomonas papulans</i>	-	-	Ak	+	+	+	+	+
<i>Erwinia amylovora</i>	+	-	+	+	+	..

* Key to symbols: +=positive reaction or growth, as pertains to the respective headings; -=negative reaction or no growth; Ak=a shift of pH from 6.2 towards alkaline side.

the organisms made visible growth in this medium even after three weeks at 25° C. Apparently, therefore, formic acid is not an energy source that these bacteria can readily utilize (table 7).

Nitrogen Source.—The influence of the nitrogen source on the characteristics of the pH change produced by the bacteria has been mentioned. Sodium nitrate, though supporting an abundant growth, was less conducive to increase in hydrogen-ion concentration than monobasic ammonium phosphate. A further study of nitrogen sources revealed that ammonium sulfate, asparagine, sodium asparaginate, and glycine were utilized by all the bacteria (table 8). The characteristics of the pH change were as follows: on ammonium sulfate a rapid hydrogen-

ion increase occurred within a few days; on sodium nitrate and glycine the increase was much slower; on asparagine and sodium asparaginate the initial pH change was towards the alkaline side and was followed by a reversal only after several days. In the degree of pH change, *Phytomonas papulans* and culture Apple II differed from the rest on sodium nitrate and glycine.

Nutrient-Dextrose Broth.—This medium was beef-extract broth to which had been added 10 grams of dextrose per liter. The pH was adjusted to 6.8. By the end of 72 hours all the bacteria except *Phytomonas papulans* and culture Apple II had produced a dense uniform turbidity of the medium and a slight, easily fragmented surface film. *Phytomonas papulans* and culture Apple II differed from the rest in producing a tough, membranous surface film and very little turbidity.

Colony Characteristics on Potato-Dextrose Agar.—The consistency, topography, and internal structure of potato-dextrose agar colonies varied greatly even in *Phytomonas cerasi* I, the descendant of a single-cell isolation.⁽⁴⁷⁾ On the whole, the colonies of all the cultures except *P. papulans* and culture Apple II were similar, being after 48 hours from 1.5 to 3 millimeters in diameter. The margins were either entire or slightly lobed. The topography was flat or slightly raised. The consistency was butyrous, except as reported earlier for *P. cerasi*;⁽⁴⁸⁾ the color slightly bluish to white. The internal structure was amorphous or broken by dark, wavy lines extending in a general radial direction. Wormald⁽⁴⁹⁾ has reported this last-named feature to be characteristic of his *P. prunicola*. The colonies of *Phytomonas papulans* and culture Apple II differed from the rest in being more opaque and somewhat slimy.

Liquefaction of Gelatin.—Stab cultures were incubated at 21° C, observations being made at 2-day intervals. The cultures of *Phytomonas cerasi* were the first to begin liquefaction, followed by those of *P. cerasi* var. *prunicola* (table 8). By the end of 168 hours all cultures, except Apple II and *P. papulans*, had produced a stratiform liquefaction to a depth of 1 inch or more. The tubes were then placed at 25° C, but culture Apple II and *P. papulans* failed to start liquefaction after 3 days at this temperature.

Reaction in Milk.—Enough litmus was added to one lot of skimmed milk to produce a distinct blue. To another lot was added enough brom thymol blue to give a distinct color. The tubes were sterilized by steaming at atmospheric pressure for 20 minutes on 4 successive days. The final pH was approximately 6.2.

In five experiments the initial reaction of all cultures was an increase

in alkalinity (table 8). Clearing of the milk accompanied by a distinct odor of peptonization began in most cases within 4 or 5 days. In this reaction *P. cerasi* was slightly more rapid than the rest. *Phytomonas papulans*, culture Apple II, and culture Bartlett I were distinguishable from the rest by their failure to produce a peptonization after 11 days.

Hydrogen Sulfide Production.—Beef-extract agar was prepared as recommended by the Society of American Bacteriologists *Manual*.⁽⁴⁴⁾ None of the bacteria under study produced hydrogen sulfide in this medium (table 8).

Starch Hydrolysis.—Two types of media were employed for these tests: (1) beef-extract agar plus 10 grams of starch per liter and (2) basal medium 2 plus 10 grams of starch per liter (table 8). Beside *Phytomonas cerasi*, *P. cerasi* var. *prunicola*, and *P. prunicola* the only other culture used was Wilder I. Good growth but no starch hydrolysis was made on the former medium; no growth was made on the latter.

Malachite Green Agar.—The growth of the bacteria on malachite green agar is reported because further differentiation of *Phytomonas papulans* and Apple II was obtained. To basal medium 2 were added 10 grams of dextrose, 15 grams of agar, and malachite green (1:100,000); the pH was adjusted to 6.8. In petri dishes this medium was distinctly green.

Phytomonas papulans and culture Apple II were differentiated from the rest because *P. papulans* failed to grow and culture Apple II grew only slightly. Although culture Bartlett I and *P. cerasi* var. *prunicola* II grew somewhat more slowly than the rest, they conformed to the characteristics of a majority of the others—namely: (1) a flat, butyrous, opalescent growth, (2) later a greenish-yellow pigment that stained the bacterial mass and diffused into the medium (*P. cerasi* I and II produced pigment earlier and in greater intensity), and (3) gradual disappearance of the malachite green after about 2 or 3 days so that by the end of 10 days the plates were usually cleared of the stain.

Differentiation of Erwinia Amylovora and the Green-Fluorescent Organisms.—Since some rapid method of distinguishing *Erwinia amylovora* from the pear-canker organism was desirable, the bacillus was included in the studies of fluorescigenesis and carbon-source utilization. Although the blast and canker cultures produced pigment on basal medium 2 in the presence of a number of carbon compounds, *E. amylovora* showed no indication of producing pigment. According to table 7, under the conditions of these tests, *E. amylovora* was distinguishable from the green-fluorescent organisms when growing on a number of carbon sources. The most rapid method of differentiating these organ-

isms, however, would consist in adding to basal medium 2, glycerol, mannitol, or peptone, carbon compounds especially favorable to pigment production.

Besides cultural methods, figure 6 shows that *Erwinia amylovora* may be distinguished from the fluorescent organisms by inoculations

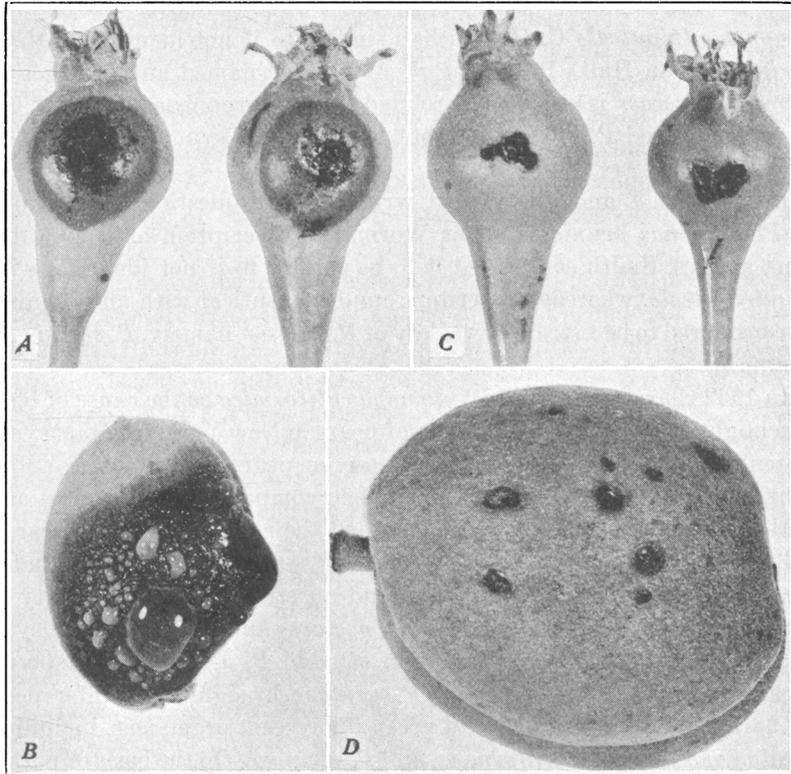


Fig. 6.—Difference in symptoms produced on pear and apricot by *Erwinia amylovora* (A, B) and by the pear-canker and blast organism (C, D). *Phytomonas cerasi* var. *prunicola* produced the same symptoms as did the pear-canker bacterium.

into young pear and apricot fruits. The fire-blight organism readily involved the entire fruit, producing a white, slimy exudate, while the pear-blast and stone-fruit organisms produced around the needle punctures only small, black, sunken pits and no visible exudate. Smith and Fawcett⁽⁴⁹⁾ have obtained with *Phytomonas citriputeale*, *P. syringae*, and *P. cerasi* the same type of black pits on a variety of fruits.

DISCUSSION

The literature contains considerable evidence that certain organisms included in this study are closely related. Although much of this evidence has been summarized elsewhere,⁽⁴⁰⁾ some recapitulation is necessary.

As Bryan,⁽⁴¹⁾ Smith,⁽⁴²⁾ and Smith and Fawcett⁽⁴³⁾ have shown, *Phytomonas citriputeale* C. O. Smith is similar to if not identical with *P. syringae* (Van Hall) Bergey *et al.* As the last-named authors further showed, *P. cerasi*⁵ is also closely related to these organisms. Elliott⁽⁶⁾ has since considered *P. citriputeale* but not *P. cerasi* synonymous with *P. syringae*.

Both Smith⁽⁴²⁾ and the present writer⁽⁴⁴⁾ have questioned the status of *Phytomonas prunicola*, after Wormald's description of it as a distinct species. Smith considered it to be similar to if not identical with *P. citriputeale*, whereas the writer found it identical with an organism he considered to be a strain or variety of *P. cerasi*—namely, *P. cerasi* var. *prunicola*.

In 1932 Clara⁽⁹⁾ described *Phytomonas utiformica* as the cause of blossom blight, fruit spot, and leaf spot of pears in New York. The same year Rosen⁽⁶⁾ found a similar blossom disease on pears in Arkansas; and a year later Rosen and Bleeker⁽⁴⁰⁾ published comparative serological and pathological tests in which they included their organism from pear, a culture of *P. syringae*, and a culture of *P. prunicola*. They concluded that these organisms were identical and questioned the advisability of considering *P. cerasi*, *P. papulans*, *P. nectarophila* (Doidge) Bergey *et al.*, *P. barkeri* (Berridge) Bergey *et al.*, and *P. utiformica* as species separated from the lilac organism, *P. syringae*, by significant differences.

Clara⁽⁹⁾ compared a number of green-fluorescent organisms, including *Phytomonas cerasi*, *P. utiformica*, and *P. syringae*. On the basis of pathogenicity to seventeen hosts he concluded that these organisms were related, but believed that certain cultural differences warranted separating them into species.

Dunegan⁽⁶⁾ has recently reported results of inoculating peach with *Phytomonas syringae* Van Hall, *P. prunicola* Wor., *P. mors-prunorum* (Wormald) Bergey *et al.*⁽⁴⁵⁾ *P. papulans*, a bacterium from apple target canker, and a bacterium from leaf spot of Italian prune. He found that when any of these bacteria were injected into leaves and green shoots, chlorotic or bleached areas surrounded by purplish zones were devel-

⁵ Smith and Fawcett's culture of *P. cerasi* was obtained in California. After corresponding with Smith the writer is convinced that it was *P. cerasi* var. *prunicola*. For description of this variety see the writer's earlier publication.⁽⁴⁷⁾

oped, symptoms differing from those produced by *P. pruni* (E. F. Smith) Bergey *et al.*, on this host.

We see, therefore, in the works of most of these authors a tendency towards clearing the literature of certain species created as the result of studying each on a limited number of hosts. Such a tendency is justifiable as far as it is founded upon a direct comparison of the organism in question. The interlinking evidence supplied by the works of Bryan,⁽¹⁾ Smith and Fawcett,⁽¹³⁾ Smith,⁽¹²⁾ Rosen and Bleeker,⁽¹⁰⁾ and the writer^(16, 17, 18) furnishes reasons for considering *P. syringae*, *P. citriputeale*, *P. cerasi* var. *prunicola*, and *P. prunicola* as either identical or differing only to a slight degree. Clara⁽⁴⁾ alone maintains that *P. syringae* and *P. cerasi*^{*} are distinct species, although he considers *P. citriputeale* and a number of other green-fluorescent pathogens synonymous with *P. syringae*.

If we examine Clara's⁽⁴⁾ reasons for regarding the three species as distinct, we see that they pertain both to pathogenic and to cultural features. He found, for example, that they differed in their ability to produce lesions on such diverse plants as *Trifolium repens*, *Holcus* sp., *Zea mays*, and *Syringa vulgaris*. On thirteen other hosts, including *Pyrus communis* and *Prunus avium*, the three organisms were indistinguishable. His results with *Syringa vulgaris* differ from those of Smith and Fawcett⁽¹³⁾ inasmuch as he found *P. cerasi* nonpathogenic to this host, whereas Smith and Fawcett obtained infection.

In cultural tests Clara found the three organisms very similar in many respects, but different in their reaction on certain carbon compounds—namely, raffinose, glycerol, salicin, acetic acid, and formic acid. Thus he reported that *Phytomonas utiformica* was the only one to “ferment” raffinose, salicin, and formic acid; that *P. cerasi* and *P. utiformica* but not *P. syringae* fermented acetic acid, whereas *P. utiformica* and *P. syringae* but not *P. cerasi* fermented glycerol. His conclusions regarding the failure of *P. cerasi* to ferment glycerol are contrary to those of Smith and Fawcett⁽¹³⁾ and of the writer,⁽¹⁷⁾ who found this compound to be an excellent energy source. Likewise his findings regarding failure of *P. cerasi* to ferment raffinose and salicin do not conform with the writer's earlier results⁽¹⁷⁾ nor with the studies presented herein, which indicate that these compounds supported fair growth. In the present study *P. utiformica* failed to make visible growth on a medium containing formic acid in the same concentration as used by Clara; acetic acid was not used.

As this brief review shows, the majority of the workers consider the organisms used in this study very closely related, and Clara's dissenting

* The culture supplied Clara was that of *Phytomonas cerasi* var. *prunicola*.

views are based upon much evidence directly contrary to that of the others.

We may now consider the results of the present work. Though certain gaps exist in the inoculation data and though cultural tests are by no means complete, the studies have yielded certain evidence:

1. The California cultures from pear differed consistently among themselves in one respect—production of fluorescent pigment on potato-dextrose agar. That is, the three cultures from limb canker of Wilder pear produced fluorescence on potato-dextrose agar, whereas those from twigs and blossoms did not, a characteristic similar to that separating *Phytomonas cerasi* from *P. cerasi* var. *prunicola*. The few instances in which the pear organisms varied in other respects could easily have resulted from experimental error.

2. The mutual pathogenic abilities, the parallel fluorescigenic variability on potato-dextrose agar, and the similar reactions in all other tests exhibited by the stone-fruit and California pear cultures give no indication that they are very different.

3. Apple II culture, although belonging to the green-fluorescent group, is definitely different from the pear cultures and from Apple I; the latter appears to be very similar to the pear cultures.

4. The culture furnished the writer by Roberts and provisionally designated by him as *Phytomonas papulans* is distinctly unlike any of the other organisms tested herein. Dunegan,⁽⁵⁾ so far as known, is the only worker who has compared *P. papulans* with one of those included in the present study.

5. *Phytomonas citriputeale*, *P. utiformica*, and the pear-blast cultures from Arkansas were pathogenically similar to the California pear cultures, and to *P. cerasi* and *P. cerasi* var. *prunicola* when inoculated into five species of stone fruits. *Phytomonas utiformica* was, furthermore, shown to produce the same type of symptoms on pear as did the California pear-canker organism. As far as the cultural studies went, this first-named group of organisms agreed with *P. cerasi* var. *prunicola* and with those California pear cultures that were not fluorescent on potato-dextrose agar.

This summary indicates that the only clear cases of differences within this group of cultures were those of *Phytomonas papulans* and Apple II. This is true as far as both the pathogenic and cultural tests are concerned. The rest of the cultures, however, though identical in inoculation tests, exhibited on media certain differences that should be mentioned. We saw that the three California cultures from pear-limb canker (Wilder I, II, and III), when grown on potato-dextrose agar, in their

production of pigment resembled *P. cerasi*, whereas the remaining California pear cultures as well as *P. citriputeale*, *P. utiformica*, and cultures Apple I and II, in their failure to produce pigment on potato-dextrose agar, resembled *P. cerasi* var. *prunicola*. As earlier comparisons⁽⁷⁾ had shown, *P. cerasi* and *P. cerasi* var. *prunicola* differed slightly in other tests. When these tests were undertaken for the present study, the similar slight differences were again evident: *P. cerasi*, for example, began to peptonize milk and to liquefy gelatin somewhat earlier than did *P. cerasi* var. *prunicola*. The pear cultures that resembled *P. cerasi* on potato-dextrose agar, on the other hand, were not so distinguished from those that resembled *P. cerasi* var. *prunicola*. Another example of separation of the cultures was afforded by sucrose in basal medium 2. Here *P. cerasi* and the three pear cultures that resembled it on potato-dextrose agar (Wilder I, II, and III) produced a smaller decrease in pH than did *P. cerasi* var. *prunicola* or any of the other cultures except *P. papulans* and culture Apple II.

Hence, except in the cases of *Phytomonas papulans* and culture Apple II, the only consistent separations of cultures were in their fluorescent capacities on potato-dextrose agar and in the degree to which they changed pH in the presence of sucrose. Host source, on the other hand, did not appear to be important as a line of cleavage. Particularly can this be said of the two stone-fruit organisms, of *P. utiformica*, of culture Winter Nelis I, and of culture Wilder I, all of which were inoculated into pear as well as five species of stone fruits. How many more slight differences can be obtained by increasing the number of tests and by refining the technique can only be surmised. Unquestionably, a number of small differences would be regarded by some as justifying the continuation of the names of existing species and the use of new names for those unnamed organisms included herein. The final disposition will, of course, depend upon more complete studies and upon the prevailing conception of species limits.

Provisionally, at least, the evidence justifies including in one species *Phytomonas cerasi*, *P. cerasi* var. *prunicola*, *P. citriputeale*, *P. utiformica*, Rosen's organism, and the California pear organism. These organisms are unquestionably very closely related to *P. syringae*. The fact that there is no recorded variation of *P. syringae* comparable with that separating *P. cerasi* and *P. cerasi* var. *prunicola* cannot be overlooked. If any changes are made, *P. cerasi* should probably retain a varietal rank.

SUMMARY AND CONCLUSIONS

The major object of this work was to establish the relation between a canker and blossom blast of pear and the bacterial canker of stone-fruit trees. Both the pear canker and pear blossom blast were known to be caused by bacteria that were on standard culture media similar to each other and to *Phytomonas cerasi*, cause of the stone-fruit bacterial canker. By observing the diseases of the two hosts for a number of seasons, information was obtained concerning the season of activity, the parts of the hosts attacked, and the character of the symptoms. To determine pathogenic similarities, the bacteria were inoculated at various times into *Pyrus* sp. and into five species of *Prunus*. By cultural tests the bacteria were compared as to growth on various standard media, growth in special media containing different carbohydrate and nitrogen sources, reactions in milk, liquefaction of gelatin, and production of hydrogen sulfide. Besides the two strains of stone-fruit organisms (*P. cerasi* and *P. cerasi* var. *prunicola*) and bacteria from pear limb cankers and blossom blast, the pathogenicity and cultural studies included the following organisms: *P. utiformica*, *P. papulans*, *P. citriputeale*, cultures obtained by Rosen from pear blossoms in Arkansas, and cultures obtained by the writer from apple in California. Incidentally, *Erwinia amylovora* was carried through certain of the cultural tests, and a method for differentiating it from the canker organisms is described.

The results of these studies afforded the following conclusions: First, the limb canker and blossom blast of pear are phases of the same disease, which also attacks dormant buds, twigs, and fruit. Second, the pear and the stone-fruit diseases exhibit similarities as to parts of the host attacked, character of symptoms, and season of activity. The bacteria from the two hosts were, furthermore, identical in the inoculation and cultural tests. Third, the inoculation and cultural tests support the view that *Phytomonas utiformica*, *P. citriputeale*, and the bacterium furnished by Rosen are identical with the stone-fruit organism. The bacterium recently isolated by Roberts and designated *P. papulans* is an unrelated species.

In the writer's opinion, therefore, these organisms, except of course *Phytomonas papulans*, should be given the same species name. The preponderance of evidence in the literature points towards *P. syringae* as the correct binomial.

LITERATURE CITED

- ¹ BRYAN, MARY K.
1928. Lilac blight in the United States. *Jour. Agr. Research* 36:225-35.
- ² BURKHOLDER, WALTER H.
1930. The genus *Phytomonas*. *Phytopathology* 20:1-23.
- ³ CLARA, FELICIANO M.
1932. A new bacterial disease of pears. *Science (n.s.)* 75:111.
- ⁴ CLARA, FELICIANO M.
1934. A comparative study of the green-fluorescent bacterial plant pathogens. *Cornell Agr. Exp. Sta. Mem.* 159:1-36.
- ⁵ DUNEGAN, JOHN C.
1934. The susceptibility of the peach to artificial inoculation with *Bacterium syringae* and some allied organisms. *Phytopathology* 24:1378.
- ⁶ ELLIOTT, CHARLOTTE.
1930. Manual of bacterial plant pathogens. 349 p. Williams and Wilkins, Baltimore.
- ⁷ ROBERTS, JOHN W.
1934. Apple target canker, measles, and rough bark. [Abstract.] *Phytopathology* 24:16.
- ⁸ ROSE, DEAN H.
1917. Blister spot of apple and its relation to a disease of apple bark. *Phytopathology* 7:198-208.
- ⁹ ROSEN, H. R.
1932. Two forms of fireblight and a new related disease. *Phytopathology* 22:23-24.
- ¹⁰ ROSEN, H. R., and W. L. BLEEKER.
1933. Comparative serological and pathological investigations of the fire-blight organism and a pathogenic fluorescent group of bacteria. *Jour. Agr. Research* 46:95-119.
- ¹¹ SMITH, C. O.
1926. Similarity of bacterial diseases of avocado, lilac, and citrus in California. *Phytopathology* 16:235-236.
- ¹² SMITH, C. O.
1931. *Pseudomonas prunicola* and *Bacterium citriputate*. *Phytopathology* 21:1091.
- ¹³ SMITH, C. O., and HOWARD S. FAWCETT.
1930. A comparative study of the citrus blast bacterium and some other allied organisms. *Jour. Agr. Research* 41:233-245.
- ¹⁴ SOCIETY OF AMERICAN BACTERIOLOGISTS.
1923. Manual of methods for pure culture study of bacteria. (Looseleaf.) Geneva, N. Y.
- ¹⁵ THOMAS, H. EARL, and P. A. ARK.
1934. Fire blight of pears and related plants. *California Agr. Exp. Sta. Bul.* 586:1-43.

- ¹⁶ WILSON, E. E.
1931. A comparison of *Pseudomonas prunicola* with a canker-producing bacterium of stone-fruit trees in California. *Phytopathology* **21**:1153-1161.
- ¹⁷ WILSON, E. E.
1933. Bacterial canker of stone-fruit trees in California. *Hilgardia* **8**(3):83-123.
- ¹⁸ WILSON, E. E.
1934. A bacterial canker of pear trees new to California. *Phytopathology* **24**:534-537.
- ¹⁹ WILSON, E. E.
1934. Variability of *Pseudomonas cerasi* in physical characteristics of growth on solid media. *Phytopathology* **24**:548-550.
- ²⁰ WORMALD, H.
1930. Bacterial diseases of stone-fruit trees in Britain II. Bacterial shoot wilt of plum trees. *Ann. Appl. Biol.* **17**:725-744.
- ²¹ WORMALD, H.
1932. Bacterial diseases of stone-fruit trees in Britain IV. The organism causing bacterial canker of plum trees. *Brit. Mycol. Soc. Trans.* **17** (Part 3):157-169.