Filbert Bacteriosis

and Its Control

By
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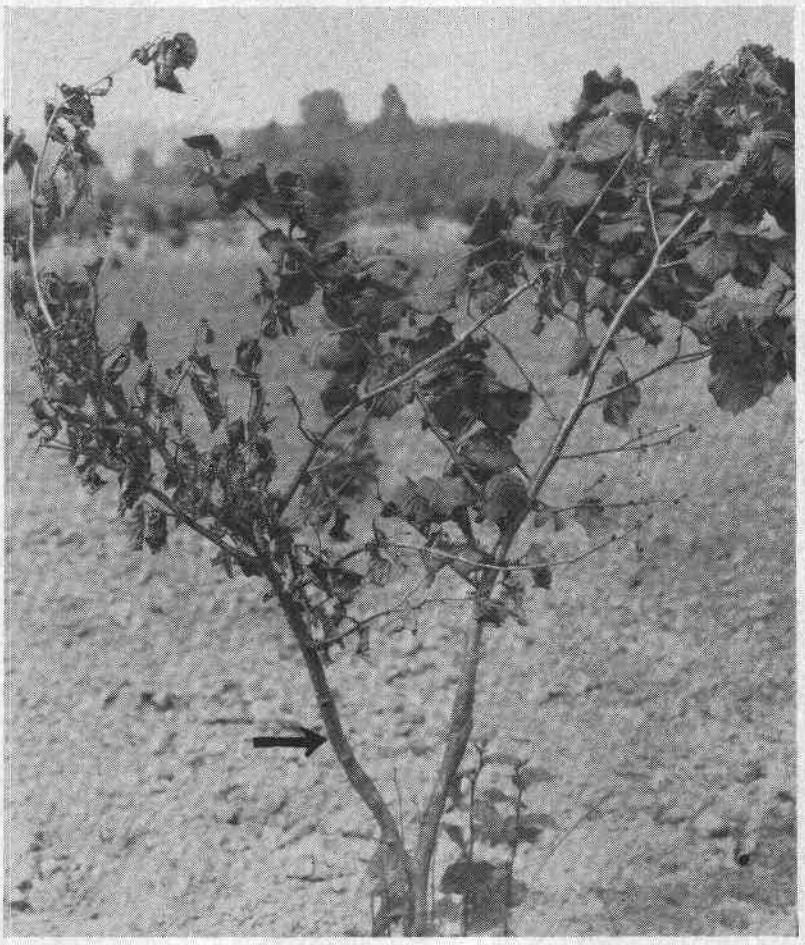

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Foreword

Filbert bacteriosis, commonly known as filbert blight, is the most destructive parasitic disease of the filbert in Oregon. It is widely distributed, occurring to a greater or lesser extent in practically all filbert orchards in the State. While the prevalence and destructiveness of the disease has varied with the season, it has caused the loss of many acres of young trees and, in older orchards, has reduced the crop substantially.

In this bulletin everything that is known about the disease has been brought together.

The research work reported herein was conducted cooperatively by personnel of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station.

Wm. A. Schoenfeld

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Filbert Bacteriosis and Its Control

By

P. W. MILLER[1], W. B. BOLLEN[2], J. E. SIMMONS[3]

BACTERIOSIS of filberts, caused by *Xanthomonas corylina* (Miller et al.) Dowson, commonly known as filbert blight, is the most serious and, insofar as is now known, the only infectious disease of filberts (*Corylus avellana* L. and *C. maxima* Mill.) of any economic importance in the Pacific Northwest. It was first discovered by H. P. Barss in 1913 (2).[4] Microscopical examinations of diseased tissues disclosed the presence of bacteria, and repeated isolations consistently yielded a bacterium of a specific type. The disease was reproduced by inoculations with pure cultures of this bacterium, and the same organism was reisolated from the lesions produced, thereby definitely establishing its causal relationship to the disease.

The first published report on the disease was made by Barss in 1915 (2). In this report the malady is described and early investigations of its cause related. In 1927 Barss (3) published a subsequent paper in which investigations on the life history of the disease and its control are related.

In 1930, the senior author initiated studies on this disease and its control in cooperation with the Oregon Agricultural Experiment Station. These investigations have been in progress for 18 years during which time reports of progress (13 to 26 inclusive) have been made periodically so that new information was made available as soon as possible to filbert growers. Although our knowledge of certain aspects of the disease is still incomplete it seems desirable to incorporate in a single technical report the results of our investigations to date. To further the reader's general knowledge of the disease in all its ramifications and to make the paper more useful, there is included a digest of the known facts concerning it.

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[4]See "Literature Cited," page 68.

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To all filbert growers who cooperated in field studies for their cordial help in making their commercial orchards and equipment available for spraying and dusting experiments and for assisting in their prosecution.

THE DISEASE

Geographical Distribution

Insofar as is known, filbert bacteriosis occurs only in Oregon and Washington. The only other reference to a bacterial disease said to be associated with *Corylus* is a report by Brzesinski in 1903 (5) of the presence of *Bacterium coryli* on hazel in Europe. The author, however, gave no adequate description of the pathogen and reported no pathogenicity tests, for which reason the name is listed in the 1939 Edition of Bergey's Manual, p. 217 (4) among those excluded because of inadequate description or unproved pathogenicity. Brzesinski reports that the organism is the same culturally and mor-

A B

Figure 1. Filbert buds infected with bacteriosis: A, diseased leaf buds, denoted by arrows; B, an infected pistillate-flower-bearing bud (at *a*).

phologically as *Bacterium mali,* an organism described by him as grayish white on agar. This would definitely place the organism in an entirely different genus from *Xanthomonas corylina,* the latter being a pale lemon-yellow organism.

Economic Importance

Bacteriosis is the most destructive disease of the filbert in the Pacific Northwest, causing greater financial loss than all of the other diseases of filbert combined. The prevalence and destructiveness of this malady varies with the age of the trees and with the season. The greatest economic losses occur in young orchards, 1 to 4 years of age, where the disease often causes the death of numerous trees. While the mortality from bacteriosis fluctuates from year to year it is estimated that during the last decade about 10 per cent of the trees planted in the Pacific Northwest died before they reached 5 years of age.

Although trees more than 4 years of age seldom die from this disease, many buds and nut-bearing twigs in the tops of the trees are attacked and killed by the causal organism, thereby reducing the yield. The average annual crop loss due to bud and twig blight has varied in the past decade from less than 1 per cent up to 10 per cent. In certain orchards, the loss has been as high as 25 per cent of the crop.

Symptoms of the Disease

The pathogen attacks the buds, leaves, branches, trunk, and occasionally the nuts. The roots are seldom invaded.

On the buds

Leaf buds and pistillate-flower-bearing buds in the axils of leaves on shoots of current growth are subject to attack by the pathogen (Figure 1, A and B). The staminate buds have so far not been found infected. The outer bud "scales" or bracts are first infected and from these the bacteria invade the central axis and subsequently the inner parts of the bud, causing them to turn brown and die. In some instances the lesions are localized in the outer bud bracts, in which case the buds do not die but partially or completely unfold in the spring. The shoots coming from these buds generally become infected, the bacteria invading the stem of the shoot from the infected bud scale (Figure 2).

On the leaves

The pathogen causes small, angular or irregularly circular, pale yellowish-green, water-soaked lesions in the leaf blades (Figure

3, A); later, the lesions turn reddish-brown (Figure 3, B). Individual leisons in the leaf blades are typically small, rarely measuring more than 3 mm. in their greatest dimension. The lesions are often grouped together near the tip of the leaf in positions where water would tend to accumulate during rainy periods.

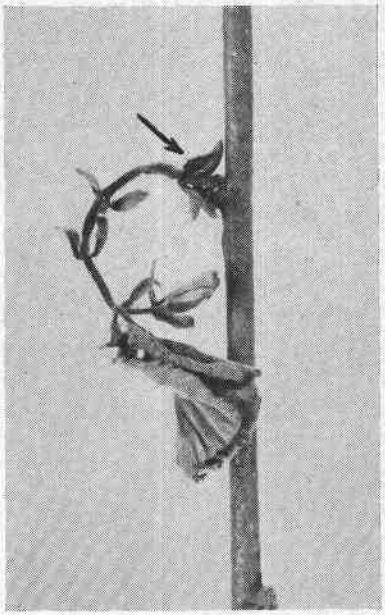


Figure 2. An infected shoot of current growth. The bacteria invaded the stem from an old, attached infected bud scale (denoted by arrow).

On stems of current growth

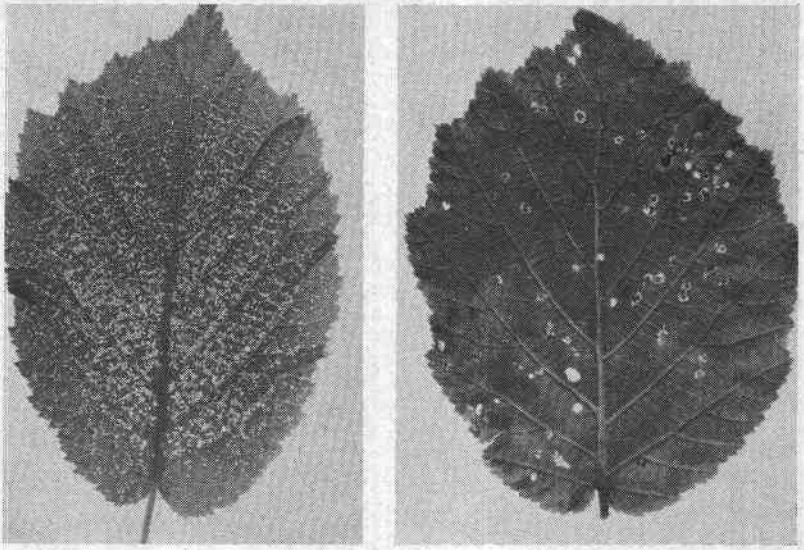
The first visible evidence of infection of the stems of shoots of the current season's growth consists in the presence of dark green, water-soaked areas in the bark; later, the infected areas turn reddish-brown (Figure 4). The lesions often encircle the stems causing the leaves on the distal parts to turn brown and die. The dead leaves generally cling to the girdled stems for some time, giving them the appearance of having been killed by fire. Infection may also occur near the base of the stem where an old diseased bud scale was attached to the shoot. In such case the stem of the shoot often breaks at the lesion and hangs downward (Figure 5), finally falling to the ground during windy periods.

On 1- to 2-year-old twigs

One- to two-year-old twigs are also attacked and killed by the pathogen. Infection of such twigs takes place indirectly either through wounds or by invasion of the bacteria from blighted buds and diseased shoots of the current season's growth (Figure 6, A). The lesions frequently girdle the twigs, causing them to die. Twig infection is of considerable economic importance as many of the twigs killed are potential bearers of nuts (Figure 7).

On the limbs and trunk

Besides the production of cankers on the stems of young shoots and twigs, the pathogen also causes lesions in the larger limbs and



A B

Figure 3. Bacteriosis lesions on filbert leaves: A, Young lesions from artificial inoculation; at this stage the infections are light yellowish-green; B, Old lesions from natural infections; the centers of the lesions are reddish-brown and a pale, yellowish-green zone typically surrounds each lesion.

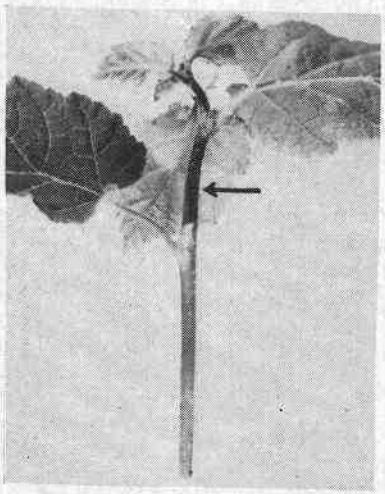


Figure 4. An infection (denoted by arrow) on stem of young filbert shoot of current growth.

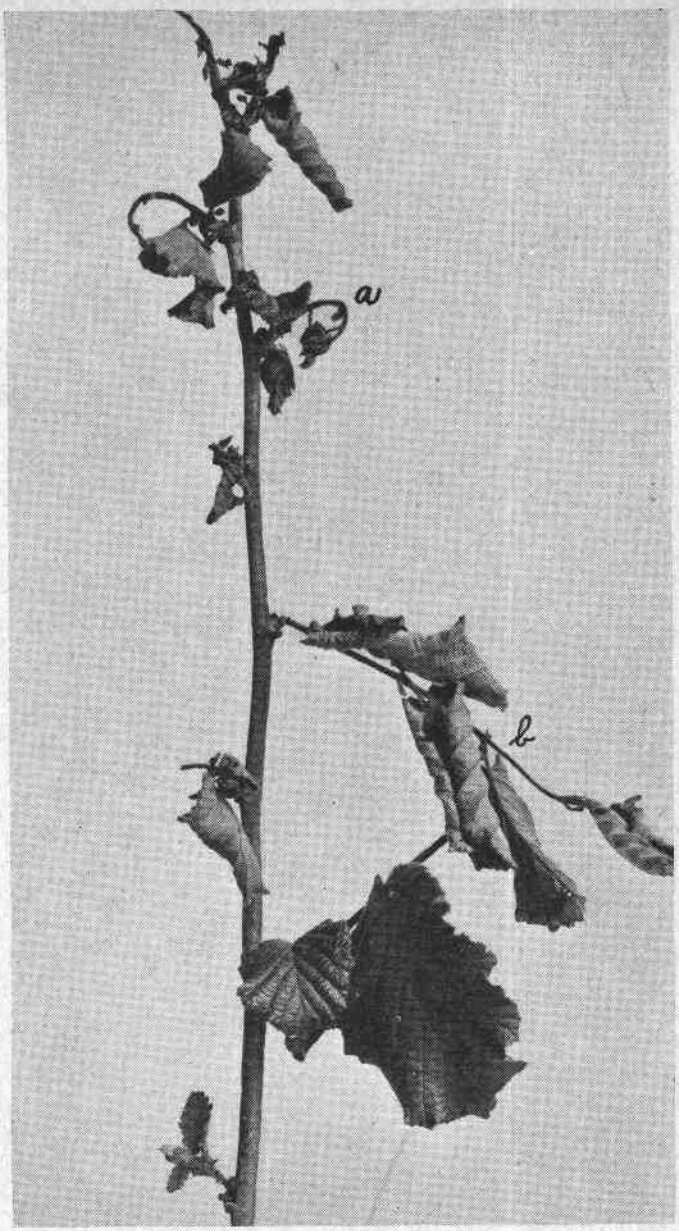
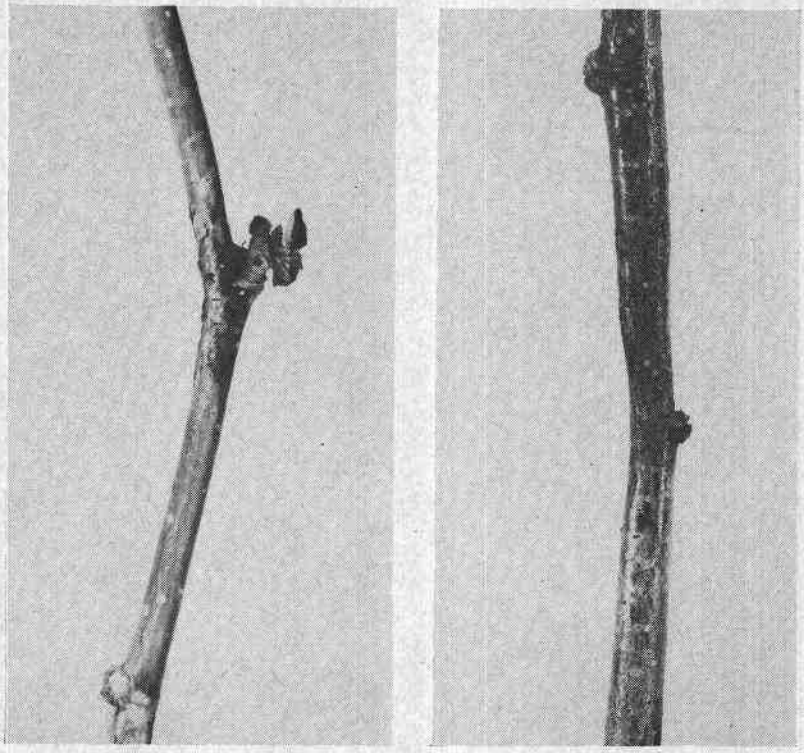


Figure 5. A branch from an infected filbert tree showing a number of blighted shoots of current growth. At *a* is shown a young shoot that was killed by bacteriosis shortly after it emerged from the bud. At *b* is shown a diseased shoot infected near the base of its stem. The shoot has broken at the point of infection and is hanging downward. (*After Barss;* Oreg. Bien. Crop Pest and Hort. Rept. 1913-14.)



A B

Figure 6. Bacteriosis cankers on filbert twigs: A, a canker on a one-year-old filbert twig originating at the base of a blighted bud; B, a canker on a two-year-old branch; note constriction of the tissues at the lesion; this is due to the cessation of growth in the infected areas.

trunk of the tree (Figure 8). Trunk infection is a particularly serious phase of the disease as the lesions frequently encircle the trunks of trees 1 to 3 years old, eventually causing them to die (Figure 9). The lesions are generally confined to the bark; the wood (xylem) is not commonly invaded. Branch and trunk cankers are generally difficult to detect as the surface does not change markedly in appearance. If the epidermis is cut away, however, the canker can be easily detected, the diseased tissues being reddish-brown to chocolate-colored with tiny white flecks scattered throughout. The cankers are at first almost oval in shape with the maximal

dimension parallel to the long axis of the branch; later, they may become irregular. The size of the cankers varies from ½ to 6 inches in their maximal dimension. The elevation of the lesion is at first

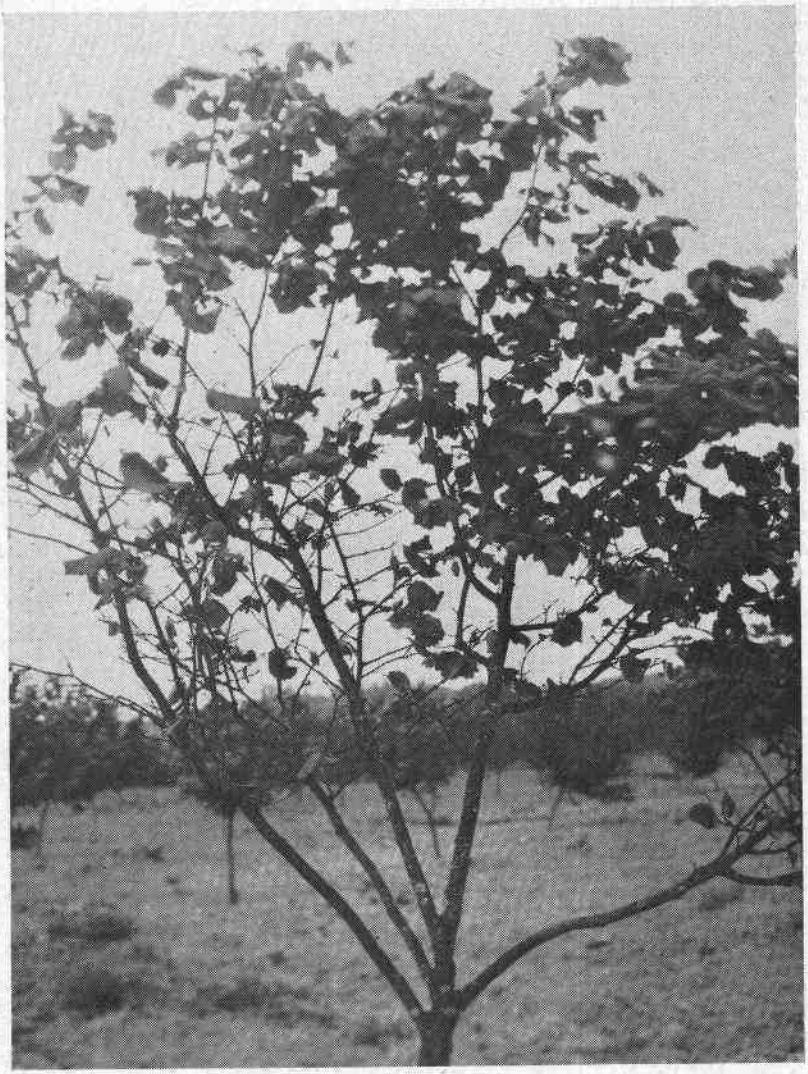


Figure 7. Bud and twig blight in a filbert tree due to bacteriosis. The killing of the buds and twigs has reduced the bearing area considerably.

Figure 8. A bacteriosis canker on a filbert branch; note that the lesion is centered about a pruning wound (denoted by arrow).

unchanged, but as the surrounding healthy tissues grow the infected areas appear sunken with poorly defined margins. Tension resulting from the growth of the surrounding healthy parts may cause longitudinal cracks in the center or at the margins of the lesions (Figure 10). The diseased areas frequently girdle the branches or trunk, causing the leaves on the parts beyond to die. The dead leaves often cling to the branches for some time before they fall.

Drops of a sticky, slimy substance, containing numerous bacteria, often ooze out of cankers during periods of high humidity (Figure 10). This bacterial ooze disperses in water and is spread to the host parts below, where, if conditions are favorable, new infections may occur.

Most of the cankers on the twigs and larger branches cease active development during the summer and the bacteria in the tissues die. In some cankers, however, the bacteria remain alive through the summer and act as sources of infection during the fall and spring.

While the pathogen does not, as a rule, attack and kill branches that are more than 3 years of age, cases are on record of 4- and 5-year-old branches being girdled and killed.

Blight cankers and dead areas on the trunk due to sunscald or cold injury are frequently confused by many growers. The only sure way of differentiating between a blight canker and sunburn or cold injury is by microscopical and cultural methods. Sunscald and cold injury generally occur on the trunks at or near the ground line on the south or southwest sides of the tree while blight cankers will be found fortuitously scattered on all sides of the trunk.

On the nuts

On the nuts, the disease produces dark brown or black, superficial spots in the shell. The lesions are typically circular, averaging

about 1 mm. in diameter (Figure 11, A). A water-soaked zone generally surrounds each young lesion. The elevation is unchanged in young lesions but slightly sunken in older ones. While the lesions generally occur on the sides of the nuts, they are sometimes found at maturity on the basal end where the nuts were attached to the stems (Figure 11, B). These basal lesions are very irregular and superficial, typically penetrating only a fraction of a millimeter into the shell.

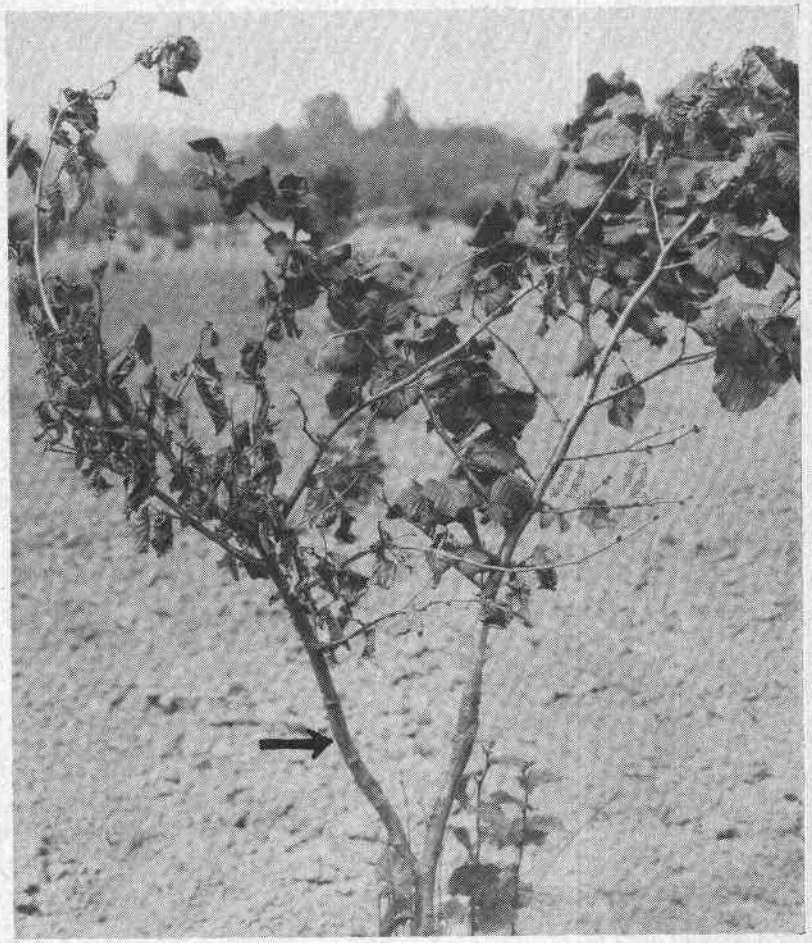


Figure 9. A young filbert tree infected with bacteriosis; the branch on the left has been girdled by a canker (location denoted by arrow).

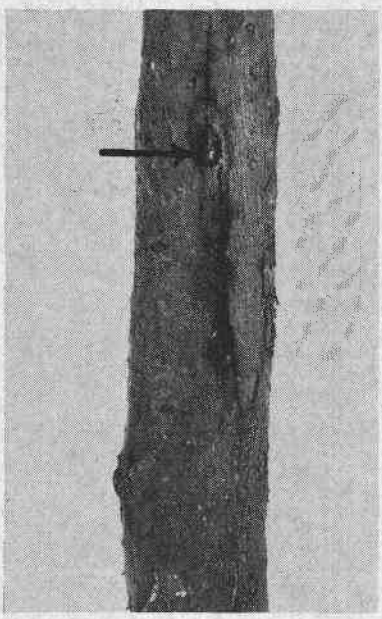


Figure 10. A canker on the trunk of a filbert tree; note bacterial ooze (denoted by arrow) coming from crack in the surface.

The disease also occurs on the husks where it produces dark brown spots in the tissues. A dark green, water-soaked zone typically surrounds each husk lesion (Figure 11, C).

Nut infection is of little, if any economic importance as the nuts are not often attacked and the disease seldom, if ever, infects the kernel.

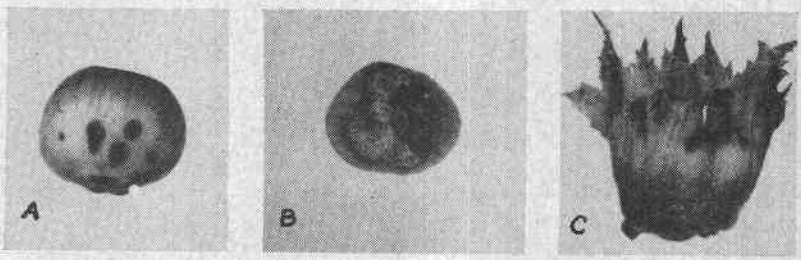


Figure 11. A and B, Lesions on nuts, from inoculations: A, Lateral infections; B, Basal infection; C, Lesions on husk.

Hosts

Bacteriosis occurs naturally on the cultivated filbert (*Corylus avellana*) in the Pacific Northwest, and we have produced the disease on leaves of the Turkish filbert (*Corylus colurna*) by artificial inoculation.

This disease has not been found on the native wild hazel (*Corylus californica*) in the Pacific Northwest.

THE CAUSAL ORGANISM

Barss (2) first isolated the causal organism in 1913 and proved its pathogenicity by inoculation. The first detailed description of the pathogen based on standard morphological and physiological tests was made by Miller et al. (19) in 1940 who named the organism *Phytomonas corylina* Miller et al. The name *Xanthomonas* proposed by Dowson (9) in 1939 for the genus *Phytomonas* has recently been accepted for this genus by competent authorities. The binomial *Xanthomonas corylina* (Miller et al.) Dowson will accordingly be used to designate the pathogen in this paper.

In an effort (a) to determine if there are different strains of the pathogen and (b) to gain a clearer knowledge of the responses of the pathogen to its environment further morphological and physiological studies of a number of different isolates have been carried on since the original studies were conducted. A detailed account of these and earlier investigations follows.

Source and Number of Isolates Studied

In all, 48 different isolates of the causal organism from widely scattered filbert orchards in Oregon and Washington were used in the present studies. While not all of these were used in all phases of the investigation, at least four different isolates from widely scattered locations were employed in each phase of the investigation.

Method of Isolation and Purification

The pathogen was isolated from diseased buds and lesions on shoots and nuts by aseptically removing small pieces of tissue from the interior of the diseased organ and plating them out by the standard poured plate dilution technique on potato-dextrose agar in petri dishes. The cultures were purified by making three successive series of dilution plates from a 24-hour-old Difco beef extract-dextrose broth culture after which subcultures were made from typical single colonies. This method has been shown by McNew (12) to yield a very high percentage of colonies which are of single-cell origin.

Morphology and Staining Reactions

Morphological characteristics of the isolates were determined from a 48-hour-old culture grown at 28° C. on Difco beef extract-dextrose agar, adjusted to pH 7.4. For form and size, negative demonstrations from smears prepared with 1 per cent nigrosine were used. Hiss' method was employed for demonstrating capsules and Cesares-Gil's flagella stain was used to determine the number and position of flagella. Gram reaction was determined according to Burke's modification of Gram's stain. Dorner's method was used as a test for spores, and Ziehl-Nielsen's method was employed to determine acid-fast properties.

Morphological Characteristics

A detailed study of four isolates, supported by numerous observations of many others, showed the pathogen to be a short rod with rounded ends, arranged singly or in pairs, and occasionally in short chains. The cells measure from 1.1 to 3.8 μ by 0.5 to 0.7 μ. The organism is motile by one polar flagellum (Figure 12, A). It is Gram-negative and is not acid fast. On nutrient-dextrose agar, the organism is heavily capsulated (Figure 12, B). No endospores are produced. It stains readily with gentian violet and carbol fuchsines,

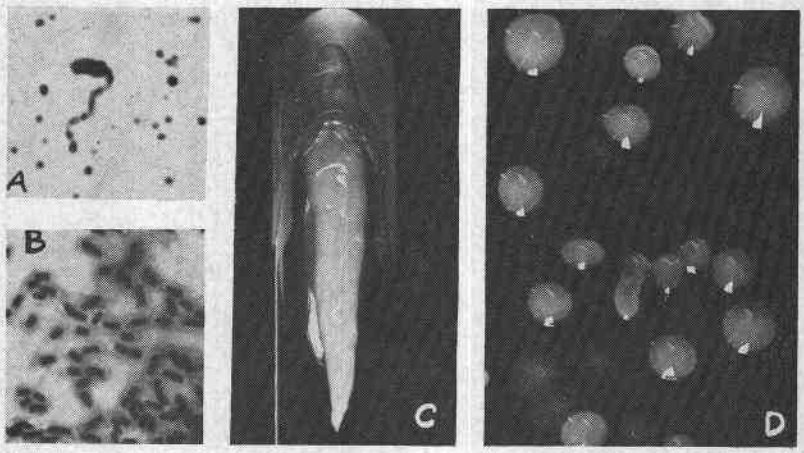


Figure 12. A and B, Photomicrographs of *Xanthomonas corylina:* A, Showing the single polar flagellum, x 2300; B, Capsule about organism, x 1475. C and D, Ten-day-old potato-dextrose agar cultures: C, Streak culture; D, Plate culture, x 1.

but only very lightly with methylene blue. In respect to these characteristics, no difference was noted between the four isolates studied.

Cultural Characteristics

Nutrient-dextrose agar streak

Growth of four isolates on Difco beef extract-dextrose agar at pH 6.6, after 48 hours at 28° C., was moderate, filiform, convex, viscid, glistening, smooth, opaque, odorless, and pale lemon-yellow (copper-yellow: No. 1 (27)).

Potato-dextrose agar streak

Growth of four isolates on Difco potato-dextrose agar at pH 6.8, after three days at an average temperature of 25° C., was abundant, filiform, convex, viscid, glistening, opaque, odorless, and a pale-lemon to light-chrome yellow (amber-yellow: No. 1 (27)) in color (Figure 12, C). The topography of the growth of all isolates was typically smooth, although a wrinkling or infolding of the surface was noted in some of the isolates after they had been in culture for several months. At room temperature, the bacterial growth covers the entire width of an agar slope at the base in from 10 to 15 days.

On potato cylinders

Growth of four isolates grown on sterilized potato cylinders after three days at 28° C. was moderate, viscid, filiform to echinulate, convex, glistening, smooth, opaque, odorless, and deep lemon to chrome-yellow. The medium was unchanged except for the formation of a partially cleared "fermentation" zone, 2 to 4 mm. in width, just beyond the margin of growth. Tests with Gram's iodine solution showed that this zone was free or nearly free from starch.

Nutrient-dextrose agar plates

Colonies of all four isolates studied became visible on Difco beef extract-dextrose agar plates in from 2 to 3 days at room temperature averaging 22° C. After 6 days' growth, the surface colonies of all isolates were circular, smooth, convex, glistening, with entire margins. The colonies imbedded in the medium were lenticular. The internal structure of the surface colonies was homogenous and finely granular. The medium remained unchanged in color. The colonies of all isolates were pale lemon-yellow (coppery yellow: No. 1 (27)) at the margins and deeper lemon to chrome-yellow at the centers. After 12 days' growth, the colonies averaged about 7 mm. in diameter.

Potato-dextrose agar plates

Colonies of four isolates on Difco potato-dextrose agar plates became visible at an average temperature of 22° C. in from 2 to 3 days. After 5 to 6 days' growth the surface colonies were circular and those imbedded in the medium, lenticular. The surface colonies of all isolates were viscid, smooth, convex to pulvinate, with entire edges (Figure 12, D). The internal structure was finely granular. In some colonies radial striations occurred at the margins. The colonies were a pale lemon to a light chrome-yellow (amber-yellow: No. 1 (27)). There was no change in the color of the medium except just in advance of the margin of each colony, where, because of the hydrolysis of the starch in the medium, there was a partly clear zone. This zone became very noticeable when the plates were flooded with Gram's iodine solution. At the end of 9 days the colonies averaged approximately 9 mm. in diameter.

Gelatin stab

Growth of four isolates in Difco nutrient-gelatin "stabs" at pH 6.6 became visible after 24 hours at an average temperature of 22° C. In all cases liquefaction began at the surface in 1 to 2 days. The liquefied portion was at first infundibuliform but soon became stratiform. In 10 days the upper half of the medium had become liquefied, was slightly turbid, with a pale yellow sediment at the bottom. Liquefaction of the lower half of the medium was very slow, requiring from four to six weeks or even longer for 10 cc. of gelatin in a 5/8-inch test tube to be entirely liquefied.

Twenty-six isolates were tested by Frazier's gelatin-plate technique (10). After 48 hours' incubation at 30° C., all cultures gave a positive reaction with tannic acid, thus indicating a considerable increase in amino-nitrogen.

Other solid media

Nine isolates were grown comparatively at the same time and under like conditions upon a number of other solid media. Growth of all isolates was sparse on Difco prune agar, Difco oat agar, Difco lima-bean agar, Difco corn-meal agar, and Difco bean-pod agar. On Endo agar, congo red agar, and Levine's eosine-methylene blue agar growth of all isolates, while normal in amount, was not sufficiently distinctive to justify detailed description.

Nutrient broth

In Difco beef extract-peptone broth at pH 6.6, all four isolates studied made a trace of growth after 16 hours at room temperature, averaging 22° C. By 48 hours a moderate clouding of the medium

was evident in all cases, but there was no sediment. After six days the medium was very turbid and a fragile ring developed where the surface of the medium met the walls of the tube. This ring was at first readily broken up into flocculent particles by agitation. Later it became more cohesive but never developed into a true pellicle. After about 10 days a pale-yellow sediment formed in the medium, which was slightly viscid on agitation. The medium cleared slightly after about 3 weeks, because of a settling of the growth. No odor was at first detectable, but after several weeks there was a slight odor resembling ammonia.

Dextrose broth

In Difco dextrose broth at pH 6.6, all four isolates studied made a trace of growth after 24 hours at 30° C. After 3 days at 30° C. all cultures showed a moderate clouding of the medium, but no sediment. By the third day a fragile ring-like growth formed at the surface which was easily broken up into flocculent particles by agitation. Later, this surface growth became more cohesive but never developed into a true pellicle. An odor resembling ammonia developed after several weeks.

PHYSIOLOGY

Methods

The recommendations of the Committee on Bacteriological Technique, Society of American Bacteriologists, as given in the Manual of Methods for Pure Culture Study of Bacteria (8), were closely followed in most of the biochemical studies. The cultures were incubated at room temperature, averaging 22° C. unless otherwise stated.

Biochemical Characteristics

Relation to free oxygen

All four of the isolates studied were strongly aerobic, as is indicated by the fact that when grown in Smith's fermentation tubes in Difco nutrient broth containing 1 per cent dextrose growth occurred only in the open arm.

Chromogenesis

All 26 isolates cultured during the course of the investigation produced a yellow pigment on Difco nutrient agar and Difco potato-dextrose agar. The color of the growth varied from the paler shades of yellow to deeper yellows, depending on the age of the cultures and

on the medium. The prevailing tone was a pale lemon-yellow (amber-yellow: No. 1 (27)). On certain media, the isolates varied somewhat in the intensity of the yellow pigment produced.

Indole production

Indole was not produced by any of the four isolates studied by standard procedure (8). To test for indole production, two-day-old cultures in tryptophane broth were tested with Ehrlich's reagent.

Hydrogen sulfide production

None of the four isolates studied produced hydrogen sulfide when grown in Difco lead acetate-agar stabs. Positive reactions for hydrogen sulfide production, however, were obtained for all the isolates when tested by the more sensitive ZoBell method (34).

Hydrolysis of starch

Tests for starch hydrolyzing ability were made by growing ten isolates on plates of potato-dextrose agar. A cleared zone, which became very noticeable when the surface of the medium was flooded with Gram's iodine solution, was produced about colonies of all the isolates.

Digestion of milk

The action of four isolates on milk was studied; each produced an enzymatic curd that was slowly digested. Peptonization occurred near the surface of all cultures after 4 to 5 days; it was more pronounced, though not complete, after 10 days.

Reduction of litmus

Four isolates were studied and found to reduce litmus slowly in litmus milk. Reduction began after one to two days, but was not complete until 1 to 2 months thereafter. Crystal formation was observed in three isolates; in three others, no crystals were noted.

Selenium reduction

Streak cultures of 26 isolates on nutrient agar containing selenium dioxide in 1: 25,000 concentration became brick-red after a short time, thus indicating intracellular reduction. This reaction is regarded by Levine (11) as a better indicaor of reducing activity than are organic dyes.

Methyl red test

Cultures of four isolates on Difco Methyl Red, Voges-Proskauer medium, adjusted to an initial pH of 6.9, became progressively more alkaline with age.

Alkali production

Alkali (ammonia) is produced when the organism is grown in a nutrient broth containing peptone as a nitrogen source. A shift in reaction from an initial pH of 6.9 to a pH of 7.5 occurred after 11 days growth in Difco nutrient broth. Tests with Nessler's reagent were positive which shows that ammonia was formed. Ammonia also was produced abundantly in a 1 per cent solution of peptone in tap water. Although an alkaline reaction in peptone-containing media and in milk is produced, they do not belong to the alkali-forming group of bacteria as defined by Ayers et al (1). Alkalinity after growth in nutrient broth is apparently induced by ammonia from amino acid breakdown. Alkaline carbonates formed by the oxidation of salts of organic acids also gave an alkaline reaction, as shown in Table 2.

Hydrogen-ion relations

The pathogen grew at a pH range of 5.2 to 10.6. It made the most rapid growth in a range of pH 6 to 8. The hydrogen-ion concentration at which growth in Difco nutrient broth was inhibited was pH 5.2 in the acid range and pH 10.6 in the alkaline range (Table 1).

Carbon metabolism

A variety of carbon sources are utilizable by the causal organism with the production of acid, but no gas. To demonstrate acid pro-

Table 1. THE RELATION OF THE REACTION OF THE CULTURAL MEDIUM TO THE GROWTH OF *Xanthomonas corylina*.

pH	Growth[1] after 10 days' incubation in beef extract-peptone broth
5.0	0[2]
5.2	0
5.4	+
5.6	+
6.0	++
6.4	++++
6.8	+++
7.0	++
7.2	++
7.6	++
8.0	++
8.4	+
8.8	+
9.2	+
10.0	+
10.4	+
10.6	0
10.8	0
11.0	0
11.2	0

[1]As indicated by the degree of turbidity.
[2]0=No growth apparent; + slight growth; ++ fair growth; +++ good growth; ++++ very good growth.

Table 2. GROWTH OF *Xanthomonas corylina* IN SYNTHETIC MEDIA CONTAINING VARIOUS SOURCES OF CARBON.

Carbon source	Days required to produce indicated changes					
	Isolate number 5092	Isolate number 5146	Isolate number 5151	Isolate number 5164	Isolate number 5170	Isolate number 5256
Dextrose	+14[1]	+10	+14	+10	+10	+10
Levulose	+21	+21	+21	+21	+21	+21
Sucrose	+28	+28	+28	+28	+28	+28
Lactose	+16	+14	+14	+14	+14	+14
Maltose	+70	+27	+33	+14	+14	+14
Raffinose	+42	+27	+42	+27	+42	+42
Arabinose	0[2]	0	0	0	0	0
Rhamnose	0	0	0	0	0	0
Xylose	+36	+32	+22	+32	+32	+32
Mannitol	+13	+13	+13	+13	+13	+13
Dulcitol	0	0	0	0	0	0
Glycerol	+32	+22	+32	+32	+32	+22
Salicin	0	0	0	0	0	0
Starch	++17[3]	++13	++13	++ 2	++ 2	++ 2
Inulin	0	0	0	0	0	0
Cellulose	0	0	0	0	0	0
Sodium acetate	0	0	0	0	0	0
Sodium benzoate	0	0	0	0	0	0
Sodium citrate	— 5[4]	— 5	— 5	— 5	— 5	— 7
Sodium formate	0	0	0	0	0	0
Sodium lactate	—13	—13	—13	—13	—13	—13
Sodium malate	— 7	— 7	— 7	— 7	— 7	— 7
Sodium salicylate	0	0	0	0	0	0
Sodium succinate	— 7	— 7	— 7	— 7	— 7	— 7
Sodium tartrate	0	0	0	0	0	0

[1]+ Indicates heavy growth and full acid color of bromcresol purple; figure following (+) sign indicates number of days required to produce full acid color of indicator.
[2](0) Indicates no growth within 60 days.
[3](++) Indicates hydrolysis of starch and full acid color of bromcresol purple; figure following indicates number of days required to produce full acid color of indciator.
[4](—) Indicates heavy growth and full alkaline color of bromthymol blue; figure following indicates number of days required to produce full alkaline color of indicator.

duction it was found necessary to employ a synthetic medium containing only inorganic nitrogen compounds, as acid production is completely masked in the presence of peptone because of ammonia production. The utilization of carbohydrates by four isolates was studied in preliminary investigations with agar slopes made with a synthetic basal medium according to Burkholder's method (6).

Sugars, alcohols, glucosides, and sodium salts of organic acids were added to the modified synthetic medium[5] of Ayers, Rupp, and Johnson (1) as recommended in *Manual of Methods for Pure Culture Study of Bacteria,* issued by the Society of American Bacteriologists (8). The basal medium was adjusted to pH7 and was sterilized by autoclaving. Bromcresol purple was added as an indicator, except in the media containing organic salts, where bromthymol blue was em-

[5]Basal medium for carbon sources:

$NaNH_4HPO_4 \cdot 4H_2O$	1.0 g.
KCl	0.2 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.
Brom-cresol purple	0.01 g.
Distilled water	1,000 cc.

Adjusted to pH 7.0 with NaOH solution.

ployed. The indicator concentration in all cases was 0.001 per cent in the basal medium. All of the carbon sources were prepared in 5 per cent concentrations with distilled water and sterilized by filtration through a glass filter (Jena G3). One cc. of the solution was added aseptically to 4 cc. of the sterilized basal medium in small test tubes, giving a concentration of 1 per cent carbon source in the final medium. All media were incubated for 5 days at 28° C. to check their sterility before using.

To test for the utilization of cellulose, strips of acid-washed filter paper were added to test tubes containing the basal medium, and these were sterilized in the autoclave. Utilization of starch was determined by streaking starch agar plates made with the basal medium and containing bromcresol purple as an indicator. The iodine method was used to detect starch digestion. Inoculations of all liquid media were made in duplicate. One loop of a distilled-water suspension prepared by mixing two loops of growth from a 24-hour-old dextrose-nutrient-agar culture with 10 cc. of sterile water was used as the inoculum. Quantitative tests showed that this inoculum averaged several hundred thousand bacteria per loop. Cultures were incubated at 28° C. and observed daily. The synthetic media permitted only relatively slow growth, from 3 to 5 days elapsing before any turbidity appeared. Tubes showing no turbidity within 60 days were considered negative for growth. Development of acidity was generally much slower than growth. The results of these carbohydrate-fermentation studies are given in Table 2.

As shown in Table 2, the most rapid change occurred with isolates numbered 5164, 5170, and 5256 on starch, full acid color of bromcresol purple being attained in two days. This was exceptional, since all the other isolates required 13 days to produce a corresponding change, which agrees closely with the average time required to ferment dextrose. The most consistent rapid fermentation by all isolates occurred with sodium citrate, only 5 to 7 days being required for development of the full alkaline color of bromthymol blue. All isolates completed the indicator change in 7 days with malate and succinate. Since the substrates in these three instances are relatively simple anions, a different fermentation mechanism is probably involved, which could explain the more rapid changes observed.

With some of the sugars the isolates showed a great variation in rate of action. Maltose, for example, was fermented to full acid color of bromcresol purple in from 14 to 70 days. Levulose, on the other hand, was fermented at a uniform rate, the indicator being fully changed by all isolates in 21 days. This is, however, an unusually slow change, levulose being typically fermented as rapidly as dex-

trose by most other bacteria (32). Good agreement in duplicate cultures was obtained; in a few cases they differed by 2 to 10 days in time required to change the indicator completely. The data in Table 1 are averages for duplicate tubes. In decreasing order of average rate of utilization by all isolates, the carbon sources can be listed as follows: citrate, malate, succinate, starch, lactate, mannitol, dextrose, lactose, levulose, maltose, glycerol, sucrose, xylose, and raffinose. A more rapid fermentation of dextrose, sucrose, lactose, raffinose, mannitol, starch, and citrate occurred. Arabinose, rhamnose, dulcitol, salicin, inulin, cellulose, acetate, benzoate, formate, salicylate, and tartrate did not permit growth of any isolate. If one isolate could grow with a given carbon source, all other isolates also could utilize it. Differences appeared only in the rate of utilization and these were not consistent, being distributed among all isolates, and among most of the carbon sources.

Nitrogen metalobism

There are a number of nitrogen compounds that can support the growth of *Xanthomonas corylina.* Availability of various nitrogen sources was determined by using 0.1 per cent concentrations in a basal synthetic medium[6] for nitrogen sources containing dextrose. The nitrogen sources were prepared in 5 per cent solutions and added aseptically to autoclaved tubes of the basal medium. The sodium nitrate was sterilized by filtration. All other nitrogen sources

Table 3. GROWTH OF *Xanthomonas corylina* IN SYNTHETIC MEDIA CONTAINING VARIOUS SOURCES OF NITROGEN.

Nitrogen source	Days required to produce heavy growth[1]					
	Isolate number 5092	Isolate number 5146	Isolate number 5151	Isolate number 5164	Isolate number 5170	Isolate number 5256
Alanine	14	10	10	10	10	10
Allantoin	12	12	12	12	12	12
Aspartic acid	10	10	10	10	10	10
Brucine	18	18	18	18	18	18
Glutamic acid	0	0	0	0	0	0
Hippuric acid	0	0	0	0	0	0
Leucine	10	10	14	10	10	10
$NaNH_4HPO_4$	14	10	14	10	10	10
$NaNO_2$	0	0	0	0	0	0
Peptone	2	2	2	2	2	2
Tyrosine	12	12	12	12	12	12
Uric acid	12	12	12	12	12	12

[1]As indicated by a turbidity comparable to that shown by a 48-hour culture of *Escherichia coli* in standard broth. All cultures indicated by (0) showed no growth within 60 days.

[6]Basal medium for nitrogen sources:

K_2HPO_4	1.0 gram
NaCl	0.2 gram
$MgSO_4 \cdot 7H_2O$	0.2 gram
Dextrose	10.0 gram

Adjusted to pH 7.0 with NaOH solution.

were autoclaved at 15 pounds pressure for 20 minutes. Inoculations were made as previously described and the cultures were incubated at 28° C. Comparisons were made on the basis of time required to produce heavy growth, as indicated by a turbidity comparable to that shown by a 48-hour-old culture of *Escherichia coli* in standard nutrient broth. The results of these studies appear in Table 3.

The data presented in Table 3 show that greater uniformity exists in the ability of the various isolates to utilize the same as well as different nitrogen sources than was the case with carbohydrate utilization. Peptone gave the most rapid growth, all isolates producing heavy turbidity in 2 days. This was to be expected from general experience with the growth of these organisms on standard laboratory media containing dextrose and peptone. Rapid growth in the presence of peptone is probably due to accompanying neutralities. Slowest growth was obtained with brucine, 18 days being required by all isolates to produce turbidity comparable to a 2-day culture in peptone. All cultures attained heavy growth in from 10 to 14 days. Listed in order according to average effect on rate of growth of all isolates, the nitrogen sources utilized are peptone, aspartic acid, alanine, leucine, sodium ammonium phosphate, allantoin, tyrosine, uric acid, and brucine. Glutamic acid, hippuric acid, and sodium nitrate did not support the growth of any isolate.

Lipolytic activity

The lipolytic activity of four isolates of *Xanthomonas corylina* was determined by using Starr and Burkholder's spirit blue agar method (29). All of the isolates tested exhibited positive lipolysis of cottonseed oil.

Temperature relations

The relation of temperature to the growth of *Xanthomonas corylina* was determined by two methods which gave similar results. In the first method, duplicate dextrose-nutrient broth cultures of the pathogen were incubated in incubators held at controlled temperatures ranging from 1° C. to 40° C., and the growth as indicated by turbidity was observed. In the second method, "giant" colonies of the pathogen were grown in quadruplicate on Difco potato-dextrose agar plates at controlled temperatures. The optimum temperature for the growth of this organism in culture is between 28° C. and 32° C., the maximum (in liquid media) approximately 37° C., and the minimum between 5° C. and 7° C.

Thermal death point

The thermal death point of *Xanthomonas corylina* is approximately 53° C. This was determined as follows: 2 ml. of a pure aqueous bacterial suspension, made by adding 10 ml. of a 20-hour-old beef extract-peptone broth culture of the pathogen to 40 ml. of sterile distilled water, was placed into each of a number of small glass test tubes, 101 mm. long, 10½ mm. in diameter, and 1 mm. in wall thickness. The tubes containing the pure water suspensions of the pathogen were then suspended in duplicate for 10 minutes in a water bath at the desired temperature. The temperature of the bacterial suspensions in the tubes was checked as to equilibrium with that in the surrounding water bath for each new adjustment.

Temperature fluctuations of the water bath during the thermal death point determinations were never greater than ± 0.5° C. At the end of the 10-minute period, the tubes were removed and immediately plunged into ice water in order to prevent further action of the heat on the bacteria. Sub-cultures in beef extract-peptone broth and potato-dextrose agar were made from the heat-treated bacterial suspensions to determine the viability of the organisms. All the bacteria in the water suspensions were killed with approximately 10 minutes' exposure at 53° C. ± 0.5° C. or above, but not below this temperature.

The thermal death time of one isolate of *Xanthomonas corylina* was found to be more than 8 minutes and less than 10 minutes at 53° C. ± 0.5° C. with a concentration of 9,000,000 bacteria per ml., in nutrient dextrose broth at pH 6.9.

Light relations

In determining the relation of light to the growth of *Xanthomonas corylina*, potato-dextrose agar streak cultures were incubated in duplicate at 25° C. in (a) diffused light, and (b) darkness. Normal development resulted in both light and darkness indicating that illumination through glass exerts no detectable influence on the development of the pathogen in culture.

Longevity in culture media

Xanthomonas corylina is apparently a relatively long-lived organism in culture media. On Difco potato-dextrose agar (pH 5.6) in screw top test tubes, incubated at room temperature, the organism was still alive after 197 days.

Biochemical Reactions of Significance

Specificity tests

Burkholder and Starr (7) have shown that certain biochemical reactions are particularly useful in determining species of *Xanthomonas*. The reactions of five isolates of *Xanthomonas corylina* to these specificity tests were studied with the following results:

Tolerance to sodium chloride: All five of the isolates studied grew rapidly in 3 per cent sodium chloride broth. However, in 4 per cent sodium chloride broth no growth occurred within 7 days.

Tyrosinase activity: All of the isolates studied were tyrosinase negative. Growth, but no red or brown color, appeared in a nutrient solution containing 0.05 per cent tyrosine after 7 days.

Utilization of sodium tartrate: Each of the five isolates studied failed to utilize sodium tartrate as a carbon source in standard synthetic medium containing this salt in 0.15 per cent concentration (7). Negative results also were obtained with 1 per cent sodium tartrate, as shown in Table 2.

Reduction of nitrates

Using reagents and methods recommended in the Manual of Methods for Pure Culture Study of Bacteria (8), nitrates were not reduced to nitrites within 7 days by any of the six isolates studied when grown in the following media: beef-extract peptone broth plus 1 per cent potassium nitrate, synthetic nitrate medium with 0.1 per cent potassium nitrate, synthetic nitrate medium with 0.1 per cent potassium nitrate plus 0.1 per cent Difco yeast extract. No gas developed nor was ammonia formed within 7 days by any of the six isolates studied. Tests for nitrates were positive throughout.

Liquefaction of sodium ammonium pectate

Ability to decompose pectic substance was tested on the sodium ammonium pectate medium, with and without the addition of 1 per cent Difco yeast extract as described by Starr (31). Four of six isolates studied produced distinct liquefaction of the media in from 5 to 15 days at 30° C.; two failed to liquefy the medium within 25 days. Except in one instance, liquefaction was slower in the medium containing yeast extract; as shown by the indicator, more acidity developed in this medium. While all cultures showed abundant growth, the amount of liquefaction varied from a small crater to a layer approximately one-half inch deep within 25 days. A culture of *Erwinia carotovora* (L. R. Jones) Holland, used for comparison, produced practically complete digestion of the pectate medium within 15 days.

Serological Relationships

The results of serological studies indicate that different strains of *Xanthomonas corylina* exist.

Methods

Forty-eight different cultures of *Xanthomonas corylina,* isolated from cankers and buds on diseased trees in widely scattered filbert orchards in Western Oregon, were used in these studies. Repeated platings were made to purify the cultures. While dissociation apparently occurs quite readily in cultures of this pathogen, only the cultures that remained smooth were used in these studies. The isolates were grown on glucose-peptone agar.

Antibody production

Ten of the isolates were selected to produce agglutinin antibodies in rabbits. The rabbits were test bled to determine whether there were any natural antibodies present but none was found. Each isolate was grown on glucose-peptone agar slopes for 48 hours at 30° C. The growth was washed off with 10 cc. of physiological salt solution. Since this pathogen produces considerable capsular material, many of the organisms remained in clumps. The suspensions were centrifuged at slow speed to remove most of the larger clumps and the suspended cells were removed and used to produce the antibodies in the rabbits.

The suspensions were standardized to approximately 500 million per ml. Generally good agglutinating serum is produced by live organisms given intravenously. The first dose consisted of 0.1 ml. of suspension. Injections were repeated at 5-day intervals until 10 or 12 had been made. Several of the rabbits lost considerable weight and developed severe shock. Some of them died suddenly after 4 or 5 injections, probably because of development of hypersensitivity to the micro-organism. In some cases the amount of the dose injected was decreased to 0.01 ml., but severe reactions continued and death resulted in several more rabbits. Additional suspensions were made to which 0.3 per cent formalin was added, and were incubated at 37° C. for 48 hours before any were used. No further severe reactions occurred and none of the rabbits died. Test bleedings were frequently made to observe titers of the serum.

The final titer of the serum from the various isolates ranged from 1 : 80 to 1 : 10,000 (Table 4). This wide range may be due to variability or difference in the antigenic qualities of the isolates to variations in the ability of the rabbits to produce antibodies. It was observed that the titer decreased very rapidly when further injections were made in some of the rabbits. When the maximum titer had

Table 4. VARIATIONS IN THE TITER OF THE RABBIT SERUM PRODUCED BY DIFFERENT ISOLATES OF *Xanthomonas corylina*.

Isolate number	Serum dilutions								
	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120	1-10240
5031	+[1]	+	+	+	—[2]	—	—	—	—
5092	+	+	—	—	—	—	—	—	—
5151	+	+	+	+	+	+	+	+	±[3]
5164	+	+	+	+	+	+	+	+	—
5146	+	+	+	+	+	+	—	—	—

[1] + = Agglutinated.
[2] — = Not agglutinated.
[3] ± = Questionably agglutinated.

been reached, each rabbit was bled from the jugular vein. About 50 ml. of serum was obtained and 0.1 per cent of merthiolate added as a preservative.

Cross agglutinations

The bacterial suspensions used in the agglutination tests were grown on glucose-peptone agar slopes for 48 hours at 30° C. Clumps were removed by slow-speed centrifuging. The suspensions were then diluted with physiological salt solution to a density of 2 on the McFarland scale (33). Dilutions up to 1 : 10,000 of the various sera were made and the suspensions of bacteria added. They were well mixed by shaking and then placed in a water bath at 37° C. for 4 hours. With one exception, the aggregates were very fine and are probably somatic. One isolate, number 5031, gave very coarse flaky clumps. This culture may have gone "rough" or the agglutinin may have been flagellar rather than somatic. This serum was the only one that agglutinated all of the isolates (Table 5). On the other hand, isolate number 5092 was agglutinated only by its own homologous serum. The agglutin-ability of the other isolates was not so limited and good agglutination could be observed when any one of several sera was used.

From the results given in Table 5 it is evident that the various isolates vary in their ability to be agglutinated. It is very probable that the antigenic structure of the various isolates contains some components not present in other isolates. This is not an unusual character since it has been observed in other species of bacteria, particularly in the *Salmonella* group. Another possible explanation might be that the isolates under study might exist in either monophasic or diphasic form.

Resemblance to Xanthomonas juglandis

Xanthomonas corylina closely resembles *Xanthomonas juglandis* (Pierce) Dowson (the cause of walnut bacteriosis), morphologically, culturally, and biochemically. So close is this resemblance that these two organisms cannot be differentiated by morphological, cultural or biochemical means. However, serological differences and differences in pathogenicity exist which prove that they are pathogenically different (19).

Table 5. SUMMARY OF THE RESULTS OF CROSS-AGGLUTINATION STUDIES OF 48 DIFFERENT ISOLATES OF *Xanthomonas corylina*.

Serum number	Number of isolates agglutinated by the indicated serum
5092	1
5146	18
5151	27
5031	48
5346	2

PATHOLOGICAL HISTOLOGY

Materials and Methods

Infected tissues selected for study were fixed in formaldehyde-acetic-alcohol.[7] The stains used to differentiate the pathogen from the host tissues were picro-aniline blue[8] and orange G, rose bengal[9] and light green, carbolthionin[10] and orange G, Giemsa and orange G, and dilute solution of safranin and light green. Of these stains, picro-aniline blue and orange G gave the best results on the widest variety of material. With this combination the bacteria stain a deep purple, the matrix in which they are generally imbedded stains a light yellow, and the cell walls of the host tissue stain a deep orange.

Invasion and Migration of the Pathogen

Filbert bacteriosis is primarily a disease of the parenchymatous tissues. The vascular tissues, if attacked at all, are invaded only in the later stages of parasitism.

Figure 13. Photomicrograph of a group of cortical cells in an active canker; note cavities within the tissues due to bacterial activity.

The bacteria gain access to the tissues through stomata and wounds. From the point of entry the bacteria invade the tissues through the intercellular spaces. At first, the organisms apparently migrate through the tissues in a free-swimming condition. Later, as the organisms increase in number they consolidate and appear to operate collectively, apparently migrating in mass. At this stage the bacteria are imbedded in a matrix, which is evidently of a different composition from the bacteria because it has different staining reactions.

A swelling and a modification in the staining reactions of the

[7] 50 per cent alcohol ..... 100 ml.
Formalin ..... 6.5 ml.
Glacial acetic acid ..... 2.5 ml.
[8] Saturated aqueous solution of aniline blue ..... 25 ml.
Saturated aqueous solution of picric acid ..... 100 ml.
[9] Rose bengal ..... 1 gram
5 per cent phenol ..... 100 ml.
[10] Thionin ..... 0.1 gram
5 per cent phenol ..... 100 ml.

host cell walls occur shortly after the tissues are invaded, followed by the appearance of openings in the cell walls that permit the bacteria to gain access to the cell lumen. Large cavities filled with bacteria imbedded in a matrix are subsequently formed in the tissues (Figure 13).

LIFE HISTORY OF THE CAUSAL ORGANISM

A knowledge of the manner in which the causal organism is carried over from one season to the next and the methods by which the inoculum is disseminated is essential to an adequate understanding of the epidemiology of the disease and to the development of satisfactory methods of control. Consequently, studies of the life history of *Xanthomonas corylina* in relation to pathogenesis and to control have been given special attention.

Overwintering of the Causal Organism

Barss (2) reported that the causal organism lives over from one season to the next in hold-over cankers and in infected buds.

Relation of branch and trunk lesions

Studies carried on over a period of 8 years show that, under Oregon conditions, *Xanthomonas corylina* lives over from one season to the next primarily in lesions on larger branches and trunks of trees as is indicated by the fact that the pathogen was isolated from such lesions in a comparatively large percentage of isolation attempts made (see Table 6, series 1 and 2).

Relation of twig lesions

Lesions on small twigs, 2 to 8 mm. in diameter, may also carry the causal organism over from one season to the next, but apparently they are not so important as cankers on the larger limbs and trunks (see Table 6, series 3). Evidently the pathogen dies out in a much larger percentage of twig lesions than it does in branch and trunk cankers.

Relation of infected buds

The pathogen may also be carried over from one season to the next in diseased buds, but evidently they are not so important as twig, branch, and trunk lesions. This conclusion is based upon the results of isolation studies, the data for which are given in Table 6, series 4.

Relation of the soil

Our studies indicate that *Xanthomonas corylina* is not carried over from one season to the next in the soil, as is shown by the fact that all attempts made to isolate the pathogen from soil collected from beneath badly infected filbert trees have been uniformly negative.

Table 6. STUDIES ON THE OVERWINTERING OF *Xanthomonas corylina;* CORVALLIS, OREGON; 1931-1939.

Series and dates of collections and platings	Number of trials	*X. corylina* isolated	
		Number	*Per cent*
Lesions on trunk of tree			
1, a January 1931	2	2	100
1, b January 1934	3	3	100
1, c March 1935	2	0	0
1, d December 1935	8	2	25
Lesions on larger branches[1]			
2, a February 1932	6	1	16.6
2, b February 1934	11	3	27.2
2, c January to March, 1935	8	2	25.0
2, d October to November, 1935	62	28	45.1
Lesions on twigs[2]			
3, a February 1931	54	3	5.5
3, b February to March, 1933	13	1	7.7
3, c January to February, 1934	81	26	32.1
3, d January to March, 1935	48	3	6.2
3, e October to November, 1935	66	7	10.6
3, f January 1939	9	3	33.3
3, g December 1939	8	0	0
Diseased buds			
4, a February 1931	43	10	23.2
4, b February 1933	14	0	0
4, c January to February, 1934	66	16	24.2
4, d November 1935	49	7	24.2
4, e January 1939	27	4	14.8
4, f[3] March 1948	7	3	42.9

[1]From 10 to 20 mm. in diameter at lesion.
[2]From 1 to 8 mm. in diameter at lesion.
[3]From inoculated potted trees in the greenhouse.

Seasonal Development of the Disease

A knowledge of the various factors associated with the development of the disease is essential to the development of effective and economical measures of control. While the early studies of Barss (2, 3) resulted in the discovery of a number of cardinal facts concerning the life history of the organism in relation to pathogenesis, gaps in our knowledge of the seasonal development of the disease still exist. Consequently, studies of the development of the disease in relation to the causal organism, its host, and the natural environment were initiated in 1931 and were actively carried on for a period of 8 years. These studies were conducted (a) in the greenhouse under partially controlled environmental conditions, and (b) in the field under natural conditions.

In greenhouse studies an attempt was made to evaluate the relative importance of the various environmental factors in the development of the disease. Approximations of the desired conditions or combination of conditions were obtained at will by suitable temperature and humidity equipment. In this way it was possible to study analytically the consequences of varying certain factors of the environment.

Seasonal Records

Field studies were carried on in a field station established in an orchard near Corvallis, Oregon. Visits were made to the station at frequent intervals throughout the season and detailed records kept of host and disease development.

Host development

The development of the Barcelona variety, the chief commercial filbert in Oregon, was followed throughout the season by observing a number of tagged shoots at approximately weekly intervals.

Disease development

Approximately 500 tagged twigs representatively situated in a number of trees were examined at approximately weekly intervals for the presence of current infections. Studies of disease development were supplemented by inoculating healthy shoots at frequent intervals throughout the season by spraying water suspensions of *Xanthomonas corylina* upon the uninjured surfaces with an atomizer.

Dissemination of the Pathogen

Relation to rainfall

Barss (2) reported that wet weather favors the spread of the causal bacteria. Our studies carried on over a period of 12 years show that rainfall is indeed one of the most important agencies concerned in the dissemination of the inoculum. As supporting evidence, the following observations and experiments are pertinent:

1. It has been observed that the greater the amount of rainfall during the infection period, the greater was the incidence of the disease. For example, in 1938, filbert bacteriosis was especially severe in Oregon. The preceding fall and winter—the critical period for infection—was abnormally wet. From September 1, 1937 to January 1, 1938, 29.05 inches of rain fell at Salem—the approximate center of the filbert industry in the state—distributed by months

as follows: September, 0.91 inch; October, 3.41 inches; November, 11.13 inches, and December, 13.6 inches. This was 11.63 inches above normal for this period in this locality.

2. Buds, leaves, and young stems were found infected in nature very early in the season at a time when there were very few, if any, insects present that might possibly be concerned in the spread of the disease.

3. Infection of leaves, buds, and stems of young shoots was induced at will by spraying with pure water suspensions of *Xanthomonas corylina* during periods of rainfall. Young shoots on potted Barcelona trees in the greenhouse were also infected at will by spraying on water suspensions of the causal organism and then placing them in a saturated atmosphere in a damp chamber for a suitable period.

4. Surveys made in commercial orchards show that trees vary considerably in the severity of the disease. In certain trees the incidence of infected twigs was quite high, while on neighboring trees it was comparatively low. Furthermore, the distribution of diseased twigs in the trees was quite variable. In some instances a high percentage of the twigs in a certain sector or part of a tree was found infected, while in other portions only a very small percentage of the twigs was diseased. If the inoculum were being disseminated by some such agency as insects, a relatively uniform distribution of the infected twigs in the trees would normally be expected with regard to both the trees infected and the distribution of diseased shoots in individual trees. These facts, however, fit in well with the conception that rain drip is the chief natural disseminating agency. The relative scarcity or abundance of sources of infection in different portions of a tree, or in different trees, could very well explain the variations noted.

5. That rain drip can indeed disseminate the pathogen was experimentally demonstrated by suspending twigs containing lesions directly above healthy young shoots on potted filbert trees in the greenhouse and then spraying water over the lesions, which then dripped upon the shoots below. Lesions developed on the leaves of some of the shoots after a suitable incubation period.

Relation to man

Field observations and experiments show that man is an important vector of the primary inoculum in young orchards 1 to 4 years old. From lesions on diseased nursery trees, the bacteria are spread to adjacent trees on unsterilized shears and knives used in pruning and suckering young trees. Numerous cankers have been

Table 7. RESULTS OF INFECTION TESTS FROM PRUNING WOUND INOCULATIONS; CORVALLIS, OREGON; 1931-1934.

Date of inoculation	Pruning wound inoculations[1]	
	Number made	Percent positive[2]
January 1933	39	46.2*
January 1934	7	14.3
February 1932	10	100.0*
February 1933	47	68.1*
February 1934	84	54.7
March 1932	9	100.0*
March 1933	41	26.8
March 1934	35	20.0
April 1933	34	11.8
April 1934	147	8.84
May 1931	17	23.5*
May 1933	55	18.2
May 1934	53	11.3
June 1933	48	18.8
July 1931	10	0.0
July 1933	33	30.3
August 1933	75	21.3
September 1931	19	74.7*
September 1933	26	46.2
October 1931	12	75.0
October 1932	30	63.3*
October 1933	22	27.3
November 1931	7	57.1*
November 1932	32	18.8*
November 1933	21	23.8
December 1931	16	75.0*
December 1932	31	58.1*
December 1933	29	51.7

[1]Lateral branches removed with pruning shears on which had been previously smeared a pure culture of *Xanthomonas corylina*.
[2]As determined by the formation of cankers about the inoculated pruning wounds.
* Isolations made from certain of the cankers and *Xanthomonas corylina* reisolated.

found about pruning wounds on young trees and lesions have been produced by cutting off branches with shears on which had been smeared a pure culture of the pathogen (see Table 7). In plantings more than 4 years of age, however, man is not an important vector. Practically all cankers present on older trees occur on twigs and branches at the base of dead buds and infected shoots and not around pruning wounds.

Relation to insects

Barss (2) was of the opinion that, under natural conditions, the pathogen is disseminated primarily by insects that feed upon infected filbert trees and then carry the germs on their contaminated mouth parts or feet from one place to another. Our studies carried on over a period of 12 years have failed to substantiate the view that insects are concerned to any significant extent in the dissemination of either the primary or the secondary inoculum. As supporting evidence, the following observations and experiments are presented:

1. No insect has been observed feeding upon or in contact with the bacterial exudate coming from lesions.

2. Attempts to transmit the disease by aphids, the most abundant form of insect life on the filbert, were negative. In certain tests a number of aphids were allowed to feed upon infected leaves on potted trees in the greenhouse for several days, after which they were transferred to young, healthy leaves on other potted plants. No infection resulted.

3. In field studies of the seasonal development of insects in relation to disease development, insect development could not be correlated with disease development. The peak of development of practically all the common insect pests of the filbert occurred long after disease development was practically over.

Mode of Entry of the Pathogen

Into buds

The pathogen gains access to the buds through stomata in the outer bud bracts. Entrance through stomata occurs only when the tissues are wet.

Into leaves

Access to the leaves occurs primarily through stomata. Wounds or mechanical injuries of various kinds in the tissues may, under some conditions, also serve as avenues of entry.

Into stems of shoots of the current season's growth

Entry into stems of shoots of the current season's growth occurs in one of two ways, either directly through stomata in the green tissues of the succulent young stem or indirectly by migration of the bacteria from an attached infected bud scale. An example of the latter type of infection is shown in Figure 2.

Into 2- to 4-year-old branches and main stem of tree

Entry into the larger branches and main stem of the tree occurs either through pruning wounds or indirectly by migration of the bacteria from attached infected buds or infected young shoots. In young trees, up to 4 years of age, entry appears to occur primarily through pruning wounds, as most of the cankers on the larger branches and main stem are located about such wounds (Figure 8). In older trees, however, most infections on the branches are apparently from the migration of the bacteria from attached infected buds and diseased shoots of the current season's growth.

Period of Incubation

In buds

The time elapsing between the inoculation of the buds and the first visible symptoms of the disease varies considerably with environmental conditions and with the stage of bud development. In experiments carried on in the greenhouse in 1935-1936, the first symptoms of infection from inoculations made just before the leaves fell became visible in from 90 to 120 days after inoculation. In nature, however, the incubation period is much longer, as buds on trees in the orchard inoculated during the latter part of September 1935 did not show any visible symptoms until 200 to 225 days after inoculation.

In leaves

In experiments carried on under greenhouse conditions the period of incubation in the leaves ranged from 8 to 23 days, depending on the incubation temperature and stage of leaf development. In general, the higher the temperature and the younger the leaves, the shorter was the period of incubation. Thus, in one experiment, incipient lesions on the leaves were noted eight days after inoculation when incubated at an average temperature of 22° C., while at 11° C. it took 17 days for the lesions to become evident.

Infection Experiments

Extensive infection experiments were performed over a period of 12 years, both in the field and under controlled greenhouse conditions. Specialized methods and temperature and humidity control equipment were devised by which approximation of desired conditions or combinations of conditions were obtained at will.

Field studies

Shoots on young trees in various stages of development were inoculated throughout the year during both rainy and dry periods by spraying them with water suspensions of *Xanthomonas corylina* with an atomizer. No post-inoculation moist treatment beyond that bestowed by nature was given the inoculated shoots. These inoculations were supplemented by wound inoculations made throughout the season by wounding the trunks and branches of young filbert trees with a sterile needle and spraying a water suspension of the pathogen into the wounds.

Greenhouse studies

One-year-old tip-layered Barcelona filbert trees were planted in soil in 8-inch pots and incubated in the greenhouse. When the young shoots on the trees had reached the desired age, they were inoculated by spraying with water suspensions of *Xanthomonas corylina*. After inoculation, the trees were placed in a damp cham-

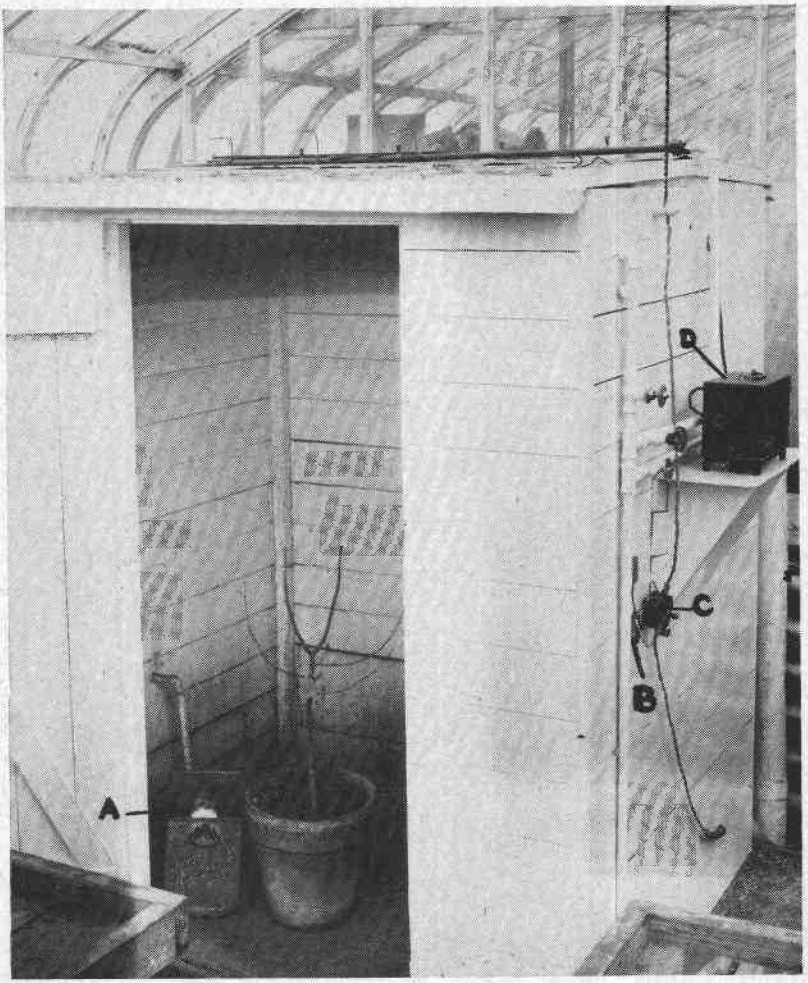


Figure 14. Inoculation chamber used for control of temperature and humidity: A, Electric heating element; B, Thermoregulator; C, Relay; D, Recording thermometer.

ber in which the desired temperature and humidity were maintained by an electric immersion heating element covered with wet absorbent cotton, the ends of which were immersed in a constantly maintained reservoir of water. (See Figure 14.) A De Kotinsky thermo-regulator and a relay hooked up in series with the electric heater maintained reasonably constant temperatures which were recorded by a thermograph.

When the electric current passed through the heating element, water vapor was given off from the moist cotton filling the chamber, thus providing a saturated atmosphere. Moist pieces of cheesecloth were suspended from troughs of water around the walls to aid in the maintenance of a saturated atmosphere. Condensation forming on the host organs under these conditions provided an adequate supply of moisture for infection. The temperature within the chamber was controllable, with variations of $\pm 2°$ C., in a range from 4° C. (in winter and spring only) to 35° C.

After inoculation the trees were moved to a greenhouse in which the temperature averaged 22° C. After suitable incubation periods, counts were made of (a) the number of leaves infected and (b) the average number of lesions per leaf infected.

Role of moisture

The results of both field and greenhouse studies conclusively show that the presence of moisture on the host parts for a suitable period after inoculation is necessary for infection. No infection occurred from simply smearing the surface growth of agar cultures of *Xanthomonas corylina* on the uninjured surfaces of young leaves and buds. Only when the inoculated plants were put in a moist chamber for a suitable period after inoculation did infection occur.

The minimal period of continuous wetting necessary for infection was found to vary with the age of the host organs, the extent of opening of the stomata, and the degree of water-soaking of the tissues.

Infection of leaves occurred with only one hour of continuous wetting when the leaves were young, the stomata wide open, and the tissues "water-congested." The older the leaves and the less the amount of water in the intercellular spaces, the longer was the minimal period of wetting required for infection. An increase in the number and size of the lesions accompanied prolongations of the moist period. The longer periods of wetting evidently improve the chances for stomatal penetration by the bacteria and also induce a water-soaking of the tissues which expedites the growth and rapid migration of the bacteria through the tissues.

Table 8. The Relation of Moisture to the Infection of Filbert Leaves and Buds by *Xanthomonas corylina*; Greenhouse, Corvallis, Oregon, 1931-1936.

Series number	Period in moist chamber		Leaves				Buds	
					Average number of lesions			
	Before inoculation	After inoculation	Number inoculated	Infected	On 10 most severely infected leaves	Per leaf infected	Number inoculated	Infected
	Hours	*Hours*		*Per cent*				*Per cent*
1, a	0	0	262	0	.....	0	....	....
1, b	0	3	375	0	.....	0	....	....
1, c	0	6	98	12	.....	4	....	....
1, d	0	48	402	15	.....	7	....	....
1, e	0	72	421	74	.....	9	....	....
2, a	0	1	136	14	.....	3	....	....
2, c	0	2	172	13	.....	2	....	....
2, d	0	8	71	28	.....	5	....	....
2, e	0	24	105	26	.....	6	....	....
3, a	0	24	13	2	2	2	....	....
3, b	0	48	172	45	251	40	....	....
3, c	0	72	317	75	570	86	....	....
4, a	0	0	347	0	0	....	....	....
4, b	0	29	339	19	260	....	....	....
4, c	24	0	359	8	33	....	....	....
4, d	24	29	312	34	685	....	....	....
5, a	12	12	540	8	19	7	....	....
5, b	24	24	431	15	213	40	....	....
5, c	48	48	502	22	398	55	....	....
6, a	0	0	.....	....	.....	....	44	0
6, b	0	1	.....	....	.....	....	81	6
6, c	0	2	.....	....	.....	....	74	6
7, a	24	24	.....	....	.....	....	199	7
7, b	48	24	.....	....	.....	....	203	10
7, c	72	24	.....	....	.....	....	207	29

Subjecting the plants to moist treatment before inoculation was found to increase the number and size of the lesions. Such treatment produces water-soaking of the tissues adjoining the stomata and facilitates connection of the surface water with that in the intercellular spaces through open stomata. The penetration and migration of the bacteria in the tissues is thereby expedited. Typical examples of data from greenhouse experiments appear in Table 8.

Role of temperature

Temperature apparently plays a role in the infection of the tissues by *Xanthomonas corylina,* although in normal range it does not appear to be as important as moisture. Relatively warm temperatures (about 20° C.) at the time of inoculation are apparently somewhat more favorable for infection of the leaves than lower temperatures. As shown by the data given in Table 9, a higher percentage of the inoculated leaves generally became infected and there was a greater number of lesions per infected leaf at 22° C. and higher than occurred at lower temperatures. Furthermore, below 20° C. the incubation period is longer than at higher temperatures. Thus, in one typical experiment, disease symptoms appeared on inoculated leaves in 8 days at an average incubation temperature of 21° C. while at 13° C. the lesions did not become evident until after 13 days.

Table 9. THE RELATION OF TEMPERATURE TO THE INFECTION OF FILBERT LEAVES BY *Xanthomonas corylina;* GREENHOUSE, CORVALLIS, OREGON; 1931-1936.

Series number	Period in moist chamber			Leaves			
						Average number of lesions	
	Average temperature	Before inoculation	After inoculation	Number inoculated	Infected	On 10 most severely infected leaves	Per leaf infected
	Degrees C.	*Hour*	*Hours*		*Per cent*		
1, a	7.5	0	48	123	13	.....	3
1, b	16.5	0	48	264	40	.....	3
2, a	16.4	48	48	674	4	78	....
2, b	25	48	48	463	60	389	....
3, a	16.5	48	24	277	26	62	....
3, b	24.8	48	24	510	14	539	....
4, a	7.8	0	24	523	11	.....	4
4, b	22.8	0	24	150	35	.....	3
5, a	7.5	0	20	330	24	.....	3
5, b	24	0	20	212	41	.....	4
6, a	13.5	0	96	227	29	.....	5
6, b	24	0	96	127	34	.....	10

Relation of reduced vigor from adverse environment conditions

The results of numerous field surveys made during the course of these investigations show that the existence of adverse growing conditions during the first few years of the life of an orchard reduces the vigor of the trees thereby predisposing them to infection. Among the most important adverse growing conditions are poor soil drainage, cold injury, sunscald, and drought.

Relation of the age of the host organs to infection

Extensive infection experiments carried on under both field and greenhouse conditions definitely show that the leaves and buds pass through a stage of maximal susceptibility into a period of ever-increasing resistance. This increased resistance is at first manifested by a longer incubation period and later by a more restricted development of the lesions.

The period for bud infection

The buds are most susceptible to infection from the time when they are about three-fourths grown until they open in the spring (i.e., during the fall, winter, and early spring). They are apparently least susceptible when they are very young, during late spring and early summer (Table 10).

The period for stem infection

The stems of young shoots of current growth are susceptible to infection through the stomata just as soon as the buds open and functional stomata develop. They are most susceptible while they are succulent and actively growing.

The shoots vary in susceptibility throughout their length. The apex of the stem remains susceptible to infection much longer than the more mature base. After the shoots cease elongating and lose their succulency they become highly resistant to stomatal infection.

The tissues of twigs and branches, 1 to 3 years of age, are most susceptible to infection during the fall, winter, and early spring. The tissues possess a considerable degree of resistance to infection during the summer (Table 10). Infection of twigs and branches more than one year of age occurs only through wounds and from migration of the bacteria from attached infected buds (see Figures 6 and 8).

The period for leaf infection

The leaves are susceptible to infection just as soon as the dormant buds open sufficiently to expose the longitudinally folded leaflets. Inoculations made at this stage result in the infection of the

midribs and veins of the leaves. The leaf lamina is most subject to infection just after the leaves unfold. As the leaves approach maturity they became increasingly resistant, as manifested through prolongation of the incubation period and restricted development of the lesions. After the leaves attain their maximal size, they are apparently no longer susceptible. The leaves on a shoot vary in susceptibility to infection with their age. Young leaves at the tip of a shoot are more susceptible than the more mature ones at the base.

THE DEVELOPMENT OF EPIDEMICS[11]

Sources of Infection

Primary: The results of extensive field studies carried on during the course of these investigations show that, in most cases, the causal organism is brought into the orchard on infected nursery stock. The disease is present in practically every nursery in the Pacific Northwest.

While most of the reliable nurserymen cull their stock and remove all visible cankered trees, many branch and trunk cankers remain undetected as the cuticle often changes but little, if any, in appearance. In addition, infected buds are frequently present in many nursery trees; these are disregarded altogether by nurserymen in culling operations.

Lesions on the branches and trunks and infected buds on these diseased nursery trees serve as primary sources of infection from which the inoculum is spread (a) by man on pruning and suckering tools and (b) by rain.

Secondary: Bacteria from primary infections on buds, branches, and trunks constitute the source of secondary infection.

Factors governing primary infection

The incidence of primary infections in an orchard is governed by the relative number of diseased nursery trees. The greater the number of diseased nursery trees the greater the number of primary infections.

Factors governing secondary infection

The intensity of secondary infection is primarily dependent on (a) the number of primary sources of infections in the trees, and

[11]The desirability of using the term "epidemic" in connection with plant diseases is questioned by some individuals who contend that the term "epiphytotic" is more explicit. However, we choose to use the term "epidemic" to denote a broad biological concept that is not limited by the kind of organism that happens to be diseased. This concept permits a similar breadth of scope for the term "epidemiology," thereby avoiding the use of the awkward term "epiphytology."

Table 10. SEASONAL SUSCEPTIBILITY OF FILBERT TREES TO INFECTION BY *Xanthomonas corylina* AS DETERMINED BY INOCULATIONS; CORVALLIS, OREGON; 1931-1936.

Date of inoculations	Stage of host development at time of inoculation	Wound inoculations		Atomizer inoculations[1]
		Number made[2]	Positive[3]	Relative number of blighted buds and shoots
			Per cent	
January 1932	Delayed dormant[4]	18	50*	
January 1935	Delayed dormant	28	100	
February 1932	Early green tips[4]	16	44*	
February 1934	Early green tips	27	22	
February 1935	Early green tips	23	100	
February 1936	Early green tips	15	93	Few
March 1933	Late green tip[4]	38	5	
March 1934	Late green tip	72	19	
March 1935	Late green tip	28	96	
March 1936	Late green tip	8	88	Few
April 1932	O.C.; $\frac{1}{8}$ leaf stage[4]	2	0	
April 1934	O.C.; $\frac{1}{8}$ leaf stage	26	0	
April 1935	O.C.; $\frac{1}{8}$ leaf stage	24	0	
April 1936	O.C.; $\frac{1}{8}$ leaf stage	25	28	None
May 1931	Leaves $\frac{1}{2}$ grown	11	36*	
May 1934	Leaves $\frac{1}{2}$ grown	13	23	
June 1933	Leaves $\frac{3}{4}$ full grown	6	18	
June 1936	Leaves $\frac{3}{4}$ full grown	27	0	None
July 1931	Leaves $\frac{3}{4}$ full grown	19	26*	
July 1932	Leaves $\frac{3}{4}$ full grown	16	6	
August 1931	Leaves full grown	28	10*	
August 1932	Leaves full grown	24	38	
August 1935	Leaves full grown	19	58	Few
September 1933	Leaves full grown	22	4	
September 1934	Leaves full grown	6	18	
September 1935	Leaves full grown	14	100	Many
October 1933	One-fourth defoliated	11	63	
October 1935	One-fourth defoliated	27	89	Very many
November 1931	One-half defoliated	25	56*	
November 1933	One-half defoliated	9	0	
November 1934	One-half defoliated	8	0	
November 1935	One-half defoliated	8	88	Very many
December 1933	Dormant	13	15	
December 1934	Dormant	10	0	
December 1935	Dormant	14	93	Few

[1]Inoculum sprayed on uninjured leaves and shoots with an atomizer during rainy periods.
[2]Made by wounding branches and trunks of 1- to 3-year-old filbert trees with a sterile needle dipped in pure water suspensions of *Xanthomonas corylina*.
[3]As determined by the formation of cankers about the point of inoculation; the asterisk indicates that isolations were made from certain of the cankers and *Xanthomonas corylina* was reisolated.
[4]Delayed dormant = buds swollen but tips of leaves not yet visible;
Early green tip = tips of leaves visible in bud cluster;
Late green tip = leaves beginning to separate in bud cluster;
O.C. = open cluster, leaves separated from bud clusters.
*Isolations made from certain of the cankers and *Xanthomonas corylina* reisolated.

(b) the extent of rainfall during the infection period. The greater the number of primary sources of infections in the trees and the greater the extent of rainfall during the infection period, the greater will be the incidence of secondary infections.

The special significance of rainfall

Rainfall is of special significance in the epidemiology of filbert bacteriosis, being the most important single natural agency concerned in determining the severity of the disease. There may be an abundance of inoculum present in the trees; yet epidemic outbreaks of

the disease will not occur unless rains of sufficient duration occur during the infection period. The greater the extent of rainfall during the infection period, the greater will be the severity of the disease.

The special significance of man

The results of numerous field observations and experiments show that man is an important vector of the primary inoculum in young orchards 1 to 4 years old. From lesions on diseased nursery trees the bacteria are spread to adjacent trees by man on shears and knives used in pruning and suckering young trees. The tools become contaminated with the bacteria by cutting through lesions on diseased trees, and the organisms are subsequently deposited on the cut surfaces of wounds made on adjacent trees.

Critical period for the development and control of epidemics

The critical period for the development and control of epidemics of filbert bacteriosis extends from the time the buds are about three-fourths grown (late summer) until they open the following spring. The forepart of this period (during the fall) appears to be the most critical in relation to control.

STUDIES OF CONTROL MEASURES

Excision Experiments

Barss (2) reported that the removal of infection sources from the tree will aid in the control of the disease.

In 1932 excision experiments were carried on in an attempt to determine whether this disease could be controlled by excision measures alone. In these experiments, all detectable sources of infection were removed during the summer from a block of infected young trees. Although there was a diminution in the incidence of the disease, considerable infection was still present in the excised trees the following season, which indicates that this disease cannot be satisfactorily controlled by excision measures alone. It is practically impossible to detect and remove all sources of infection, particularly diseased buds.

In connection with the use of the excision measures as a means of controlling the disease, the question arose as to whether it would be possible to kill bacteria within the lesions by painting the surface with a penetrating disinfectant without injuring the host tissues. A 1 per cent solution of copper nitrate—a chemical recommended by Owens (28) for the control of bacterial gummosis of cherries—was selected for trial. The solution was painted on the surface of a

number of cankers after first scarifying and slitting the bark in a number of places to expedite entrance of the disinfectant. After an 8-month interval the treated cankers were examined for evidence of extension of the diseased areas. A number of the treated cankers were still active at the end of this period; indicated by the further extension of the canker margins. It would seem from these limited studies that the use of this particular chemical on the surface of filbert cankers will not kill the pathogen within the tissues.

Varieties in Relation to the Disease

While varietal immunity to filbert bacteriosis apparently does not exist, varieties do differ considerably in the degree of susceptibility to this disease. Barss (2) first called attention to this fact. In 1926 he reported that the Aveline types are more susceptible than the Barcelona variety.

Observations and experiments made over a 15-year period indicate that the Barcelona, Du Chilly, White Aveline, and Brixnut are the most susceptible of the more important commercial varieties grown in the Pacific Northwest, and the Daviana and Bolwyller the most resistant.

In this connection, it is noteworthy that the Turkish filbert (*Corylus colurna*) appears to possess a high degree of resistance to this disease, as is indicated by the fact that puncture (wound) inoculations made into 1-, 2-, and 3-year-old stems were uniformly negative. However, even this species is apparently not immune to the disease; lesions have been produced on Turkish filbert leaves by inoculations with an atomizer.

Spraying and Dusting Experiments

Barss first suggested that spraying with bordeaux mixture in the fall might aid in controlling this disease (2).

Studies of the control of the disease by spraying and dusting were initiated by the senior author in 1935 and have been actively carried on in cooperation with the Oregon Agricultural Experiment Station and a number of Oregon filbert growers for 12 seasons, 1935-1947. A detailed discussion of methods, materials, and results of investigations follows:

Field tests of spray and dust materials

The investigations carried on have been concerned primarily with the following problems:

A. The comparative efficacy of various spray and dust materials.

B. The effect of varying the number and timing of the applications.
C. The possibility of combining insect and disease control programs.

Spray and dust materials tested

Home-made bordeaux mixture: Prepared by mixing together dilute solutions of commercial copper sulfate and quick (caustic) lime or fresh hydrated lime in designated proportions.

Yellow cuprous oxide spray: A commercial dry preparation containing from 82 to 86 per cent, by weight, of metallic copper.

Monohydrated copper sulfate and lime dust: Factory-mixed dusts containing from 25 to 30 per cent monohydrated copper sulfate and varying amounts of hydrated lime and various types of "extenders" and powdered spreaders and stickers to improve the physical properties and adhesiveness of the dust. In some instances, 40 per cent lead arsenate was added for the control of the filbert worm.

Cuprous oxide dust: Factory-mixed dusts composed of 10 per cent yellow cuprous oxide with varying amounts of talc, bentonite, diatomacious earth and other similar materials added to improve the physical properties and adhesiveness of the dusts. In some instances, 40 per cent of lead arsenate was added for the control of the filbert worm.

Wetting and adhesive agents used

Casein spreaders: The following casein-type spreaders were used: "Fluxit," powdered casein, and skim milk powder.

B-1956 spreader: A proprietary liquid wetting and adhesive agent, the active ingredient of which is glycerol phthalic resin.

Penetrol: A commercial liquid wetting and adhesive agent, composed of 90 per cent of sulfonated, oxidized petroleum hydrocarbons (32°34° Bé).

Grasseli spreader-sticker: A proprietary liquid spreading and adhesive agent; chemically, it is sodium oleyl sulfate and a synthetic resinous sticker.

Method of determining efficacy of spray and dust materials

From 1930 to 1939, the comparative incidence of the disease in the various plots was determined by comparisons of the numbers of blighted buds and twigs in a representative number of trees—generally 4 or more—in a plot. From 1940 to 1947, the percentage of infected buds and shoots in a plot was determined by examining, as

one moved around the trees, a number of twigs and buds in, generally, four or more trees located near the middle of the plot, so as to minimize the possibility of spray or dust contamination from adjoining plots. The numbers of healthy and infected shoots and buds found were recorded on hand tally registers.

Table 11. THE EFFICACY OF VARIOUS SPRAY MATERIALS FOR THE CONTROL OF FILBERT BACTERIOSIS, WESTERN OREGON AND WASHINGTON, 1935-1939.

Year, plot number, orchard, material and concentration	Number of applications	Number of trees in plot	Results		
			Trees with small amount of bud and twig blight[1]	Trees with moderate amount of bud and twig blight[2]	Trees with a great amount of bud and twig blight[3]
			Per cent	*Per cent*	*Per cent*
1935-36					
(J. J. Doerffler, Silverton, Oregon)					
1 Bordeaux, 8-8-100	2	74	89	8	3
2 Not treated	0	60	38	43	18
(H. M. Bibby, Salem, Oregon)					
3 Bordeaux, 8-8-100	2	21	76	24	0
4 Not treated	0	65	38	45	17
(O.S.C., Corvallis, Oregon)					
5 Bordeaux, 8-8-100	3	6	83	17	0
6 Not treated	0	14	0	43	57
(H. Quick, Chehalis, Washington)					
7 Bordeaux, 8-8-100	2	55	82	11	7
8 Not treated	0	114	6	33	61
(McLoughlin, Woodburn, Oregon)					
9 Bordeaux, 8-8-100	2	27	93	7	0
10 Not treated	0	72	14	54	32
1936-37					
(McNary and Stoltz, Salem, Oregon)					
11 Bordeaux, 8-8-100	2	72	86	14	0
12 Not treated	0	89	36	57	7
(McLoughlin, Woodburn, Oregon)					
13 Bordeaux, 8-8-100	1	54	93	7	0
14 Not treated	0	55	65	32	3
(Ringo, Albany, Oregon)					
15 Cuprous oxide, 1-100	1	22	100	0	0
16 Bordeaux, 8-8-100	1	14	93	7	0
17 Not treated	0	21	60	40	0
1937-38					
(McLoughlin, Woodburn, Oregon)					
18 Bordeaux, 8-8-100	1	17	94	6	0
19 Not treated	0	35	11	60	29
(Ringo, Albany, Oregon)					
20 Bordeaux, 8-8-100	2	16	94	6	0
21 Not treated	0	15	13	20	67
1938-39					
(Beagles, Newberg, Oregon)					
22 Bordeaux, 8-4-100	1	52	71	29	0
23 Copper oxalate, 3-100	1	9	33	44	23
24 Not treated	0	54	31	38	31

[1] 1 to 25 diseased buds and/or shoots per tree.
[2] 25 to 75 diseased buds and/or shoots per tree.
[3] 75 or more diseased buds and/or shoots per tree.

Table 12. The Efficacy of Various Spray and Dust Materials for the Control of Filbert Bacteriosis, Western Oregon, 1939-1946.

Year, plot number, orchard, material and concentration	Number of applications	Buds or young shoots		
		Examined	Infected	
		Number	*Number*	*Per cent*
1939-40				
(Ringo, Albany, Oregon)				
1 Not treated	0	8,199	644	7.8
2 Bordeaux, 8-4-100	2	6,237	89	1.4
(McLoughlin, Woodburn, Oregon)				
3 Not treated	0	5,425	318	5.8
4 Bordeaux, 12-6-100	1	6,383	121	1.9
5 Bordeaux, 8-4-100	1	5,241	78	1.4
(Harper, Junction City, Oregon)				
6 Not treated	0	5,593	1,035	18.5
7 Bordeaux, 8-4-100	2	5,160	307	5.9
1940-41				
(Beagles, Newberg, Oregon)				
8 Not treated	0	5,373	516	9.6
9 Bordeaux, 8-4-100	1	5,364	78	1.4
(Harper, Junction City, Oregon)				
10 Not treated	0	12,757	1,607	12.6
11 Bordeaux, 8-4-100	1	10,328	367	3.5
1941-42				
(Beagles, Newberg, Oregon)				
12 Not treated	0	679	33	4.8
13 Yellow Cuprous Oxide, 2-100	1	992	5	0.5
(McLoughlin, Woodburn, Oregon)				
14 Not treated	0	1,655	67	4.0
15 Bordeaux, 8-4-100	1	2,025	29	1.4
(O.S.C., Corvallis, Oregon)				
16 Not treated	0	609	21	3.4
17 Bordeaux, 8-4-100	2	437	3	0.6
(Ringo, Albany, Oregon)				
18 Not treated	0	1,636	45	2.7
19 Bordeaux, 8-4-100	1	1,928	16	0.8
(Harper, Junction City, Oregon)				
20 Not treated	0	2,822	221	7.8
21 Bordeaux, 8-4-100	2	1,251	16	1.2
1942-43				
(Groshong, Albany, Oregon)				
22 Not treated	0	891	27	3
23 Yellow cuprocide 1½-100 + lead arsenate 3-100	1	1,238	2	0.1
(Harper, Junction City, Oregon)				
24 Not treated	0	1,037	38	3.6
25 Bordeaux, 8-4-100	2	989	3	0.3
1943-44				
(Harper, Junction City, Oregon)				
26 Not treated	0	485	10	2.0
27 Bordeaux, 6-2-100 + lead arsenate 3-100	1	659	11	1.6
(Groshong, Albany, Oregon)				
28 Not treated	0	554	24	4.3
29 Yellow cuprocide 1½-100 + lead arsenate 3-100	1	576	4	0.7
1944-45				
(Groshong, Albany, Oregon)				
30 Not treated	0	1,680	56	3.3
31 Yellow cuprocide + lead arsenate (10-40) dust	1	1,348	9	0.6
(O.S.C., Corvallis, Oregon)				
32 Not treated	0	597	31	5.2
33 Bordeaux, 8-4-100	1	464	1	0.2
1945-46				
(Groshong, Albany, Oregon)				
34 Not treated	0	549	12	2.1
35 Copper + lime + lead arsenate (20-30-40) dust	1	528	5	0.9
(O.S.C., Corvallis, Oregon)				
36 Not treated	0	652	21	3.2
37 Copper + lime + lead arsenate (25-35-40) dust	1	515	1	0.2

Spraying and dusting experiments here reported were performed in 12 widely separated commercial orchards in western Oregon. The experimental plots were located in the portion of that orchard that normally had the most disease. Each plot generally contained five or more trees.

Comparative efficacy of various sprays and dusts

Data on the comparative efficacy of various spray and dust materials tested are given by years in Tables 11 and 12.

As is shown by the data in these two tables a significant reduction in the incidence of buds and twig infection followed the application of a sufficient number of timely sprays of bordeaux mixture or yellow cuprous oxide. Of these two materials, bordeaux mixture is the cheaper and apparently has a longer duration of effectiveness.

In limited tests carried on under mild to moderate disease conditions, a monohydrated copper sulfate + lime + lead arsenate (25-35-40) dust and a yellow cuprous oxide + lead arsenate (10-40) dust also gave indications of control.

Comparative efficacy of different concentrations of bordeaux mixture

The results of investigations carried on for a 12-year period under a wide range of local and seasonal conditions indicate that a 6-3-100 bordeaux mixture is practically as effective as stronger concentrations. Typical examples of the data showing the efficiency of different concentrations of bordeaux mixture are given in Tables 11 and 12.

Effect of varying the number and timing of applications

Investigations on the effect of varying the number and timing of the applications on the control of the disease were carried on for 12 seasons under a wide range of environmental conditions. Resulting data are given in Tables 13 and 14.

One spray application made in late summer before the first fall rain occurred controlled the disease satisfactorily in a normal season. In seasons when the fall, winter, and early spring were exceptionally rainy, however, three applications made in (a) the late summer, (b) the late fall when the leaves are about three-fourths off the trees, and (c) the early spring when the buds are opening, were required to control the disease satisfactorily.

Table 13. The Effect of Varying the Number and Time of the Spray Applications on the Control of Filbert Bacteriosis; Western Oregon and Washington, 1935-1938.

Year, plot number, orchard, and material	Number of applications	Dates of applications	Number of trees in plot	Results		
				Trees with small amount of bud and twig blight[1]	Trees with moderate amount of bud and twig blight[2]	Trees with great amount of bud and twig blight[3]
1935-36				*Per cent*	*Per cent*	*Per cent*
(Doerffler, Silverton, Oregon)						
1 Not treated	0		62	64	34	2
2 Bordeaux, 8-8-100	2	(1) September 7, 1935 (2) March 22, 1936	42	95	5	0
3 Bordeaux, 8-8-100	1	September 7, 1935	57	96	4	0
4 Bordeaux, 8-8-100	1	March 22, 1935	27	75	21	4
(Bibby, Salem, Oregon)						
5 Not treated	0		65	38	45	17
6 Bordeaux, 8-8-100	2	(1) August 24, 1935 (2) March 2, 1936	21	76	24	0
7 Bordeaux, 8-8-100	1	March 2, 1936	32	69	31	0
(McLoughlin, Woodburn, Oregon)						
8 Not treated	0		72	14	54	32
9 Bordeaux, 8-8-100	3	(1) August 30, 1935 (2) March 7, 1936 (3) April 4, 1936	24	88	12	0
10 Bordeaux, 8-8-100	2	(1) August 30, 1935 (2) March 7, 1936	53	89	11	0
11 Bordeaux, 8-8-100	2	(1) August 30, 1935 (2) April 4, 1936	27	93	7	0
12 Bordeaux, 8-8-100	2	(1) March 7, 1936 (2) April 4, 1936	26	27	69	4
13 Bordeaux, 8-8-100	1	August 30, 1935	72	88	12	0
14 Bordeaux, 8-8-100	1	March 7, 1936	61	21	67	12
15 Bordeaux, 8-8-100	1	April 4, 1936	50	34	56	10
(Quick, Chehalis, Washington)						
16 Not treated	0		114	6	34	60
17 Bordeaux, 8-8-100	2	(1) September 5, 1935 (2) April 4, 1936	55	82	11	7
18 Bordeaux, 8-8-100	1	September 5, 1935	51	67	29	4
19 Bordeaux, 8-8-100	1	April 4, 1936	78	37	49	14

Table 13 (*Concluded*). The Effect of Varying the Number and Time of the Spray Applications on the Control of Filbert Bacteriosis; Western Oregon and Washington, 1935-1938.

Year, plot number, orchard, and material	Number of applications	Dates of applications	Number of trees in plot	Results: Trees with small amount of bud and twig blight[1]	Results: Trees with moderate amount of bud and twig blight[2]	Results: Trees with great amount of bud and twig blight[3]
1936-37				*Per cent*	*Per cent*	*Per cent*
(Ringo, Albany, Oregon)						
20 Not treated	0		21	60	40	0
21 Bordeaux, 8-8-100	3	(1) September 1, 1936 (2) November 23, 1936 (3) March 16, 1937	11	91	9	0
22 Bordeaux, 8-8-100	2	(1) September 1, 1936 (2) March 16, 1937	21	90	10	0
23 Bordeaux, 8-8-100	1	September 1, 1936	14	93	7	0
(O.S.C., Corvallis, Oregon)						
24 Not treated	0		14	0	43	57
25 Bordeaux, 8-8-100	3	(1) August 20, 1936 (2) November 11, 1936 (3) March 4, 1937	6	83	17	0
26 Bordeaux, 8-8-100	2	(1) August 20, 1936 (2) March 4, 1937	8	75	25	0
27 Bordeaux, 8-8-100	1	August 20, 1936	7	100	0	0
28 Bordeaux, 8-8-100	1	November 14, 1936	8	62	38	0
1937-38						
(McLoughlin, Woodburn, Oregon)						
29 Not treated	0		52	5	46	49
30 Bordeaux, 8-4-100	3	(1) August 20, 1937 (2) December 6, 1937 (3) February 24, 1938	27	100	0	0
31 Bordeaux, 8-4-100	2	(1) August 20, 1937 (2) December 6, 1937	22	90	10	0
32 Bordeaux, 8-4-100	2	(1) August 20, 1937 (2) February 24, 1938	21	100	0	0
33 Bordeaux, 8-4-100	2	(1) December 6, 1937 (2) February 24, 1938	32	29	50	21
34 Bordeaux, 8-4-100	1	August 20, 1937	21	90	10	0
35 Bordeaux, 8-4-100	1	December 6, 1937	26	11	50	39
36 Bordeaux, 8-4-100	1	February 24, 1938	32	34	50	16
(Ringo, Albany, Oregon)						
37 Not treated	0		15	13	20	67
38 Bordeaux, 8-8-100	3	(1) September 3, 1937 (2) December 7, 1937 (3) February 25, 1938	28	100	0	0
39 Bordeaux, 8-8-100	2	(1) September 3, 1937 (2) December 7, 1937	16	94	6	0
40 Bordeaux, 8-8-100	2	(1) September 3, 1937 (2) February 25, 1938	15	87	13	0
41 Bordeaux, 8-8-100	1	September 4, 1937	13	92	8	0
42 Bordeaux, 8-8-100	1	February 25, 1938	16	19	31	50

[1] 1 to 25 diseased buds and/or shoots per tree.
[2] 25 to 75 diseased buds and/or shoots per tree.
[3] 75 or more diseased buds and/or shoots per tree.

Table 14. The Effect of Varying the Number and Time of the Spray Applications on the Control of Filbert Bacteriosis, Western Oregon, 1937-1947.

Year, plot number, orchard, and material	Number of applications	Dates of application	Number of trees in plot	Results		
				Buds or young shoots		
				Number examined	Infected	
					Number	*Per cent*
1939-40						
(Ringo, Albany, Oregon)						
1 Not treated	0	.....	10	8,199	644	7.8
2 Bordeaux, 8-4-100	2	(1) September 1, 1939 (2) November 29, 1939	10	6,237	89	1.4
3 Bordeaux, 8-4-100	1	November 29, 1939	10	10,004	208	2.0
(Harper, Junction City, Oregon)						
4 Not treated	0	.....	15	6,628	1,035	15.6
5 Bordeaux, 8-4-100	3	(1) May 29, 1939 (2) August 21, 1939 (3) November 25, 1939	14	5,160	307	5.9
6 Bordeaux, 8-4-100	1	May 29, 1939	11	6,025	580	9.6
(Hoover, Hillsboro, Oregon)						
7 Not treated	0	.....	10	3,990	158	3.9
8 Bordeaux, 12-6-100	4	(1) August 26, 1939 (2) December 2, 1939 (3) March 16, 1940 (4) May 3, 1940	10	2,867	35	1.2
9 Bordeaux, 12-6-100	2	(1) August 26, 1939 (2) December 2, 1939	10	3,433	59	1.7
10 Bordeaux, 12-6-100	1	August 26, 1939	10	5,593	84	1.5
1940-41						
(Harper, Junction City, Oregon)						
11 Not treated	0	.....	10	12,757	1,607	12.6
12 Bordeaux, 8-4-100	4	(1) August 5, 1940 (2) November 18, 1940 (3) February 19, 1941 (4) March 21, 1941	10	9,545	335	3.5
13 Bordeaux, 8-4-100	3	(1) August 5, 1940 (2) November 18, 1940 (3) February 19, 1941	10	10,863	418	3.8
14 Bordeaux, 8-4-100	2	(1) August 5, 1940 (2) November 18, 1940	10	11,044	529	4.8
15 Bordeaux, 8-4-100	1	August 5, 1940	10	10,328	367	3.5

Table 14 (*Concluded*). The Effect of Varying the Numrer and Time of the Spray Applications on the Control of Filbert Bacteriosis, Western Oregon, 1937-1947.

Year, plot number, orchard, and material	Number of applications	Dates of application	Number of trees in plot	Results: Buds or young shoots examined	Results: Infected Number	Results: Infected Per cent
1941-42						
(Harper, Junction City, Oregon)						
16 Not treated	0		10	2,822	221	7.8
17 Bordeaux, 8-4-100	4	(1) February 19, 1941 (2) March 21, 1941 (3) July 15, 1941 (4) March 16, 1942	10	1,241	12	0.9
18 Bordeaux, 8-4-100	2	(1) March 21, 1941 (2) July 15, 1941	10	1,251	16	1.2
(Hoover, Hillsboro, Oregon)						
19 Not treated	0		10	1,086	110	10.1
20 Bordeaux, 8-4-100	4	(1) February 22, 1941 (2) July 15, 1941 (3) November 25, 1941 (4) March 11, 1942	10	1,017	6	0.5
21 Bordeaux, 8-4-100	2	(1) July 15, 1941 (2) November 25, 1941	10	1,154	17	1.4
(O.S.C., Corvallis, Oregon)						
22 Not treated	0		8	609	21	3.4
23 Bordeaux, 8-4-100	2	(1) August 22, 1941 (2) March 10, 1942	5	437	3	0.6
24 Bordeaux, 8-4-100	1	August 22, 1941	5	570	4	0.7
1942-43						
(Hoover, Hillsboro, Oregon)						
25 Not treated	0		5	2,133	73	3.4
26 Bordeaux, 8-4-100 + lead arsenate 3-100	2	(1) July 17, 1942 (2) April 1943	5	2,062	8	0.3
27 Bordeaux, 8-4-100 + lead arsenate 3-100	1	July 17, 1942	5	1,871	10	0.5
1943-44						
(Hoover, Hillsboro, Oregon)						
28 Not treated	0		5	959	64	6.6
29 Bordeaux, 8-4-100 + lead arsenate 3-100	2	(1) July 18, 1943 (2) February, 1944	5	843	5	0.6
30 Bordeaux, 8-4-100 + lead arsenate 3-100	1	July 18, 1943	5	934	13	1.4
1946-47						
(O.S.C., Corvallis, Oregon)						
31 Not treated	0		5	652	21	3.2
32 Copper + lime + lead arsenate (25-35-40) dust	1	August 2, 1946	5	516	1	.02
33 Copper + lime + lead arsenate (25-35-40) dust	1	March 15, 1947	5	461	18	3.9
34 Copper + lime + lead arsenate (25-35-40) dust	2	(1) February 8, 1947 (2) March 15, 1947	5	696	11	1.6
35 Copper + lime + lead arsenate (25-35-40) dust	3	(1) August 2, 1946 (2) February 8, 1947 (3) March 15, 1947	5	650	3	0.4

Supplementary Control Measures

One of the most serious phases of filbert bacteriosis is the formation of cankers on the trunks, which frequently girdle and kill young trees. In the sections to follow there is presented a discussion of studies of supplementary control measures designed to prevent infection of young trees, thereby reducing tree losses from this disease.

Location of orchard

The results of extensive field observations indicate that filberts are predisposed to bacterial blight infection by planting the trees on low, poorly drained, or waterlogged soils.

The roots of a filbert tree normally make quite an extensive growth during the winter months in western Oregon, but if the soil is water-soaked, growth may be reduced or stopped entirely. This is due to the filling of the normal air spaces between the soil particles with water, thereby reducing the available oxygen supply. Without oxygen the roots cannot grow and function; without root growth, top growth is not possible.

In a waterlogged soil the root system of the tree is not extensive enough to provide adequately for its needs; as a result the tree is undernourished and readily succumbs to bacterial blight infection.

Planting stock

Numerous field surveys carried on during the course of these investigations indicate that losses of trees are less when only well-rooted, first-quality nursery stock is planted. A first-quality tree has an extensive system of lateral roots at the base of the trunk massed within a space of from 2 to 4 inches. Trees that have but few, or no, lateral roots should not be planted. The trees should also be free from bacterial cankers—denoted by dark brown, flattened or sunken areas in the bark of the trunk. Such stock is often responsible for the introduction of the disease into a planting.

Resistant root-stocks

The results of field surveys indicate that tree losses from bacteriosis can be greatly reduced by the use of Turkish filbert root-stocks which apparently posses a high degree of resistance to the disease. The control possible from the use of Turkish rootstocks was shown strikingly in a partly grafted and partly layered orchard located near Silver Creek, Washington. Less than 1 per cent of the trees grafted on Turkish filbert rootstocks succumbed to filbert bacteriosis before the fifth year, whereas approximately 25 per cent of

the layered trees (*ie.,* trees on their own roots) died from bacteriosis.

The advisability of using the Turkish filbert as a rootstock has been questioned by some filbert growers on the assumption that the Turkish root dwarfs the top. Observations made in an old grafted and layered orchard, set out in 1919 by the late A. Quarnberg, near Vancouver, Washington, however, indicate that there is no inherent dwarfing of the top from the use of Turkish rootstocks. In this orchard a row of 27-year-old Barcelona filberts grafted on Turkish roots is growing beside a row of Barcelonas growing on their own roots. These trees were planted at the same time and they have been growing under the same soil and cultural conditions. The grafted trees are just as large as the layered Barcelonas.

While grafting trees on Turkish rootstocks apparently reduces losses of young trees from bacteriosis, such grafted trees should not be planted on poorly drained land or on land with a high water table during the winter months. Grafted trees, like layered ones, will not thrive under such conditions.

Time of planting

In numerous field surveys made during the course of these investigations it was noted that tree losses from bacteriosis in orchards planted in the late fall or early winter were generally much less than in those planted in the spring, especially late spring. Planting the orchard early permits the root system to become well established before top growth begins in the spring. Trees with a well-established root system planted early make a vigorous, strong growth the first season while trees planted in the late spring generally make comparatively little growth and are more likely to succumb to drought or other unfavorable conditions.

Protection of tree trunks

The results of numerous surveys and experiments carried on over a period of 15 years indicate that protection of the tree trunks by trunk protectors during the first 3 or 4 years of their life will reduce tree losses appreciably. Such protectors lessen damage from sunscald and cold. Tissues weakened by sunscald or cold injury evidently offer little, if any, resistance to infection and extensive invasion by the bacteria. Any other unfavorable factor, such as poor drainage or drought, that lessens the vigor of the trees predisposes them to infection and serious damage by the causal organism.

Among the materials that make good trunk protectors are newspaper mats, extra heavy wrapping paper, and yucca boards (Figure 15).

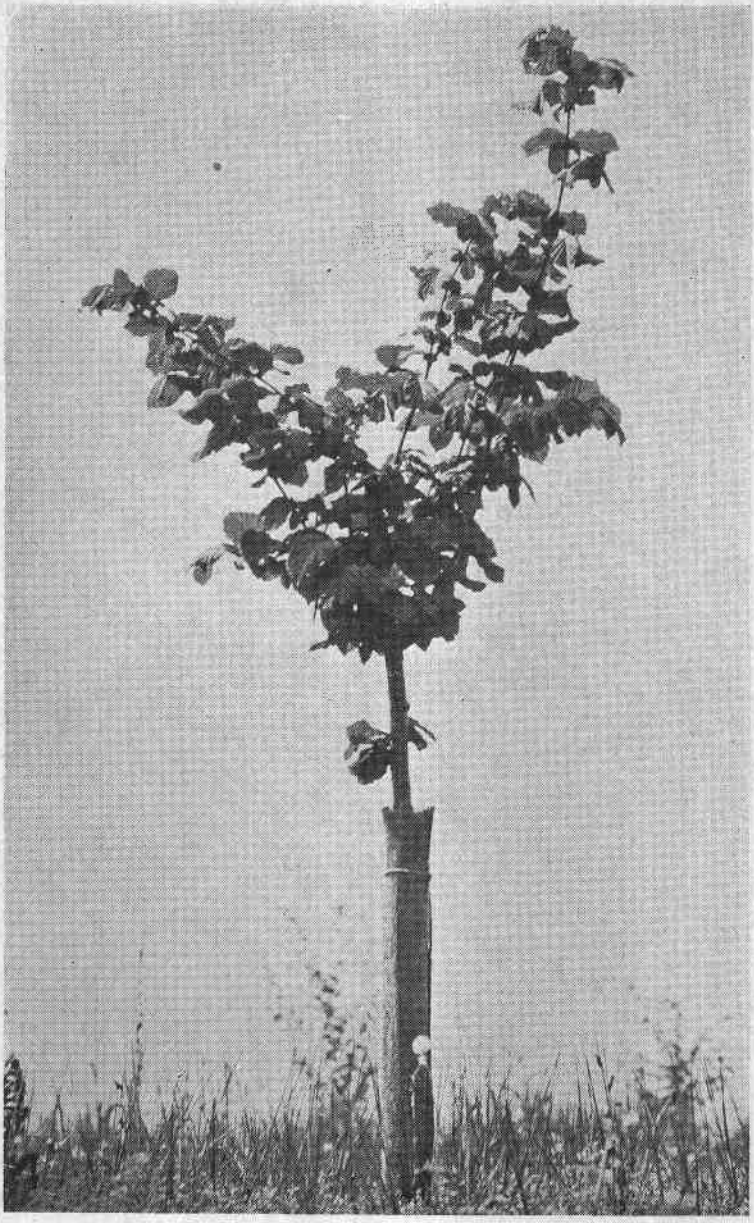


Figure 15. A yucca board protector about the trunk of a filbert tree. (Photograph by the late O. T. McWhorter; Oreg. Agr. Sta. Bull. 428.)

Sterilization of pruning tools

Extensive observations and experiments carried on during the course of these investigations show the tools used in pruning and suckering young trees should be sterilized at frequent intervals with an effective sterilizing agent, such as bichloride of mercury 1-1,000, to prevent contamination and spread of the bacteria. The necessity of sterilizing the tools was pointed out by finding numerous cankers about pruning wounds and by the experimental production of lesions about cuts made with shears on which had been smeared a pure culture of the causal organism (see Figure 7).

The use of a sterilizing solution on the tools is particularly necessary when suckering and pruning young trees, 1 to 4 years of age, as infection of the trunks during this period frequently results in girdling and death of the trees. After the fourth year the use of a sterilizing agent on the tools is not so important, as the tissues of the trunk become increasingly resistant with age. While it is impracticable to sterilize the tools after every cut, they should at least be sterilized between trees.

Recommendations for Control of Filbert Bacteriosis

Filbert bacteriosis varies so greatly in its severity of occurrence under different seasonal and local conditions that no single control program can be recommended that will be applicable to all situations and conditions likely to be experienced. The following recommendations are accordingly offered not as a fixed program but as a general guide subject to intelligent modification to meet exceptional seasonal conditions.

The recommended spray materials

Bordeaux mixture is recommended for general use for the control of filbert bacteriosis in the Pacific Northwest as it is the cheapest effective material available.

As a possible substitute, yellow cuprous oxide spray may be used where convenience is a more important consideration than cost.

The spray formula

Bordeaux mixture: The 6-3-100 concentration (6 pounds of copper sulfate, 3 pounds of hydrated lime, and 100 gallons of water) is recommended for general use. The addition of an efficient wetting agent, such as B 1956 Spreader or Grasselli spreader-sticker, is advised to increase the efficacy of the spray material.

Yellow cuprous oxide: This material should be used at the rate of 1½ pounds in 100 gallons of water. The addition of a compatible, efficient spreader-sticker, such as B 1956 spreader, will increase the efficacy of this material.

Combination spray program for control of filbert bacteriosis and filbert worm

The spray material for the control of filbert bacteriosis may be combined with that for the control of the filbert worm, thereby saving both time and labor. The combination mixture consists of bordeaux mixture 6-3-100 plus 3 pounds of lead arsenate in 100 gallons, plus an efficient and compatible spreader-sticker. In preparing this mixture, the 6-3-100 bordeaux mixture should be made up first; then 3 pounds of lead arsenate per 100 gallons, and a suitable amount of spreader should be added in the order named. This spray mixture should be applied after the filbert moths begin to lay eggs but before any hatch (about the middle of July in a normal season).

The use of a combination spray control program is contingent on the amount of bud and twig blight present in the orchard. In many orchards, not enough disease is present to warrant the extra expense of using a combination spray program. If considerable bud and twig blight is present, however, a combination spray program is justifiable.

Number and timing of the spray applications

In a normal season, one spray application made in the late summer (August) before the first fall rain occurs will control the disease satisfactorily. In seasons when the fall and winter are exceptionally rainy, however, three applications made (a) in late summer, (b) in late fall, when the leaves are about three-fourths off the trees, and (c) in early spring, when the leaf buds are opening, appear to be necessary to control the disease satisfactorily.

The dust program

The results of limited tests carried on under mild to moderate disease conditions indicate that a sufficient number of properly timed copper dust treatments will appreciably reduce the incidence of bud blight and twig blight due to *Xanthomonas corylina.* It remains to be determined if dusts will control the disease under epidemic conditions.

For those who are only equipped to dust, the following dust program is tentatively recommended.

The dusts to use: A dust composed of 25 per cent monohydrated copper sulfate, 50 per cent hydrated lime, 5 per cent benton-

ite, 18.5 per cent talc, and 1.5 per cent light mineral oil is advised as first choice of possible dust formulas.

As an alternative, a dust composed of 10 per cent yellow cuprous oxide, 83.5 per cent talc, 5 per cent bentonite, and 1.5 per cent light mineral oil may be used.

Number and timing of applications: In a normal season, one dust application made in the late summer (August) before the first fall rain occurs appears to be sufficient to control the disease. In seasons when the fall, winter, and early spring are exceptionally rainy, however, three dust applications made (a) in late summer, (b) in late fall, when the leaves are about three-fourths off the trees, and (c) in early spring, when the buds are opening, may be necessary to control the disease satisfactorily.

Combination dust program for control of filbert bacteriosis and filbert worm

The dust material for the control of filbert bacteriosis may be combined with that for the control of the filbert worm.

The combination dust mixture consists of 25 per cent monohydrated copper sulfate, 33.5 per cent hydrated lime, 40 per cent lead arsenate, and 1.5 per cent light mineral oil.

As an alternative, a mixture consisting of 10 per cent yellow cuprous oxide, 40 per cent lead arsenate, 48.5 per cent talc, and 1.5 per cent light mineral oil may be used.

The combination dust mixture should be applied after the filbert moths begin to lay eggs but before any hatch (about the middle of July in a normal Pacific Northwest season).

Here again, the combination dust mixture should only be used when there is sufficient filbert bacteriosis present to warrant the extra expense involved in its application.

Other supplementary control measures

Losses of young trees from the disease may be reduced by the following measures:

1. Planting the orchard only on well-drained land.
2. Planting only well-rooted, first-quality nursery stock.
3. Planting the trees in late fall or early winter.
4. Protecting the tree trunks from sunscald during the first 3 or 4 years with trunk protectors.
5. Sterilizing, between trees, the tools used in pruning and suckering.

SUMMARY AND CONCLUSIONS

Filbert bacteriosis, caused by *Xanthomonas corylina* (Miller et al.) Dowson is the only infectious disease of filberts of any economic importance in the Pacific Northwest.

The disease has so far been found only in Oregon and Washington.

The malady attacks the leaves, leaf buds, and pistillate-flower buds, branches, tree trunks, and occasionally the nuts.

The disease occurs naturally only on the cultivated filbert (*Corylus avellana* L. and *C. maxima* Mill.). It has been artificially induced by inoculations on leaves of the Turkish filbert (*Corylus colurna* L.).

The causal organism is a short capsulated rod 1.1 to 3.8 μ by 0.5 to 0.7 μ, arranged singly or in pairs, with rounded ends; motile by 1 polar flagellum; without endospores; gram-negative and not acid-fast; stains readily with gentian-violet and carbol-fuchsin, but only very lightly with methylene blue; growth at room temperature (about 22° C.) on nutrient-dextrose agar streaks is abundant, filiform, convex, glistening, smooth, opaque, pale lemon-yellow, viscid, and odorless; medium unchanged; dextrose agar surface colonies are circular, over 1 mm. in diameter, round, glistening, pale lemon-yellow, convex, entire margins, and finely granular within; growth in nutrient broth is abundant and a ring forms at the surface after 2 to 5 days; on gelatin stabs growth is best at the top, liquidation is stratiform, beginning in 1 day but not complete until from 4 to 6 weeks, the medium is unchanged except for a slight turbidity; aerobic; gas is not formed; no indole or hydrogen sulfide is produced; milk (casein) is slowly digested and rennin is produced; selenium dioxide and litmus in litmus milk are reduced; starch is hydrolized; ammonia is produced in nutrient broth containing peptone. At 28° C. acid, but no gas, is produced from dextrose, levulose, galactose, lactose, sucrose, maltose, xylose, raffinose, mannitol, glycerol, or starch; no acid or gas is produced from arabinose, rhamnose, dulcitol, salicin, inulin, or cellulose; alkali is produced from citrate, lactate, malate, and succinate. At 28° C., acetate, benzoate, formate, salicylate, and tartrate are not fermented. Nitrogen sources utilized at 28° C., in the order of their availability, are peptone, aspartic acid, alanine, leucine, sodium ammonium phosphate, allantoin, tyrosine, uric acid, and brucine; glutamic acid and sodium nitrite are not utilized; positively lipolytic when tested on spirit blue cottonseed-oil agar; grows rapidly in 3 per cent sodium chloride broth but fails to grow in 4 per cent sodium chloride broth; is tyrosinase negative; cannot utilize sodium tartrate as a carbon source; nitrates in beef extract-peptone broth containing 1 per cent potassium nitrate are not reduced to nitrites;

however, nitrites are formed in abundance within 3 days in a synthetic medium; sodium ammonium pectate is slowly liquefied; optimum temperature for growth is between 28° and 32° C.; maximum about 37° C., minimum between 5° and 7° C.; thermal death point between 53° and 55° C.; and pH range for growth is pH 5.2 to 10.5, optimum reaction for growth pH 6 to 8; serological studies indicate the existence of strains of the pathogen.

Filbert bacteriosis is primarily a disease of the parenchymatous tissues, the vascular bundles being only occasionally invaded. In the initial stages of parasitism, the bacteria are intercellular, but later they gain access to the cell lumen and develop intracellularly. Large cavities are subsequently formed which are filled with bacteria imbedded in a slimy matrix.

The causal organism is brought into the orchard on infected nursery stock.

Cankers on the larger branches and trunks of the trees were found to be the most important sources of primary inoculum. Infected buds and lesions on small twigs were found to be less important sources of inoculum.

The inoculum is spread by man on tools used in pruning and suckering and by rain-drip.

Insects do not appear to be concerned in the dissemination of the bacteria.

The pathogen gains access to the host tissues through stomata and wounds.

The presence of moisture on the host organs for a suitable period after inoculation is a prerequisite to infection. The minimal period of wetting necessary for infection was found to vary with the age of the host organs and the extent of water-congestion of the tissues. Infection of the leaves occurred with only one hour of wetting following inoculation when they were young and the tissues water-soaked. While only short minimal periods of wetting are necessary to infect the young leaves and buds when water-soaked, an increase in the incidence and extent of infection accompanied prolongations of the moist period to the limits reached in these investigations. Subjecting the plants to a pre-inoculation moist treatment increased the incidence and extent of infection.

Infection took place over a relatively wide range of temperature ranging from 7° to 25° C. Relatively warm temperatures (above 25° C.) at the time of inoculation were somewhat more favorable for infection than lower temperatures.

The incubation period was longer at lower temperatures (below 20° C.) than at higher ones. The period of incubation as determined

by inoculation experiments on leaves varied from 8 to 13 days, depending on the age of the host organs and the environmental conditions. In general, the younger the host organs and the higher the temperature, the shorter was the incubation period.

The host organs pass through a stage of maximal susceptibility into a period of increasing resistance. They were most susceptible when young and succulent.

Rainfall and man were found to be of special significance in the epidemiology of filbert bacteriosis.

Man is an important agent in spreading the inoculum in young orchards, 1 to 4 years of age, by disseminating the bacteria on tools used in pruning and suckering.

Rainfall is the most important natural agency concerned in the spread of the inoculum. The greater the number of sources of primary inoculum and the more frequent and abundant the rainfall during the period for infection, the greater the incidence of infection. Bacteria from primary infections are the source of secondary infections. The occurrence of secondary infection was governed by (a) the relative abundance of primary sources of infection, and (b) the frequency and amount of rainfall during the infection period.

The infection period was found to extend from the time the buds are about three-fourths grown (*ie.*, late summer) until they open the following spring. The forepart of this period (during the fall) is the most critical in relation to control.

The existence of adverse growing conditions during the first few years of the life of an orchard was found to reduce the vigor of the trees, which predisposes them to infection. Among the most prevalent and important adverse growing conditions are poor soil drainage, cold injury, sunscald and drought.

Control experiments performed during the years 1935 to 1947 inclusive are reported and discussed.

In young orchards, 1 to 4 years old, fall or winter planting of well-rooted, first-quality nursery trees in a deep, well-drained soil, placing protectors about the trunks, using a good disinfecting agent on pruning tools, and spraying with a protectant bactericide reduced tree losses from this disease greatly.

In older orchards, the only practical method of controlling the disease was found to consist in spraying or dusting with protectant bactericides. Of the spray materials tested, home-made bordeaux mixture was found to be the cheapest and most effective. Yellow cuprous oxide approximated bordeaux mixture in effectiveness but is more costly.

The 6-3-100 concentration of bordeaux mixture and the 1½-100 concentration of yellow cuprous oxide gave as good control as stronger concentrations.

Tests indicate that it is possible to combine in one mixture the material for the control of bacteriosis with that for the control of filbert worm, thus saving both time and labor. This combination spray consists of bordeaux mixture 6-3-100 plus 3 pounds of lead arsenate in 100 gallons, and a suitable amount of an efficient and compatible spreader-sticker. It should be applied after the moths begin to lay eggs but before any hatch.

In limited tests carried on under mild disease conditions, dusting with copper dusts also gave indications of control. Of the dusts tested, a copper + lime + lead arsenate (25-30-40) dust and a yellow cuprous oxide + lead arsenate (10-40) dust gave the best control. It remains to be determined whether these dusts will control the disease under epidemic conditions.

In a normal season, one spray application made in the late summer before the first fall rain occurs (August) has controlled the disease satisfactorily. In seasons when the fall, winter, and early spring are exceptionally rainy, however, three applications made (a) in late summer, (b) in late fall, when the leaves are about three-fourths off the trees, and (c) in early spring, when the leaf buds are opening, appear to be necessary to control the disease satisfactorily.

The Barcelona, Du Chilly, White Aveline, and Brixnut varieties are the most susceptible of the more important commercial varieties grown in the Pacific Northwest, and Daviana and Bolwyller are the most resistant.

The Turkish filbert (*Corylus colurna*) appears to be more resistant to the disease than the cultivated filbert (*Corylus avellana*).

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