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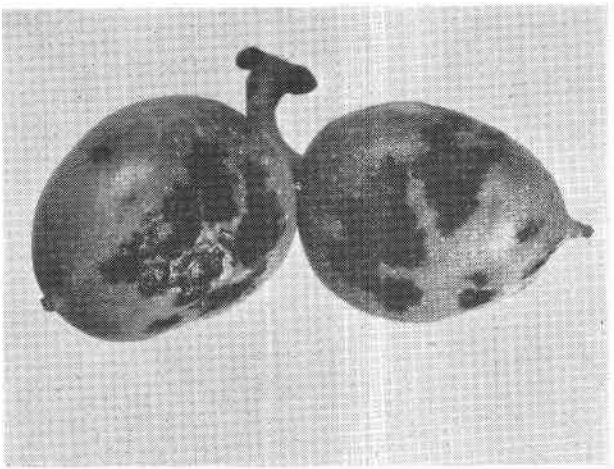
Walnut Bacteriosis and Its Control

By

P. W. MILLER and W. B. BOLLEN



United States Department of Agriculture
Bureau of Plant Industry, Soils and Agricultural Engineering
in cooperation with the
Agricultural Experiment Station
Oregon State College, Corvallis

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P. W. MILLER, plant pathologist,
Division of Fruit and Vegetable Crops and Diseases,
Bureau of Plant Industry, Soils, and Agricultural Engineering,
Agricultural Research Administration
and
W. B. BOLLEN, associate bacteriologist,
Oregon Agricultural Experiment Station*

I. INTRODUCTION

WALNUT BACTERIOSIS, popularly known as walnut blight, is the most destructive disease of the Persian (English) walnut (*Juglans regia* L.) on the Pacific Coast. The disease occurs at its worst where the climate is humid in the spring and early summer. It is believed to have been introduced into the United States on imported nursery stock. It was first observed in Los Angeles County, California, about 1891 (*110*).† Shortly thereafter its presence was reported in Orange County, California (*110*). The disease spread rapidly to neighboring regions and became increasingly destructive.

The first published reference to the disease occurs in the annual report of the Chief of the Division of Vegetable Pathology, United States Department of Agriculture, for the year 1893 (*40*) in which is given a brief description of the disease. By this time the disease was suspected to be of bacterial origin, but inoculations to prove definitely the causal relationship of the bacterium in question to the disease had apparently not been made. In 1896, Pierce wrote a letter to the editor of the *California Fruit Grower* in which he gave the first detailed description of the disease. This letter was later published in the *California Fruit Grower* (*77*). From the contents of this letter, it is inferred that by this time inoculations with pure cultures of the bacterium had been made and its pathogenicity proved. In later articles, that were mostly written in popular style and were practically devoid of experimental data (*77, 78, 79, 80*), Pierce discussed the life history of the causal organism in relation to pathogenesis and reported on the results of limited attempts to control the disease. In 1901, Pierce (*81*) published a technical description of the causal organism, giving it the name *Pseudomonas juglandis,* n. sp.

It was not long until bacteriosis became so destructive that the California Walnut Growers Association offered a reward of $20,000 to anyone who could

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† Reference is made by italicized number in parentheses to LITERATURE CITED, page 102.

find a practical method of control. In 1905, the California State Legislature, in response to urgent requests from walnut growers of the state for help, granted an annual appropriation of $4,000 for a study of this disease and its control. As a result of investigations carried on under this grant, Ramsey (*82*), and Smith and his associates (*107* to *111*) confirmed Pierce's earlier studies on the etiology of the disease, and added materially to the existing knowledge of the morphology and physiology of the pathogen and its life history in relation to pathogenesis. However, little or no success attended their efforts to find satisfactory measures of control. During the next decade, further studies of the disease were carried on by Batchelor (*21*), C. O. Smith (*100, 101, 102*), Fawcett and Batchelor (*37*), Doidge (*32*), McMurran (*49, 50*), and others, but attempts to find economical measures of control were without success. It was not until 1928 that any advancement was made in the development of a satisfactory control program, when Rudolph (*86, 87*) began an extensive series of spraying tests that were carried on during the growing season instead of in the dormant period as had been the case in most of the earlier work. He concluded from these and later studies (*88, 89, 90, 91, 92, 93, 95*) that the disease can be controlled by timely spraying with bordeaux mixture or with red cuprous oxide during the forepart of the growing season.

In Oregon, attempts made in 1930 and 1931 to control walnut bacteriosis by the use of Rudolph's spray program met with variable results (*20, 53*). Good control of the disease followed its use under certain conditions, while under others it failed to control the disease satisfactorily. Moreover, the use of bordeaux mixture at the recommended concentration resulted in definite injury to young walnut leaves (*20, 53, 54*). The injurious effects of the spray and the variability in control led to further studies of the disease and its control under Pacific Northwest conditions. It was deemed necessary first to acquire a clearer understanding of the epidemiology of the disease in relation to this variability in effectiveness and to bring this information to bear upon the problem of control. The present investigations have been in progress now for 16 years, from 1930 to 1945 inclusive, during which time reports of progress (*53* to *64,* and *66* to *72,* inclusive) have been made so that the findings were made available as soon as possible to Pacific Northwest walnut growers. Although knowledge of some phases of the problem is still incomplete, it seemed 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.

II. THE DISEASE

GEOGRAPHICAL DISTRIBUTION

Walnut bacteriosis is widely distributed, the range of its occurrence closely paralleling that of walnut culture throughout the world. Its presence has been definitely reported in the following countries, which are listed in chronological order of the reported appearance of the disease: United States (*40*), New Zealand (*23*), Russia (*2, 44*), Canada (*41*), Tasmania (*84*), Mexico (*101, 110*) Australia (*26, 8*), Chile (*25*), South Africa (*32*), Italy (*98*), Holland (*116*), Switzerland (*73*), England (*120, 121*), France (*122*), West Indies (*119*), and Rumania (*117*). Stapp (*112*) described it in a German handbook of plant diseases, referring to it as *Bakterienbrand der Walnusse.*

In the United States the disease has been reported from the following states which are given in chronological order: California (*40, 76, 109, 4, 5, 52, 3*), Oregon (*48, 18, 4, 13, 15, 16, 19, 14, 53, 54, 56*), Texas (*99, 118, 115, 3*), Louisiana (*118, 50, 115*), Maryland (*118, 50, 115, 4*), Pennsylvania (*49, 50, 115, 4*), Delaware (*49, 50, 115, 5, 4, 9*), New Jersey (*49, 29, 7, 28, 4, 5*), New York (*50, 115, 5, 4*), Virginia (*50, 39, 4*), District of Columbia (*50, 4*), Alabama (*7*), Georgia (*4, 6*), Washington (*4, 5, 9, 93, 3*), Arkansas (*85*), Mississippi (*75*), and Michigan.*

ECONOMIC IMPORTANCE

Bacteriosis is the most destructive disease of the Persian walnut on the Pacific Coast, causing greater financial loss than all of the other diseases of walnuts combined. In years of severe disease outbreaks as much as 75 per cent of the crop has been lost from this disease. In 1903, for example, this disease caused an estimated loss of approximately 50 per cent of the California walnut crop. In 1912, Smith (*110*) stated "it is probably conservative to state that in the seedling groves of southern California an average loss of at least 50 per cent of the crop which would otherwise have been harvested has been caused by the blight during the past 10 years." Rudolph (*88*) declared that "frequently the average annual losses vary from 75 to 80 per cent (*sic*) in the San Francisco Bay region as well as in the important walnut growing communities of the San Joaquin and Sacramento valleys, particularly when climatic conditions are anyways favorable to the disease. For the state of California as a whole, since the disease was first introduced, the loss to growers can be estimated conservatively at many millions of dollars."

The disease has also been very destructive in the Pacific Northwest, causing great financial loss in epidemic years. In 1927 Barss (*17*) reported that at least one-half of the crop that set on the trees in Oregon in 1925, 1926, and 1927 was attacked by bacteriosis. In recent years the average crop loss in the Pacific Northwest from this disease has varied according to the season from a negligible amount up to 35 per cent. In the past decade, there have been 7 years when crop losses from bacteriosis have averaged 20 per cent or greater. The years of relatively severe disease outbreaks during this period and the estimated average crop losses in each are as follows: 1936, 35 per cent; 1937, 25 per cent; 1940, 20 per cent; 1941, 35 per cent; 1942, 40 per cent; 1943, 35 per cent; 1944, 20 per cent; and 1945, 15 per cent. However, even in those years when the average crop loss was less than 20 per cent, comparatively severe losses ranging from 50 to 75 per cent occurred in individual orchards in certain localities.

SYMPTOMS OF THE DISEASE

Walnut bacteriosis attacks the current growth only. After the host organs pass through one growing season they are highly resistant to further infection. The disease would be of no economic importance if it were not for the destruction of the nuts, as it has never been known to kill a bearing tree and causes but little, if any, premature defoliation.

On the Leaves

All parts of the leaf may be attacked by the pathogen. The disease generally appears first at the margins of the leaflets as circular to irregularly cir-

* As determined by specimens received from this state for diagnosis.

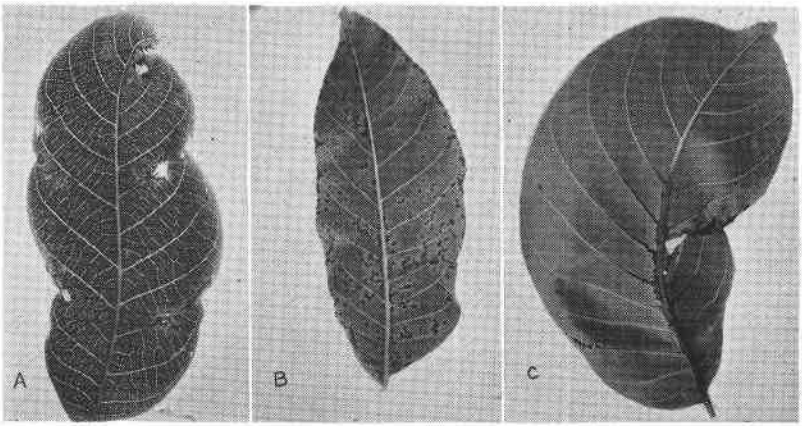

Figure 1. Walnut leaflets affected by bacteriosis: A, marginal lesions; B, interveinal lesions; C, lesions on the midribs and lateral veins.

cular, pale yellowish-green, water-soaked, translucent areas which later turn a vinaceous-brown (*83*). The presence of lesions at the margins usually causes the leaflets to be distorted in shape due to a checking of the growth in the infected areas (Figure 1, A). The majority of lesions of this type are of the preceding year's origin, infection taking place while in bud.

Leaf lesions of current origin typically appear later in the spring in the lamina or on the rachis, petiole, midrib, lateral veins, or veinlets. In the leaf lamina, the isolated lesions are typically small, rarely measuring more than 4 millimeters in their greatest dimension. When fully developed, they are angular to irregularly circular and reddish to vinaceous-brown (Figure 1, B). The lesions occur miscellaneously scattered over the surface or they may be and often are grouped near the apical end of the leaflet, in positions where water would tend to accumulate during rainy periods. Lesions in the lamina do not, as a rule, cause a distortion in the shape of the leaf. On the midribs, veins, rachis, and petiole the lesions are dark brown or black and slightly depressed (Figure 1, C). The entire vein or veinlet may be invaded before the disease runs its course. From the veins the bacteria frequently work their way into the contiguous parenchymatous tissues where reddish- to vinaceous-brown areas are formed.

Bacteriosis causes little, if any, defoliation of the trees even in seasons especially favorable for its development. The infected leaves typically persist on the trees until the time of normal leaf fall unless the disease attacks and kills the petiole or rachis in which case the leaf or leaflet dependent upon it may die and fall prematurely.

On the Nuts

If the nut is infected during the prebloom or blooming period the lesions are typically located at the apical or blossom end. The first macroscopic symptom of infection consists in the appearance of small, circular or irregularly circular, water-soaked areas in the bracts, bracteoles, or involucre at the apical end (Figure 2, A). The infected areas enlarge as the bacteria invade the surrounding tissues and turn black (Figure 2, B). During the active stages of

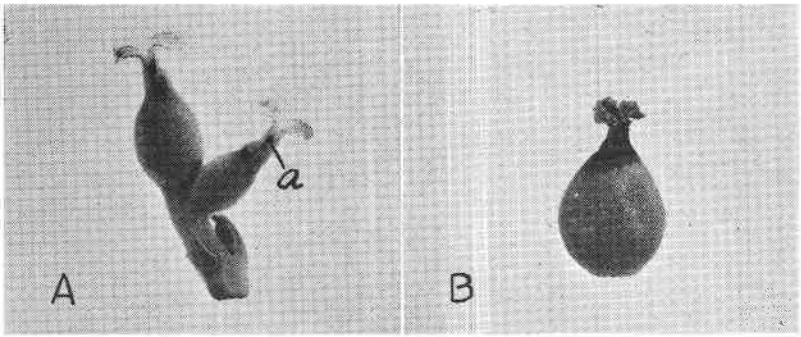

Figure 2. Young walnuts affected by bacteriosis: A, Lesion (a) on a bract; B, a lesion at the apical end of a nut.

parasitism, a narrow, peripheral, water-soaked zone denoting advanced bacterial action may frequently be seen surrounding the discolored areas. With a cessation in bacterial activity, this water-soaked zone usually disappears and the line between the diseased and healthy areas becomes more or less sharply defined. The elevation of the lesion is at first unchanged; later, it becomes depressed. Drops of a black, slimy exudate containing myriads of bacteria and decomposed cellular materials may ooze out of the lesions during periods of high humidity. This ooze is water-soluble and rains spread the bacteria in it to the host organs below, giving rise to secondary infections.

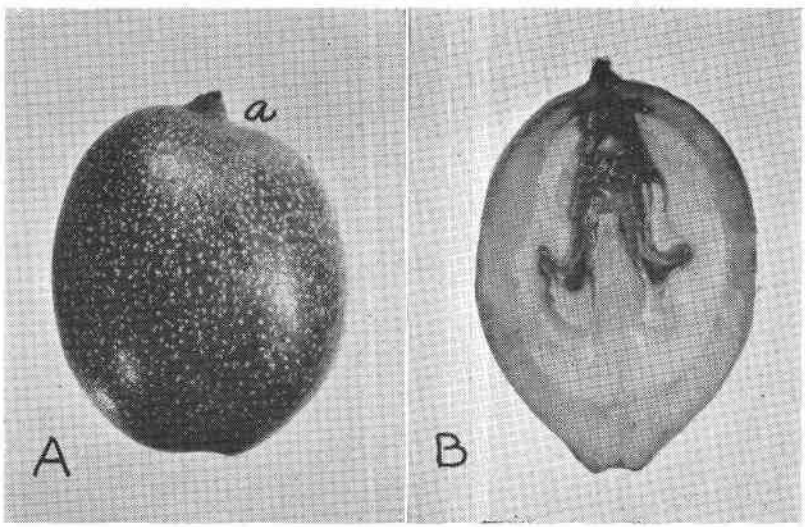

Figure 3. Walnuts affected internally by bacteriosis: A, An internally infected nut as seen from the exterior. The only visible evidence of infection is a slight depression (a) in the tissues at the apex. B, A longitudinal, medium section through an internally infected nut. Note the extent of invasion within.

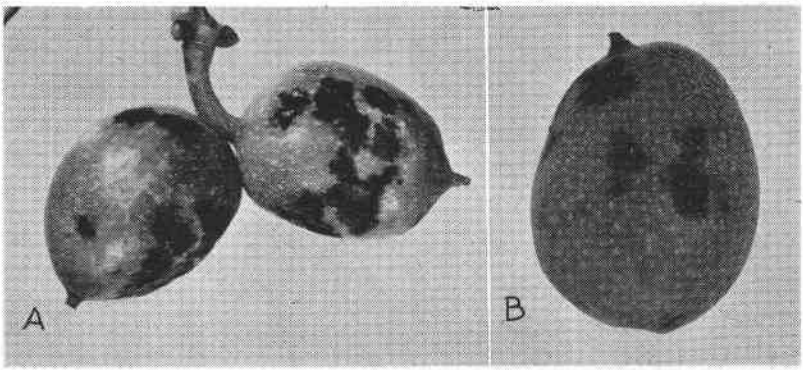

Figure 4. Mature lateral lesions on walnuts. A bacterial exudate is coming from several of the lesions shown in B.

Although the lesions are generally visible from the outside, the interior of the nut may be infected without the disease being outwardly plainly perceptible. The only visible evidence of infection in cases of this sort is a slight depression in the tissues at the apex (Figure 3, A). If such a nut is cut open, the central tissues will be found badly diseased (Figure 3, B).

Nuts that are infected early in their development commonly fail to mature, as the bacteria generally gain access to the interior and parasitize the tissues within, and the nut drops prematurely. In some years especially favorable for disease development, a large portion of the crop may be lost from early infection. It is not an unusual occurrence in epidemic years for the ground beneath badly infected trees to be literally covered with "blighted" nuts. However, not all of the nuts that drop are infected with bacteriosis. Some drop may occur as a result of lack of pollination or from faulty nutrition. Nuts that drop from either of these last two causes are usually free from black spots on the outside, and on the inside the tissues are yellowish or slightly brown and not black.

Infections that occur after bloom are generally located on the sides of the nut. When fully developed, the lateral lesions appear as depressed, circular to irregularly circular, black areas with well-defined margins, ranging from a few millimeters in diameter to half the surface area of the nut, the ultimate size and extent of the lesions depending on the age of the nut at the time of infection (Figure 4). Infections occurring in the interval from the end of the blooming period to the time the nuts are about the size of olives frequently penetrate to the shell and, in some instances, succeed in gaining access to the ovary in which case the nuts generally fall to the ground prematurely, though some of them may persist on the trees until maturity. If infection occurs after the nuts are about one-half grown, the lesions are usually confined to the outer portion of the fleshy hull, but in some cases the bacteria may succeed in reaching the shell, in which case the infected parts usually stick tightly to the shell at maturity (Figure 5, A). A brown stain which is not removable by ordinary bleaching agents is left on the shell when the lesions are scraped off thereby reducing the marketability of the nut (Figure 5, B).

Under certain conditions a type of late infection, describable as a "ring-spot," may occur in the nuts. In this type of infection a narrow, circular band

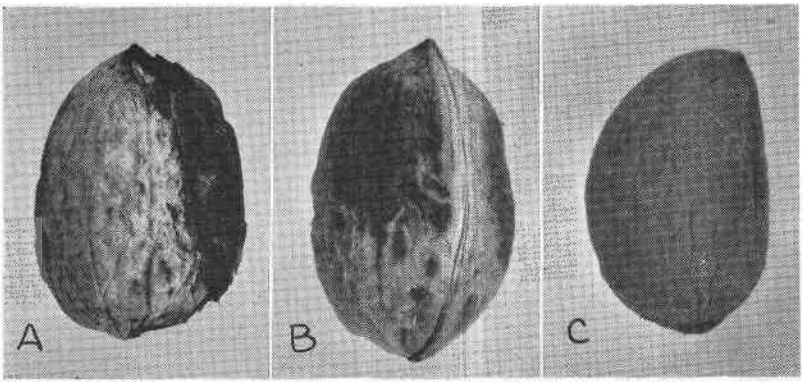

Figure 5. The effect of bacteriosis on the shape and quality of the nuts: A, Diseased part of hull adhering tightly to shell at maturity; B, brown stain left on shell after a lesion in the hull is scraped off; C, misshapen nut caused by presence of a lesion in the hull.

of blackened tissue, penetrating only a few millimeters into the fleshy hull, encircles a live, green area, producing the effect shown in Figure 6, A.

The continued growth of the live, green tissues delimited by this circular band of infected tissue frequently results in a wart-like protuberance. In some instances, the delimited area remains green and continues to function until the nut matures, though in most instances the central tissues eventually turn yellow and die. While this type of lesion seldom reaches and stains the shell, it may interfere with the normal development of the shell, causing it to be misshapen at maturity (Figure 5, C).

Infections that occur relatively late in the season when the nuts are from ½ to ¾ grown are typically very small, rarely more than 3 millimeters in diameter,

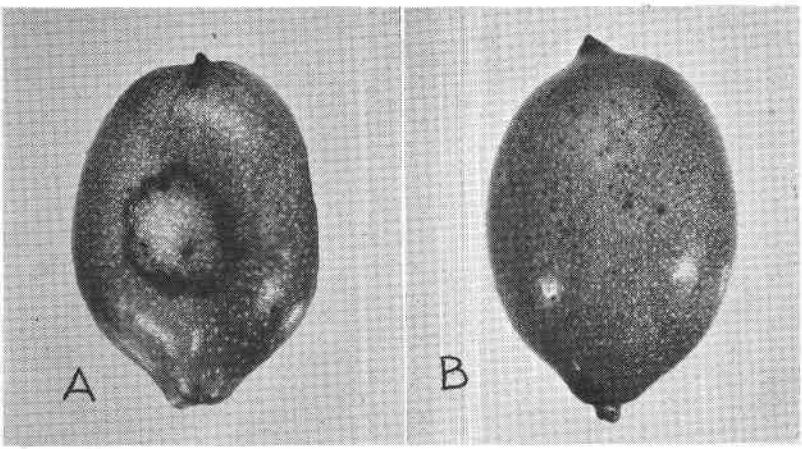

Figure 6. Late infections on walnuts: A, a "ring-spot" type of lesion from a late infection; B, lesions in outer portion of the hull from artificial inoculations made when the nut was about three-fourths grown.

and seldom penetrate more than a few millimeters into the fleshy hull. When fully developed, they are dark brown to black, circular, and only very slightly depressed (Figure 6, B). Such infections are probably of no economic importance as they apparently do not affect the shell or the kernel within.

On the Twigs

The stems of young shoots of current growth are commonly infected early in the season—mostly in the prebloom and blooming periods—when the tissues are young and succulent. As the stems grow and become "woody," their susceptibility to infection diminishes. Different portions of the stem will vary in the degree to which they are subject to infection. The base of the stem may be quite woody and consequently highly resistant to infection while the apical part will be succulent and correspondingly very susceptible. Laterally borne shoots generally become woody throughout and consequently resistant to infection much earlier in the season than terminally borne ones. After a shoot has passed through one growing season, it is apparently no longer susceptible to infection as is indicated by the fact that all attempts to infect twigs and branches one year of age or older have been uniformly negative.

The first macroscopic symptom of the disease on young, succulent shoots consists of minute, dark-green, water-soaked, circular areas, not more than a few millimeters in diameter. The lesions enlarge as the bacteria invade the tissues, the greatest development commonly being parallel to the long axis of the shoot. As the lesions develop, the central areas turn dark brown or black. During the active stages of parasitism a narrow, dark-green, water-soaked zone, denoting advanced bacterial action, surrounds the discolored areas. During periods of high humidity, a dark brown or black, water-soluble, bacterial exudate sometimes appears on the surface of the lesions. The elevation of the lesion is at first unchanged but later is depressed. When fully developed, stem lesions are typically irregularly oval, dark brown to black, depressed, with well-defined margins (Figure 7, A). They are quite variable in size, ranging from a few millimeters to several inches in their maximal dimension. In some in-

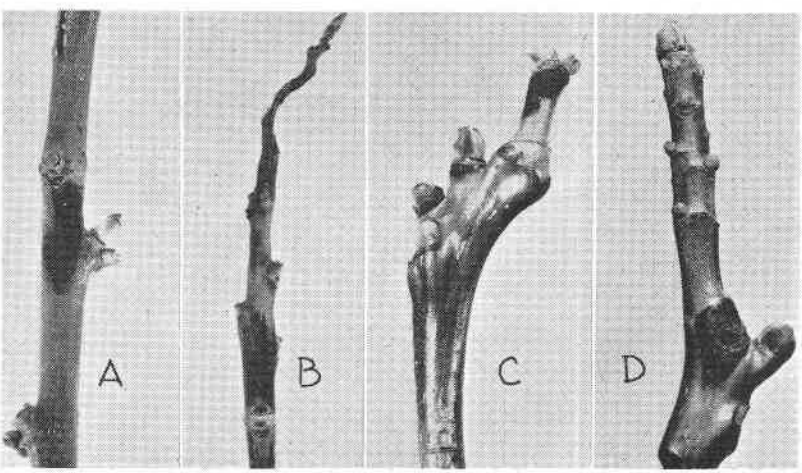

Figure 7. Walnut shoots affected by bacteriosis: A, Lesion on a shoot; B, shoot girdled by a lesion; C, lesion at base of a blighted bud; D, lesion around a nut "scar."

stances the lesion extends completely around the shoot, causing the distal portion to die (Figure 7, B). However, bacteriosis is not responsible for the death of all shoots that die during the growing season. There are a number of nonparasitic agencies that may also cause a dying-back of the shoots.

The lesions may be quite superficial involving only the outer portion of the bark or they may extend inwardly to the pith. They may occur scattered over the surface of the stem, about infected buds (Figure 7, C), or around "scars" where the peduncles of diseased nuts were attached (Figure 7, D). Stem cankers about infected buds and nut "scars" are formed secondarily by invasion of the bacteria from diseased buds and nuts, respectively.

The majority of stem lesions become inactive at or near the end of the first season and the process of healing is inaugurated. However, in a comparatively small percentage of shoot lesions the bacteria remain alive through the winter and constitute one source of primary inoculum. By the end of the second season, however, the bacteria die in practically all of the stem cankers.

On the Buds

Leaf buds and staminate and pistillate flower-bearing buds in the axils of leaves on young shoots are also attacked by the disease. Infection of the buds generally occurs very early in the growing season, principally in the prebloom and blooming stages, when they are still green and succulent. The first macroscopic evidence of infection of the buds consists of small, dark green, water-soaked areas in the outer bud "scales" or bracts. As the lesions develop, the infected parts turn dark brown. From the outer bracts the bacteria invade the central axis and subsequently the inner parts of the bud. Buds infected early in the growing season ordinarily die by the end of the season and turn dark brown (Figure 8).

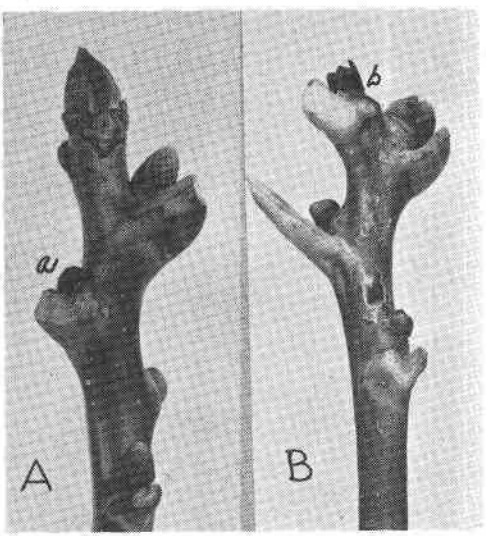

Figure 8. Walnut buds affected by bacteriosis; inoculated in early spring and photographed in September of the same year: A, An infected catkin bud (a); B, a diseased leaf bud (b).

In some instances, however, infection is localized in the bud bracts in which case the buds do not die but unfold at the normal time the following spring. The leaves of the shoots coming therefrom generally have lesions on the margins of the leaflets (Figure 1, A). Such lesions constitute one source of primary inoculum. Bud infection is an important phase of the disease, as it has been found that the causal organism overwinters principally in diseased buds under Pacific Northwest conditions. Bud blight is also of considerable ecomonic importance, since many of those killed are pistillate flower-bearing buds.

On the Catkins

The staminate buds may

be infected by the bacteria and killed either in the same year they are formed while in the bud stage (Figure 8, B) or the following spring after they have broken dormancy and are partially to completely elongated. In the latter case, the infected parts turn black and die. In most cases, only local areas in the catkin are attacked, though occasionally the whole catkin may be diseased (Figure 9). The infected catkins frequently assume abnormal shapes due to a cessation of growth in the diseased areas. However, not all the catkins that turn black are diseased. Late frosts and certain other adverse conditions, such as prolonged periods of rain during the time when the staminate buds begin to grow and during flowering, are also responsible for the death of catkins.

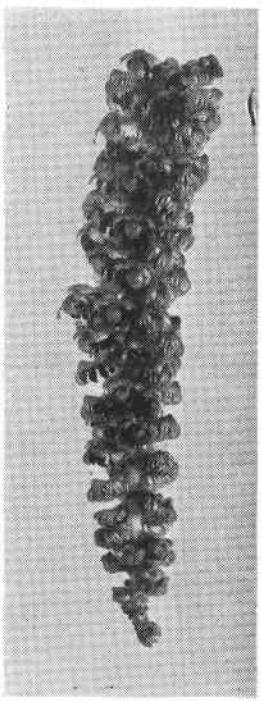

Figure 9. A catkin affected by walnut bacteriosis.

HOSTS

Bacteriosis has been reported as occurring naturally on *Juglans regia* L., on leaves of *J. hindsii* Jepson and of Paradox hybrids (crosses between *J. californica* S. Wats or *J. hindsii* and *J. regia*) (*93, 110*), and on the nuts of *J. cordiformis* Maxim. var. *ailantifolia* (Carr.) Rehd. (*6*). It has also been found occurring naturally in Oregon on leaves and stems of sprouts coming from Northern California black walnut rootstocks (*J. hindsii*) topworked to Persian walnuts.

The disease has been artificially produced by C. O. Smith (*100, 102, 110*) on tender shoots and leaves of *Juglans nigra, J. californica, J. hindsii, J. cinerea, J. cordiformis* Maxim, *J. cordiformis* Maxim var. *ailantifolia,* Paradox hybrids, and Royal hybrids (hybrids between *J. nigra* and *J. californica* or *J. hindsii*). We have also produced the disease by artificial inoculation on leaves of *J. nigra* and on leaves, stems and nuts of *J. hindsii.*

III. THE CAUSAL ORGANISM

Pierce (*76*) first isolated and described the causal organism, naming it *Pseudomonas juglandis.* A verbatim copy of Pierce's original description of the pathogen follows:

> *Pseudomonas juglandis,* n. sp.—A short, rod-shaped micro-organism with rounded ends, actively motile, bearing a single long polar usually wavy flagellum. Length of organism, taken from colony in acid gelatin, set directly from walnut, and stained with gentian violet, 1-2 μ, according to whether the germ has just divided or has elongated but not yet divided. Just before separation a pair of germs will usually average about 2 μ in length. Average breadth of organism about 0.5 μ. Occurs singly or in pairs and sometimes in shorter or longer chains. Produces a bright chrome-yellow growth on potato and many other media. When growing normally on potato the starch is so acted upon by a diastatic ferment produced by the organism, that it is altered throughout a wide band beyond the margin of the culture of organisms. This band of converted starch may extend 0.5-1 cm. or more beyond the margin of the growth of germs, appears white to the eye, fails to show normal starch reaction to iodine, yields marked grape sugar reactions, has an exceedingly sharp and well defined limiting outline, often passing so sharply through a cell as to include only the starch grains on one side of the cell. This broad and distinct ferment band distinguishes this organism at once from *Pseudomonas stewarti* and *P. hyacinthi,* as well as from the more nearly related *P. campestris,*

which occasionally forms a weak but much narrower band, and from all other uniflagellate organisms studied. Organism prefers neutral or acid reaction of culture medium, a moderate degree of alkalinity inhibiting growth; it liquefies neutral and acid gelatin. Produces no gas in fermentation tubes of sugar solutions; growth confined to neck and bulb of tube, hence aerobic, no growth under mica plate. Colonies in malic acid potato gelatin and agar circular; at first clear but soon decidedly yellow, margin sharp. This organism is distinguished from *P. campestris*, the most nearly related species of the genus, aside from the characters already assigned, in producing an abundant and bright yellow pigment on the surface of extracts of the leaves of the following plants, while *P. campestris* produces little or no pigment upon such extracts: walnut (*Juglans regia*), magnolia (*Magnolia macrophylla*), fig (*Ficus carica*), castor bean (*Ricinus communis*), loquat (*Eriobotrya japonica*).

In 1905, E. F. Smith changed the name of the pathogen to *Bacterium juglandis* (Pierce) E.F.S. to conform to his system of nomenclature and later a Committee of the Society of American Bacteriologists reclassified it according to a more modern system of nomenclature, assigning to it the name *Phytomonas juglandis* (Pierce) Bergey et al. (*30*). In 1937, Elliott (*36*) called attention to the fact that the name *Phytomonas* was first used by Donovan in 1909 as a name for a group of flagellate protozoans in the latex of plants. The name *Xanthomonas*, proposed by Dowson (*34*) in 1939 for this genus, has recently been accepted by competent authorities in lieu of the name *Phytomonas*. The binomial *Xanthomonas juglandis* (Pierce) Dowson will accordingly be used to designate the organism in this paper.

Following Pierce's (*81*) pioneer studies on the causal organism, a number of other investigators, notably Smith (*110*), Doidge (*32*), Wormald (*120*), and Burkholder (*24*), have conducted and reported additional studies on its morphology and physiology. An amended description of the pathogen based upon the results of these more recent as well as earlier studies is given by Elliott (*35*) and by Stapp (*112*). We have also carried on morphological and physiological studies of a number of isolates of the causal organism 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 under controlled conditions in order to gain a better understanding of its behavior in nature. A preliminary report of these studies has been published in part elsewhere (*65*). There is presented herewith a more complete report including certain studies not previously reported.

SOURCE AND NUMBER OF ISOLATES STUDIED

Three different isolates of *Xanthomonas juglandis* from widely scattered walnut orchards in the Pacific Northwest were used in most of the morphological and biochemical studies. In certain phases of the work as many as 17 different isolates from different orchards in California, Oregon, and Washington were employed.

METHOD OF ISOLATION AND PURIFICATION OF THE ISOLATES

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 was shown by McNew (*51*) to yield a very high percentage of colonies that 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.

The causal organism is a capsulate rod, 1.1 μ to 3.8 μ by 0.5 μ to 0.7 μ (averaging 1.86 μ by 0.58 μ), arranged singly or in pairs, with rounded ends; motile by one polar flagellum (Figure 10, A). Gram negative and not acid fast; no endospores; stains readily with gentian violet and carbol fuchsine but only very lightly with methylene blue. The 3 different isolates of the pathogen

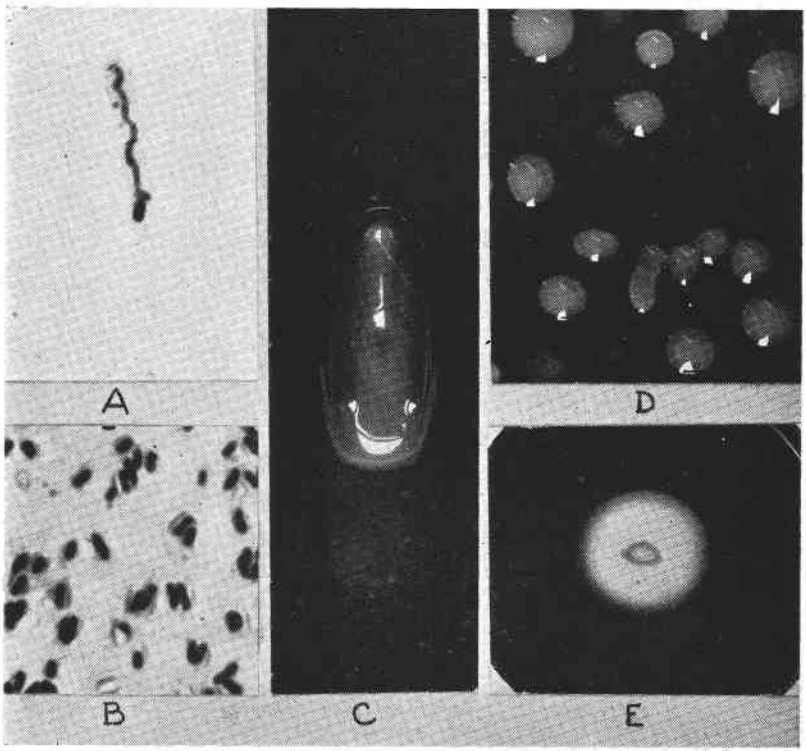

Figure 10. A, B. Photomicrographs of *Xanthomonas juglandis,* showing: A, the single polar flagellum, x 2300; B, capsule about organism, x 1475; C, D, ten-day-old potato dextrose agar cultures of the same organism; C, streak culture; D, plate culture; E, starch-free zone about a bacterial colony demonstrated by flooding the surface of the medium with iodine and potassium iodide, xl. *Photographs A and B by F. P. McWhorter.*

studied comparatively had similar morphological characteristics, as did numerous other isolates studied periodically during the course of these investigations.

The data given are at variance in certain respects, with those of Smith (*110*) who found the pathogen to vary from 1.5 μ to 3.01 μ in length and from 0.3 μ to 0.51 μ in diameter. The smaller size reported by Smith may be due to using "positive" staining methods, whereas in this investigation the more precise "negative" staining technique was used. Smith states that *Xanthomonas juglandis* does not possess a true capsule. We find *X. juglandis* to be definitely capsulated when grown on Difco beef extract-dextrose agar (Figure 10, B). Smith also reported that *X. juglandis* is Gram positive; all the isolates used in these studies were found to be Gram negative.

PHYSIOLOGY

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 (*30*) were closely followed in the biochemical studies reported herewith.

Cultural Characteristics of Media

Although *Xanthomonas juglandis* grows well on a wide range of bacteriological media, it was found to make the best and most distinctive growth on potato-dextrose agar. A description of the pathogen on this and other common cultural media follows.

Potato-dextrose agar streak. On tubes of freshly slanted Difco potato-dextrose agar at pH 6.8 and at an average temperature of 25° C., growth first became noticeable about 24 hours after inoculation. After 3 days' incubation, the bacterial growth covered from ¼ to ⅓ the surface of the slant. The streak was filiform, convex, viscid, glistening, opaque, odorless, and a pale lemon to amber yellow (*74*) (Figure 10, C). The topography of the growth was smooth. At 25° C. the bacterial growth covered the entire surface in from 10 to 15 days. Seventeen different isolates of the pathogen studied during the course of these investigations showed no significant difference in growth characteristics on this medium.

Beef extract-dextrose agar streak. Growth after 3 days at 28° C. on Difco beef-extract-dextrose agar slants at pH 6.6 was moderate, filiform, convex, viscid, glistening, smooth, opaque, odorless, and pale lemon to coppery yellow (*74*). Seventeen different isolates of the pathogen studied comparatively on this medium behaved similarly.

On potato cylinders. Growth 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 practically so from starch. The behavior of three different isolates of the causal organism studied comparatively on this medium was the same.

Beef extract-dextrose agar plates. Colonies of *Xanthomonas juglandis* on Difco beef extract-dextrose agar plates became visible in from 2 to 3 days at 22° C. After 6 days' growth, the surface colonies were circular, smooth,

convex, glistening, with entire margins. The colonies imbedded in the medium were lenticular. The internal structure of the surface colonies was homogeneous, and finely granular. The medium remained unchanged in color. The colonies were pale lemon or coppery yellow (*74*) at the margins and deeper lemon to chrome yellow at the centers. After 12 days' growth at 22° C. the colonies averaged about 7 mm. in diameter. Three different isolates of the pathogen studied comparatively behaved similarly on this medium.

Potato-dextrose agar plates. Growth of *Xanthomonas juglandis* on Difco potato-dextrose plates at an average temperature of 22° C. became visible in from 2 to 3 days. After 5 to 6 days' growth the surface colonies were viscid, smooth, convex to pulvinate, with entire edges. The internal structure was finely granular. Radial striations occurred at the margins of some colonies. The colonies were a pale lemon to an amber yellow (*47*). There was no change in the color of the medium except for the production of a partially cleared zone just in advance of the margin of each colony due to the hydrolysis of the starch in the medium. 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 (Figure 10, D). Three different isolates of the causal organism studied comparatively showed no differences in growth characteristics on this medium.

Gelatin stab. Growth in Difco nutrient gelatin "stabs" at pH 6.6 became visible after 24 hours at an average temperature of 22° C. Liquefaction began at the surface in from 1 to 2 days. The liquefied portion was at first infundibuliform but soon became stratiform. In 10 days the upper half of the medium was liquefied. The liquefied portion was slightly turbid and a pale yellow sediment was evident at the bottom. Liquefaction of the lower half of the medium was very slow, requiring from 4 to 6 weeks, or even longer, for 10 cc. of gelatin in a $\frac{5}{8}$-inch test tube to be entirely liquefied. A positive reaction with tannic acid after 48 hours' incubation at 30° C. was obtained when tested by Frazier's gelatin plate technique (*38*), indicating a considerable increase in amino-nitrogen. Three isolates of the pathogen studied comparatively behaved in a like manner on this medium.

Other solid media. *Xanthomonas juglandis* was also grown and studied on 10 other solid media, as follows: Difco prune agar, Difco lima bean agar, Difco corn meal agar, Difco bean pod agar, dextrose-phosphate agar, malt agar, Endo agar, Congo red agar, and Levine's eosine-methylene blue agar. On the first 5 of these media, growth was very sparse while on the latter 5 the growth was not sufficiently distinctive to justify detailed description.

Nutrient broth. In Difco beef extract-peptone broth at pH 6.6 the causal organism made a trace of growth after 16 hours at 22° C. By 48 hours a moderate clouding of the medium was evident but there was no sediment. After 6 days the medium became very turbid and a fragile ring developed where the surface of the medium meets 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, slightly viscid sediment was formed in the medium. After about 3 weeks the medium cleared slightly due to a settling of the growth. No odor was at first detectable but after several weeks a slight odor resembling ammonia was present. Three isolates of the pathogen studied comparatively had similar growth characteristics in this medium.

Dextrose broth. In Difco beef extract-peptone-dextrose broth at pH 6.6, *Xanthomonas juglandis* made a trace of growth after 24 hours at 30° C. After 3 days at 30° C. the 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. The behavior of three different isolates of the causal organism studied comparatively in this was the same.

Biochemical Characteristics of Media

Nitrate reduction. Nitrates were not reduced to nitrites by *Xanthomonas juglandis* nor was any gas formed in nitrate broth. To test for the reduction of nitrates Trommsdorf reagent was added to 1-, 2-, 4-, and 7-day-old cultures, respectively. Three different isolates of the pathogen studied comparatively reacted similarly.

Chromogenesis. *Xanthomonas juglandis* produced a yellow pigment on Difco beef extract-dextrose agar and Difco potato-dextrose agar as has been previously stated. The color of the growth varied from a pale lemon yellow to a deep chrome yellow, depending on the age of the cultures and on the medium. The prevailing color was a pale lemon or amber yellow (*74*). Seventeen different isolates of *X. juglandis* studied comparatively showed no significant differences in chromogenic characteristics.

Indole production. Indole is not produced by *Xanthomonas juglandis*. To test for indole production, a 2-day-old culture in tryptophane broth was treated with Ehrlich's reagent, as recommended by the Committee on bacteriological technique (*30*). Three different isolates of the pathogen studied comparatively showed no difference in this regard. Smith (*110*), using a different procedure reported a strong but delayed indole reaction at 75° C. to 80° C. when cultures of *X. juglandis* in Dunham's solution, 2 weeks of age, were tested with 0.02 per cent sodium nitrate and a few drops of concentrated sulphuric acid.

Hydrogen sulphide production. *Xanthomonas juglandis* does not produce hydrogen sulphide, as indicated by a negative response when the pathogen was grown in Difco lead acetate agar stabs. Three different isolates of the pathogen studied comparatively behaved similarly on this medium.

Hydrolysis of starch. *Xanthomonas juglandis* hydrolyzes starch. Tests for starch hydrolyzing ability were made by growing the pathogen on plates of starch or potato dextrose agar. A cleared zone, that became noticeable when the surface of the medium was flooded with Gram's iodine solution, was produced about the colonies (Figure 10, E). The behavior of 3 different isolates of the pathogen was similar on this medium.

Digestion of milk. When grown in milk, *Xanthomonas juglandis* produces an enzymatic curd that is slowly digested. Peptonization occurred near the surface after 4 or 5 days; it was more pronounced though not complete after 10 days. Three different isolates studied comparatively behaved similarly on this medium.

Reduction of litmus. *Xanthomonas juglandis* slowly reduces litmus in litmus milk. Reduction began after 1 to 2 days but was not complete until 1 to

2 months thereafter. The behavior of 3 different isolates of the pathogen was the same on this medium.

Selenium reduction. Streak cultures of the pathogen on nutrient agar containing selenium dioxide in 1:25,000 concentration became brick red after a short time, indicating intracellular reduction. This reaction is regarded by Levine (*47*) as a better indicator than organic dyes for reducing activity. Three different isolates of the causal organism behaved similarly on this medium.

Methyl red test. *Xanthomonas juglandis* on Difco M.R.-V.P. medium (for the performance of the Methyl red and Voges-Proskauer tests) adjusted to an initial pH of 6.9, became progressively more alkaline with age. Two different isolates of the pathogen behaved alike on this medium.

Relation to free oxygen. *Xanthomonas juglandis* is strongly aerobic as indicated by the fact that absolutely no growth of the organism occurred on potato-dextrose agar plates or slants when the cultures were incubated for 10 days or longer in an oxygen-free atmosphere created by burning phosphorus in an air-tight glass jar. Potato-dextrose agar cultures incubated outside that jar under laboratory or aerobic conditions made a luxuriant growth in the same time. Moreover, when the pathogen was grown in Smith's fermentation tubes in Difco beef extract-peptone broth containing 1 per cent dextrose, growth occurred only in the open arm of the tube.

Relation to reaction of medium. The relation of the reaction of the medium to the growth of *Xanthomonas juglandis* was determined by growing the organism in tubes of Difco beef extract-peptone broth adjusted to various hydrogen-ion concentrations ranging from pH 5 to pH 11.5. In Table 1 the data for the relation of the reaction of the medium to the growth of the pathogen in these cultures are given.

The data presented in Table 1 show that *Xanthomonas juglandis* can grow at a pH range of from 5.4 to 10.8, the most favorable range being

Table 1. THE RELATION OF THE REACTION OF THE CULTURE MEDIUM TO THE GROWTH OF *Xanthomonas juglandis*

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	+
9.6	+
10.0	+
10.4	+
10.6	+
10.8	+
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.

pH 6.4 to pH 7.2. No growth was apparent in 10 days at and below pH 5.2 nor at or above pH 11.

Alkali production. Alkali (ammonia) is produced by *Xanthomonas juglandis* when grown in a nutrient broth containing peptone as a nitrogen source. A shift in reaction from an initial pH value of 6.8 to pH 7.5 occurred during 11 days' growth in Difco beef extract-peptone broth. Tests with Nessler's reagent were positive showing that ammonia is formed. Ammonia was also produced abundantly in a 1 per cent solution of peptone in tap water. Although the pathogen produces an alkaline reaction in peptone-containing media and in milk, it does not belong to the alkali-forming group of bacteria as defined by Ayers, et al. (*12*). Alkali production in nutrient broth by *X. juglandis* is apparently due to the breakdown of amino acid to form ammonia. In this regard these studies are at variance with those of Dowson (*34*) who reported that *X. juglandis* does not produce ammonia in nitrate-peptone broth.

Carbon metabolism. A variety of carbon sources are utilizable by *Xanthomonas juglandis* with the production of acid but no gas. To demonstrate acid production, it was found necessary to employ a synthetic medium containing only inorganic nitrogen compounds since acid production is completely masked in the presence of peptone, due to ammonia production. The utilization of carbohydrates by the pathogen was studied in preliminary investigations using agar slopes made with a synthetic basal medium containing various sources of carbon as per the procedure used by Burkholder (*24*). After 10 to 14 days' incubation at 28° C., it was observed that all isolates slowly produced acid from dextrose, but not from sucrose. In a subsequent study, however, using 12 different isolates of the causal organism, acid was produced by all cultures on sucrose as well as on dextrose media when the incubation period was extended to 60 days though there was considerable variation between isolates in the amount of acid produced. Since the agar slopes dried out extensively during prolonged incubation, the experiment was repeated, using liquid media. Sugars, alcohols, glucosides, and sodium salts of organic acids were sterilized by filtration and added aseptically to a sterile basal synthetic liquid medium.*

Bromcresol purple was added as an indicator except in media containing organic salts, where brom-thymol blue was employed. 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 milliliter of the solution was added aseptically to 4 ml. of the sterilized basal medium in small test tubes, giving a concentration of 1 per cent carbon source in the final medium. Before using, all media were incubated for 5 days at 28° C. to check their sterility. To test for 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. All liquid media were inoculated in duplicate. One loop of a distilled water sus-

*Sodium ammonium phosphate ($NaNH_4HPO_4 4H_2O$) ..... 1.0 gram
Potassium chloride (KCl) ..... 1.0 gram
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) ..... 0.2 gram
Bromcresol purple ..... 0.01 gram
Distilled water ..... 1,000 ml.
Adjusted to pH 7.

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

Carbon source	Time required to produce indicated changes				
	Isolate number 5056	Isolate number 5080	Isolate number 5154	Isolate number 5171	Isolate number 5184
	Days	*Days*	*Days*	*Days*	*Days*
Dextrose	+10[1]	+14	+14	+14	+30
Levulose	+21	+21	+21	+21	+21
Sucrose	+31	+28	+33	+28	+28
Lactose	+33	+14	+14	+33	+27
Maltose	+27	+27	+32	+27	+14
Raffinose	+42	+27	+42	+52	+42
Arabinose	*[2]	*	*	*	*
Rhamnose	*	*	*	*	*
Xylose	+22	+32	+32	+32	+32
Mannitol	+17	+13	+13	+13	+13
Dulcitol	*	*	*	*	*
Glycerol	+32	+22	+22	+32	+22
Salicin	*	*	*	*	*
Starch	++13[3]	++13	++13	++13	++ 2
Inulin	*	*	*	*	*
Cellulose	*	*	*	*	*
Sodium acetate	*	*	*	*	*
Sodium benzoate	*	*	*	*	*
Sodium citrate	— 5[4]	— 5	— 7	— 5	— 5
Sodium formate	*	*	*	*	*
Sodium lactate	—13	—13	—13	—13	—13
Sodium malate	— 7	— 7	— 7	— 7	— 7
Sodium salicylate	*	*	*	*	*
Sodium succinate	— 7	— 7	— 7	— 7	— 7
Sodium tartrate	*	*	*	*	*

[1]+=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]*=No growth within 60 days.

[3]++=Hydrolysis of starch and full acid color of bromcresol purple; figure following indicates number of days required to produce full acid color of indicator.

[4]—=Heavy growth and full alkaline color of bromthymol blue; figure following indicates number of days required to produce full alkaline color of indicator.

pension prepared by mixing 2 loops of the growth from a 24-hour-old dextrose nutrient agar culture with 10 cc. of sterile water, was used as the inoculum. Quantitative tests showed the inoculum used contained between 200,000 and 300,000 bacteria. Cultures were incubated at 28° C. and observed daily. The pathogen made only relatively slow growth on these synthetic media, 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 the appearance of turbidity. In Table 2 data are given for the growth of 5 isolates of *Xanthomonas juglandis* in synthetic media containing various carbon sources.

It would appear from the data given in Table 2 that *Xanthomonas juglandis* can use the following carbon sources (listed in decreasing order of average rate of utilization): citrate, malate, succinate, starch, lactate, mannitol, dextrose, lactose, levulose, maltose, glycerol, sucrose, xylose, and raffinose. Arabinose, rhamnose, dulcitol, salicin, inulin, cellulose, acetate, benzoate, formate, salicylate, and tartrate were not utilized. The most rapid rate of acid production occurred with isolate number 5184 on starch, the full acid color of bromcresol purple being attained in 2 days. This was exceptional, since all the other isolates required 13 days to produce a corresponding change which is comparable to the average time required to ferment dextrose. Sodium citrate was fermented most rapidly by all isolates, only 5 to 7 days being required for development of the full alkaline color of bromthymol blue. All isolates com-

pleted the indicator change in 7 days with malate and succinate. Since the carbon sources in these three instances are relatively simple anions, a different fermentation mechanism is probably involved which could explain the more rapid changes observed. The isolates showed great variation in the rate of action on some of the sugars; maltose, for example, was fermented to full acid color of bromcresol purple in from 14 to 32 days. Levulose, on the other hand, was fermented at a uniform rate, the indicator being fully changed by all isolates in 21 days. This, however, is an unusually slow change, levulose often being fermented more rapidly than dextrose by other bacteria (*113*). Good agreement in duplicate cultures was obtained. In a few cases, there was a difference of from 2 to 10 days in the time required completely to change the indicator.

If 1 of the 5 isolates could grow on a given carbon source, all the other isolates could utilize it also. Differences appeared only in the rate of utilization, and these were not consistent, being fortuitously distributed among all isolates and among most of the carbon sources. These results are at variance in certain respects with those of Dowson (*34*) who reports that *Xanthomonas juglandis* produces no acid from sucrose, xylose, and glycerol. The short incubation period—less than 4 weeks—used by Dowson may account for this discrepancy. The incubation temperature, while not specifically stated by him, inferentially was 27° C.

Nitrogen metabolism. *Xanthomonas juglandis* can utilize a relatively wide variety of nitrogen sources. Availability of various nitrogen sources was determined by using a 0.1 per cent concentration of each in a basal synthetic liquid medium† containing dextrose. The nitrogen sources were prepared in 0.5 per cent solution and sterilized separately; 1 cc. portions were then added aseptically to autoclaved tubes containing 4 cc. of the basal-medium. The sodium nitrite was sterilized by filtration; all other nitrogen sources were autoclaved at 15 pounds pressure for 20 minutes. Inoculations were made as

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

Nitrogen source	Time required to produce heavy growth[1]				
	Isolate number 5056	Isolate number 5080	Isolate number 5154	Isolate number 5171	Isolate number 5184
	Days	*Days*	*Days*	*Days*	*Days*
a-Alanine	10	10	14	14	10
Allatoin	12	12	12	12	12
Aspartic acid	10	10	10	10	10
Brucine	18	18	18	18	18
d-Glutamic acid	*	*	*	*	*
Hippuric acid	*	*	*	*	*
l-Leucine	14	14	10	10	10
Sodium ammonium phosphate	10	14	14	14	30
Sodium nitrite	*	*	*	*	*
Peptone (Difco)	2	2	2	2	2
l-Tyrosine	12	12	12	12	12
Uric acid	12	12	12	12	12

[1]As indicated by a turbidity comparable to that shown by a 48-hour culture of *Escherichia coli* in Difco beef extract-peptone broth. * = no growth within 60 days.

†Dibasic potassium phosphate (K_2HPO_4) ..... 1.0 gram
Sodium chloride (NaCl) ..... 0.2 gram
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) ..... 0.2 gram
Dextrose ..... 10.0 grams
Distilled water ..... 1,000 ml.
* Adjusted to pH 7.0 with NaOH solution.

previously described, and the cultures were incubated at 28° C. Comparisons were made on the basis of the 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 Difco beef extract-peptone broth. In Table 3 data are given for the growth of 5 isolates of *X. juglandis* in synthetic liquid media containing various sources of nitrogen.

These data show that *Xanthomonas juglandis* can utilize the following nitrogen sources: peptone, aspartic acid, a-alanine, l-leucine, sodium ammonium phosphate, allantoin, tyrosine, uric acid, and brucine. Hippuric acid, d-glutamic acid, and sodium nitrite did not support the growth of any of the isolates. It is noteworthy that greater uniformity exists in the rate at which the various isolates utilize a given nitrogen source than was the case with carbohydrates, and there is less variation in time requirement for the various sources. Peptone gave the most rapid growth, all isolates producing heavy turbidity in 2 days. Rapid growth in the presence of peptone is probably due to accompanying "nutrilites." Slowest growth was obtained with brucine, 18 days being required by all isolates to produce turbidity comparable to a 2-day old culture in peptone; this is excepting the 30-day period required by one isolate on sodium ammonium phosphate. All other cultures attained heavy growth in from 10 to 14 days.

Lipolytic activity. Since lipolytic activity of phytopathogenic bacteria has been shown to be of taxonomic value by Starr and Burkholder (*113*), their spirit-blue agar method was applied to 6 of our isolates. Two of these had been in culture for 5 years, while the others were of more recent origin. All exhibited active lipolysis of cottonseed oil. Similar results were obtained by Starr and Burkholder for *Xanthomonas juglandis*. The walnut bacteriosis pathogen is thus shown to possess the lipolytic character common to *Xanthomonas* species generally.

Enzyme production. Pierce (*81*) reported that *Xanthomonas juglandis* produces a diastatic ferment when grown on potato. Smith (*110*) stated that "the walnut organism produces at least 4 enzymes: diastatic (starch-destroying), cytohydrolytic (cellulose-dissolving), rennet (casein-forming), and proteolytic (peptonizing)." As a knowledge of the enzymes produced by the pathogen is important to an adequate understanding of the relations of *X. juglandis* to its host, further enzymotic studies were conducted. In our investigations no attempt was made to determine the whole range of enzymes produced by this organism. Rather, an effort was made to demonstrate the production of those enzymes that might conceivably be concerned in the development of the pathological condition. The production of pectinase, diastase, rennin, and proteinase was experimentally demonstrated. A discussion of the methods used to demonstrate enzyme production and the results follows.

PECTIC ENZYMES. To demonstrate pectinase* production, *Xanthomonas juglandis* was grown on pectin agar† and in a liquid medium‡ each containing

* Pectinase is apparently a group of specific enzymes acting upon certain linkages of the pectin molecule [Kertesz (*46*)].

†Composition as follows:

Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.25	gram
Dibasic potassium phosphate (K_2HPO_4)	0.25	gram
Calcium carbonate ($CaCO_3$)	5.0	gram
Calcium pectate (Certo)	10.0	ml.
Agar	20.0	grams
Distilled water	1,000	ml.

Sterilized by autoclaving 20 minutes at 15 pounds.

‡ Basal medium described under carbon metabolism plus 0.5 per cent pectin.

pectin as a source of carbon. On pectin agar plates, after 20 days' incubation at 22° C., colonies of *X. juglandis* averaged 8 mm. in diameter, while on the same medium with pectin omitted no detectable growth had occurred. However, pectin is apparently not so good a source of carbon as starch or dextrose, as is shown by the fact that after 20 days' incubation at the same temperature upon potato dextrose agar plates, colonies of *X. juglandis* averaged 18 mm. in diameter, or 2 to 3 times larger colonies than on pectin agar.

Although the best pectin available commercially is considered to be free from reducing substances or other fermentable material aside from pectin itself, it was deemed advisable to avoid autoclaving and thus prevent possible hydrolysis. Since high viscosity precluded the use of filtration, sterilization was accomplished by alternate freezing and thawing. A 2.5 per cent distilled water solution of pectin (Pfanstiehl, technical, citrus) was alternately frozen in the freezing element of a refrigerator and incubated at 37° C. on 3 successive days; the solution was frozen over night and incubated on the following day, this procedure being repeated three times. One milliliter of this solution was then added to each of a series of tubes containing 4 ml. of autoclaved basal medium, giving a final pectin concentration of 0.5 per cent. These tubes were then incubated at 28° C. for 5 days to check upon their sterility. Five different isolates of *Xanthomonas juglandis* were transferred in duplicate to this medium and incubated at 28° C. Full acid color of the indicator appeared in 14 days with 4 of the isolates, but only after 24 days with the fifth. Control tubes showed no change. It would appear, therefore, that *X. juglandis* produces an enzyme, or enzymes, classifiable in the pectinase group, which hydrolyzes pectin to simple fermentable components.

Evidence that *Xanthomonas juglandis* produces protopectinase was also obtained from certain biological studies in which pieces of carrots were used to test for pectinase production. In these studies, *X. juglandis* was grown in carrot decoction* for 9 days after which tests were made for the presence of protopectinase. Small pieces of tissue were removed aseptically from the root of a healthy carrot and placed (a) in a 19-day-old carrot juice culture of *X. juglandis,* (b) in a 19-day-old carrot juice culture to which had been added 0.5 ml. of toluol to inactivate the bacteria but not any exo-enzymes which might be present, (c) in a tube of sterile carrot juice to which had been added 0.5 ml. of toluol, and (d) in a tube of sterile carrot juice. Ten days after the carrot tissue was added to these cultures, a difference in the consistency or softness of the carrot pieces in certain of them was noted. The pieces of carrot in the untreated cultures of *X. juglandis* (a) were noticeably softer than the pieces in any of the other solutions. The carrot tissues in the toluol-treated cultures (b) were not quite so soft as those in the untreated bacterial cultures but they were noticeably softer than those in the controls. However, no marked rotting of the tissues, such as would be the case with *Erwinia carotovorus,* the bacterium causing the soft rot disease of carrots, occurred in any of these cultures. After 58 days the carrot pieces in both the toluol-treated and untreated cultures were definitely softer than those in the control solutions, but even after this interval no marked dissolution or rotting of the tissues had taken place.

A microscopic examination of the tissues in the various solutions showed that the cell walls of the carrot pieces in the toluol-treated and in the untreated bacterial cultures were more swollen and the intercellular spaces larger than those in the controls. Essentially the same results were obtained in Difco beef

*Peeled carrots ..... 40 grams
Tap water ..... 200 ml.

extract-peptone broth cultures of the pathogen. These results are interpreted as indicating that an exo-enzyme of the nature of protopectinase is produced by *Xanthomonas juglandis*, which acts upon the middle lamella and other pectin-like substances normally present in the cell walls, softening and partially dissolving them, with the result that intercellular penetration by the bacteria is facilitated.

DIASTASE. Pierce (*76*) and Smith et al. (*110*) found that a diastatic ferment is produced by *Xanthomonas juglandis*. Our studies further show that diastase is produced in relatively large quantities by this organism. Thus, when *X. juglandis* is grown on potato dextrose agar or starch agar*, a definite fermentation zone is produced about the bacterial colonies. This fermentation zone becomes quite apparent when the surface of the medium is flooded with a weak solution of iodine and potassium iodide (Figure 12, E). Furthermore, starch in a synthetic medium is hydrolized by the organism as pointed out in the section on utilization of carbon sources (see Table 1).

RENNIN. Smith, et al. (*110*) reported that rennin also is produced by *Xanthomonas juglandis*. We have likewise demonstrated the production of rennin as indicated by the coagulation of casein in milk. However, only limited amounts of rennin apparently are produced, as separation of casein in milk does not occur until 4 or 5 days after inoculation.

PROTEINASE. Smith, et al. (*110*) found that a proteolytic enzyme is produced by *Xanthomonas juglandis*. Our studies confirm Smith's findings as the rennin curd of milk is digested by the organism. However, only comparatively small amounts of this enzyme apparently are produced, as peptonization of milk occurs very slowly.

CELLULASE. Smith, et al. (*110*) reported that *Xanthomonas juglandis* also produces a "cytohydrolytic (cellulose-dissolving)" enzyme. However, we have been unable to demonstrate experimentally the production of an enzyme of the nature of cellulase. In our studies pure cultures of *X. juglandis* were grown on cellulose agar† at room temperature for as long as 43 days. Moreover, filter papers were exposed to the action of beef extract-peptone broth cultures of *X. juglandis* for as long as 30 days without noticeable effect. Furthermore, cellulose in synthetic medium was not fermented even at the end of 60 days (see Table 2). The results of these studies would seem to indicate, therefore, that cellulase in measurable amounts is not produced by *X. juglandis* in pure

*Agar	10.0	grams
Dibasic potassium phosphate (K_2HPO_4)	0.2	gram
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.2	gram
Potassium carbonate (K_2CO_3)	0.4	gram
Calcium chloride (fused) ($CaCl_2$)	0.02	gram
Ferric sulphate ($Fe_2(SO_4)_3$	0.02	gram
Sodium chloride (NaCl)	0.02	gram
Peptone	0.50	gram
Starch solution (Made by adding 800 ml. of boiling water to 10 gm. of potato starch suspended in a little cold water, and concentrating by boiling to 500 ml.)	500.00	ml.
Tap water	500.00	ml.
†Agar	15.0	grams
Cellulose	2.5	grams
Dibasic potassium phosphate (K_2HPO_4)	0.2	gram
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.2	gram
Potassium carbonate (K_2CO_3)	0.2	gram
Calcium chloride ($CaCl_2$)	0.2	gram
Ferric sulphate ($Fe_2(SO_4)_3$)	0.02	gram
Sodium chloride (NaCl)	0.02	gram
Peptone (Difco)	0.02	gram
Distilled water	1,000	ml.

culture on artificial media of the kinds used in these studies. There still remains the possibility that the pathogen produces an enzyme of the nature of cellulase in association with its live hosts, for unless the production of small amounts of some cellulose-dissolving substance in the invaded tissues either by the causal organism or by the host under the stimulus of bacterial invasion is postulated, the evidence presented by histological studies of diseased tissues is without adequate interpretation.

Relation of temperature. Studies on the relation of temperature to the growth of *Xanthomonas juglandis* indicate that the optimum temperature for growth in culture is about 28° C., the maximum (in liquid media) between 35° C. and 37° C. and the minimum about 1° C. The relation of temperature to the growth of *Xanthomonas juglandis* was determined by 2 methods which gave similar results. In the first method, duplicate dextrose-nutrient broth

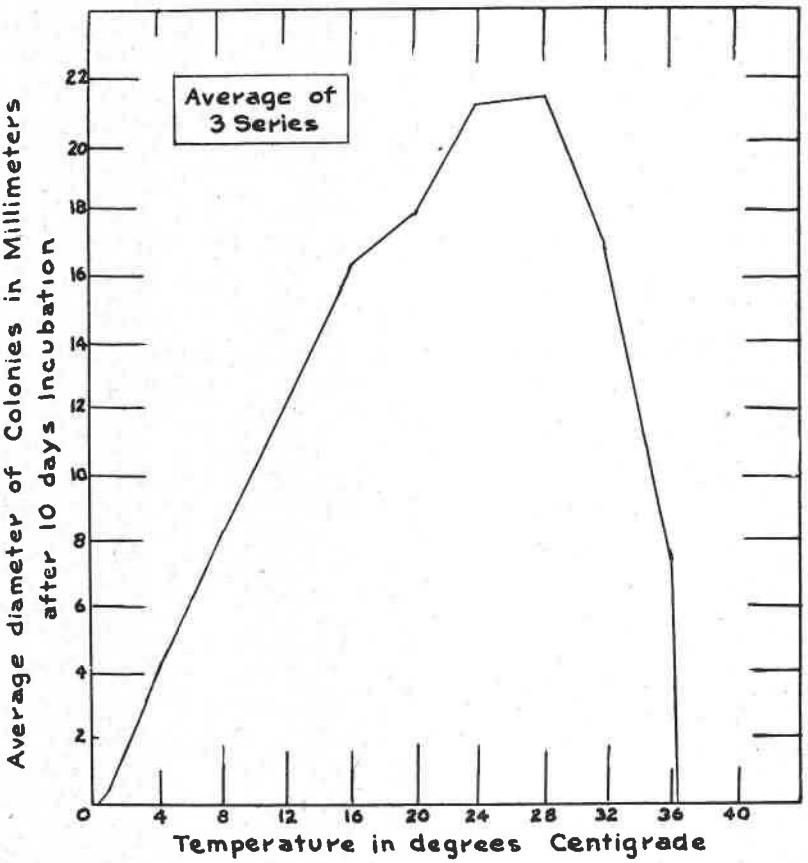

Figure 11. The relation of temperature to the growth of *Xanthomonas juglandis* on potato dextrose agar.

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 the aforementioned controlled temperatures; averaged results of 3 different series with 2 different isolates grown for 10 days are shown graphically in Figure 11.

Thermal death point. The thermal death point of *Xanthomonas juglandis* 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 after 10 minutes' exposure at 53° C. ± 0.5° C. or above, but not below.

The thermal death time of one isolate of *Xanthomonas juglandis* was found to be more than 8 minutes and less than 10 minutes at 53° C. ± 0.2° C. with a concentration of 9,000,000 bacteria per ml., in nutrient dextrose broth at pH 6.9.

Relation of light. The results of studies on the relation of light to the growth of *Xanthomonas juglandis* indicate that illumination through glass exerts no detectable influence on the development of the pathogen in culture. In determining the relation of light to the growth of the causal organism, 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.

Longevity in culture media. Smith, et al. (*110*) found that *Xanthomonas juglandis* is a relatively long-lived organism in culture media. In milk cultures, he found viable organisms after 8 months. On potato cylinders, the pathogen was still alive after 9½ months. We have also found *Xanthomonas juglandis* to be a relatively long-lived organism in culture media, the average life of potato-dextrose agar cultures at room temperature being approximately 4 months.

Longevity in the soil. Smith (*103*) reported that the pathogen does not survive long when inoculated into either sterilized or unsterilized soil. In sterilized soil, he recovered the organism 18 days after inoculation but in unsterilized soil, incubated at 20° C., it could not be re-isolated after 9 days. We have likewise found the pathogen to be short-lived in unsterilized soil, viable blight bacteria being recovered from inoculated, unsterilized soil 4 days after inoculation but not thereafter. However, in moist, inoculated autoclaved field soil the organism was still alive after 160 days at room temperature, but not

after 165 days. The increased longevity of the pathogen in sterilized soil is thought to be associated with freedom from antagonistic or competitive influences due to the absence of other soil organisms.

Effect of desiccation. Smith, et al. (*110*) found *Xanthomonas juglandis* to remain alive for as long as 73 days when held at room temperature in an air-dried condition in the dark, but not to withstand more than 4 days of desiccation in the light. Our studies further indicate that *Xanthomonas juglandis* cannot withstand desiccation long, as is indicated by the fact that no viable organisms could be recovered from beef-extract peptone broth cultures of one isolate of *Xanthomonas juglandis* air-dried on cover glasses and held in the dark at room temperature for 8 days.

Duration of pathogenicity in culture. *Xanthomonas juglandis* apparently retains its virulence for relatively long periods as is shown by the fact that a typical isolate of the pathogen maintained continuously in culture at room temperature for 1,310 days without re-passage through the host was still pathogenic at the end of this time, producing lesions on leaves and nuts when used as inoculum.

IV. PATHOLOGICAL HISTOLOGY

There is practically no published information on the relation of *Xanthomonas juglandis* to the host tissues. A large cavity in the tissues of a diseased walnut in which bacteria are present is shown in a figure in one of Smith's publications (*110*) but no discussion of the relation of the pathogen to its host accompanies it. In view of the lack of information on this subject, studies were made of the relation of *Xanthomonas juglandis* to the host tissues.

MATERIALS AND METHODS

Infected tissues selected for study were fixed in formalin-acetic-alcohol.* The stains used to differentiate the pathogen from the host tissues were picro-aniline blue† and orange G, rose bengal‡ and the light green, carbol-thionin§ and orange G, Giemsa and orange G, and a 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 stain a deep orange. A triple stain employing rose bengal, carbol-thionin, and orange G also gave good results, particularly with nut tissues.

INVASION AND MIGRATION OF THE PATHOGEN

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

The bacteria gain access to the tissues through stomata. From the point of entry, the bacteria invade the tissues through the intercellular spaces. At

*50 per cent alcohol ..... 100 ml.
Formalin ..... 6.5 ml.
Glacial acetic acid ..... 2.5 ml.

†Saturated aqueous solution of aniline blue ..... 25 ml.
Saturated aqueous solution of picric acid ..... 100 ml.

‡Rose bengal ..... 1 gram
5 per cent phenol ..... 100 ml.

§Thionin ..... 0.1 gram
5 per cent phenol ..... 100 ml.

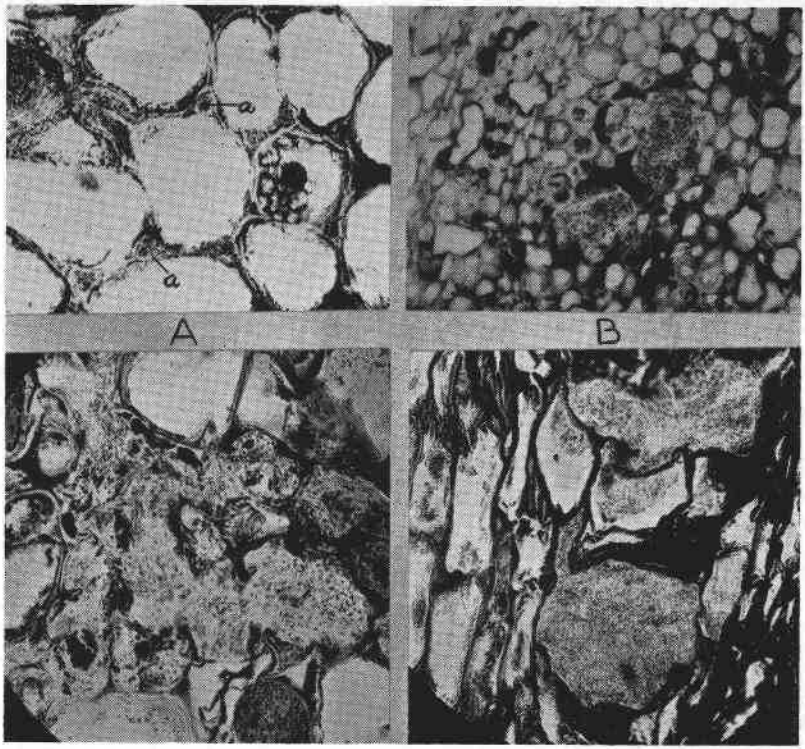

Figure 12. Photomicrographs showing the mode of migration and effect of *Xanthomonas juglandis* on the tissues: A, Bacteria (a) in the intercellular spaces; B, The pathogen within the cells; C, D, Cavities within the tissues due to bacterial activity.

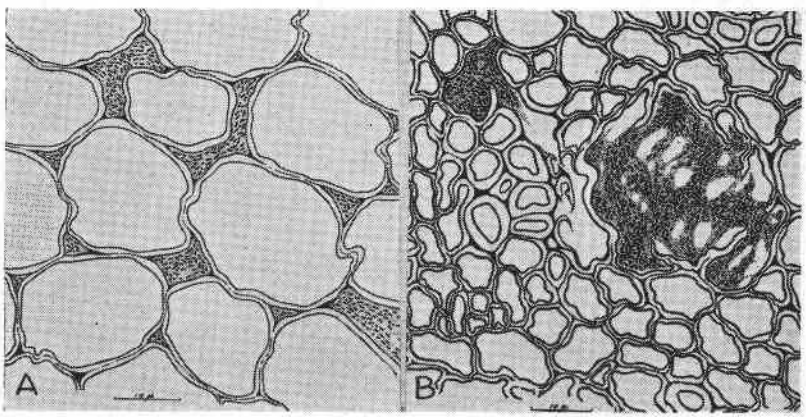

Figure 13. Camera lucida line drawings of host tissues invaded by *Xanthomonas juglandis:* A, Bacteria in the intercellular spaces; the organisms are imbedded in a matrix that is denoted by fine stipling. B, Bacteria within the cells; the organisms are shown migrating from one cell to another through openings or rifts in the cell walls.

first, the organisms apparently migrate through the tissues in a free-swimming condition (Figure 12, A).

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, for it has different staining reactions (Figure 13, A).

A swelling and a modification in the staining reactions of the 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 (Figures 12, B and 13, B). Large cavities filled with bacteria imbedded in a matrix are subsequently formed in the tissues (Figures 12, C, D; and 13, B).

Enzymes produced by the pathogen are evidently quite important in the physiology of parasitism. As pointed out elsewhere, diastase, rennin, proteolase and pectinase are produced by the pathogen in culture. Although the production of cellulase could not be experimentally demonstrated, histological evidence strongly indicates that such an enzyme is produced either by the pathogen or by the host cells under the stimulus of bacterial action since openings in the cell walls bounded by well rounded edges and large lysigenous cavities in which bacteria were present in great numbers (Figures 12, C, D; and 13, B) were found in the invaded areas.

Histological evidence indicates that certain physical forces may also act on the host cells. Internal pressure from the multiplication of the bacteria in the intercellular spaces and the possible osmotic properties of the slime in which they are imbedded are perhaps among these forces. Such histological evidence as is presented in Figures 12, D and 13, B would seem to support such a view, for the walls of the cells bordering the bacterial masses appear to have been telescoped together as if under pressure.

Osmotic pressure may also be concerned in the death of the cells. Numerous cases of apparent plasmolysis of the cell protoplast have been observed in tissues infected by *Xanthomonas juglandis*. It seems possible that the extraction of water from the cells due possibly to the osmotic properties of the bacterial slime present in the intercellular spaces may be responsible for the observed plasmolysis of the protoplast, though it may be that other factors are also involved.

The possibility exists that asphyxiation of the cells may also be a factor. In healthy tissues, the intercellular spaces normally contain air from which the cells obtain oxygen for respiration. When the bacteria invade the tissues, the intercellular spaces soon become filled with bacteria and the metabolic products of bacterial activity with the possible result that the supply of oxygen is used up or materially reduced; thus creating anaerobic conditions which, if continued over a sufficiently long period, would likely result in the asphyxiation and death of the cells.

V. LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS

A knowledge of the modes of overwintering of the causal organism and the methods by which the inocula are 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 juglandis* in relation to pathogenesis and to control have been given special consideration.

OVERWINTERING OF THE CAUSAL ORGANISM

Pierce (*80*) reported that the causal organism lives overwinter in lesions on the twigs and in diseased nuts and leaves on the ground and probably in the soil. Smith (*103*) and Smith, et al. (*110*) concluded from their studies that the pathogen is carried overwinter principally in old lesions on the branches. Smith (*103*) found live blight bacteria on the surface of healthy leaf and catkin buds before growth started in the spring. He states, "It is not known just how significant the presence of the organism on the surface of the buds may be, but the same source could furnish sufficient inoculum to infect the tissues if the buds have begun to expand into leaves." He did not consider old diseased nuts on the ground to be an important source of infection since in most well-cared-for orchards they are plowed under before the trees leaf out in the spring. Rudolph (*91, 93*) states that the pathogen overwinters principally in lesions on the previous year's growth and in the husks of diseased nuts which persist on the trees through the winter.

From studies carried on in England, Hammond (*42*) concluded that the pathogen overwinters principally in diseased shoots of the previous year, though diseased bud scales were also considered to be a source of primary infection.

Relation of Infected Buds

Our studies carried on over a period of 5 years conclusively show that, under Pacific Northwest conditions, *Xanthomonas juglandis* lives over from one season to the next principally in diseased buds. This conclusion is based on extensive surveys of the relative prevalence of infection sources in the trees and upon data from extensive isolation studies (Table 4).

It is evident from the data given in Table 4 that diseased buds are a very important source of primary inoculum under Pacific Northwest conditions since *Xanthomonas juglandis* was isolated in a relatively large percentage of infected buds collected during the winter months. Local infections in live as well as dead buds can evidently carry the pathogen overwinter. The causal organism apparently lives in the dead tissues of blighted buds for at least 2 and possibly as many as 5 years after infection occurs, as is indicated by the fact that *X. juglandis* was isolated from dead buds that were infected from 2 to 5 years prior to the date of isolation attempts (Table 4: series 4-7, 17-23, and 29-30).

Additional evidence of the importance of diseased buds in carrying the pathogen overwinter was obtained from extensive surveys of the comparative prevalence of dead buds and twig lesions in the trees. It was found that the dead buds far outnumber the twig lesions present. Thus, in one survey made in an orchard near Corvallis, Oregon, there were over 14 times as many dead buds as stem lesions present on a given number of representative twigs in the trees.

Relation of Twig Lesions

Lesions on twigs of the previous year's growth may also carry the pathogen overwinter under Pacific Northwest conditions but they apparently are not as important a source of primary inoculum as are infected buds. This conclusion is based upon isolation studies the data for which are given in Table 5 and on the surveys mentioned in the preceding paragraph.

It is evident from these data that the pathogen overwinters in a relatively large percentage of the lesions on twigs of current growth. Stem infections

Table 4. Results of Attempts to Isolate *Xanthomonas juglandis* from Walnut Buds; Corvallis, Oregon, 1930-1936

Series	Dates of collections and plantings	Parts from which isolation made	Trials	*X. juglandis* isolated	
			Number	*Number*	*Per cent*
1	December 1930	Dead leaf[1] buds on twigs of 1930 growth	44	9	20
2	January 1931	" " " "	32	20	62
3	February 1931	" " " "	34	24	71
4	December 1931	Dead leaf buds on twigs originating in 1930[2]	19	6	32
5	January 1932	" " " "	28	4	14
6	February 1932	" " " "	26	14	54
7	March 1932	" " " "	25	6	24
8	November 1931	Dead leaf buds on twigs of 1931 growth	42	9	21
9	December 1931	" " " "	36	9	25
10	January 1932	" " " "	28	11	39
11	February 1932	" " " "	30	8	27
12	March 1932	" " " "	16	7	44
13	January 1932	Dead catkin buds on twigs of 1931 growth	2	1	50
14	March 1932	" " " "	4	2	50
15	October 1932	Dead leaf buds on twigs of 1932 growth	3	1	33
16	November 1932	" " " "	8	5	62
17	March 1933	Dead leaf buds on twigs originating in 1928	5	1	20
18	January 1933	Dead leaf buds on twigs originating in 1929	3	1	33
19	March 1933	" " " "	6	2	33
20	January 1933	Dead leaf buds on twigs originating in 1930	14	5	36
21	March 1933	" " " "	19	11	58
22	January 1933	Dead leaf buds on twigs originating in 1931	31	17	55
23	March 1933	" " " "	10	5	50
24	January 1933	Dead leaf buds on twigs of 1932 growth	8	4	50
25	March 1933	" " " "	22	13	59
26	January 1933	Dead catkin buds on twigs of 1932 growth	10	8	80
27	February 1933	" " " "	12	5	42
28	March 1933	" " " "	10	10	100
29	February 1934	Dead leaf buds on twigs originating in 1930	2	0	0
30	February 1934	Dead leaf buds on twigs originating in 1931	3	0	0
31	January 1936	Dead leaf buds on twigs of 1936 growth	8	2	25
32	December 1931	Live[3] leaf buds on twigs of 1931 growth	33	7	21
33	January 1932	" " " "	26	4	15
34	February 1932	" " " "	20	2	10
35	March 1932	" " " "	21	3	14
36	December 1931	Live catkin buds on twigs of 1931 growth	11	2	18
37	January 1932	" " " "	19	4	21
38	February 1932	" " " "	17	3	18
39	March 1932	" " " "	8	6	75
40	November 1932	Live leaf buds on twigs of 1932 growth	6	1	17
41	December 1932	" " " "	16	4	25
42	January 1933	" " " "	13	1	8
43	November 1932	Live catkin buds on twigs of 1932 growth	5	0	0
44	December 1932	" " " "	9	1	11
45	January 1933	" " " "	14	1	7

[1]Some of the leaf buds were pistillate flower-bearing buds. These are listed as leaf buds since it is impossible macroscopically to segregate them in the dormant stage.

[2]Artificial inoculation studies show that the buds are susceptible to infection only during the year of their formation; hence, a blighted bud on a twig which originated in 1930 was infected during the spring or early summer of the same year and at the time platings were made had probably been dead about 1½ years.

[3]These buds were alive at the time the platings were made, as is shown by the presence of live, green tissues within; in some cases, local lesions were present in some of the outer or inner scales, as is indicated by the presence of brown areas.

Table 5. Results of Attempts to Isolate *Xanthomonas juglandis* from Lesions on Walnut Twigs; Corvallis, Oregon, 1930-1934

Series	Dates of collections and platings	Source from which isolation attempts made	Location of lesions on stems	Trials	*X. juglandis* isolated	
				Number	*Number*	*Per cent*
1	Jan. 1930-Apr. 1930	Lesions on twigs of 1929 growth	Distributed over surface	48	6	12
2	Dec. 1930-Feb. 1931	Lesions on twigs of 1930 growth	"	24	7	29
3	January 1931	" " "	About nut scars[1]	9	3	33
4	November 1931	Lesions on twigs of 1931 growth	"	4	1	25
5	December 1931	" " "	"	23	1	4
6	January 1932	" " "	"	24	5	21
7	February 1932	" " "	"	37	1	3
8	March 1932	" " "	"	12	2	17
9	January 1931	Lesions on twigs of 1930 growth	About dead buds[2]	65	6	9
10	November 1931	Lesions on twigs of 1931 growth	"	14	1	7
11	December 1931	" " "	"	26	5	19
12	January 1932	" " "	"	21	3	14
13	February 1932	" " "	"	45	3	7
14	March 1932	" " "	"	17	6	35
15	November 1931	Lesions on twigs of 1931 growth	Distributed over surface	6	1	17
16	December 1931	Lesions on twigs of 1931 growth	"	4	1	25
17	January 1932	" " "	"	2	1	50
18	February 1932	" " "	"	17	2	12
19	March 1932	" " "	"	3	2	67
20	March 1933	Lesions on twigs of 1932 growth	"	16	1	6
21	March 1933	" " "	About nut scars	17	2	12
22	April 1933	" " "	"	4	0	0
23	October 1932	" " "	Distributed over surface	10	8	80
24	November 1932	Lesions on twigs of 1932 growth	Distributed over surface	4	4	100
25	February 1933	" " "	"	6	3	50
26	March 1933	" " "	"	12	1	8
27	January 1933	Lesions on twigs of 1931 growth	"	2	0	0
28	March 1933	" " "	"	5	0	0
29	April 1933	Lesions on twigs of 1932 growth	"	22	1	4
30	February 1934	Lesions on twigs of 1931 growth	"	1	0	0
31	February 1934	Lesions on twigs of 1933 growth	"	4	3	75

[1]Lesions formed by migration of the pathogen from infected nuts attached to the twigs.
[2]Lesions formed by invasion of the bacteria from infected buds.

Table 6. STUDIES ON THE LOCATION OF *Xanthomonas juglandis* IN OVERWINTERING LESIONS ON WALNUT TWIGS; CORVALLIS, OREGON, 1932-1934

Series	Dates of collections and platings	Part of lesion from which platings were made and results of trials								
		Dead tissues in center of the lesion			Discolored marginal areas			Living tissues 1-4 mm. beyond margin		
		Trials	*X. juglandis* isolated		Trials	*X. juglandis* isolated		Trials	*X. juglandis* isolated	
		Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
1.....	Oct. 1932	10	7	70	10	2	20	10	0	0
2.....	Nov. 1932	4	1	25	4	4	100	4	0	0
3.....	Feb. 1934	4	3	75	4	0	0	....	....	....

resulting from direct stomatal invasion are apparently more concerned in carrying the pathogen overwinter than are stem lesions around nut scars or about diseased buds. The causal organism evidently does not live for more than 2 years in stem lesions as all attempts to isolate it from lesions 2 years of age or older have been uniformly negative.

The pathogen apparently overwinters only in the discolored areas in the lesions and not in the surrounding live tissues (Table 6).

Relation of Persistent Infected Nut Mummies and Hulls

Old "blighted" nut mummies and hulls that persist in the trees are apparently not concerned to any significant extent in carrying *Xanthomonas jug-*

Table 7. RESULTS OF ATTEMPTS TO ISOLATE *Xanthomonas juglandis* FROM VARIOUS MATERIALS IN THE TREES AND ON THE GROUND; CORVALLIS, OREGON, 1932-1934

Series	Dates of collections and platings	Sources from which platings were made	Trials	*X. juglandis* isolated	
			Number	*Number*	*Per cent*
1.....	January, 1932	Persistent "blighted" nut mummies	11	2	18
2.....	February, 1932	" " "	22	3	14
3.....	March, 1932	" " "	14	4	29
4.....	March, 1933	" " "	23	1	4
5.....	April, 1933	" " "	30	1	3
6.....	March, 1933	Persistent hulls[1]	10	0	0
7.....	February, 1932	"Blighted" nuts on ground	10	0	0
8.....	March, 1932	" "	18	1	6
9.....	February, 1933	" "	10	1	10
10.....	March, 1933	" "	15	1	7
11.....	November, 1933	Infected leaves on ground[2]	9	1	11
12.....	December, 1932	" "	12	1	8
13.....	January, 1933	" "	10	2	20
14.....	February, 1933	" "	12	1	8
15.....	March, 1933	" "	17	1	6
16.....	April, 1933	" "	10	0	0
17.....	May, 1933	" "	7	0	0
18.....	February, 1934	" "	8	4	50
19.....	November, 1931	Soil from under diseased walnut trees	1	0	0
20.....	December, 1931	" " "	1	0	0
21.....	February, 1932	" " "	1	0	0
22.....	March, 1932	" " "	1	0	0
23.....	December, 1932	" " "	1	0	0
24.....	January, 1933	" " "	4	0	0
25.....	February, 1933	" " "	3	0	0
26.....	March, 1933	" " "	3	0	0
27.....	April, 1933	" " "	1	0	0
28.....	February, 1934	" " "	7	0	0

[1]Hulls that remained attached to the twigs after harvest.
[2]Diseased walnut leaves collected during the fall of 1932 and placed in screen wire cages on ground out of doors at Corvallis, Oregon.

landis overwinter under Pacific Northwest conditions as most of the walnut orchards have very few, if any, persistent, "blighted" nut mummies in the trees and hulls left by the time growth starts in the spring. Moreover, only a very small percentage of those that do persist appear to be active in carrying the pathogen overwinter as indicated by the comparatively small percentage of positive results from isolation attempts (Table 7, series 1 to 6).

Relation of Blighted Nut Mummies and Old Infected Leaves on the Ground

Old blighted nuts and infected leaves on the ground do not appear to be of any importance in carrying the pathogen overwinter under Pacific Northwest conditions as only a comparatively small percentage of them were found to contain viable bacteria by the time the trees come into leaf in the spring (Table 7, series 7-18). Even had the pathogen been found viable in a large percentage of old blighted nut mummies and infected leaves on the ground it is difficult to comprehend how bacteria from these sources could be transferred up into the trees from the ground unless it is postulated that wind or some insect is concerned in disseminating the inoculum which is not in accord with the evidence.

Relation of the Soil

Pierce (*79*) reported that the causal organism probably overwinters in the soil. Smith (*103*) concluded that the soil is not an important source of primary inoculum as he found that the pathogen does not survive long when inoculated in either sterilized or unsterilized soil. Rudolph (*93*) failed to isolate *Xanthomonas juglandis* from soil collected from under badly diseased walnut trees. Our studies further indicate that *X. juglandis* is not carried over from one season to the next in the soil as all of our attempts made by the standard soil bacteriological dilution plate technique to isolate the pathogen from soil collected from under diseased walnut trees have been uniformly negative (Table 7, series 19 to 28). That the soil does not constitute an important source of primary inoculum under Pacific Northwest conditions was further indicated by the studies on the longevity of *X. juglandis* in unsterilized soil. No viable blight organisms could be recovered from inoculated, unsterilized soil after 4 days at room temperature.

SEASONAL DEVELOPMENT OF THE DISEASE IN RELATION TO THE HOST AND NATURAL ENVIRONMENT

The seasonal development of the disease has received attention from Pierce (*80, 81*), Smith (*103, 105, 110*), Rudolph (*89, 90, 91, 93*), and others. While the studies of these workers resulted in the discovery of a number of cardinal facts concerning the life history of the causal organism in relation to pathogenesis, gaps in the knowledge of the seasonal development of the disease still existed.

The most striking aspect of the epidemiology of walnut bacteriosis is the wide variation in severity of the disease under different seasonal and local conditions. An intimate knowledge of all the factors associated with "blight" development and their relation to disease outbreaks is essential to the development of effective and economical measures of control. Consequently, studies of the development of the disease in relation to the causal organism, its host, and to the natural environment were initiated in 1930 and intensively carried on for a period of 4 years. These studies were carried on in (a) the greenhouse under

controlled environmental conditions, and (b) in the field under natural conditions.

In studies of the disease carried on in the greenhouse under controlled conditions, an attempt was made to evaluate the relative importance of the various environmental factors in the development of the disease. By use of suitable temperature and humidity equipment approximations of the desired conditions or combinations of conditions were obtained at will. In this way it was possible to study analytically the consequences of varying certain factors of the environment. It is recognized that a measure of artificiality attends experimentation with plants grown under controlled conditions. Life processes are so integrated that consequences observed to follow a given environmental factor under certain controlled conditions may not necessarily be governed by the action of this factor alone, and in another environment it may perhaps be followed by other consequences. The results have, therefore, been interpreted conservatively and checked against field evidence where possible.

Seasonal Records

To facilitate the studies under field conditions, a station was maintained in 1930 and 1931 in a commercial Franquette walnut orchard near Lebanon, Oregon. In 1932 and 1933, field studies were carried on in a Franquette orchard near Aumsville, Oregon. Visits were made to the field stations at 2- to 3-day intervals throughout the season and detailed records were kept of the daily rainfall, relative humidity, air temperature and host and disease development. Where possible, records were taken on a quantitative basis.

Host Development

The seasonal development of the Franquette, the chief variety in the Pacific Northwest, was followed for 4 years. The development of 50 tagged, representative pistillate flowers was recorded at approximately weekly intervals during the growing season. Descriptive notes were supplemented by photographs. During the first 2 years, shoot and leaf development was also followed by frequent measurements. Graphic summaries of the data obtained are given in Figures 14 to 17.

Development of the Disease

Approximately 1,000 tagged nuts, representatively situated on a number of unsprayed trees, were examined at 2- to 3-day intervals for the presence of incipient blight infections. Studies of natural disease development were supplemented by inoculating healthy nuts at frequent intervals throughout the season by spraying water suspensions of *Xanthomonas juglandis* upon the uninjured surfaces with an atomizer. During the latter part of the growing period, wound inoculations were also made. A moisture-supplying device designed by Keitt (*45*) was used to provide favorable infection conditions during dry periods in the summer. The resulting data are graphically shown in Figures 14 to 17.

Meteorological Records

A continuous record of the relative humidity and air temperature was obtained at the field station by means of a hygrothermograph housed in a standard instrument shelter set in a centrally located point in the orchard. Records of rainfall were taken by means of a standard rain gauge. The resulting data are graphically presented in Figures 14 to 17.

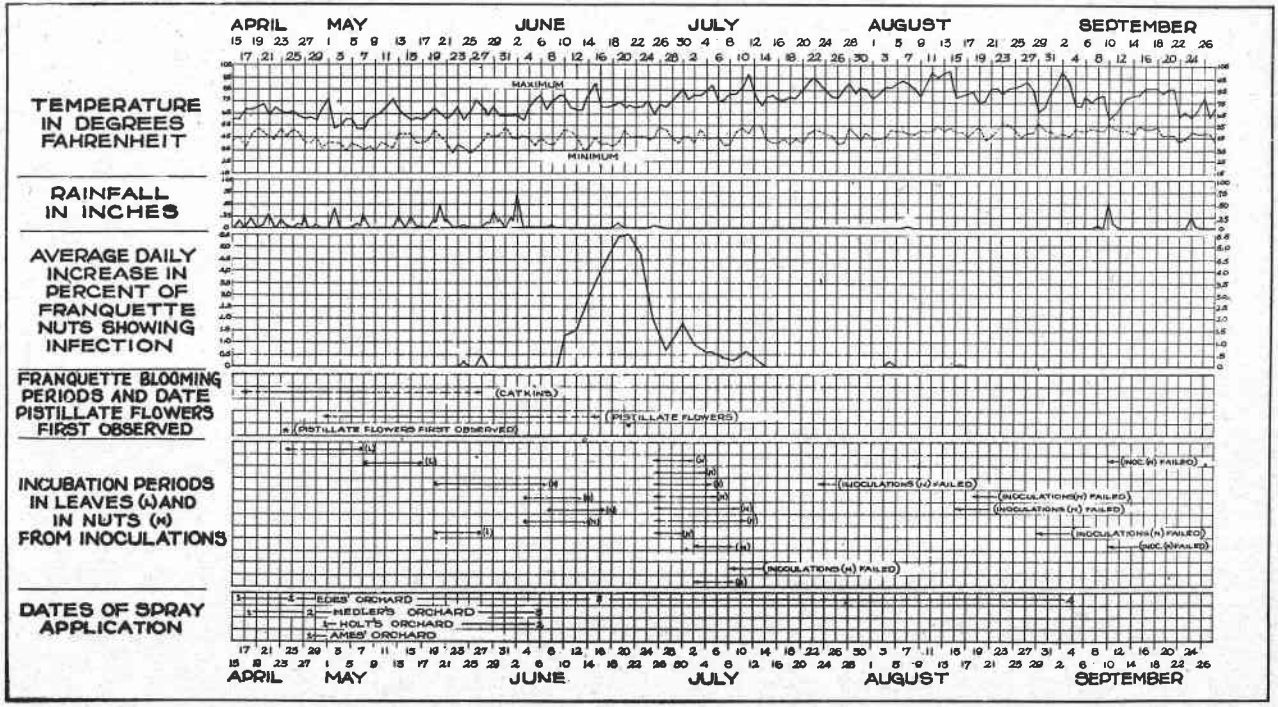

Figure 14. Graphic summary of certain records pertaining to the epidemiology and control of walnut bacteriosis, Lebanon, Oregon, 1930.

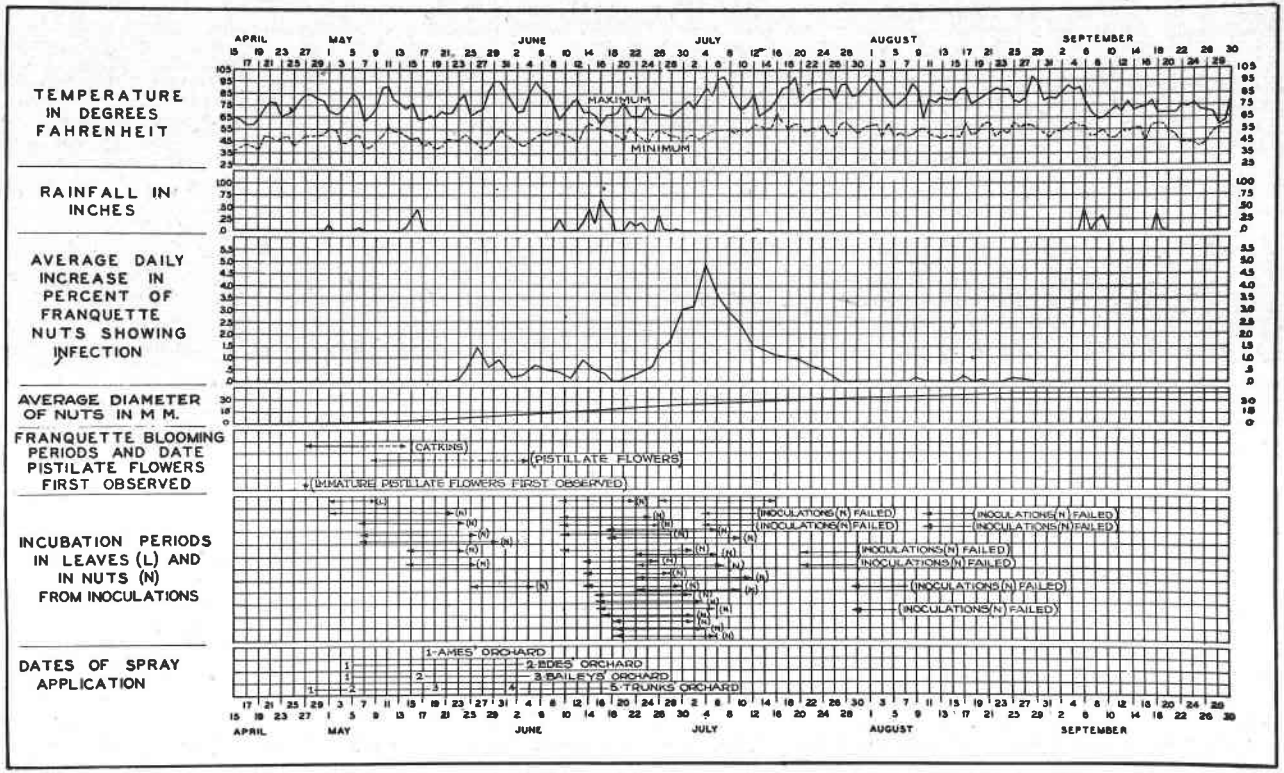

Figure 15. Graphic summary of certain records pertaining to the epidemiology and control of walnut bacteriosis, Lebanon, Oregon, 1931.

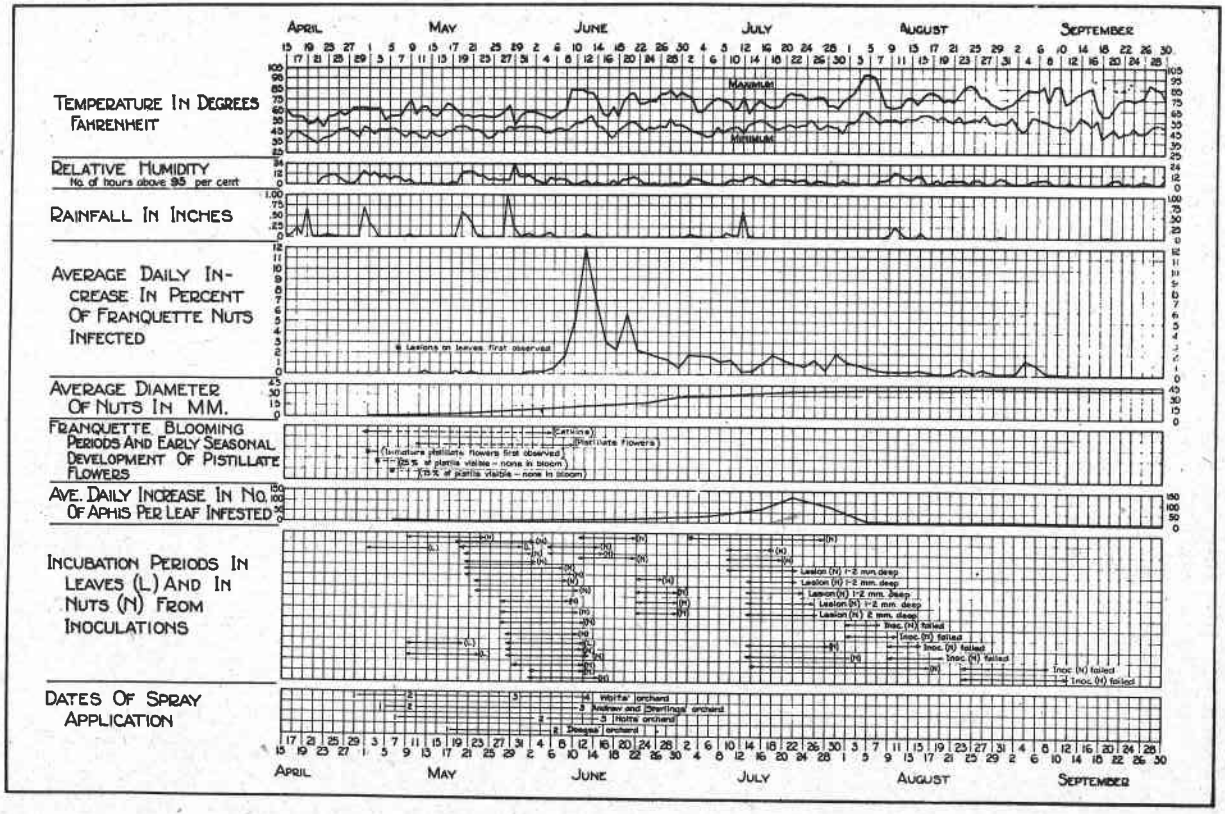

Figure 16. Graphic summary of certain records pertaining to the epidemiology and control of walnut bacteriosis, Aumsville, Oregon, 1932.

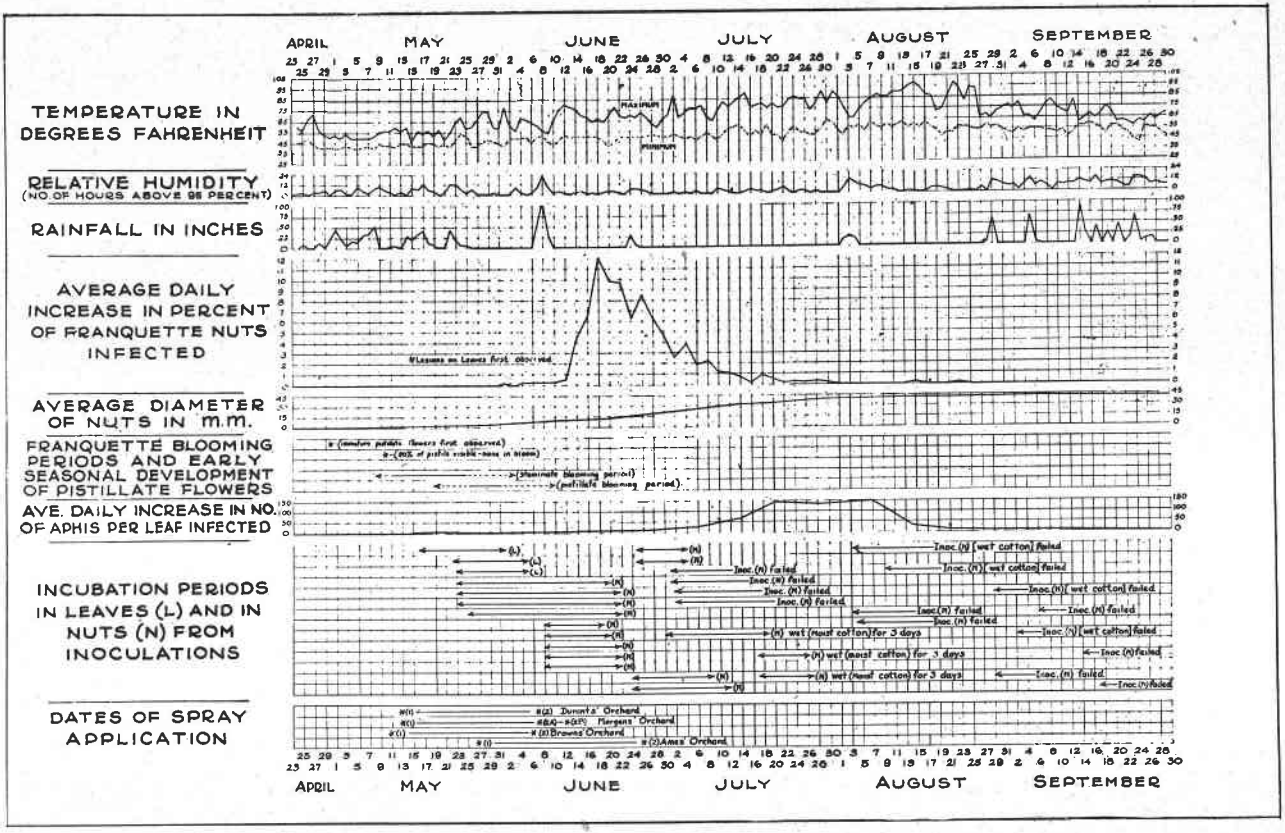

Figure 17. Graphic summary of certain records pertaining to the epidemiology and control of walnut bacteriosis, Aumsville, Oregon, 1933.

Discussion of Seasonal Records

These data show that the extent of rainfall during the critical period for infection plays a leading role in determining the intensity of the disease and difficulty in its control. They have disclosed the critical periods in the epidemiology of the disease and have thus contributed materially towards the development of a more efficient spray program.

Relation of Rainfall, Insects, and Infected Pollen

Relation of rainfall. Smith (*103, 104, 110*), Doidge (*33*), Rudolph (*90, 91, 93*), Barss (*17*), Hammond (*42*), and others have pointed out that this disease is worse in wet seasons than in dry ones. Our studies carried on over a period of 14 years show that rainfall is the most important, if not the only, significant agency concerned, under Pacific Northwest conditions, in the dissemination of the primary and secondary inocula. As supporting evidence, the following observations and experiments are pertinent:

1. The greater the amount of rainfall during the infection period, the greater the incidence of the disease. For example, in Oregon in 1935 losses from this disease did not exceed 5 per cent of the potential crop, whereas in 1936 approximately 35 per cent of the crop was lost. In 1935 only 0.77 inch of rain fell at Salem during the critical period for infection: 0.41 inch in May and 0.36 inch in June—a departure from the normal for these months of 1.56 and 0.86 inch, respectively. In 1936, on the other hand, 4.52 inches of rain fell during this same period: 3.41 inches in May and 1.11 inches in June—a departure from the normal of 1.60 inches and 0.10 inch, respectively.

2. In field studies of the seasonal development of the disease on a number of tagged nuts, increases in the incidence of infection appeared only after periods of rainfall following a normal incubation period, as was found from the results of artificial inoculations made during the rainy periods in question. Lesions appeared on the inoculated nuts at approximately the same time as they became evident on tagged nuts in nature, and occurred in successive "waves" traceable to specific rainy periods (Figures 14 to 17).

3. Surveys in commercial orchards showed that the trees varied considerably in the severity of the disease. In certain trees the incidence of the disease on the nuts would be quite high, while on neighboring trees it would be comparatively low. Furthermore, the distribution of infected nuts in the trees was quite variable. In some instances, a high percentage of the nuts in a certain sector or part of a tree was found infected, while in other portions only a very small percentage of nuts was found diseased. If the inoculum were being disseminated by some such agency as wind, insects, or pollen, as has been suggested by certain other investigators, a relatively uniform distribution of the infected nuts in the trees with regard both to the trees infected and the distribution of diseased nuts in individual trees would normally be expected. However, the observed facts fit in well with the conception that rain drip is the major 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 found.

4. Infection has been induced at will by spraying healthy leaves and nuts with pure water suspensions of *Xanthomonas juglandis* during periods of rainfall. Young, healthy leaves and fruits on potted Franquette walnut trees in the greenhouse were also infected at will by spraying with water

suspensions of the causal organism and placing them in a saturated atmosphere in a damp chamber for a suitable period. Uninoculated leaves and nuts subjected to the same conditions remained healthy.

5. Infection of healthy nuts on potted walnut trees has been induced at will by suspending over them walnut twigs bearing infected buds, cut from trees in the field, and spraying with a fine stream of water; the drip therefrom falling upon the young leaves and nuts on the potted trees below. After a suitable incubation period lesions developed on the leaves and fruit, the incidence of infection being greatest on the leaflets that were directly beneath the dead buds.

Relation of insects. Smith, et al. (*110*) and Rudolph (*93, 96, 97*) report that aphids, blister mites, and certain other insect species may spread the causal organism. Our studies indicate that, under Pacific Northwest conditions, insects are not concerned to any significant extent, if at all, in the dissemination of either the primary or secondary inoculum. As supporting evidence, the following observations are given:

1. 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 walnuts in Oregon occurred either a long time before disease development or after it was largely over. Graphic summaries of the seasonal development of the walnut aphis (*Chromaphis juglandicola* Kalt.), the most abundant insect pest on walnuts in Oregon, in relation to disease development, are presented in Figures 16 and 17.

2. The walnut blister mite (*Eriophyes tristriatus erineus Nalepa*) recently reported by Rudolph (*96, 97*) as a carrier of *Xanthomonas juglandis* in California is not found to any extent on the Franquette variety in Oregon. Yet despite the scarcity of this insect, this variety "blights" badly in years of abundant rainfall during the critical period for infection.

3. No insects have ever been observed actually feeding upon diseased buds or lesions on twigs, and only a relatively few insects (mostly various species of flies) have been noted on diseased nuts in contact with the exudate which sometimes oozes from lesions during periods of high humidity.

4. Attempts to transmit the disease by aphids have been uniformly negative. In numerous trials aphids were allowed to feed upon infected leaves on potted walnut trees for several days, after which they were transferred to healthy leaves on other potted plants; no infections resulted.

5. Attempts made to isolate *Xanthomonas juglandis* from the bodies of walnut aphis collected from walnut leaves containing numerous blight lesions, were uniformly negative.

Relation of infected pollen. Smith (*103, 104, 105*) and Ark (*10*) report having isolated the pathogen from pollen collected from infected catkins and suggest that infected pollen from diseased catkins may disseminate the inoculum under California conditions. Our studies do not indicate that infected pollen is concerned to any significant extent in dissemination under Pacific Northwest conditions. In this regard, the following observations and experiments are pertinent:

1. In those years when the seasonal development of the disease was closely followed, there was little or no correlation between the peak of disease development and the time of most abundant pollen shedding (Figures 14-17).

2. If pollen is an important disseminating agency, the disease should

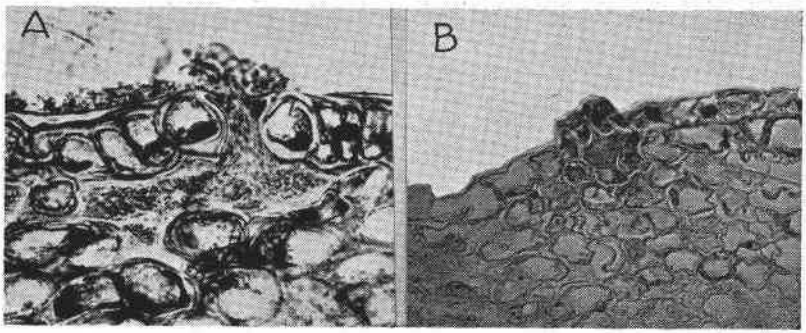

Figure 18. Photomicrographs showing mode of entry of *Xanthomonas juglandis* into (A) a nut and (B) a leaf. The bacteria are shown in the stomatal openings and substomatal cavities.

be severe practically every year irrespective of weather conditions, which is not the case.

3. If pollen is concerned to any extent in disseminating the inoculum, there should be a more or less uniform distribution of infected nuts in the trees with regard both to the trees infected and the distribution of diseased nuts in individual trees, which is not in accordance with the facts.

4. A limited number of attempts to isolate the pathogen from field-collected pollen have been uniformly negative..

MODE OF ENTRY OF THE PATHOGEN

Xanthomonas juglandis enters the nuts primarily through stomata and, to a lesser extent, through mechanical injuries. Numerous cases of stomatal penetration have been found in nuts inoculated by spraying with suspensions of the pathogen (Figure 18, A).

Figure 19. Lesions on a nut about hail injuries.

If infection occurs in the prebloom or blooming periods, the bacteria enter primarily through stomata located in the bracteole lobes or in the contiguous tissues at the tips of the nuts (Figure 2, A). Rudolph (*93*) states that the nut is usually infected at the blossom end by the way of the stigma that is "constitutionally adapted to easy attack by the organism." In none of the inoculated flowers that we have sectioned have the bacteria been observed gaining access to the nut through the stigmatic surface. In fact, in our preparations we have been unable to find even natural avenues of entry in the stigmatic surfaces through which access to the tissues could be gained.

After bloom, access to the tissues occurs principally through the stomata in the sides of the nut, giving rise to lateral infections.

The scarcity of lateral infections before bloom is thought to be associated with the fact that comparatively few differentiated stomata normally occur on the sides of young nuts prior to bloom. It is only after bloom that any appreciable number develop along the sides.

Access to the tissues may also occasionally occur through abrasions due to limb rubbing, hail, and other mechanical injuries (Figure 19).

Xanthomonas juglandis enters the leaves primarily through stomata, although wounds occasionally serve as portals of entry. Numerous cases of stomatal penetration have been found in unwounded leaves inoculated by atomizing with water suspensions of *X. juglandis* (Figure 18, B).

The pathogen was found to gain access to the buds chiefly through stomata in the outer bud bracts and occasionally through mechanical injuries of various kinds.

PERIOD OF INCUBATION

The incubation period varies with the environmental conditions and the age of the nuts. In general, the higher the temperature and the younger the nuts the shorter the incubation period.

In nature the incubation period for nuts ranged from 5 to 34 days, generally 10 to 15 days. On the leaves it ranged from 8 to 18 days, generally 10 to 12 days (Figures 14 to 17).

INFECTION EXPERIMENTS

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

Field Studies

Nuts in various stages of development on young Franquette trees in the field were inoculated during both rainy and dry periods by spraying them with water suspensions of *Xanthomonas juglandis* with an atomizer. No post-inoculation moist treatment beyond that bestowed by nature was given young nuts in the prebloom and early postbloom stages as it was found that infection occurred without it. However, nuts inoculated during the late postbloom stage were generally given a moist treatment after inoculation by the use of a moisture supplying device.

Greenhouse Studies

Two- to four-year-old Franquette walnut trees grafted on *Juglans hindsii* rootstocks were planted in soil in 14-inch pots or kegs and placed in a cool-temperature greenhouse to root. When they reached the desired age, the host organs were inoculated by spraying water suspensions of *Xanthomonas juglandis* on the uninjured parts with an atomizer. After inoculation they were placed in a damp chamber in which the desired temperature and humidity were maintained by an electric water-heating element covered by wet, absorbent cotton, the ends of which were immersed in a constantly maintained reservoir of water (Figure 20). A De Kotinsky thermo-regulator and a relay hooked up in a series with the electric water heater maintained reasonably constant temperatures that were recorded by a thermograph.

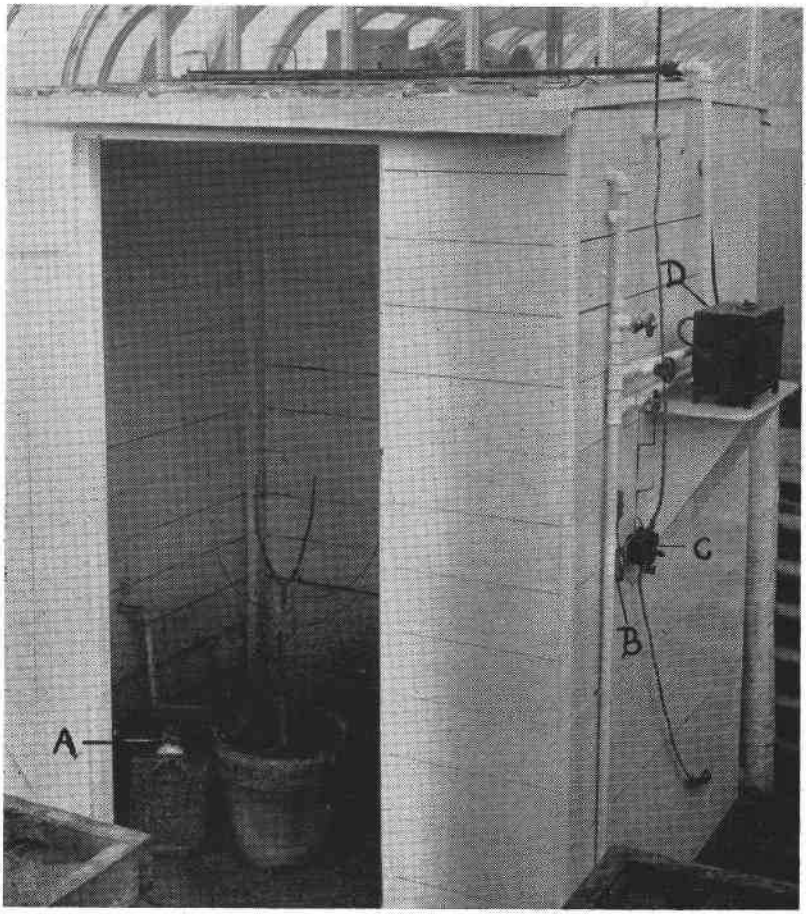

Figure 20. Inoculation chamber used for control of temperature and humidity: A, electric heating element; B, thermo-regulator; C, relay; D, recording thermometer.

When the electric current passed through the heating element, water-vapor was given off from the moist cotton, filling the chamber and 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 water 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 ± 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 the number of nuts or leaves infected and the number of lesions on each.

Relation of Environment to Infection

Relation of moisture. Both field and greenhouse studies definitely show that the presence of moisture on the host organs for a suitable period after inoculation is a prerequisite to infection. No infection ever occurred in any of the numerous experiments carried on where the surface growth from agar cultures of *Xanthomonas juglandis* was smeared on the uninjured surfaces and dried immediately thereafter. Only when moisture was present after inoculation did infection take place.

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 the nuts and leaves occurred after only 5 and 15 minutes, respectively, of continuous wetting when these organs were very young, the stomata wide open, and the tissues "water-congested." The older the host organs, the less the amount of water in the tissues, and the smaller the stomatal opening, 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 enhanced the chances of stomatal penetration by the bacteria and also induced a water-soaking of the tissues which undoubtedly expedited the growth and rapid migration of the bacteria through the tissues.

Subjecting the trees to a moist treatment before inoculation was found to increase the number and size of the lesions. Such treatment produced a water-soaking of the tissues adjoining the stomata and facilitated connection of the surface water with that in the intercellular spaces through open stomata. The

Table 8. THE RELATION OF MOISTURE TO THE INFECTION OF THE LEAVES AND NUTS BY *Xanthomonas juglandis*; GREENHOUSE, CORVALLIS, OREGON, 1931-1940

Series	Period in moist chamber		Nuts			Leaves			
	Before inoculation	After inoculation	Inoculated[1]	Infected		Inoculated	Infected		Average number of lesions per leaf infected
	Minutes	*Minutes*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*
1	0	15	.....	.....	.....	50	22	44	3
	0	60	.....	.....	.....	132	85	64	5
	0	120	.....	.....	.....	115	82	71	8
	0	240	.....	.....	.....	108	103	95	11
2	0	30	.....	.....	.....	44	29	66	2
	0	60	.....	.....	.....	54	27	50	2
	0	120	.....	.....	.....	123	122	99	5
3	0	0	.....	.....	.....	43	0	0	0
	0	15	.....	.....	.....	54	30	56	3
4	0	5	3	3	100	.....	.....	.....	.....
	0	10	3	3	100	.....	.....	.....	.....
	0	15	26	19	73	.....	.....	.....	.....
	0	30	2	2	100	.....	.....	.....	.....
5	0	5	31	25	81	.....	.....	.....	.....
	0	10	23	22	96	.....	.....	.....	.....
	0	15	6	6	100	.....	.....	.....	.....
6	0	15	4	4	100	.....	.....	.....	.....
	0	30	5	5	100	.....	.....	.....	.....
	0	60	4	4	100	.....	.....	.....	.....
	0	120	2	2	100	.....	.....	.....	.....

[1]The nuts were in the early prebloom to early postbloom stages of development when inoculated.

Table 9. THE RELATION OF TEMPERATURE TO THE INFECTION OF THE LEAVES AND NUTS BY *Xanthomonas juglandis*; GREENHOUSE, CORVALLIS, OREGON, 1931-1940

Series	Inoculation conditions		Nuts[1]			Leaves			
	Average temperature	Period in moist chamber	Inoculated	Infected		Inoculated	Infected		Average number of lesions per leaf infected
	Degrees C	*Minutes*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	
1	4	30	....	....	.....	454	34	7	1
	10.5	30	....	....	.....	32	1	3	1
	20	30	....	....	.....	44	29	66	2
	22.5	30	....	....	.....	127	110	87	4
	26	30	....	....	.....	112	94	84	5
	30	30	....	....	.....	213	96	45	3
2	10.5	60	....	....	.....	30	7	23	1
	14.5	60	....	....	.....	61	3	5	2
	20.5	60	....	....	.....	54	27	50	2
	22.5	60	....	....	.....	132	85	64	5
	28	60	....	....	.....	153	117	76	6
3	11.5	120	....	....	.....	24	5	21	1
	14.5	120	....	....	.....	221	38	17	3
	20	120	....	....	.....	123	122	99	5
	22.5	120	....	....	.....	115	82	71	8
4	8	15	1	1	100	.....	.....	....	....
	13.5	15	4	4	100	.....	.....	....	....
	17	15	26	19	73	.....	.....	....	....
	22.5	15	2	1	50	.....	.....	....	....
5	7.5	30	2	1	50	.....	.....	....	....
	22.5	30	4	4	100	.....	.....	....	....
	26	30	6	5	83	.....	.....	....	....
6	7.5	240	1	1	100	.....	.....	....	....
	14.6	240	4	4	100	.....	.....	....	....
	19	240	1	1	100	.....	.....	....	....
	22.5	240	2	2	100	.....	.....	....	....
	24.6	240	2	2	100	.....	.....	....	....
	26.5	240	9	4	44	.....	.....	....	....
7	5	1,440	1	1	100	.....	.....	....	....
	16.6	1,440	1	1	100	.....	.....	....	....
	21.1	1,440	3	3	100	.....	.....	....	....
	23	1,440	1	1	100	.....	.....	....	....
	27	1,440	2	2	100	.....	.....	....	....

[1]The nuts were in the early prebloom to early postbloom stages of development when inoculated.

penetration and migration of the bacteria in the tissues were thereby expedited. Typical examples of the data from numerous field and greenhouse experiments appear in Table 8.

Relation of temperature. The temperature at the time of, and following, inoculation seems to be important in the infection of the leaves but not with the nuts. The data in Table 9 show that a higher percentage of the leaves became infected and that there was a greater number of lesions per infected leaf at 20° C. and higher than occurred at lower temperatures. In the case of the nuts, temperatures ranging from 5° C. to 27° C. seemed to be without effect except to hasten the appearance of the lesions. In one typical experiment, disease symptoms appeared on inoculated leaves in 6 days at an average incubation temperature of 21° C. while at 16° C. the lesions did not become evident until after 13 days. The incubation period in the nuts was likewise shorter at higher incubation temperatures than at lower ones. In a typical experiment, lesions on the nuts became evident 4 days after inoculation

Table 10. The Relation of the Stage of Nut Development to Infection by *Xanthomonas juglandis*, Corvallis, Oregon

Series	Place experiment performed	Stage of nut development at time of inoculation	Diameter of nuts: When inoculated	Diameter of nuts: At maturity	Results of inoculations	Nuts: With lesions in hull: Confined to outer part of hull	Nuts: With lesions in hull: Extending to shell, staining same, but kernel within unaffected	Nuts: Infected internally
			Millimeters	*Millimeters*				
1, A	Greenhouse	D[1]	.....	.....	0[2]	.....	.....	.....
B	"	GT	.....	.....	0	.....	.....	.....
C	"	EP	1	*[3]	+	.....	.....	+
D	"	EP	1.5	*	+	.....	.....	+
E	"	LP	3	*	+	.....	.....	+
F	"	EPB	12	*	+	.....	.....	+
G	"	EPB	16	*	+	.....	.....	+
H	"	MPB	28	53.5	+	.....	+	.....
I	"	LPB	46	51	+	+	.....	.....
J	"	LPB	43	43.5	0	.....	.....	.....
2, A	"	D	.....	.....	0	.....	.....	.....
B	"	GT	.....	.....	0	.....	.....	.....
C	"	EP	1	*	+	.....	.....	+
D	"	EP	1.5	*	+	.....	.....	+
E	"	LP	2.5	*	+	.....	.....	+
F	"	FB	3	*	+	.....	.....	+
G	"	EPB	6	*	+	.....	.....	+
H	"	EPB	6	35	+	.....	+	.....
I	"	MPB	29.5	40	+	+	.....	.....
J	"	LPB	39	42	+	+	.....	.....
K	"	LPB	50	50.5	0	.....	.....	.....
3, A	Field	EP	2	*	+	.....	.....	+
B	"	LP	3	*	+	.....	.....	+
C	"	FB	4	*	+	.....	.....	+
D	"	EPB	9	*	+	.....	.....	+
E	"	EPB	15.5	*	+	.....	.....	+
F	"	MPB	22	47	+	.....	.....	.....
G	"	MPB	33	47	+	+	.....	.....
H	"	MPB	32	46	+	+	.....	.....
I	"	MPB	36	41	+	+	.....	.....
J	"	LPB	41	49	0	.....	.....	.....
K	"	LPB	42.5	44	0	.....	.....	.....
L	"	LPB	45	48.5	0	.....	.....	.....
M	"	LPB	45	47.5	0	.....	.....	.....
4, A	"	EP	1.5	*	+	.....	.....	+
B	"	LP	2	*	+	.....	.....	+
C	"	FB	2.5	*	+	.....	.....	+
D	"	EPB	16	*	+	.....	.....	+
E	"	MPB	21	42.5	+	+	.....	.....
F	"	MPB	31	42.5	+	+	.....	.....
G	"	MPB	36	45	+	.....	+	.....
H	"	LPB	39.5	42.5	+	+	.....	.....
I	"	LPB	43	46	0	.....	.....	.....
J	"	LPB	44.5	45	0	.....	.....	.....

[1]D=dormant (winter-bud); GT=green tip; EP=early preblossom; LP=late preblossom; FB=full blossom; EPB=early postblossom; MPB=middle postblossom; LPB=late postblossom.

[2]0=negative; +=positive.

[3]*=nut dropped prematurely

at an average incubation temperature of 27° C., while at 15° C. the symptoms did not appear until after 8 days.

Relation of the Age of the Host Organs

Extensive infection experiments over a 10-year period under both field and greenhouse conditions definitely show that the leaves and nuts pass through a stage of maximal susceptibility into a period of ever-increasing resistance. This increased resistance is first manifested by a longer incubation period and later by a more restricted development of the lesions.

The period of nut infection. The nuts were found susceptible from the time the buds unfold sufficiently to allow the bacteria to gain access to the interior and come in contact with the rudimentary pistillate flowers until the nuts are about seven-eighths grown. Inoculations made during the prepollination and blooming stages resulted in the infection of a much greater percentage of the nuts than did inoculations made after bloom. Moreover, infections occurring from inoculations made before and during bloom were almost always fatal, resulting in a premature dropping of the nuts, while lesions resulting from inoculations made after bloom were generally confined to the hull, though in some cases they did succeed in penetrating to and staining the shell. After the nuts were three-quarters grown, the lesions were confined to the outer portion of the hull. Inoculations made after the nuts were seven-eighths grown were almost all negative. Typical examples of the data from field and greenhouse experiments appear in Table 10.

The period of leaf infection. The leaves were found susceptible to infection just as soon as the dormant buds opened sufficiently to expose the longitudinally folded leaflets. Inoculations made at this stage resulted in the infection of the midribs and veins of the leaflets. The leaf lamina was found most subject to infection just after the leaflets unfolded. As the leaves approached maturity they became increasingly resistant, as manifested through prolongation of the incubation period and restricted development of the lesions. After the leaves attained their maximal size, the inoculations were negative. The leaves on a shoot varied in their susceptibility to infection with their age. Young leaves at the tip of a shoot were found to be more susceptible than the more mature ones of the base. The same situation prevailed with respect to the individual leaflets of a leaf. When a half-grown leaf was inoculated very few, if any, lesions occurred on the basal pair of leaflets; the lesions that did develop occurred on the terminal (younger) leaflets.

The period of bud infection. The buds were found subject to infection just as soon as the old winter buds of the previous year's growth opened sufficiently to permit the bacteria to gain access to the interior and to come in contact with the young, rudimentary buds on the growing shoots and in the axils of the unfolding leaves. At this time the axillary buds were very small, not exceeding 2 mm. in cross-sectional diameter and green and succulent. As the buds approached maturity and lost their greenish cast and succulency, they became increasingly resistant to infection. Buds in the winter or dormant stage are apparently not susceptible to infection, as all attempts to infect them have been uniformly negative.

The period of stem infection. The stems of young, new shoots were found subject to infection just as soon as the dormant or winter buds opened. They were most susceptible during the prebloom stage. This is the period

when they are most succulent and contain the greatest number of functional stomata. The stem of a new shoot was found to vary in susceptibility throughout its entire length. The apex of a shoot was found to remain susceptible to infection much longer than the more mature base. After the shoots ceased elongating and lost their succulency they became very resistant to infection. Twigs 1 year of age and older are apparently immune as all attempts made to infect them have been uniformly negative.

THE DEVELOPMENT OF EPIDEMICS*

Sources of Infection

Primary. Infected buds were found to be the most important source of primary infection under Pacific Northwest conditions. Lesions on twigs of the preceding year's growth and "latent" infections at the margins of young leaflets from diseased buds of the preceding year's origin are other sources of primary inoculum, but these are of lesser importance.

Secondary. Bacteria from primary lesions on the leaves, nuts and other parts of current growth constitute the source of secondary infection.

Factors Governing Primary Infection

Under Pacific Northwest conditions, the occurrence and intensity of primary infection was found to be governed by (a) the relative abundance of the sources of primary inoculum in the trees and (b) the extent of rainfall during the forepart of the infection period. The greater the number of primary sources of inoculum and the greater the extent of rainfall during the forepart of the critical period for infection, the greater was the number of primary infections.

Factors Governing Secondary Infection

The intensity of secondary infection was found to be dependent on (a) the number of primary infections in the trees and (b) the extent of rainfall during the latter part of the infection period. The greater the number of primary infections in the trees and the greater the extent of rainfall during the latter part of the infection period, the greater was the incidence of secondary infections. Secondary infection occurred in successive "waves" traceable to specific rainy periods.

The Special Significance of Rainfall

Rainfall is of special significance in the epidemiology of walnut bacteriosis, being the most important single factor 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 frequent rains of sufficient duration occur during the infection period. The greater the extent of rainfall during the critical period for infection the greater will be the incidence of the disease.

Critical Periods for the Development and Control of Epidemics

The critical period for the development and control of epidemics of walnut bacteriosis was found to extend from the time the buds open sufficiently to

* The propriety of the use of the term "epidemic" in connection with plant diseases is questioned by some individuals who contend 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 "epiphytotiology."

allow the bacteria to come in contact with the rudimentary pistillate flowers until the nuts are about half-grown. The forepart of this period (the pre-bloom and blooming periods), was found to be the most critical in relation to control for the following reasons:

1. The pistillate flowers were found to be most susceptible to infection at this stage, and the natural resistance of the tissues to invasion is at its lowest level. Most of the fatal type of nut infection takes place during this period.
2. The nuts expand most rapidly during this time, necessitating frequent spraying in order to keep them adequately protected.

An adjustment of the control program to the critical period for infection is obviously essential to the efficient control of this disease.

VI. STUDIES OF CONTROL MEASURES

EXCISION EXPERIMENTS

Pierce (*76*) suggested on apparently "a priori" grounds that the removal of all infected twigs from the trees would aid in the control of walnut bacteriosis. In 1915-1916, Fawcett and Batchelor (*37*) carried on experiments in two different orchards in California in an attempt to control this disease by the excision of twig lesions with no apparent success.

In 1930, we carried on excision experiments in Oregon in an attempt to determine whether or not the disease could be controlled by the removal of twig lesions. All dead and cankered twigs that could be found were removed during the early spring from four 20-year-old Franquette walnut trees. Eighteen per cent of the nuts examined on the "excised" trees were found infected after the period of disease development was over, whereas in adjoining, non-excised trees an average of 17.2 per cent of the nuts were found diseased. There was also no significant difference in the incidence of infected nuts the following year. At the time of this experiment the important role played by diseased buds in carrying the pathogen overwinter was not known. Consequently, excision failed because no attempt was made to remove diseased buds from the trees. A program involving the detection and removal of all infected buds as well as twig lesions is manifestly a practical impossibility. Hence, it is believed that excision of the sources of infection holds no promise of itself as a practical measure for controlling this disease.

VARIETIES IN RELATION TO THE DISEASE

Varietal immunity to walnut bacteriosis has apparently not been found, but it has long been observed that certain varieties and individual seedling trees are much less affected than others. Pierce (*76, 77, 80*) was the first investigator to call attention to this fact. Differences in the degree to which certain varieties are attacked by the disease under natural conditions have also been noted by R. E. Smith, et al (*108, 110*), C. O. Smith (*102, 105*), Batchelor (*21*), Rudolph (*91, 93*), and others (*9*). Among the varieties reported to be most susceptible to bacteriosis under California conditions are the Payne, Chase, Placentia, and Santa Rosa, while the Eureka, Franquette, San Jose, and Ehrhardt are said to be affected the least. In practically all seedling orchards there are certain trees that are severely attacked almost every year while others are seldom badly infected. It is noteworthy, however, that on the Pacific Coast many of the varieties and individual seedling trees that escape serious infection come into

leaf relatively late in the spring, thus missing many of the early spring rains by which the inoculum is disseminated. That their comparative freedom from infection is due to the possession of some such trait rather than to an inherent resistance is indicated by the results from inoculations made by Smith (*104*) in 1924. He inoculated nuts of a number of the more important commercial varieties which do not "blight" badly under California conditions with the result that well-defined lesions, comparable in size and extent, were produced on the nuts of all varieties.

In the Pacific Northwest, as in California, considerable variation exists in the degree to which the various commercial varieties and individual seedling trees are attacked. It is significant that some of the varieties, as the Eureka and El Monte, that are said to escape severe infection under California conditions are among the most susceptible under Oregon conditions. Likewise, some of the varieties, like the Franquette, that do not "blight" badly in California are severely attacked by the disease in the Pacific Northwest. Such reversal of performance under different environmental conditions gives further support to the idea that so-called resistance is, in reality, a matter of disease escape due to the possession of certain attributes, such as late foliation, in relation to the local climate. Under Oregon conditions, the Parisienne variety seems to be the least affected of the commercial varieties grown in the State. The situation that prevailed in 1933, a very severe "blight" year in Oregon, demonstrates well the variation in the degree to which the commercial varieties are attacked by the disease in Oregon. In that year, the Parisienne in a varietal orchard on the Oregon State College experimental farm at Corvallis was only slightly infected, the incidence of infection on the nuts being 3.3 per cent, whereas under the same conditions the incidence of nut infection on the El Monte, Eureka, Meyland, Mayette, Westphal, and Franquette varieties was 85.2, 81.4, 76, 60.9, 59.4, and 45.2 per cent, respectively. It is significant that the Parisienne variety foliates quite late in the Pacific Northwest, thereby escaping many of the early spring rains.

SPRAYING AND DUSTING EXPERIMENTS

Pierce (*79*) was the first to carry on spraying experiments for the control of walnut bacteriosis. He reported good results from the combined use of excision measures and bordeaux mixture before the trees come into leaf and again in early summer if weather conditions warranted. Following these studies, Smith and his associates (*107, 110, 111*) carried on further spraying experiments with bordeaux mixture and lime-sulphur. They concluded that spraying for the control of walnut bacteriosis was not only of doubtful value but was impractical due to the extreme height of the trees which made thorough spraying with the equipment then available difficult and costly. In 1916, Fawcett and Batchelor (*37*) sprayed trees with bordeaux mixture and lime-sulphur when the buds were swelling with little, or no, beneficial results. In the same year, Cole (*27*) applied bordeaux mixture to walnuts for the control of bacteriosis under Australian conditions with unsatisfactory results.

In 1917, spraying experiments for the control of the disease were initiated in Oregon by Barss who reported (*20*) a reduction in the incidence of infection from 5 spring applications of bordeaux mixture, but the benefit derived was considered insufficient to pay for the cost of the materials. Further spraying and dusting tests were carried on in Oregon from 1918 to 1926 with little or no apparent benefit (*20*). However, in practically all these trials, the sprays

or dusts were applied either in the fall or during the delayed-dormant or green-tip stages of development. In 1927, Ames (*1*) reported beneficial results from three applications of bordeaux mixture 10-10-100 applied (a) just before the buds opened, (b) as the catkins fell, and (c) when the first leaves were full grown. During the same year, Rudolph (*86, 87*) initiated extensive spraying experiments in California. He reported promising results from one application of bordeaux mixture (16-8-100) applied when the catkins were fully elongated and about to shed pollen. Further confirmation of the efficacy of timely spraying with bordeaux mixture was obtained by Rudolph in tests carried on in 1929 and 1930 (*86, 87*). Bordeaux 16-8-100 gave the best control of any strength tested. Basic copper acetate and ammoniacal copper carbonate also gave comparatively good control. At the same time, spraying tests for the control of this disease were also carried on in Oregon by Barss, et al (*20*). In these experiments bordeaux mixture 10-10-100 or 16-8-100, applied at the time pollen was being shed and the nuts were setting, gave definite indications of control but the spray injured the foliage, and in certain instances the set of nuts was reported to have been adversely affected.

In 1929, the Federal government appropriated funds for further studies of the control of this disease under Pacific Northwest conditions. Control studies under this grant were initiated in 1930 and have been actively carried on by the United States Department of Agriculture in cooperation with the Oregon Agricultural Experiment Station and a number of Oregon walnut growers for 16 seasons, 1930 to 1945. A detailed discussion of methods, materials, and results of investigations follows:

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

1. The comparative efficacy and phytocidal effect of various spray and dust materials.
2. The effect of varying the number and timing of the applications.
3. The comparative efficacy of different concentrations of bordeaux mixture.
4. The effect of the addition of certain supplements to bordeaux mixture on its efficacy and phytotoxicity.
5. The effect of varying the lime component in bordeaux mixture and the addition of certain supplements to the mixture on the incidence and severity of foliage spray injury.

Spray Materials Tested

Home-made bordeaux mixture. Prepared by mixing together dilute solutions of commercial copper sulphate and freshly hydrated lime in designated proportions.

Zinc sulphate + lime. Prepared by mixing together dilute solutions of commercial zinc sulphate and freshly hydrated lime in designated proportions.

Copper sulphate + zinc sulphate + lime. Prepared by mixing together in designated proportions dilute solutions of freshly hydrated lime, commercial zinc sulphate, and commercial copper sulphate in the order named.

Home-made ammoniacal copper carbonate. Prepared by mixing together solutions of commercial copper carbonate (containing 55 per cent metallic copper) and ammonia water in the following proportions: copper carbonate, 10 ounces; ammonia water (26° Baumé), 6 pints; water, 100 gallons.

Commercial ammoniacal copper carbonates. Two forms were tested; namely, (a) a commercial dry form containing 17 per cent, by weight, of metallic copper, and (b) a commercial, liquid product containing 3 per cent of metallic copper.

Burgundy mixture. Prepared by mixing together dilute solutions of commercial copper sulphate and sodium carbonate in designated proportions.

Copper phosphate + bentonite + lime. Prepared by mixing together dilute solutions of commercial copper phosphate (containing 50 per cent, by weight, of metallic copper), bentonite, and hydrated lime in designated proportions.

Commercial bordeaux mixture. Commercial dehydrated forms of bordeaux mixture, containing from 12½ per cent to 26 per cent, by weight, of metallic copper.

Basic copper sulphate. A commercial dry product containing 50 per cent, by weight, of metallic copper.

Tribasic copper sulphate. A proprietary dry copper material containing 53 per cent, by weight, of metallic copper.

Copper carbonate. A commercial dry product containing 52 per cent, by weight, of metallic copper.

Zinc-copper ammonium silicate. Two forms of this fungicide were used, viz.: (a) a commercial dry product containing 25 per cent, by weight, of metallic copper and 5 per cent, by weight, of metallic zinc and (b) a dry form containing 19 per cent, by weight, of metallic copper and 19 per cent, by weight, of metallic zinc.

Copper phosphate. A commercial dry product containing 50 per cent, by weight, of metallic copper.

Copper oxalate. Two commercial dry forms were tested; namely, (a) an extended form containing 19 per cent, by weight, of metallic copper, and (b) a pure form containing 40 per cent, by weight, of metallic copper.

Cuprous oxide. Two commercial dry forms were tested; namely, (a) a red form, and (b) a yellow form. These 2 materials have the same chemical formula but they differ physically, the particles in the so-called yellow form being many times smaller. Stabilizers or extenders were added to both forms by the manufacturer, the final materials containing from 47 to 86 per cent, by weight, of metallic copper.

Cupric oxide. A proprietary dry product, sold under the name "Brown cupric oxide," containing 80 per cent, by weight, of metallic copper.

Grasselli organic copper compound IN877-A-6. A commercial dry organic copper compound containing 25 per cent, by weight, of metallic copper.

Copper oxychloride. A commercial dry product containing 45 per cent, by weight, of metallic copper.

Copper zeolite. A commercial dry material containing 25 per cent, by weight, of metallic copper.

Sodium-copper polyphosphate. A commercial dry copper fungicide containing 25 per cent, by weight, of metallic copper.

Manganese "Bordo." A proprietary dry material containing 17 per cent, by weight, of metallic copper and 3 per cent, by weight, of manganese.

Copper hydroxide. A commercial dry product containing 26 per cent, by weight, of metallic copper.

Copper resinate. Two commercial liquid preparations containing 0.3 per cent of copper resinate in (a) pine tar oil, and (b) a petroleum oil, respectively, were used.

"Elgetol." A proprietary liquid-eradicant fungicide containing 34 per cent of sodium dinitro-ortho-cresolate.

"Thiosan." A commercial dry powder containing 50 per cent of tetramethyl thiuram disulphide.

"Fermate." A proprietary dry powder containing 70 per cent of ferric dimethyl dithiocarbamate.

"Isothan Q-15." A proprietary liquid organic compound containing 20 per cent lauryl isoquinolinum bromide.

"Dithane D-14." A proprietary liquid organic material, the active ingredient of which is disodium ethylene bisdithio-carbamate.

"Puratized N5D." A commercial liquid, organic material containing 20 per cent phenyl mercuri triethanol ammonium lactate.

Wettable sulphur (Kolofog). A proprietary dry, bentonite-sulphur preparation containing from 30 to 40 per cent of sulphur, made by fusing sulphur with bentonite.

Boric acid. Commercial granulated crystals.

Dusting Materials Tested

Monohydrated copper sulphate + lime dusts. Factory-mixed dusts containing from 20 to 30 per cent of monohydrated copper sulphate and varying amounts of hydrated lime together with various types of "extenders" and powdered spreaders and stickers to improve the physical properties and adhesiveness. Ferrous sulphate, ferric oxide, zinc arsenite, dusting sulphur and mineral oil, respectively, were added to some of the mixtures in an effort to increase their effectiveness.

Commercial bordeaux mixture dusts. Factory-mixed dusts composed of from 33 to 97 per cent of commercial powdered bordeaux mixture (containing from 12 to 22 per cent, by weight, of metallic copper) and varying amounts of hydrated lime, bentonite, and powdered spreaders and stickers of different types added to improve the physical properties and adhesiveness.

Cuprous oxide dusts. Factory-mixed dusts composed of from 7 to 10 per cent of cuprous oxide in either the red or yellow form with varying amounts of "extenders" of various types added to improve the physical properties and adhesiveness of the dusts. Beginning in 1941, 15 per cent of dusting sulphur was added to the mixtures in an effort to increase their effectiveness.

In 1945, 2 per cent of a light mineral oil was added to the dust mixtures to increase adherence.

Copper oxalate dusts. Factory-mixed dusts composed of from 20 to 50 per cent of copper oxalate with varying amounts of "extenders" and "stickers" of various types added to improve the physical properties and adhesiveness of the mixtures.

Basic copper sulphate dusts. Factory-mixed dusts composed of from 10 to 50 per cent of basic copper sulphate (containing 50 per cent, by weight, of metallic copper) with varying amounts of "extenders" and "stickers" of various types added to improve the physical properties and adhesiveness of the mixtures.

Copper hydroxide dusts. Factory-mixed dusts composed of from 25 to 50 per cent of copper hydroxide (containing 26 per cent, by weight, of metallic copper) with varying amounts of "extenders" of various types added to improve the physical properties and adhesiveness of the dust mixtures. In 1945, dusting sulphur and a light mineral oil were added in an effort to increase adherence and effectiveness of the dust.

Copper ammonium silicate dust. A factory-mixed dust composed of 97 per cent of copper ammonium silicate (containing 19 per cent, by weight, of metallic copper) and 3 per cent of glue dust.

Copper phosphate dust. A commercially mixed dust composed of 50 per cent of copper phosphate (containing 50 per cent by weight of metallic copper), 47 per cent of diatomaceous earth, and 3 per cent of glue dust.

Zinc-copper ammonium silicate dusts. Factory-mixed dusts composed of from 25 to 33 per cent of zinc-copper ammonium silicate (containing 19 per cent by weight of metallic copper and 19 per cent by weight of metallic zinc) with varying amounts of "extenders" and "spreaders" of various types added to improve the physical properties and adhesiveness of the mixtures. In 1944, 15 per cent of dusting sulphur was added in an effort to increase their effectiveness. In 1945, 2 per cent of a light mineral oil was added to the dust mixture to increase its adherence.

Tribasic copper sulphate dust. A factory-mixed dust composed of 10 per cent of tribasic copper sulphate, 5 per cent of dusting sulphur, 74.75 per cent of talc, 10 per cent of diatomaceous earth, and 0.25 per cent of powdered spreader.

Wetting and Adhesive Agents Tested

Mineral oils and oil emulsions. Commercial petroleum or mineral oils and oil emulsions of various viscosities (from 50 to 110 seconds Saybolt) and sulphonation valves (from 45 to 95). The oils and oil emulsions were slowly added to freshly prepared bordeaux mixture with the tank agitator in motion, and thoroughly mixed.

Fish oils. Both crude and refined salmon and herring oils were used. The refined herring oil was clear, liquid at 65° F., slow-drying, with an iodine number of between 120 and 145.

Linseed oil. Commercial raw linseed oil.

Casein spreaders. The following casein-type spreaders were used: "Kayso," "Fluxit," "Colloidal Z-1," skim milk powder, powdered casein and casein-lime. The last product was composed of 1 part casein and 2 parts hydrated lime.

Blood albumin. A commercial powdered product.

Penetrol. A proprietary liquid spreading agent, composed of 90 per cent of sulphonated, oxidized petroleum hydrocarbons (32°-34° Bé).

"B 1956 Spreader." A proprietary wetting and adhesive agent, the essential ingredient of which is glycerol phthalic resin.

"Tergitol penetrant No. 7." A proprietary liquid spreader; chemically it is a sodium sulphate of a higher synthetic secondary alcohol.

"Grasselli spreader-sticker." A commercial liquid spreading and sticking agent; chemically, it is sodium oleyl sulphate and a synthetic resinous sticker.

"Vatsol (O.T.)." A proprietary dry spreading agent, the chemical composition of which is a dioctyl ester of sodium sulpho-succinate.

"Vatsol (O.S.)." A commercial dry spreader, the chemical composition of which is a sodium salt of an alkyl naphthalene-sulphonic acid.

Lignin pitch. Commercial powdered and liquid materials.

Rosin-fish oil. A home made spreader-sticker composed of rosin, caustic potash, fish oil, and water.

Rosin-emulsion. A commercial product composed of rosin, caustic potash, and water.

Orthex spreader adhesive. A commercial complex mineral oil emulsion containing certain highly refined oils.

Ortho liquid spreader. A proprietary liquid spreading and wetting agent.

Ortho adhesive. A commercial emulsive type of mineral oil.

Bordo No. 101 spreader-sticker. A proprietary wetting and sticking agent whose essential ingredient is one of the higher sulphonated secondary alcohols. A resin emulsion was added as an adhesive.

Dresinate. A proprietary powdered spreader-sticker whose essential ingredient is sodium resinate.

Dreft. A proprietary spreading agent whose active ingredient is sodium lauryl sulphate.

Glue. A dry, finely ground product.

Soybean flour. A commercial finely ground preparation.

Bentonite. A commercial spreading and sticking agent used in both pellet and powdered forms.

"Earlonite." A commercial powdered clay used as a dilutent in certain dust mixtures.

"Frianite." A commercial powdered clay used as a dilutent in certain dust mixtures.

Manganese sulphate. A commercial product containing 65 per cent of manganese sulphate.

Techniques of Application

The sprays were applied with portable power sprayers having capacities ranging from 8 to 45 gallons per minute. The pressures used varied according to the individual machines from 250 pounds to 600 pounds per square inch. Prior to 1933, the applications were made with both spray guns and rods; in 1933 and thereafter, by spray guns exclusively. Brush (broom) spray guns having from 3 to 8 nozzles were used in most of the later spraying operations. The sprays were applied from both the ground and a tower on top of the spray tanks, and the trees were sprayed from two opposite sides.

Dust applications were made with standard portable power dusters operated along rows as nearly parallel to the wind direction as feasible. Where wind conditions permitted, each row was dusted from two sides.

Methods of Determining the Comparative Efficacy of the Spray and Dust Materials

The efficacy of the various materials was ascertained by a comparison of the percentages of infected nuts on a representative number of trees in the various plots, or by a comparison of the yields therefrom. Where and when conditions permitted, both methods were used.

The percentage of infected nuts in a given plot was determined by examining, as the operator moved around the trees, a number of nuts on usually five or more vigorous trees, located near the middle of the plot to minimize the possibility of spray or dust contamination from adjoining plots. The number of healthy and infected nuts found was recorded on hand tally registers. Counts were made after the peak of disease development was over. To minimize the error associated with variability in the distribution of infected nuts in the trees, a total of not less than 100 and usually 200 or more nuts, from all sides of each tree were examined. From 1939 to 1944, counts were made on an individual tree basis in most of the experimental plots and the standard error determined by the formula $E = \frac{s}{\sqrt{N}}$ in which s represents the standard deviation and N the number of trees.

In some years, yield records from a representative number of trees per plot in certain selected orchards were obtained at harvest. Replication and randomization of the plots, following either the randomized-block or Latin-square method of experimental plot design, were found to be the only means of compensating for differences due to variability in tree size.

A spray or dust material was tested for at least three years at widely scattered locations before making final deductions with respect to its efficacy and phytodical effect. In this way a wide range of conditions was encountered and the reliability of the results correspondingly enhanced.

Spraying and dusting experiments here reported were performed in 45 widely separated, commercial orchards in western Oregon. The experimental plots were located in the most uniform section of the orchard with respect to

topography and to size and vigor of the trees. The size of the plots varied according to the size of the orchard and to the amount of spray material available for trial. The majority of the plots contained 10 or more trees each. However, in certain preliminary, small-scale trials of new spray materials the plots contained as few as 3 trees. The plots were replicated at least twice in most instances. At the Aumsville, Oregon, branch station the more important plots were replicated five times and randomized according to the Latin-square system of plot arrangement. In addition to replications made in the individual orchards, the more important plots were replicated in a number of widely separated commercial orchards in western Oregon, thereby subjecting the spray material in question to test under a wider range of conditions than would have been possible at any one location.

The Comparative Efficacy of Various Spray Materials

Data on the comparative efficacy and phytocidal effect of the various spray materials tested are given by years in Table 11.

It is evident from the data in Table 11 that home-made bordeaux mixture gave more consistently effective control than any other material tested. Where an adequate number of properly timed and thorough treatments were made, it never failed to control the disease satisfactorily. It is, moreover, the cheapest of any of the spray materials tested.

Of the fixed or "low-soluble" copper compounds tested, copper oxalate and yellow cuprous oxide were the most consistently effective, comparing favorably with bordeaux mixture under moderate disease conditions, but they were not quite so effective when conditions for control were difficult. Where thoroughness of application did not meet experimental standards, the differential in favor of bordeaux mixture increased. A number of the other "fixed" copper materials and certain of the organic compounds tested gave some indication of control under slight to moderate disease conditions but were not so effective as bordeaux mixture under epidemic conditions. The application of an "eradicant" fungicide ("Elgetol") in the dormant period failed completely to control the disease.

Bordeaux mixture caused more foliage injury than any of the other materials tested. None of the "fixed" copper compounds tested caused any significant foliage injury.

The Effect of Varying the Number and Timing of the Applications on the Control of the Disease

Investigations on the effect of varying the number and timing of the applications on the control of the disease were carried on for 15 seasons under a wide range of environmental conditions. Typical examples of the data obtained are given in Table 12.

As shown by the data given in Table 12, the number of applications needed to control the disease satisfactorily varied from 1 to 3 depending on local and seasonal conditions. In seasons when very little rain fell during the critical period for infection, one properly timed application was sufficient to control the disease while in very rainy seasons as many as 3 applications made in (a) the early preblossom stage, (b) the late preblossom stage, and (c) the early postblossom stage were required to control the disease satisfactorily. The applications that were most effective varied according to the distribution of rains in relation to the stage of pistillate flower development. Generally speaking, the early preblossom and late preblossom applications were the most effective in the majority of seasons. The early postblossom application was effective only

Table 11. The Comparative Efficacy of Various Spray Materials for the Control of Walnut Bacteriosis and Their Phytocidal Effects; Western Oregon, 1930-1945

Year and orchard—plot number, material and concentration	Metallic copper content of material	Relative amount of foliage injury	Number of applications	Stages of pistillate flower development at time of applications	Nuts			
					Examined	Infected		
	Per cent				*Number*	*Number*	*Per cent*	*E*[1]
1930 (Holt)								
1 None	....	None	0	.....	3,855	1,220	31.6	.....
2 Bordeaux 8-8-100	25	Considerable	2	LP, EPB[2]	1,499	74	4.9	.....
1930 (Ames)								
3 None	....	None	0	.....	1,822	1,173	64.3	.....
4 Bordeaux 10-10-100	25	Appreciable	2	EP, EPB	5,163	935	18.1	.....
1931 (Edes)								
5 None	....	None	0	.....	6,339	1,230	19.4	.....
6 Zinc sulphate + lime 8-8-100	0	None	2	LP, MPB	1,869	144	7.7	.....
7 Copper sulphate + zinc sulphate + lime 2-6-12-100	25	None	2	"	2,445	44	1.7	.....
8 Bordeaux 8-8-100	25	Severe	2	"	3,650	16	0.4	.....
1931 (Bailey)								
9 None	....	None	0	.....	1,868	1,442	77.1	.....
10 Bordeaux 8-8-100	25	Appreciable	3	LP, EPB, MPB	4,770	198	4.1	.....
1932 (Andrew & Sterling)								
11 None	....	None	0	.....	1,830	597	32.6	.....
12 Bordeaux 6-6-100	25	Moderate	3	EP, LP, MPB	1,429	80	5.5	.....
1932 (Doege)								
13 None	....	None	0	.....	1,375	624	45.3	.....
14 Bordeaux 6-6-100	25	Moderate	2	LP, EPB	2,182	57	2.6	.....
1933 (Brown)								
15 None	....	None	0	.....	1,400	757	54.0	.....
16 Bordeaux 6-6-100	25	Considerable	2	LP, EPB	1,614	58	3.5	.....
1933 (Wolf)								
17 None	....	None	0	.....	2,185	1,769	80.9	.....
18 Copper carbonate 8-100	52	None	3	LP, EPB, MPB	2,822	858	30.4	.....
19 Copper sulphate + zinc sulphate + lime 2-4-6-100	25	Slight	3	"	2,868	835	29.1	.....
20 Zinc sulphate + lime 16-16-100	0	None	3	"	1,280	367	28.6	.....
21 Ammoniacal copper carbonate 4-100	17	Slight	3	"	3,100	490	15.8	.....
22 Bordeaux 6-6-100	25	Appreciable	3	"	1,506	188	12.4	.....
1933 (Brown)								
23 None	....	None	0	.....	2,981	626	20.9	.....
24 Copper carbonate 2-100	52	None	2	LP, EPB	3,212	122	3.7	.....
25 Ammoniacal copper carbonate 6-10-100[3]	17	Trace	2	"	2,645	63	2.3	.....
26 Basic copper sulphate 6-100	50	None	2	"	2,556	51	1.9	.....
27 Bordeaux 4-4-100	25	Moderate	2	"	2,456	4	0.1	.....

Table 11 (*Continued*). THE COMPARATIVF EFFICACY OF VARIOUS SPRAY MATERIALS FOR THE CONTROL OF WALNUT BACTERIOSIS AND THEIR PHYTOCIDAL EFFECTS; WESTERN OREGON, 1930-1945

Year and orchard—plot number, material and concentration	Metallic copper content of material	Relative amount of foliage injury	Number of applications	Stages of pistillate flower development at time of applications	Nuts			
					Examined	Infected		
	Per cent				*Number*	*Number*	*Per cent*	E[1]
1934 (Wolf)								
28 None	....	None	0	.....	2,498	1,002	40.1	.....
29 Zinc-copper ammonium silicate 3-100	15	None	3	LP, EPB, MPB	2,781	308	11.0	.....
30 Copper phosphate + bentonite + lime 4-4-8-100	50	None	3	"	2,273	250	10.9	.....
31 Ammoniacal copper carbonate[3] 6-10-100	17	None	3	"	2,622	168	6.4	.....
32 Basic copper sulphate 6-100	50	None	3	"	2,622	168	6.4	.....
33 Copper carbonate 12-100	52	None	3	"	2,412	131	5.4	.....
34 Bordeaux 6-6-100	25	Considerable	3	"	2,555	31	1.2	.....
1935 (Andrew and Sterling)								
35 None	....	None	0	.....	1,145	671	58.6	.....
36 Basic copper sulphate 4-100	50	None	2	LP, EPB	1,131	600	53.0	.....
37 Burgundy mixture 8-8-100	25	Slight	2	"	1,010	490	48.5	.....
38 Red cuprous oxide 4-100	86	None	2	"	1,109	161	14.5	.....
39 Bordeaux 4-4-100	25	Moderate	2	"	1,166	89	7.6	.....
40 Copper oxalate 4-100	40	None	2	"	1,060	46	4.3	.....
1936 (Brown)								
41 None	....	None	0	.....	2,166	1,229	56.7	.....
42 Copper phosphate 2-100	50	None	2	LP, EPB	2,518	568	22.5	.....
43 Copper hydroxide 2-100	26	None	2	"	2,508	490	19.5	.....
44 Basic copper sulphate 4-100	50	None	2	"	2,583	488	18.8	.....
45 Zinc-copper ammonium silicate 2-100	15	None	2	"	2,733	510	18.6	.....
46 Bordeaux 4-4-100	25	Considerable	2	"	2,324	130	5.5	.....
1936 (Woodford)								
47 None	....	None	0	.....	2,458	1,406	57.2	.....
48 Copper phosphate 4-100	50	None	2	LP, EPB	1,704	527	30.9	.....
49 Copper hydroxide 8-100	26	None	2	"	1,932	347	17.9	.....
50 Copper oxalate 4-100	40	None	2	"	987	140	14.1	.....
51 Red cuprous oxide 4-100	86	None	2	"	2,151	263	12.2	.....
52 Bordeaux 6-6-100	25	Appreciable	2	"	2,279	136	5.9	.....
1936 (Leonard)								
53 None	....	None	0	.....	3,069	2,813	91.6	.....
54 Copper hydroxide 4-100	26	None	2	LP, EPB	2,898	1,820	62.8	.....
55 Red cuprous oxide 2-100	86	None	2	"	3,617	2,078	57.4	.....
56 Commercial bordeaux 8-100	12.5	None	2	"	3,239	1,846	56.9	.....
57 Basic copper sulphate 4-100	50	None	2	"	4,438	2,294	51.6	.....
58 Copper oxalate 4-100	40	None	2	"	5,217	1,640	31.4	.....
59 Bordeaux 4-4-50	25	Moderate	2	"	3,645	729	20.0	.....
1937 (Wolf)								
60 None	....	None	0	.....	2,026	1,671	82.4	.....
61 Copper zeolite 3-100	25	None	3	EP, LP, EPB	1,915	1,088	56.8	.....
62 Copper oxychloride 4-100	45	None	3	"	1,658	276	16.6	.....
63 Bordeaux 4-2-100	25	Moderate	3	"	3,600	542	15.0	.....
64 Copper oxalate 2-100	40	None	3	"	1,933	276	14.2	.....
65 Copper oxalate 4-100	40	None	3	"	1,909	106	5.5	.....

1937 (Holt)										
66	None	....	None	0	.....	1,618	487	30.0	.....	
67	Bordeaux 4-4-100	25	Moderate	2	EP, EPB	1,386	135	9.7	.....	
68	Copper oxalate 2-100	40	None	2	"	1,758	102	5.8	.....	
1937 (Dale)										
69	None	....	None	0	.....	1,563	883	56.4	.....	
70	Red cuprous oxide 1-100	86	None	2	"	1,545	327	21.1	.....	
71	Bordeaux 4-4-100	25	Appreciable	2	"	1,386	135	9.7	.....	
1938 (Wolf)										
72	None	....	None	0	.....	2,302	784	34.0	.....	
73	Zinc-copper ammonium silicate 2-100	15	None	3	EP, LP, EPB	1,713	323	18.8	.....	
74	Zinc-copper ammonium silicate 4-100	15	None	3	"	1,065	141	13.2	.....	
75	Zinc-copper ammonium silicate 3-100	15	None	3	"	1,617	207	12.8	.....	
76	Bordeaux 4-4-100	25	Appreciable	3	"	1,530	162	10.5	.....	
77	Copper oxalate 4-100	40	None	3	"	1,753	146	8.3	.....	
78	Copper oxalate 3-100	40	None	3	"	1,601	129	8.0	.....	
1938 (Holt)										
79	None	....	None	0	.....	1,535	256	16.6	.....	
80	Bordeaux 4-4-100	25	Considerable	3	EP, LP, EPB	979	5	0.5	.....	
81	Copper oxalate 3-100	40	None	3	.....	1,176	3	0.2	.....	
1938 (Leonard)										
82	None	....	None	0	.....	1,273	462	36.2	.....	
83	Bordeaux 4-4-100	25	Moderate	3	EP, LP, EPB	1,146	158	13.7	.....	
84	Copper oxalate 3-100	19	None	3	"	639	47	7.3	.....	
1939 (Duncan)										
85	None	....	None	0	.....	1,290	279	21.6	.....	
86	Copper oxalate 3-100	19	None	3	EP, LP, EPB	1,044	30	2.8	.....	
87	Copper oxalate 4-100	19	None	3	"	1,262	17	1.3	.....	
88	Bordeaux 6-2-100 + a "heavy" mineral oil emulsion, 1 pt.-100	25	Trace	3	"	1,093	6	0.5	.....	
1939 (Dale)										
89	None	....	None	0	.....	782	244	31.2	.....	
90	Bordeaux 6-2-100	25	Slight	3	EP, LP, EPB	642	5	0.7	.....	
91	Copper oxychloride 3-100	45	None	3	"	658	3	0.4	.....	
92	Copper oxalate 3-100	19	None	3	"	638	1	0.1	.....	
1939 (Brown)										
93	None	....	None	0	.....	1,937	703	36.2	.....	
94	Copper oxalate 3-100	19	None	2	EP, LP	739	88	11.9	.....	
95	Copper oxalate 4-100	19	None	2	"	711	7	0.9	.....	
96	Bordeaux 4-1-100	25	Slight	2	"	2,758	25	0.9	.....	
97	Yellow cuprous oxide 3-100 + rosin emulsion, ½ pt.-100	47	None	2	"	925	8	0.8	.....	
1939 (Wolf, orchard "A")										
98	None	....	None	0	.....	887	424	47.8	4.04	
99	Brown cupric oxide 4-100	80	None	3	EP, LP, EPB	649	131	20.1	2.80	
100	Commercial copper fungicide 4-100	36	None	3	"	501	58	11.5	2.37	
101	Yellow cuprous oxide 2-100	47	None	3	"	825	55	6.6	0.87	
102	Grasselli copper compound IN877-A-6, 3-100	25	None	3	"	531	21	3.9	1.34	
103	Copper oxalate 3-100	19	None	3	"	542	16	2.9	0.79	
104	Yellow cuprous oxide 3-100	47	None	3	"	380	8	2.1	0.83	
105	Bordeaux 6-2-100	25	Slight	3	"	1,206	18	1.4	0.43	
1939 (Wolf, orchard "B")										
106	None	....	None	0	.....	3,069	1,380	44.9	0.20	
107	Zinc-copper ammonium silicate 3-100	19	None	3	EP, LP, EPB	3,284	369	11.2	1.24	
108	Copper oxalate 3-100	19	None	3	"	3,272	134	4.0	0.61	
109	Copper oxychloride 3-100	45	None	3	"	3,266	128	3.9	0.39	
110	Bordeaux 6-2-100	25	Slight	3	"	3,217	125	3.8	0.46	
111	Copper oxalate 3-100	40	None	3	"	1,127	16	1.4	0.41	

Table 11 (*Continued*). The Comparativf Efficacy of Various Spray Materials for the Control of Walnut Bacteriosis and Their Phytocidal Effects; Western Oregon, 1930-1945

Year and orchard—plot number, material and concentration	Metallic copper content of material	Relative amount of foliage injury	Number of applications	Stages of pistillate flower development at time of applications	Nuts			
					Examined	Infected		
	Per cent				*Number*	*Number*	*Per cent*	*E*[1]
1940 (Dale)								
112 None	....	None	0	.....	1,941	719	37.0	3.88
113 Copper oxychloride 3-100	45	None	2	EP, LP	2,411	175	7.2	0.89
114 Yellow cuprous oxide 3-100	47	None	2	"	2,395	65	2.7	0.55
115 Bordeaux 6-2-100 + oil, 1 pt.-100	25	Trace	2	"	2,030	24	1.1	0.37
116 Copper oxalate 4-100	19	None	2	"	2,757	17	0.6	0.27
1940 (Duncan)								
117 None	....	None	0	.....	1,356	687	50.6	2.54
118 Copper oxalate 3-100 + rosin emulsion 1 pt.-100	19	None	3	EP, LP, EPB	2,110	65	3.0	0.62
119 Bordeaux 4-1-100 + a "heavy" mineral oil emulsion 1 qt.-100	25	Slight	3	"	1,692	19	1.1	0.16
1940 (Bashford)								
120 None	....	None	0	.....	1,095	329	30.0	5.14
121 Yellow cuprous oxide 3-100	47	None	3	EP, LP, EPB	1,901	12	0.6	0.80
122 Copper oxalate 3-100 + "Orthex" sticker, 1 pt.-100	20	None	3	"	1,566	7	0.4	0.26
123 Copper oxalate 4-100	20	None	3	"	3,023	13	0.4	0.34
124 Bordeaux 6-2-100 + oil	25	Slight	3	"	1,620	4	0.2	1.43
1940 (Brooks)								
125 None	....	None	0	.....	677	152	22.4	1.40
126 Bordeaux 6-2-100	25	Trace	2	EP, LP	735	20	2.7	0.84
127 Copper oxalate 4-100	20	None	2	"	504	9	1.7	0.86
128 Copper oxychloride 3-100	45	None	2	"	461	7	1.5	2.29
1940 (Schaad)								
129 None	....	None	0	.....	1,491	289	19.3	1.88
130 Red cuprous oxide 2-100 + "Orthex" sticker, 1 qt.-100	54	None	3	EP, LP, EPB	3,382	1,018	30.1	2.23
131 Copper oxalate 3-100	19	None	3	"	3,617	137	3.7	0.74
1940 (Brown)								
132 None	....	None	0	.....	2,136	602	28.1	2.41
133 Manganese "Bordo" 3-100	17	None	2	EP, LP	1,407	250	17.7	1.82
134 Yellow cuprous oxide 1.5-100	47	None	2	"	1,255	77	6.1	1.44
135 Copper oxalate 3-100	20	None	2	"	1,402	59	4.2	0.59
136 Copper oxychloride 3-100	45	None	2	"	1,264	53	4.1	0.47
137 Bordeaux 6-1.5-100	25	Slight	2	"	1,684	44	2.6	0.39
1940 (Brown and McClure)								
138 None	....	None	0	.....	2,277	674	29.6	2.59
139 Red cuprous oxide 2-100 + "Orthex" sticker, 1 qt.-100	54	None	2	EP, LP	1,642	242	14.7	1.47
140 Grasselli copper compound IN877-A-6	25	None	2	"	1,629	208	12.7	1.14
141 Yellow cuprous oxide 2-100	47	None	2	"	1,628	153	9.3	1.15
142 Bordeaux 6-2-100 + mineral oil emulsion, 1 pt.-100	25	Slight	2	"	1,129	81	7.1	0.83
143 Copper oxalate 4-100	20	None	2	"	1,486	72	4.8	0.77
144 Copper oxalate 3-100	40	None	2	"	1,429	55	3.8	0.41

1941 (Bashford)									
145	None	....	None	0		1,854	855	46.1	4.76
146	Bordeaux 6-2-100 + oil, 1 pt.-100	25	Slight	3	EP, LP, EPB	2,064	37	1.7	0.09
147	Yellow cuprous oxide 2-100	86	Trace	3	"	1,591	13	0.8	0.08
1941 (Trunk)									
148	None	....	None	0		5,637	1,319	23.3	2.08
149	Sodium copper polyphosphate 3-100	25	None	3	EP, LP, EPB	5,775	189	3.2	0.38
150	Bordeaux 6-2-100 + oil, 1 pt.-100	25	Slight	3	"	5,134	73	1.4	0.06
151	Copper oxalate 3-100	20	Trace	3	"	5,455	65	1.1	0.06
1941 (Brown and McClure)									
152	None	....	None	0		4,014	961	23.9	1.88
153	Sodium copper polyphosphate 3-100 + "Vatsol" 2 oz.-100	25	Trace	2	EP, LP	3,152	205	6.5	0.75
154	Copper oxalate 3-100 + "Vatsol" 2 oz.-100	20	Trace	2	"	3,694	79	2.1	0.08
155	Yellow cuprous oxide, 1½-100 + "Vatsol" 2 oz.-100	86	Trace	2	"	3,452	82	2.3	0.40
156	Bordeaux 6-2-100 + "heavy" mineral oil emulsion, 1 pt.-100	25	Slight	2	"	3,452	49	1.4	0.06
1941 (Wolf)									
157	None	....	None	0		6,149	3,633	59	1.58
158	"Elgetol" ½ gal.-100	0	None	1	D	636	493	77.5	3.06
159	Sodium copper polyphosphate 3-100 + "B 1956" spreader, ½ pt.-100	25	Trace	4	EP, MP, LP, EPB	5,446	893	16.3	1.54
160	Copper oxalate 3-100 + "B 1956" spreader, ½ pt.-100	20	Slight	4	"	5,311	326	6.1	1.01
161	Yellow cuprous oxide + "B 1956" spreader, ½ pt.-100	86	Trace	4	"	5,481	169	3	0.30
162	Bordeaux 6-2-100 + oil, 1 pt.-100	25	Moderate	4	"	5,867	180	3	0.06
1941 (Gale)									
163	None	....	None	0		3,815	649	17.0	2.47
164	"Elgetol" ½ gal.-100	0	None	1	D	1,714	300	17.5	3.73
165	Copper oxalate 3-100	20	Trace	3	EP, LP, EPB	5,885	47	0.7	0.11
166	Bordeaux 6-2-100 + "heavy" mineral oil emulsion, 1 qt.-100	25	Slight	3	"	5,816	11	0.1	0.19
1941 (Johnson)									
167	None	....	None	0		1,900	1,091	57.4	6.24
168	Copper oxalate 2-100 + Grasselli sticker-spreader, ¼ pt.-100	20	None	3	MP, LP, EPB	2,421	16	0.6	0.25
169	Bordeaux 6-2-100 + oil, 1 pt.-100	25	Slight	3	"	3,834	22	0.5	0.44
1941 (Hamre)									
170	None	....	None	0		1,197	878	73.3	.....
171	Copper oxychloride 2-100 + Grasselli spreader-sticker ¼ pt.-100	45	None	3	MP, LP, EPB	2,332	61	2.6	.....
172	Bordeaux 6-2-100 + oil, 1 pt.-100	25	Slight	3	"	4,998	101	2.0	.....
1941 (Fellows)									
173	None	....	.....	0		714	313	43.8	.....
174	Bordeaux 6-2-100 + oil, 1 pt.-100	....	Slight	2	EP, LP	2,459	159	6.4	.....
175	Yellow cuprous oxide 1½-100 + casein spreader ½ lb.-100	86	None	2	"	3,149	176	5.5	.....
1941 (Vanderspek)									
176	None	....	.....	0		1,502	220	14.6	4.12
177	"Elgetol" ½ gal.-100	0	None	1	.....	2,017	252	12.4	2.59
178	Bordeaux 6-2-100 + oil, 1 pt.-100	25	Trace	2	EP, LP	2,294	45	1.9	0.27

Table 11 (*Continued*). THE COMPARATIVF EFFICACY OF VARIOUS SPRAY MATERIALS FOR THE CONTROL OF WALNUT BACTERIOSIS AND THEIR PHYTOCIDAL EFFECTS; WESTERN OREGON, 1930-1945

Year and orchard—plot number, material and concentration	Metallic copper content of material	Relative amount of foliage injury	Number of applications	Stages of pistillate flower development at time of applications	Nuts			
					Examined	Infected		
	Per cent				*Number*	*Number*	*Per cent*	*E*[1]
1942 (Bamford)								
179 None	....	.....	0	.....	1,032	719	69.6	.....
180 Bordeaux 6-2-100 + oil, 1 pt.-100	25	Trace	3	EP, MP, LP	333	32	9.6	.....
181 Yellow cuprous oxide 1-100 + Ortho adhesive, 1 pt.-100	86	None	3	"	632	46	7.2	.....
182 Yellow cuprous oxide 1½-100 + Ortho adhesive, 1 pt.-100	86	None	3	"	532	23	4.3	.....
1942 (Woodford)								
183 None	....	.....	0	.....	1,032	719	69.6	.....
184 Yellow cuprous oxide 1-100 + Ortho adhesive, 1 pt.-100	86	None	2	MP, EPB	1,148	49	4.2	.....
185 Bordeaux 4-2-100 + oil, 1 pt.-100	25	Slight	2	"	1,149	23	2.0	.....
186 Copper oxalate 3-100 + casein-lime spreader 1-100	20	None	2	"	1,021	14	1.3	.....
1942 (Brown and McClure)								
187 None	....	.....	0	.....	1,336	439	32.8	2.93
188 Yellow cuprous oxide ¾-100 + B-1956 spreader, ¼ pt.-100	86	None	2	EP, LP	1,166	72	6.1	1.11
189 Bordeaux 6-2-100 + "heavy" mineral oil emulsion, 1 pt.-100	25	Trace	2	"	1,173	57	4.8	0.79
1943 (Brown and McClure)								
190 None	....	.....	0	.....	691	211	30.5	2.42
191 Fermate 1-100 + B-1956 spreader, ¼ pt.-100	0	None	2	MP, LP	928	188	20.2	2.58
192 Thiosan 1½-100 + B-1956 spreader, ¼ pt.-100	0	None	2	"	571	64	11.2	3.79
193 Tribasic copper sulphate 2-100 + "Bordo" spreader No. 101, 1 pt.-100	53	Trace	2	"	598	39	6.5	1.71
194 Yellow cuprous oxide 1-100 + B-1956 spreader, ¼ pt.-100	86	Slight	2	"	596	39	6.5	1.44
195 Copper oxalate 2-100 + B-1956 spreader, ¼ pt.-100	20	None	2	"	549	30	5.4	1.85
196 Zinc-copper ammonium silicate 4-100 + Ortho liquid spreader, 1 pt.-100	19	None	2	"	742	32	4.3	1.59
197 Bordeaux 4-2-100	25	Moderate	2	"	782	18	2.3	0.27
1943 (Dale)								
198 None	....	.....	0	.....	694	340	48.9	4.00
199 Thiosan 1-100	0	None	2	MP, EPB	757	301	39.7	3.50
200 Fermate 1-100	0	None	2	"	798	294	36.8	5.46
201 Yellow cuprous oxide 1½-100	86	None	2	"	838	117	13.9	3.19
202 Bordeaux 4-2-100 + oil, 1 pt.-100	25	Slight	2	"	1,091	65	5.9	1.21
1943 (Landstrom)								
203 None	....	.....	0	.....	922	550	59.6	4.05
204 Fermate 1-100 + B-1956 spreader, ¼ pt.-100	0	None	1	LP	1,169	316	27.0	6.18
205 Yellow cuprous oxide 1-100 + B-1956 spreader, ¼ pt.-100	86	Slight	1	LP	1,188	97	8.1	1.61
1943 (Withycombe)								
206 None	....	.....	0	.....	761	470	61.7	4.69
207 Thiosan 1-100	0	None	2	LP, PB	1,073	326	30.3	6.01
208 Bordeaux 4-3-100 + oil, 1 pt.-100	25	Trace	2	"	1,089	137	12.5	1.07

1944 (Bamford)									
209 None	....	.....	0	.....	1,037	477	45.9	4.57
210 Tribasic copper sulphate 3-100 + "Bordo" 101 spreader, 1 pt.-100	53	None	3	EP, LP, EPB	790	38	4.8	1.40
211 Bordeaux 6-3-100 + Ortho adhesive, 1 pt.-100	25	Trace	3	"	747	5	0.6	0.01
1944 (Wylie)								
212 None	....	.....	0	.....	930	311	33.4	4.13
213 Yellow cuprous oxide 1½-100 + B-1956 spreader, ¼ pt.-100	86	Trace	3	EP, LP, EPB	852	8	0.9	0.48
1944 (Withycombe)								
214 None	....	.....	0	.....	1,241	496	39.9	4.33
215 Tribasic copper sulphate 3-100 + Ortho adhesive, 1 pt.-100	53	None	3	MP, LP, EPB	1,443	245	16.9	1.11
216 Bordeaux 4-2-100 + oil, 1 pt.-100	25	Slight	3	"	1,317	84	6.3	1.02
1944 (Brown and McClure)								
217 None	....	.....	0	.....	898	292	32.5	.....
218 Yellow cuprous oxide ½-100 + Ortho adhesive, 1 pt.-100	83	None	2	MP, LP	728	51	7.0	1.12
219 Yellow cuprous oxide 1-100 + Ortho adhesive, 1 pt.-100	83	Trace	2	"	703	8	1.1	0.13
220 Copper hydroxide 4-100 + colloidal Z-1 spreader ¼ lb.-100 + summer oil emulsion, 1 pt.-100	26	Trace	2	"	611	16	2.6	0.17
221 Tribasic copper sulphate 3-100 + Bordo No. 101 spreader, 1 pt.-100	53	None	2	"	634	10	1.5	0.63
222 Copper oxychloride 3-100 + zinc sulphate 1-100 + lime ½-100	45	None	2	"	710	5	0.7	0.14
223 Bordeaux 4-2-100 + summer oil emulsion, 1 pt.-100..	25	Slight	2	"	715	8	1.1	0.02
224 Zinc-copper ammonium silicate 3-100 + Ortho adhesive 1 pt.-100	19	None	2	"	707	1	0.1	0.004
225 Ammoniacal copper carbonate 2 gals.-100	17	None	2	"	612	1	0.1	0.01
1945 (Brown and McClure)								
226 None	....	.....	0	.....	711	184	25.4	2.01
227 Puratized Agricultural spray, ½ lb.-100	0	None	2	EP, LP	818	67	8.1	1.30
228 "Isothane Q-15," 1 gal.-800	0	None	2	"	1,309	65	4.9	1.39
229 "Dithane D14," 2 qts.-100 + basic zinc sulphate 1-100	0	None	2	"	1,102	25	2.2	0.13
230 Copper hydroxide 4-100 + "Colloidal Z-1" spreader ¼ lb.-100	26	None	2	"	1,113	21	1.8	1.09
231 Zinc-copper ammonium silicate 4-100 + colloidal Z-1 spreader, ¼ lb.-100	19	None	2	"	1,059	9	0.8	0.13
234 Yellow cuprous oxide ¾-100	83	Trace	2	"	1,176	9	0.7	0.10
235 Bordeaux 4-2-100 + Summer oil emulsion, 1 pt.-100	25	Slight	2	"	820	2	0.2	0.06

[1]E = Standard error of the mean.

[2]Stages of pistillate flower development are referred to by letter as follows: D = dormant; EP = early preblossom; MP = middle preblossom; LP = late preblossom; EPB = early postblossom; MPB = middle postblossom.

[3]Copper carbonate 10 oz., ammonia 6 pts., water 100 gals.

[4]Except where otherwise stated, the oil used in all tests recorded in this table was a "heavy" mineral spray oil having a viscosity of between 90 and 120 seconds Saybolt, and sulphonation test of from 50 to 70.

Table 12. The Effect of Varying the Number and Time of Spray Applications on the Control of Walnut Bacteriosis; Western Oregon, 1931-1945

Year and orchard—plot number, and material	Number of applications	Stages of pistillate flower development at time of applications	Nuts		
			Examined	Infected	
			Number	*Number*	*Per cent*
1931 (Edes)					
1 None	0		6,339	1,230	19.4
2 Bordeaux 8-8-100	1	EP[1]	4,413	337	7.6
3 Bordeaux 8-8-100	2	EP, EPB	3,650	16	0.4
1931 (Bailey)					
4 None	0		1,243	663	53.3
5 Bordeaux 8-8-100	2	EP, MPB	800	85	10.6
6 Bordeaux 8-8-100	3	EP, EPB, MPB	2,745	129	4.6
1932 (Andrew and Sterling)					
7 None	0		1,830	597	32.6
8 Bordeaux 6-6-100	1	EP	1,502	240	15.9
9 Bordeaux 6-6-100	1	MPB	1,642	473	28.8
10 Bordeaux 6-6-100	2	LP, MPB	2,679	195	7.2
11 Bordeaux 6-6-100	3	EP, LP, MPB	1,429	80	5.5
1933 (Durant)					
12 None	0		2,294	1,399	60.9
13 Bordeaux 6-6-100	1	EP	1,905	782	41.0
14 Bordeaux 6-6-100	2	EP, EPB	2,361	191	8.0
1933 (Brown)					
15 None	0		2,410	1,363	56.5
16 Bordeaux 6-6-100	2	EP, MPB	1,650	520	31.5
17 Bordeaux 6-6-100	2	LP, MPB	2,004	599	29.8
18 Bordeaux 6-6-100	2	EPB, MPB	2,073	313	15.0
19 Bordeaux 6-6-100	2	LP, EPB	1,907	120	6.2
20 Bordeaux 6-6-100	2	EP, EPB	1,880	88	4.6
21 Bordeaux 6-6-100	4	EP, LP, EPB, MPB	1,371	32	2.3
1933 (Morgan)					
22 None	0		926	780	84.2
23 Bordeaux 6-6-100	2	LP, EPB[2]	2,266	1,168	51.5
24 Bordeaux 6-6-100	2	LP, EPB	2,481	136	5.4
1934 (Wolf)					
25 None	0		2,640	1,232	46.6
26 Bordeaux 6-6-100	1	MPB	2,592	917	35.3
27 Bordeaux 6-6-100	1	EPB	2,285	332	14.5
28 Bordeaux 6-6-100	1	LP	1,385	145	10.4
29 Bordeaux 6-6-100	2	EPB, MPB	2,506	369	14.7
30 Bordeaux 6-6-100	2	LP, MPB	2,434	220	9.0
31 Bordeaux 6-6-100	2	EP, EPB	1,788	107	5.9
32 Bordeaux 6-6-100	2	LP, EPB	2,298	25	1.0
33 Bordeaux 6-6-100	3	LP, EPB, MPB	1,323	18	1.3
34 Bordeaux 6-6-100	4	EP, LP, EPB, MPB	1,627	27	1.6
1935 (Andrew and Sterling)					
35 None	0		1,145	671	58.6
36 Bordeaux 4-4-100	1	EP	1,289	286	22.1
37 Bordeaux 4-4-100	1	LP	605	81	13.3
38 Bordeaux 4-4-100	2	LP, EPB	1,166	89	7.6
39 Bordeaux 4-4-100	3	EP, LP, EPB	398	15	3.7
1936 (Brown and McClure)					
40 None	0		2,166	1,229	56.7
41 Bordeaux 4-4-100	1	EPB	2,454	356	14.5
42 Bordeaux 4-4-100	1	LP	2,437	228	9.3
43 Bordeaux 4-4-100	2	EP, EPB	2,603	205	7.8
44 Bordeaux 4-4-100	2	LP, EPB	2,324	130	5.5
45 Bordeaux 4-4-100	3	EP, LP, EPB	2,601	68	2.6
1937 (Brown and McClure)					
46 None	0		1,694	1,236	72.9
47 Bordeaux 4-4-100	1	LP	1,769	959	54.2
48 Bordeaux 4-4-100	1	EP	1,638	800	48.8
49 Bordeaux 4-4-100	2	LP, EPB	1,825	623	34.1
50 Bordeaux 4-4-100	2	EP, EPB	1,751	302	17.2
51 Bordeaux 4-4-100	3	EP, LP, EPB	1,740	185	10.6

Table 12 (*Continued*). The Effect of Varying the Number and Time of Spray Applications on the Control of Walnut Bacteriosis; Western Oregon, 1931-1945

Year and orchard—plot number, and material	Number of applications	Stages of pistillate flower development at time of applications	Nuts		
			Examined	Infected	
			Number	*Number*	*Per cent*
1938 (Brown and McClure)					
52 None	0		2,156	106	4.9
53 Bordeaux 6-2-100	1	LP	1,719	74	4.3
54 Bordeaux 6-2-100	1	EPB	1,585	55	3.4
55 Bordeaux 6-2-100	1	EP	1,585	5	0.3
56 Bordeaux 6-2-100	2	LP, EPB	1,843	82	4.4
57 Bordeaux 6-2-100	2	EP, EPB	1,461	1	0.06
58 Bordeaux 6-2-100	3	EP, LP, EPB	1,727	6	0.3
1939 (Wolf)					
59 None	0		3,069	1,380	44.9
60 Bordeaux 6-2-100	1	EP	902	310	34.3
61 Bordeaux 6-2-100	1	LP	922	266	28.8
62 Bordeaux 6-2-100	1	EPB	650	160	24.6
63 Bordeaux 6-2-100	2	EP, LP	1,123	275	24.4
64 Bordeaux 6-2-100	2	EP, EPB	920	100	10.8
65 Bordeaux 6-2-100	3	EP, LP, EPB	3,217	125	3.8
1940 (Brown and McClure)					
66 None	0		1,533	446	29.0
67 Bordeaux 6-1.5-100	1	EP	1,543	257	16.6
68 Bordeaux 6-1.5-100	1	LP	1,562	330	21.1
69 Bordeaux 6-1.5-100	2	EP, LP	1,449	134	9.2
70 Bordeaux 6-1.5-100	3	EP, LP, EPB	1,353	85	6.2
1940 (Brown)					
71 None	0		2,277	674	29.6
72 Bordeaux 6-2-100 + oil[3]	1	EP	576	29	5.0
73 Bordeaux 6-2-100 + oil	1	LP	619	64	10.3
74 Bordeaux 6-2-100 + oil	2	EP, LP	1,102	44	3.9
1941 (Wolf)					
75 None	0		6,149	3,633	59.0
76 Bordeaux 4-1-100 + oil	1	EP	704	40	5.6
77 Bordeaux 4-1-100 + oil	1	MP	916	220	24.0
78 Bordeaux 4-1-100 + oil	1	LP	517	151	29.2
79 Bordeaux 4-1-100 + oil	2	EP, MP	667	211	31.6
80 Bordeaux 4-1-100 + oil	2	EP, LP	706	173	24.5
81 Bordeaux 4-1-100 + oil	2	EP, EPB	688	281	40.8
82 Bordeaux 4-1-100 + oil	2	MP, LP	705	112	15.8
83 Bordeaux 4-1-100 + oil	2	MP, EPB	844	191	22.6
84 Bordeaux 4-1-100 + oil	2	LP, EPB	531	97	18.2
85 Bordeaux 4-1-100 + oil	3	EP, MP, LP	779	135	17.3
86 Bordeaux 4-1-100 + oil	3	EP, LP, EPB	592	123	20.7
87 Bordeaux 4-1-100 + oil	3	EP, MP, EPB	800	86	10.7
88 Bordeaux 4-1-100 + oil	3	MP, LP, EPB	559	34	6.0
89 Bordeaux 4-1-100 + oil	4	EP, MP, LP, EPB	2,584	79	3.0
1941 (Stump)					
90 None	0		936	462	49.3
91 Bordeaux 6-2-100 + oil	2	EP, LP	7,059	161	2.2
92 Bordeaux 6-2-100 + oil	3	EP, MP, LP	5,645	40	0.7
1941 (Withycombe)					
93 None	0		1,639	479	29.2
94 Bordeaux 6-2-100 + oil	1	EP	1,633	100	6.1
95 Bordeaux 6-2-100 + oil	3	EP, MP, LP	1,548	30	1.9
96 Bordeaux 6-2-100 + oil	4	EP, MP, LP, EPB	1,556	13	0.8
1941 (Chambers)					
97 None	0		6,829	3,211	47.0
98 Bordeaux 6-2-100 + oil	2	EP, LP	5,255	405	7.7
99 Bordeaux 6-2-100 + oil	2	LP, EPB	4,625	85	1.8
1941 (Young)					
100 None	0		1,117	342	30.6
101 Bordeaux 6-2-100 + oil	3	EP, LP, EPB	1,051	29	2.7
102 Bordeaux 6-2-100 + oil	3	EP, MP, LP	1,399	25	1.7
1942 (Withycombe)					
103 None	0		762	287	37.6
104 Bordeaux 6-2-100 + oil	3	EP, MP, PB	755	42	5.5
105 Bordeaux 6-2-100 + oil	3	EP, MP, LP	412	19	4.6
106 Bordeaux 6-2-100 + oil	4	EP, MP, LP, EPB	550	2	0.3

Table 12 (*Continued*). THE EFFECT OF VARYING THE NUMBER AND TIME OF SPRAY APPLICATIONS ON THE CONTROL OF WALNUT BACTERIOSIS; WESTERN OREGON, 1931-1945

Plot, orchard, and material	Number of applications	Stages of pistillate flower development at time of applications	Nuts		
			Examined	Infected	
			Number	*Number*	*Per cent*
1942 (Duncan and Miller)					
107 None	0		990	742	74.9
108 Bordeaux 6-2-100 + oil, 1 pt.-100	1	EPB	586	148	25.2
109 Bordeaux 6-2-100 + oil, 1 pt.-100	2	EP, EPB	987	109	11.0
1942 (Landstrom)					
110 None	0		667	403	60.4
111 Yellow cuprous oxide, 1½-100 + Ortho Adhesive, 1 pt.-100	1	EP	126	19	15.0
112 Yellow cuprous oxide, 1½-100 + Ortho Adhesive, 1 pt.-100	1	LP	95	17	17.8
113 Yellow cuprous oxide, 1½-100 + Ortho Adhesive, 1 pt.-100	2	EP, LP	495	29	5.8
1942 (Withycombe)					
114 None	0		762	287	37.6
115 Yellow cuprous oxide, 1½-100 + Ortho Adhesive, 1 pt.-100	1	EP	604	152	25.1
116 Yellow cuprous oxide, 1½-100 + Ortho Adhesive, 1 pt.-100	2	EP, MP	129	17	13.1
117 Yellow cuprous oxide, 1½-100 + Ortho Adhesive, 1 pt.-100	2	MP, EPB	519	8	1.5
118 Yellow cuprous oxide, 1½-100 + Ortho Adhesive, 1 pt.-100	3	EP, MP, EPB	566	15	2.6
1942 (Brown and McClure)					
119 None	0		1,336	439	32.8
120 Bordeaux 4-2-100 + oil, 1 pt.-100	1	LP	1,004	62	6.1
121 Bordeaux 4-2-100 + oil, 1 pt.-100	2	EP, LP	1,452	78	5.3
122 Bordeaux 4-2-100 + oil, 1 pt.-100	2	LP, EPB	936	28	2.9
1943 (Woodford and Raymond)					
123 None	0		1,354	778	57.4
124 Bordeaux 4-2-100 + oil, 1 pt.-100	1	MP	411	49	11.9
125 Bordeaux 4-2-100 + oil, 1 pt.-100	2	MP, EPB	1,042	9	0.8
1944 (Brown and McClure)					
126 None	0		898	292	32.5
127 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	1	MP	715	25	3.4
128 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	1	LP	681	9	1.3
129 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	2	MP, LP	666	5	0.7
1945 (Brown and McClure)					
130 None	0		904	157	17.3
131 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	2	EP, LP	1,065	7	0.6
132 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	3	EP, LP, EPB	1,045	8	0.7

[1]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; MP = middle preblossom; LP = late preblossom; EPB = early postblossom; MPB = middle postblossom.

[2]A prolonged rainy period intervened before the early postbloom application could be applied to this part of the plot.

[3]Except where otherwise stated, the oil used in all tests recorded in this table was a "heavy" mineral spray oil having a viscosity of between 90 and 120 seconds Saybolt and a sulphonation test of from 50 to 70.

in those years when prolonged rains occurred after bloom. In a few seasons when the rainfall before bloom was exceptionally heavy a middle preblossom treatment applied about a week after the early preblossom application was worth while.

The proper timing of the spray applications in relation to the stage of pistillate flower development and to the occurrence of rainfall is extremely important to successful control. The importance of timing the applications properly is clearly illustrated by the results obtained in 1933 in a sprayed Franquette orchard near Roseburg, Oregon. Approximately one-half of the orchard in question received the early postblossom application just prior to a prolonged rainy period and the remainder of the orchard could not be sprayed until five days later. The incidence of infection in the portion sprayed prior to this rainy period was only 5.4 per cent, while the incidence of infection in the part sprayed afterwards was 51.5 per cent (Table 12, plots 22 to 24).

The Comparative Efficiency of Different Concentrations of Bordeaux Mixture

Rudolph (*87, 93*) reported that, under California conditions, bordeaux mixture 16-8-100 was more effective than any other formula. The results of our work over a 16-year period under a wide range of local and seasonal conditions indicate that relatively weak concentrations of bordeaux mixture are practically as effective under Pacific Northwest conditions as stronger concentrations and cause less injury. Typical examples of the data showing the comparative efficiency of different concentrations of bordeaux mixture for the control of the disease and their phytocidal effects are given in Table 13.

As shown by the data given in Table 13, the 4-2-100 or the 4-4-100 concentration of bordeaux mixture generally gave practically as good control as more concentrated mixtures and caused proportionately less injury. Concentrations weaker than 4-2-100 did not always give satisfactory control.

Effect of Adding Supplements to Bordeaux Mixture on Its Efficacy and Phytotoxicity

In an effort to increase the efficacy of bordeaux mixture and decrease its phytotoxicity, a number of supplements were added to the mixture. Typical examples of the data obtained from these studies are given in Table 14.

It is evident from the data given in Table 14 that none of the supplements used significantly increased the efficacy of bordeaux mixture. However, the addition of fish oil, vegetable oil, or mineral oil or oil emulsions to bordeaux mixture generally reduced the severity of foliage injury, though it was not entirely eliminated.

Comparative Effectiveness of Sprays versus Dusts

Studies on the effectiveness of a number of dusts were carried on for a period of 15 years under a wide range of seasonal and local conditions. Where conditions permitted, comparative tests with liquid sprays were made. Various kinds of detergents, adhesives, adsorbents, and extenders were used with a number of the dusts in an effort to improve their physical properties and increase their efficacy. Data showing the comparative control obtained from these dusts and liquid sprays are given in Table 15.

It is evident from the data given in Table 15 that liquid bordeaux mixture was consistently more effective than any of the dusting materials tested. However, some of the dusts gave what may be regarded as commercial control where a sufficient number of properly timed applications were made. Of the

Table 13. The Comparative Effectiveness of Different Concentrations of Bordeaux Mixture for the Control of Walnut Bacteriosis and Their Phytocidal Effects; Western Oregon, 1930-1945

Year and orchard—plot number, material and concentration	Relative amount of foliage injury	Number of applications	Stages of pistillate flower development at time of applications	Nuts		
				Examined	Infected	
				Number	*Number*	*Per cent*
1930 (Ames)						
1 None		0		1,822	1,173	64.3
2 Bordeaux 10-10-100		2	EP, EPB[1]	5,163	935	18.1
3 Bordeaux 16-8-100		2	"	4,540	768	16.9
1931 (Edes)						
4 None	None	0		6,339	1,230	19.4
5 Bordeaux 6-6-100	Moderate	2	LP, EPB	4,371	41	0.9
6 Bordeaux 8-8-100	Appreciable	2	"	3,650	16	0.4
7 Bordeaux 16-8-100	Considerable	2	"	5,196	22	0.4
1931 (Bailey)						
8 None	None	0		1,243	663	53.3
9 Bordeaux 8-8-100	Moderate	3	EP, EPB, MPB	2,745	129	4.6
10 Bordeaux 16-4-100	Considerable	3	"	1,311	41	3.1
1932 (Holt)						
11 None	None	0		2,511	879	35.0
12 Bordeaux 2-2-100	Slight	3	EP, EPB, MPB	2,320	201	8.6
13 Bordeaux 4-4-100	Moderate	3	"	2,409	72	2.9
14 Bordeaux 6-6-100	Severe	3	"	2,461	58	2.3
1933 (Brown)						
15 None	None	0		1,400	757	54.0
16 Bordeaux 2-2-100	Slight	2	LP, EPB	1,490	142	9.5
17 Bordeaux 4-4-100	Moderate	2	LP, EPB	1,742	105	6.0
18 Bordeaux 6-6-100	Appreciable	2	"	1,614	58	3.5
19 Bordeaux 12-6-100	Considerable	2	"	1,570	43	2.7
1934 (Brown)						
20 None	None	0		2,981	626	20.9
21 Bordeaux 3-3-100	Slight	2	LP, EPB	2,485	8	0.3
22 Bordeaux 4-4-100	Moderate	2	"	2,456	4	0.1
23 Bordeaux 6-6-100	Considerable	2	"	2,376	2	0.08
24 Bordeaux 16-8-100	Appreciable	2	"	1,980	2	0.1
1934 (Wolf)						
25 None	None	0		2,640	1,232	46.6
26 Bordeaux 3-3-100	Trace	2	LP, EPB	2,468	77	3.1
27 Bordeaux 4-4-100	Slight	2	"	2,509	45	1.7
28 Bordeaux 6-6-100	Considerable	2	"	2,781	73	2.6
29 Bordeaux 12-6-100	Moderate	2	"	2,364	38	1.6
1936 (Brown)						
30 None	None	0		2,166	1,229	56.7
31 Bordeaux 2-2-100	Trace	2	LP, EPB	2,192	209	9.5
32 Bordeaux 4-4-100	Moderate	2	"	2,324	130	5.5
33 Bordeaux 8-8-100	Severe	2	"	2,321	144	6.2

1937 (Brown and McClure)							
34 None	None	0		1,613	1,397	86.6
35 Bordeaux 2-1-100	Trace	3	EP, LP, EPB	1,801	537	29.8
36 Bordeaux 4-1-100	Slight	3	"	3,485	366	10.5
37 Bordeaux 8-4-100	Moderate	3	"	1,634	94	5.7
1937 (Neibert)						
38 None	None	0		1,233	902	73.1
39 Bordeaux 4-4-100	Moderate	3	EP, LP, EPB	1,581	169	10.6
40 Bordeaux 8-8-100	Appreciable	3	"	1,899	114	6.0
1937 (Brown)						
41 None	None	0		1,699	1,022	60.1
42 Bordeaux 4-2-100	Slight	2	LP, EPB	1,622	103	6.3
43 Bordeaux 8-4-100	Moderate	2	"	1,635	192	11.7
44 Bordeaux 16-8-100	Appreciable	2	"	1,466	32	2.1
1938 (Wolf)						
45 None	None	0		2,384	419	17.5
46 Bordeaux 4-4-100	Moderate	3	EP, LP, EPB	2,198	115	5.2
47 Bordeaux 8-8-100	Appreciable	3	"	2,359	186	7.8
1938 (Neibert)						
48 None	None	0		1,347	336	24.9
49 Bordeaux 4-4-100	Moderate	3	EP, LP, EPB	1,794	185	10.3
50 Bordeaux 6-6-100	Considerable	3	"	1,782	106	5.9
1939 (Wolf)						
51 None	None	0		3,069	1,380	44.9
52 Bordeaux 4-4-100	Moderate	3	EP, LP, EPB	1,610	134	8.3
53 Bordeaux 6-6-100	Considerable	3	"	1,983	85	4.2
1939 (Brown and McClure)						
54 None	None	0		1,937	703	36.2
55 Bordeaux 4-4-100	Moderate	2	EP, LP	609	9	1.4
56 Bordeaux 6-6-100	Appreciable	2	"	731	14	1.9
57 Bordeaux 8-8-100	Severe	2	"	691	9	1.3
1939 (Duncan)						
58 None	None	0		2,253	453	20.1
59 Bordeaux 4-1-100	Slight	3	EP, LP, EPB	1,037	21	2.0
60 Bordeaux 6-2-100	Slight	3	"	902	4	0.4
1940 (Brooks)						
61 None	None	0		677	152	22.4
62 Bordeaux 4-1-100	Trace	2	EP, LP	1,067	11	1.0
63 Bordeaux 6-2-100	Trace	2	"	735	20	2.7
1940 (Wilson)						
64 None	None	0		1,003	226	22.5
65 Bordeaux 4-1-100 + oil[2] 1 qt.-100	Trace	2	EP, LP	2,390	197	8.2
66 Bordeaux 6-2-100 + oil, 1 qt.-100	Trace	2	"	2,288	152	6.6
67 Bordeaux 8-2-100 + oil, 1 qt.-100	Slight	2	"	1,850	129	6.9
1941 (Young)						
68 None	None	0		1,117	342	30.6
69 Bordeaux 4-2-100 + oil, 1 pt.-100	Slight	3	EP, LP, EPB	1,391	75	5.3
70 Bordeaux 6-2-100 + oil, 1 pt.-100	Moderate	3	"	1,051	29	2.7
1941 (Dale)						
71 None	None	0		2,158	632	29.2
72 Bordeaux 4-1-100 + oil, 1 pt.-100	Trace	2	EP, LP	4,684	126	2.6
73 Bordeaux 6-2-100 + oil, 1 pt.-100	Slight	2	"	2,360	9	0.3

Table 13 (*Continued*). The Comparative Effectiveness of Different Concentrations of Bordeaux Mixture for the Control of Walnut Bacteriosis and Their Phytocidal Effects; Western Oregon, 1930-1945

Year and orchard—plot number, material and concentration	Relative amount of foliage injury	Number of applications	Stages of pistillate flower development at time of applications	Nuts		
				Examined	Infected	
				Number	*Number*	*Per cent*
1941 (Wilson)						
74 None	None	0		1,134	309	27.2
75 Bordeaux 6-2-100 + oil, 1 pt.-100	Slight	3	EP, LP, EPB	2,613	85	3.2
76 Bordeaux 8-4-100 + oil, 1 pt.-100	Slight	3	"	3,277	52	1.5
1941 (Bamford)						
77 None	None	0		2,632	1,404	53.3
78 Bordeaux 6-2-100 + oil, 1 pt.-100	Slight	4	EP, MP, LP, EPB	3,640	22	0.6
79 Bordeaux 8-4-100 + oil, 1 pt.-100	Slight	4	"	3,030	59	1.9
1941 (Wolf)						
80 None	None	0		6,149	3,633	59.0
81 Bordeaux 4-1-100 + oil, 1 pt.-100	Slight	4	EP, MP, LP,	2,584	79	3.0
82 Bordeaux 6-2-100 + oil, 1 pt.-100	Moderate	4	"	5,867	180	3.0
1941 (Brown and McClure)						
83 None	None	0		4,014	961	23.9
84 Bordeaux 6-2-100 + oil emulsion, 1 qt.-100	Slight	2	EP, LP	4,430	81	1.8
85 Bordeaux 8-4-100 + oil emulsion, 1 qt.-100	Slight	2	"	4,294	41	0.9
1942 (Gale and Duncan)						
86 None	.....	0		990	742	74.9
87 Bordeaux 6-2-100 + oil emulsion, 1 pt.-100	.....	2	MP, EPB	987	109	11.0
88 Bordeaux 4-2-100 + oil emulsion, 1 pt.-100	.....	2	"	581	42	7.2
1943 (Brown and McClure)						
89 None	None	0		1,229	407	33.1
90 Bordeaux 6-3-100 + light oil emulsion, 1 pt.-100	Moderate	2	EP, LP	820	16	1.9
91 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	Slight	2	"	595	10	1.6
1944 (Brown and McClure)						
92 None	None	0		898	292	32.5
93 Bordeaux 6-3-100 + light oil emulsion, 1 pt.-100	Slight	2	MP, LP	715	2	0.2
94 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	Slight	2	"	767	7	0.9
1945 (Brown and McClure)						
95 None	None	0		711	181	25.4
96 Bordeaux 6-3-100 + light oil emulsion, 1 pt.-100	Moderate	2	EP, LP	1,042	3	0.2
97 Bordeaux 4-2-100 + light oil emulsion, 1 pt.-100	Slight	2	"	820	2	0.2

[1]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; MP = middle preblossom; LP = late preblossom; EPB = early postblossom; MPB = middle postblossom.

[2]Except where otherwise stated, the oil used in all tests recorded in this table was a "heavy" mineral spray oil having a viscosity of between 90 and 120 seconds Saybolt and a sulphonation test of from 5 to 70.

Table 14. The Effect of the Addition of Certain Supplements to Bordeaux Mixture on Its Phytotoxicity and Its Efficacy for the Control of Walnut Bacteriosis; Western Oregon, 1933-1944

Year and orchard—plot number, material, and stages of development when applied	Relative amount of foliage injury	Nuts		
		Examined	Infected	
		Number	*Number*	*Per cent*
1933 (Brown)				
1 None	.....	1,400	757	54.0
2 Bordeaux 6-6-100: LP, EPB[1]	Severe	1,607	89	5.5
3 Bordeaux 6-6-100 + skim milk powder, 1¼ lbs.-100: LP, EPB	Severe	1,614	58	3.5
4 Bordeaux 6-6-100 + rosin-fish oil, 1 pt.-100: LP, EPB	Moderate	853	25	2.9
5 Bordeaux 6-6-100 + light mineral oil emulsion, ½ gal.-100: LP, EPB	Trace	1,517	75	4.9
1934 (Wolf)				
6 None	.....	2,498	1,002	40.1
7 Bordeaux 6-6-100 + casein-lime ¼ lb.-100: LP, EPB, MPB	Moderate	2,555	31	1.2
8 Bordeaux 6-6-100 + rosin-fish oil, 1 gal.-100: LP, EPB, MPB	Trace	1,218	32	2.6
9 Bordeaux 6-6-100 + lignin pitch, 6 lb.-150: LP, EPB, MPB	None	2,205	74	3.3
10 Bordeaux 6-6-100 + salmon oil, ½ gal.-150: LP, EPB, MPB	None	2,305	41	1.7
11 Bordeaux 6-6-100 + light mineral oil emulsion, 1 qt.-150: LP, EPB, MPB	None	2,482	49	1.9
12 Bordeaux 6-6-100 + light mineral oil, 1½ gal.-150: LP, EPB, MPB	Trace	2,087	43	2.0
13 Bordeaux 6-6-100 + raw linseed oil, ½ gal.-150: LP, EPB, MPB	None	2,364	43	1.8
1935 (Brown)				
14 None	.....	1,638	125	7.6
15 Bordeaux 4-4-100: LP, EPB	Severe	1,513	2	0.1
16 Bordeaux 4-4-100 + light mineral oil, 1 pt.-100: LP, EPB	Slight	1,588	2	0.1
17 Bordeaux 4-4-100 + herring oil, 1 pt.-100: LP, EPB	Slight	1,531	1	0
18 Bordeaux 4-4-100 + heavy mineral oil, 1 pt.-100: LP, EPB	Trace	1,653	1	0
19 Bordeaux 4-4-100 + raw linseed oil, ½ pt.-100: LP, EPB	Trace	1,625	1	0
20 Bordeaux 4-4-100 + light mineral oil emulsion, 1 qt.-100: LP, EPB	None	1,533	1	0
1936 (Brown)				
21 None	.....	2,559	1,441	56.3
22 Bordeaux 4-4-100: LP, EPB	Severe	2,168	133	6.1
23 Bordeaux 4-4-100 + blood albumen, ¼ lb.-100: LP, EPB	Moderate	2,959	121	4.0
24 Bordeaux 4-4-100 + lignin pitch, ½ lb.-100: LP, EPB	Moderate	2,263	39	1.7
25 Bordeaux 4-4-100 + light mineral oil, 1 pt.-100: LP, EPB	Trace	2,325	106	4.5
26 Bordeaux 4-4-100 + light mineral oil, 1 qt.-100: LP, EPB	Trace	2,483	77	3.1
27 Bordeaux 4-4-100 + heavy mineral oil, 1 pt.-100: LP, EPB	Trace	2,252	48	2.1
28 Bordeaux 4-4-100 + heavy mineral oil, 1 qt.-100: LP, EPB	Trace	2,307	37	1.6
1937 (Brown and McClure)				
29 None	.....	1,694	1,236	72.9
30 Bordeaux 4-2-100: EP, LP, EPB	Moderate	3,393	189	5.5
31 Bordeaux 4-2-100 + soy bean flour, 1 lb.-100: EP, LP, EPB	Severe	1,666	122	7.3
32 Bordeaux 4-2-100 + heavy mineral oil, 1 pt.-100: EP, LP, EPB	Trace	1,634	157	9.6
33 Bordeaux 4-2-100 + heavy mineral oil, 1 qt.-100: EP, LP, EPB	Trace	1,607	137	8.5
34 Bordeaux 4-2-100 + heavy mineral oil emulsion, 1 pt.-100: EP, LP, EPB	Trace	1,634	141	8.6
35 Bordeaux 4-2-100 + heavy mineral oil emulsion, 1 qt.-100: EP, LP, EPB	None	1,678	169	10.0
1938 (Bashford)				
36 None	.....	971	129	13.2
37 Bordeaux 6-6-100: EP, LP, EPB	Severe	1,714	39	2.2
38 Bordeaux 6-6-100 + salmon oil, 1 pt.-100: EP, LP, EPB	Slight	1,850	5	0.2
39 Bordeaux 6-6-100 + light mineral oil, 1 pt.-100: EP, LP, EPB	None	1,597	19	1.1

Table 14 (*Continued*). The Effect of the Addition of Certain Supplements to Bordeaux Mixture on Its Phytotoxicity and Its Efficacy for the Control of Walnut Bacteriosis; Western Oregon, 1933-1944

Year and orchard—plot number, material, and stages of development when applied	Relative amount of foliage injury	Nuts		
		Examined	Infected	
		Number	*Number*	*Per cent*
1939 (Wolf, orchard "A")				
40 None	.....	887	424	47.8
41 Bordeaux 6-6-100: EP, LP, EPB	Moderate	1,319	24	1.8
42 Bordeaux 6-6-100 + heavy mineral oil, 1 pt.-100: EP, LP, EPB	Trace	1,147	73	6.3
43 Bordeaux 6-6-100 + heavy mineral oil, 1 qt.-100: EP, LP, EPB	None	1,663	79	4.7
1939 (Brown and McClure)				
44 None	.....	1,937	703	36.2
45 Bordeaux 6-6-100: EP, LP	Severe	837	30	3.5
46 Bordeaux 6-6-100 + "Vatsol" 2 oz.-100: EP, LP	Moderate	731	14	1.9
47 Bordeaux 4-1-100: EP, LP	Slight	2,758	25	0.9
48 Bordeaux 4-1-100 + "Vatsol," 2 oz.-100: EP, LP	Trace	2,091	21	1.0
49 Bordeaux 4-1-100 + ferrous sulphate, ½ lb.-100: EP, LP	Severe	622	12	1.9
50 Bordeaux 4-1-100 + "Tergitol Penetrant No. 7" ½ pt.-100: EP, LP	Trace	743	10	1.3
51 Bordeaux 4-1-100 + salmon oil, 1 pt.-100: EP, LP	Trace	659	13	1.9
52 Bordeaux 4-1-100 + "Penetrol" 1 pt.-100: EP, LP	Trace	735	10	1.3
53 Bordeaux 4-1-100 + zinc arsenite, 1 lb.-100: EP, LP	Slight	682	1	0.1
1939 (Wolf, orchard "B")				
54 None	.....	3,069	1,380	44.9
55 Bordeaux 6-6-100: EP, LP, EPB	Moderate	1,983	85	4.2
56 Bordeaux 6-6-100 + "Tergitol Penetrant No. 7," ½ pt.-100: EP, LP, EPB	Slight	826	61	7.3
57 Bordeaux 6-6-100 + heavy mineral oil, 1 pt.-100: EP, LP, EPB	Trace	891	45	5.0
58 Bordeaux 4-4-100: EP, LP, EPB	Moderate	1,610	134	8.3
59 Bordeaux 4-4-100 + zinc arsenite, 1 lb.-100: EP, LP, EPB	Trace	869	77	8.8
1940 (Brown)				
60 None	.....	1,392	314	22.5
61 Bordeaux 6-1.5-100: EP, LP	Slight	1,684	44	2.6
62 Bordeaux 6-1.5-100 + heavy mineral oil emulsion, 4 gal.-100: EP: + heavy oil emulsion, 1 gal-100: LP	Moderate	1,593	39	2.4
1941 (Neibert)				
63 None	.....	2,356	1,557	66.1
64 Bordeaux 6-2-100: EP, LP, EPB	.....	1,113	68	6.1
65 Bordeaux 6-2-100 + heavy mineral oil, 1 pt.-100: EP, LP, EPB	.....	4,912	124	2.5
1941 (Wolf, orchard "A")				
66 None	.....	6,149	3,633	59.0
67 Bordeaux 4-1-100: EP, MP, LP, EPB	Slight	1,832	83	4.5
68 Bordeaux 4-1-100 + heavy mineral oil, 1 pt.-100: EP, MP, LP, EPB	Trace	2,584	79	3.0
1941 (Wolf, orchard "B")				
69 None	.....	2,767	1,488	53.7
70 Bordeaux 6-2-100: EP, MP, LP, EPB	Moderate	1,359	43	3.1
71 Bordeaux 6-2-100 + heavy mineral oil, ½ pt.-100: EP, MP, LP, EPB	Slight	1,516	42	2.7
72 Bordeaux 6-2-100 + heavy mineral oil, 1 qt.-100: EP, MP, LP, EPB	Trace	1,948	64	3.2
1941 (Brown and McClure)				
73 None	.....	4,014	961	23.9
74 Bordeaux 6-6-100: EP, LP	Severe	2,220	38	1.7
75 Bordeaux 6-6-100 + heavy mineral oil, 1 pt.-100: EP, LP	Moderate	2,350	68	2.8
1944 (Brown and McClure)				
76 None	.....	898	292	32.5
77 Bordeaux 4-2-100 + light mineral oil emulsion: MP, LP	Trace	671	5	0.7
78 Bordeaux 4-2-100 + zinc sulphate 1-100: MP, LP	Slight	656	5	0.7
79 Bordeaux 4-2-100 + wettable sulphur 3-100: MP, LP	Moderate	677	2	0.3
80 Bordeaux 4-2-100 + soy bean flour 1-100: MP, LP	Slight	816	14	1.7
81 Bordeaux 4-2-100 + "Dresinate" ¼-100: MP, LP	Slight	687	0	0
82 Bordeaux 4-2-100 + boric acid 2-100	Severe	649	20	3.0
83 Bordeaux 4-2-100 + boric acid 2-100 + light mineral oil emulsion, 1 pt.-100	Trace	756	10	1.3

[1]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; MP = middle preblossom; LP = late preblossom; EPB = early postblossom; MPB = middle postblossom.

Table 15. The Comparative Efficacy of Certain Dusts and Spray Mixtures for the Control of Walnut Bacteriosis; Western Oregon, 1931-1945

Year and orchard—plot number, material, and stages of development when applied	Number of applications	Nuts			
		Examined	Infected		
		Number	*Number*	*Per cent*	*E*[1]
1931 (Trunk)					
1 None	0	7,126	3,439	48.2	.....
2 Copper + lime[2] (20-80) dust: EP, MP, LP, EPB, MPB[3]	5	4,301	846	19.6	.....
1932 (Trunk)					
3 None	0	5,466	732	13.3	.....
4 Copper + lime (20-80) dust: EP, LP, EPB, MPB	4	5,703	343	6.0	.....
5 Bordeaux[4] dust: EP, LP, EPB, MPB	4	5,441	273	5.0	.....
1933 (Trunk)					
6 None	0	2,438	1,099	45.0	.....
7 Bordeaux[5] + lime (33-67) dust: LP, EP, EPB, MPB	4	2,404	699	29.0	.....
8 Copper + lime (20-80) dust: LP, EB, EPB, MPB	4	2,316	578	24.9	.....
1934 (Young)					
9 None	0	2,480	505	20.3	.....
10 Copper + lime (20-80) dust: EP, MP, EB, EPB	4	2,811	48	1.7	.....
1934 (Wolf)					
11 None	0	2,640	1,232	46.6	.....
12 Copper + lime (20-80) dust: EP, LP, EPB, MPB	4	2,023	298	14.7	.....
13 Bordeaux mixture 6-6-100: EP, LP, EPB, MPB	4	1,627	27	1.6	.....
1935 (Hendricks)					
14 None	0	275	78	28.3	.....
15 Basic copper sulphate dust: EP, EB, EPB	3	357	33	9.2	.....
16 Copper ammonium silicate + glue (97-3) dust: EP, EB, EPB	3	111	10	9.0	.....
17 Bordeaux[5] + bentonite + blood albumen (50-45-5) dust: EP, EB, EPB	3	237	18	7.5	.....
18 Bordeaux[5] + glue (97-3) dust: EP, EB, EPB	3	220	15	6.8	.....
19 Copper + lime (20-77-3) dust: EP, EB, EPB	3	317	6	1.8	.....
20 Bordeaux mixture 4-4-100: EP, EB, EPB	3	1,073	3	0.2	.....
1936 (Wolf)					
21 None	0	716	576	80.4	.....
22 Copper hydroxide + diatomaceous earth + glue (50-47-3) dust: EP, EB, EPB	3	649	388	59.7	.....
23 Copper phosphate + diatomaceous earth + glue (50-47-3) dust: EP, EB, EPB	3	482	249	51.6	.....
24 Red cuprous oxide (7-93) dust[6]: EP, EB, EPB	3	747	358	47.9	.....
25 Copper + lime + glue (30-67-3) dust: EP, EB, EPB	3	641	221	34.4	.....
1936 (Hendricks, orchard "A")					
26 None	0	856	773	90.3	.....
27 Copper ammonium silicate dust: EP, EB, EPB	3	329	140	42.5	.....
28 Copper hydroxide + diatomaceous earth + glue (50-47-3): EP, EB, EPB	3	276	114	41.3	.....
29 Copper phosphate + diatomaceous earth + glue (50-47-3): EP, EB, EPB	3	526	217	41.2	.....
30 Copper + lime + glue (30-67-3) dust: EP, EB, EPB	3	605	87	14.3	.....
1936 (Hendricks, orchard "B")					
31 None	0	239	191	79.9	.....
32 Red cuprous oxide (7-93) dust[6]: EP, EB, EPB	3	417	187	44.8	.....
33 Basic copper sulphate + lime (50-50) dust: EP, EB, EPB	3	514	160	31.1	.....
34 Copper hydroxide + diatomaceous earth + glue (50-47-3) dust: EP, EB, EPB	3	419	115	27.4	.....
35 Bordeaux[5] + lime (50-50) dust: EP, EB, EPB	3	631	136	21.5	.....
36 Copper + lime (20-80) dust: EP, EB, EPB	3	425	92	21.6	.....
1937 (Seavy)					
37 None	0	847	314	37.0	.....
38 Copper + lime (25-75) dust: EP, LP, EPB	3	829	121	14.5	.....
1938 (Wolf)					
39 None	0	2,384	419	17.5	.....
40 Copper + lime + ferric oxide (30-68-2) dust: EP, LP, EPB	3	1,256	122	9.7	.....
41 Copper oxalate[7] + bentonite (50-50) dust: EP, LP, EPB	3	897	79	8.8	.....
42 Bordeaux mixture 4-4-100: EP, LP, EPB	3	2,732	159	5.8	.....

Table 15 (*Continued*). The Comparative Efficacy of Certain Dusts and Spray Mixtures for the Control of Walnut Bacteriosis; Western Oregon, 1931-1945

Year and orchard—plot number, material, and stages of development when applied	Number of applications	Nuts			
		Examined	Infected		
		Number	*Number*	*Per cent*	*E*[1]
1939 (Wolf)					
43 None	0	887	424	47.8	4.06
44 Copper + lime + diatomaceous earth + Vatsol (20-67-10-3) dust: EP, LP, LPB	3	409	43	10.5	0.93
45 Copper + lime + diatomaceous earth + Vatsol (25-62-10-3) dust: EP, LP, EPB	3	904	94	10.3	2.39
46 Zinc-copper ammonium silicate[8] + talc + diatomaceous earth + Vatsol (33-33-33-1) dust: EP, LP, EPB	3	556	46	8.2	2.76
47 Copper oxalate[9] + talc + diatomaceous earth + Vatsol (20-67-10-3) dust: EP, LP, EPB	3	531	40	7.5	2.27
48 Bordeaux mixture 6-2-100: EP, LP, EPB	3	1,206	18	1.4	0.45
1940 (Brown and McClure)					
49 None	0	1,533	446	29.0	2.60
50 Copper + lime (25-75) dust: EP, LP	2	1,149	148	12.8	1.50
51 Bordeaux mixture 4-1-100 + heavy oil emulsion, 1 pt.-100: EP, LP	2	1,154	58	5.0	0.64
1940 (Seavy et al.)					
52 None	0	380	172	45.2	.....
53 Copper + lime + talc (25-63-10) dust: MP	1	1,141	204	17.8	.....
54 Bordeaux mixture 6-2-100 + heavy mineral oil: MP, EPB	2	2,067	22	1.0	.....
1941 (Wolf)					
55 None	0	2,767	1,488	53.7	3.10
56 Copper + lime + diatomaceous earth (30-60-10) dust: EP, MP, LP, EPB	4	2,170	318	14.6	5.13
57 Copper oxalate + talc + diatomaceous earth (25-65-10): EP, MP, LP, EPB	4	1,946	109	5.6	1.49
58 Yellow cuprous oxide + soapstone + sulphur + diatomaceous earth (10-65-15-10) dust: EP, MP, LP, EPB	4	1,438	62	4.3	0.86
59 Bordeaux mixture 6-2-100 + heavy mineral oil, 1 qt.-100: EP, MP, LP, EPB	4	1,948	64	3.2	0.58
1941 (Seavy et al.)					
60 None	0	2,632	1,404	53.3	2.07
61 Copper + lime + sulphur + talc (25-55-10-10) dust: MP, LP	2	3,716	94	2.5	0.41
62 Bordeaux mixture 6-2-100 + heavy mineral oil: EP, MP, LP	3	2,322	28	1.2	0.21
1942 (Trunk)					
63 None	0	790	406	51.3	4.71
64 Yellow cuprous oxide + sulphur + soapstone + diatomaceous earth (10-15-65-10) dust; EP, LP, EPB	3	657	51	7.7	1.54
65 Bordeaux mixture 6-2-100 + heavy mineral oil: EP, LP, EPB	3	928	22	2.3	0.03
1942 (Seavy et al.)					
66 None	0	1,032	719	69.6	.....
67 Yellow cuprous oxide + sulphur + soapstone + diatomaceous earth (10-15-65-10) dust: EP, LP	2	1,565	345	22.0	.....
68 Copper + lime + sulphur + "Frianite" (25-56-9-10) dust: EP, LP	2	1,731	128	7.3	.....
69 Bordeaux mixture 4-2-100 + heavy mineral oil: MP, EPB	2	1,149	23	2.0	.....
1942 (Davis)					
70 None	0	244	134	54.9	.....
71 Yellow cuprous oxide + sulphur + soapstone + diatomaceous earth (10-15-65-10) dust: EP, MP, LP	3	580	103	17.7	.....
1942 (Blake)					
72 None	0	2,945	1,270	43.1	.....
73 Yellow cuprous oxide + sulphur + soapstone + diatomaceous earth (10-15-65-10) dust: MP, LP	2	1,111	212	19.0	.....
1943 (Davis et al.)					
74 None	0	1,354	778	57.4	4.00
75 Copper + lime + sulphur + "Frianite" (25-56-9-10) dust: LP, EPB	2	1,083	181	16.7	3.71

Table 15 (*Continued*). The Efficacy of Certain Dusts and Spray Mixtures for the Control of Walnut Bacteriosis; Western Oregon, 1931-1945

Year and orchard—plot number, material, and stages of development when applied	Number of applications	Nuts			
		Examined	Infected		
		Number	*Number*	*Per cent*	*E*[1]
1943 (Harper)					
76 None	0	1,262	303	24.0	5.39
77 Copper + lime + sulphur + "Frianite" + diatomaceous earth (25-25-9-25-15.75) dust: MP, EPB	2	1,401	125	8.9	1.98
78 Bordeaux mixture 4-2-100 + oil: LP, EPB	2	616	26	4.2	2.61
1943 (Chambers)					
79 None	0	1,966	760	38.6	4.06
80 Copper + lime + sulphur + "Frianite" + spreader (25-55.75-9-10-0.25) dust: MP, EPB	2	2,010	265	13.1	1.41
1944 (Groner et al.)					
81 None	0	898	292	32.5	6.72
82 Tribasic copper sulphate + sulphur + talc + diatomaceous earth + "Dreft" (10-5-74.75-10-0.25) dust: MP, LP, FB, EPB	4	833	134	16.0	2.62
83 Zinc-copper ammonium silicate + sulphur + diatomaceous earth + "Frianite" + "Dreft" (20-40-10-10-19.25-0.25) dust: MP, LP, FB, EPB	4	846	108	12.7	1.83
84 Yellow cuprous oxide + sulphur + "Frianite" + diatomaceous earth (8-15-67-10) dust: MP, LP, FB, EPB	4	833	47	5.6	1.46
85 Copper + lime + sulphur + diatomaceous earth + "Frianite" + "Dreft" (20-40-10-10-19.25-0.25) dust: MP, LP, FB, EPB	4	1,019	50	4.9	1.32
86 Bordeaux mixture 6-2-100 + summer oil emulsion, 1 pt.-100: MP, LP, EPB	3	721	14	1.9	.008
1944 (Caywood et al.)					
87 None	0	199	82	41.2	.....
88 Copper + lime + sulphur + "Frianite" (25-50-10-15) dust: EP, MP, LP, EPB	4	366	10	2.7	.....
1944 (Stump)					
89 None	0	498	124	24.8	.....
90 Copper + lime + "Frianite" + diatomaceous earth + "Dreft" (25-50-14.75-10-0.25) dust: EP, MP, LP, FB, EPB	5	554	5	0.9	.....
1944 (Chambers)					
91 None	0	838	253	30.1	1.66
92 Yellow cuprous oxide + sulphur + "Frianite" (8-15-67) dust: EP, MP, LP	3	977	87	8.9	1.68
1945 (Marnach et al.)					
93 None	0	526	216	41.0	1.47
94 Copper + lime + sulphur + "Frianite" (20-40-10-30) dust: EP, LP, EPB	3	1,032	60	5.8	3.19
95 Copper + lime + sulphur + "Frianite" (20-40-10-30) dust: EP, MP, LP, EPB	4	522	11	2.1	0.06
1945 (Groner et al.)					
96 None	0	711	181	25.4	2.01
97 Zinc coposil + sulphur + "Frianite" + oil (25-15-58-2) dust: EP, MP, LP, EPB	4	835	55	6.5	2.10
98 Copper hydro + sulphur + talc + oil (30-15-53-2) dust: MP, LP, EPB	3	757	40	5.2	1.60
99 Copper + lime + sulphur + "Frianite" (20-40-10-30) dust: EP, MP, LP, EPB	4	792	43	5.4	1.63
100 Copper + lime + sulphur + "Frianite" + oil (20-40-10-28-2) dust: EP, MP, LP, EPB	4	724	16	2.2	0.27

[1]Standard error.
[2]Copper = monohydrated copper sulphate; lime = hydrated lime.
[3]Stages of pistillate flower development are referred to by letter as follows: DD = delayed dormant; EP = early preblossom; MP = middle preblossom; LP = late preblossom; EB = early blossom; FB = full blossom; EPB = early postblossom; MPB = middle postblossom.
[4]A commercial dehydrated bordeaux mixture containing 12 per cent, by weight, metallic copper.
[5]A commercial dehydrated bordeaux mixture containing 22 per cent by weight metallic copper.
[6]Seven per cent red cuprous oxide (containing 86 per cent by weight of metallic copper) and 93 per cent filler ("Earlonite").
[7]Containing 19 per cent, by weight, of metallic copper.
[8]Containing 17 per cent, by weight, of metallic copper and 19 per cent, by weight, of metallic zinc.
[9]Containing 40 per cent, by weight, of metallic copper.

Table 16. The Relation of the Control of Walnut Bacteriosis to the Amount of "Blight" Culls in the Crop; Western Oregon, 1930-1939

Year and orchard—plot number, material, and stages of development when applied	Nuts		
	Examined	With "blight" stains on shell or adhering diseased parts of hull	
	Number	*Number*	*Per cent*
1930 (Edes)			
1 None	15,514	685	4.4
2 Bordeaux 8-8-100: DD, EP, EPB, LPB[1]	16,750	313	1.8
1930 (Holt)			
3 None	14,216	954	6.7
4 Bordeaux 8-8-100: EP, EPB	6,691	169	2.5
1931 (Edes)			
5 None	832	35	4.2
6 Bordeaux 8-8-100: EP, EPB	834	11	1.3
1932 (Andrew and Sterling)			
7 None	1,021	170	16.6
8 Bordeaux 6-6-100: EP, EPB	1,000	16	1.6
1932 (Wolf)			
9 None	1,000	92	9.2
10 Bordeaux 6-6-100: LP, EPB	1,000	17	1.7
1932 (Doege)			
11 None	500	57	11.4
12 Bordeaux 6-6-100: LP, EPB	500	6	1.2
1933 (Brown)			
13 None	1,580	233	14.7
14 Bordeaux 6-6-100: LP, EPB	1,603	35	2.1
1933 (Durant)			
15 None	1,498	104	6.9
16 Bordeaux 6-6-100: LP, EPB	1,413	26	1.8
1933 (Wolf)			
17 None	2,036	332	16.3
18 Bordeaux 6-6-100: LP, EPB	1,694	106	6.2
1934 (Wolf)			
19 None	1,285	136	10.5
20 Bordeaux 6-6-100: LP, EPB	1,160	42	3.6
1935 (Andrew and Sterling)			
21 None	500	52	10.4
22 Bordeaux 6-6-100: LP, EPB	500	7	1.4
1935 (Brown)			
23 None	1,120	17	1.5
24 Bordeaux 4-4-100: LP, EPB	1,121	6	0.5
1936 (Brown and McClure)			
25 None	2,210	353	15.9
26 Bordeaux 4-4-100: EP, LP, EPB	2,279	63	2.7
1936 (Holt)			
27 None	1,144	134	11.7
28 Bordeaux 4-4-100: LP, EPB	1,043	49	4.6
1936 (Wolf)			
29 None	211	147	69.6
30 Bordeaux 4-4-100: EP, LP, EPB	791	172	21.7
1937 (Brown and McClure)			
31 None	900	226	25.1
32 Bordeaux 4-2-100: EP, LP, EPB	550	48	8.7
1938 (Wolf)			
35 None	1,106	67	6.0
36 Bordeaux 8-4-100: EP, LP, EPB	1,192	25	2.0
1939 (Brown and McClure)			
37 None	2,000	79	3.9
38 Bordeaux 6-6-100: EP, LP	1,972	17	0.8

[1]Stages of pistillate flower development are referred to by letters as follows: DD = delayed dormant; EP = early preblossom; LP = late preblossom; EPB = early postblossom.

Table 17. The Effect of Walnut Bacteriosis Control on the Yield, Quality, and Market Value of Nuts; Western Oregon, 1931-1941

Year and orchard—plot number, material, and stages of pistillate flower development when applied	Number of trees per plot	Weight of nuts[1]			Grade summary										Market value of crop
					Graded stock							Culls			
		From plot	Per tree	E[2]	Large Oregons[3]	Fancy Oregons[4]	Medium Oregons[5]	Large Cascades[6]	Fancy Cascades[7]	Medium Cascades[8]	Total	Blower[9]	Grader[10]	Total	
		Pounds	*Pounds*		*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	
1931 (Edes)															
1 None	5	934[1]	186.8[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2 Bordeaux 8-8-100: EP, EPB[11]	5	1,162[1]	232.4[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1931 (Bentley)															
3 None	37	2,331[1]	63[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4 Bordeaux 8-8-100: EP, MP, LP, FB, EPB	37	3,914[1]	105.7[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1932 (Wolf)															
5 None	27	1,265[1]	46.8[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6 Bordeaux 8-8-100: EP, LP, EPB, MPB	27	1,572[1]	58.2[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1933 (Wolf)															
7 None	14	261[1]	18.6[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8 Bordeaux 6-6-100: LP, EPB, MPB	14	517[1]	36.9[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1934 (Wolf)															
9 None	7	590[1]	84.2[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10 Bordeaux 6-6-100: LP, EPB, MPB	7	742[1]	106[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1934 (Holt)															
11 None	9	517[1]	57.4[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12 Bordeaux 4-4-100: LP, EPB, MPB	10	782[1]	78.2[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1936 (Holt)															
13 None	2	142[1]	71[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14 Bordeaux 4-4-100: LP, EPB	2	198[1]	99[1]	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1937 (Brown and McClure)															
15 None	10	225	22.5	....	116	11	1	27	10	1	166	.....	.....	59	$19.58
16 Bordeaux 4-4-100: EP, LP, EPB	10	527	52.7	....	303	22	1	107	20	7	460	.....	.....	67	51.01
1938 (Wolf)															
17 None	10	469	46.9	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
18 Bordeaux 4-4-100: EP, LP, EPB	10	539	53.9	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1939 (Wolf)															
19 None	20	437	21.8	1.4	78	122	141	0	0	0	341	1	95	96	32.57
20 Bordeaux 6-2-100: EP, LP, EPB	20	594	29.7	1.6	131	204	185	0	0	0	520	5	69	74	49.43
21 Copper oxalate 3-100: EP, LP, EPB	20	538	26.9	1.6	117	169	167	0	0	0	453	2	83	85	43.41

Table 17. (*Continued*). THE EFFECT OF WALNUT BACTERIOSIS CONTROL ON THE YIELD, QUALITY, AND MARKET VALUE OF NUTS; WESTERN OREGON, 1931-1941

Year and orchard—plot number, material, and stages of pistillate flower development when applied	Number of trees per plot	Weight of nuts[1]			Grade summary										
					Graded stock							Culls			
		From plot	Per tree	E[2]	Large Oregons[3]	Fancy Oregons[4]	Medium Oregons[5]	Large Cascades[6]	Fancy Cascades[7]	Medium Cascades[8]	Total	Blower[9]	Grader[10]	Total	Market value of crop
		Pounds	*Pounds*	*Pounds*		*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1940 (Trunk)															
22 None	15	483	32.2	1.1	163	30	7	102	18	5	425	.....	.....	58	73.43
23 Bordeaux 6-2-100: EP, LP, EPB	15	822	54.8	1.0	511	40	6	143	28	7	735	.....	.....	87	128.80
24 Copper oxalate 4-100: EP, LP, EPB	15	708	47.2	1.4	433	30	8	143	23	6	643	.....	.....	65	113.36
25 Red cuprous oxide 2-100: EP, LP, EPB	15	572	38.1	2.4	360	30	7	103	18	5	523	.....	.....	49	91.98
1940 (Duncan)															
26 None	10	182	18.2	1.3	136	3	2	0	9	2	152	4	26	30	26.96
27 Bordeaux 4-1-100: EP, LP, EPB	10	317	31.7	1.0	181	65	21	0	0	0	267	7	43	50	46.02
1940 (Wolf)															
28 None	5	79	15.9	1.7	30	5	2	12	13	1	63	16	0	16	11.01
29 Copper oxalate 3-100: EP, LP, EPB	5	234	46.8	4.0	95	33	8	25	31	6	198	32	4	36	33.98
1941 (Duncan)															
30 None	6	247	41.1	....	91	45	12	11	11	4	174	7	66	73	30.47
31 Bordeaux 6-2-100: EP, LP, EPB	6	471	78.5	....	119	203	63	5	6	3	399	6	66	72	68.48
1941 (Wolf)															
32 None	20	492	24.6	....	27	106	158	0	8	25	324	56	112[12]	168	63.24
33 Bordeaux 6-2-100: EP, MP, LP, PB	20	835	41.7	....	39	228	293	1	10	29	600	96	139[12]	235	112.76
34 Yellow cuprous oxide 1½-100: EP, MP, LP, EPB	20	759	37.9	....	54	234	229	1	10	32	560	62	137[12]	199	106.78
35 Copper oxalate 3-100: EP, MP, LP, EPB	20	709	35.4	....	48	203	238	1	10	20	520	59	130[12]	189	99.56

[1]The weights indicated by an asterisk are "green" (wet) weights; all others are dry weights.
[2]Standard error.
[3]First quality; of a diameter too large to pass through a round opening 79/64 inches in diameter.
[4]First quality; of a diameter too large to pass through a round opening 74/64 inches in diameter.
[5]First quality; of a diameter too large to pass through a round opening 60/64 inches in diameter.
[6]Second quality; of a diameter too large to pass through a round opening 79/64 inches in diameter.
[7]Second quality; of a diameter too large to pass through a round opening 74/64 inches in diameter.
[8]Second quality; of a diameter too large to pass through a round opening 60/64 inches in diameter.
[9]Culls removed by suction.
[10]Culls removed by hand grading.
[11]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; LP = late preblossom; MP = middle preblossom; FB = full blossom; EPB = early postblossom; MPB = middle postblossom.
[12]The large quantity of grader culls in this crop was due to a very hot period during July which caused perforations in the shells, and was not due to blight.

Table 18. THE RELATION OF SPRAYING FRANQUETTE WALNUTS FOR THE CONTROL OF WALNUT BACTERIOSIS TO THE QUALITY AND QUANTITY OF THE NUT KERNELS. 1941.

Year and orchard—material, and stages of pistillate flower development when applied	Number of trees per plot	Dry weight of the kernels		Grade summary			
		From plot	Per tree	Light halves[1]	Light pieces[2]	Light ambers[3]	Market value of crop
1941 (Wylie)		*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	
1 Untreated	5	60	12	26	17	17	$25.39
2 Bordeaux 6-2-100: EP, LP, EPB[4]	5	100	20	33	29	38	$41.25
3 Yellow cuprous oxide 2-100: EP, LP, PB	5	100	20	38	29	33	$41.76

[1]Highest quality; light-colored kernels in halves.
[2]Light-colored kernels in smaller pieces.
[3]Second quality; amber or dark-colored kernels.
[4]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; LP = late preblossom; EPB = early postblossom.

dusts tested, a monohydrated copper sulphate + lime + sulphur + oil (20-40-10-2) dust and a yellow cuprous oxide + sulphur (8-15) dust were the most effective under the widest variety of conditions.

Relation of Spraying for the Control of the Disease to the Yield

Yield records show that a significant increase in the quantity and quality of the crop follows the control of walnut bacteriosis resulting from the application of a sufficient number of properly timed and thoroughly applied bordeaux mixture sprays. Typical examples of the data resulting from these studies are given in Tables 16 to 18.

It is apparent from the study of the data given in Tables 16 to 18 that an increased yield of nuts having a superior quality followed timely and thorough spraying with bordeaux mixture, the net increase being proportionate to the severity of occurrence of the disease and the efficiency of control measures employed. In more limited tests, copper oxalate and yellow cuprous oxide when properly used also gave significant increases in the yield and quality of nuts produced.

Spraying Costs and Its Benefits

The cost of spraying walnuts for the control of walnut bacteriosis varies with the size of the trees and the cost of labor and spray materials. In 1939-1940, the average total annual cost of applying 3 bordeaux mixture (4-2-100) spray applications to 9 representative walnut orchards in Oregon, 20 to 25 years of age, ranging in size from 8 to 115 acres and totaling 484 acres, was $4.81 per acre.* Of this, $1.72 or 36 per cent was for spray materials, $1.25 or 26 per cent was for labor, and $1.84 or 38 per cent was for tractor and sprayer operation. Under current (1946) conditions, the annual total cost will be about 88 per cent higher or approximately $9.07 per acre.

The net gain from spraying due to increased yields, better quality, and more marketable nuts will vary from year to year with the incidence of infection. The data given in Tables 17 and 18 are indicative of what one might expect from proper spraying. These and other data which could be cited if space permitted indicate that in a normal season a five-to-ten-fold return on one's investment for spraying costs may be reasonably expected. In years

* Acknowledgment is due Professor G. W. Kuhlman, of the Farm Management Department of Oregon State College, for valued assistance in assembling and working up these records.

Table 19. THE CUMULATIVE BENEFITS FROM SPRAYING WALNUTS OVER A PERIOD OF YEARS; ROSEBURG, OREGON, 1931

Plot	Treatment in 1931	Previous treatment	Nuts		
			Examined	Infected	
			Number	*Number*	*Per cent*
1	None	None	1,243	663	53.3
2	Bordeaux 8-8-100: EP, EPB, MPB[1]	None	856	82	9.5
3	Bordeaux 8-8-100: EP, EPB, MPB	Bordeaux, 1929-1930	690	25	3.6

[1]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; EPB = early postblossom; MPB = middle postblossom.

when epidemic outbreaks of the disease occur spraying for the control of bacteriosis is most profitable.

The benefits from spraying walnuts with bordeaux mixture are apparently cumulative, as the data presented in Table 19 indicate.

It will be noted that the best results from spraying in 1931 occurred in the portion of the orchard which also had been sprayed in 1929 and 1930. This is interpreted as indicating a gradual diminution in the number of sources of inocula because of control by spraying in previous years. The necessity for and advantages of continuous annual spraying are obvious.

STUDIES OF POSSIBLE HOST INJURIES ASSOCIATED WITH SPRAYING

Effect of Bordeaux Mixture on the Set of Nuts

Barss (*20*) and Rudolph (*93*) state that a reduction in the set of nuts may follow the application of bordeaux mixture to walnut flowers which are receptive to pollen. As this, if true, would be a matter of considerable economic importance, a series of experiments was undertaken to determine if a reduction in the set follows spraying under Pacific Northwest conditions. A number of pistillate-flower-bearing buds were placed under paper bags while they were still dormant. Approximately half of these bagged flowers were sprayed with bordeaux mixture when they were in the early- to full-blossom stage and the remainder left untreated. Both the sprayed and unsprayed flowers were hand-pollinated when they were receptive. When the nuts were from one-half to three-fourths grown, the percentages of sprayed and of unsprayed nuts which had set were determined. The data from these studies are presented in Table 20.

A significant reduction in the set seemingly resulted from spraying with bordeaux mixture during the period of full bloom in experiments carried on in 1931. However, additional studies carried on in 1932, 1934, 1935, and 1938 failed to confirm the results of this experiment. In these latter studies, walnut trees in full bloom were thoroughly sprayed with bordeaux mixture and a number of pistillate flowers on representative sprayed and unsprayed trees were tagged and later the percentage of nuts that set determined. There was no significant difference in the percentages of sprayed and unsprayed nuts that set. Extensive observations made in seedling orchards sprayed during bloom also failed to show any significant reduction in the set from spraying. It would appear, therefore, that spraying walnuts with bordeaux mixture has but little, if any, detrimental effect on the set of nuts. However, in consideration of the possibility that under certain conditions bordeaux mixture might possibly have

Table 20. THE EFFECT OF SPRAYING FRANQUETTE WALNUTS WHILE IN BLOOM ON THE SET OF THE NUTS; WESTERN OREGON, 1931-1938

Year and orchard—series, and material	Stage of development at time of spraying	Number of pistillate flowers	Nuts set	
			Number	*Per cent*
1931 (Oregon State College)				
1 None		151	86	56.9
2 Bordeaux 8-8-100	Full bloom	156	38	24.3
1932 (Oregon State College)				
3 None		133	35	26.3
4 Bordeaux 8-8-100	Full bloom	166	44	26.5
1934 (Oregon State College)				
5 None		74	18	24.3
6 Bordeaux 4-4-100	Early to full bloom	112	39	34.8
1935 (Wolf)				
7 None		82	74	90.2
8 Bordeaux 4-4-100	Early to full bloom	79	70	88.6
1938 (Brown)				
9 None		77	70	90.9
10 Bordeaux 4-4-100	Early to full bloom	80	73	91.2

some deleterious effect, it seems advisable to avoid spraying during the period when the majority of pistillate flowers are receptive.

Effect of Bordeaux Mixture on the Foliage

Barss (*2*) and Rudolph (*93, 94*) report injury to walnut leaves from the application of bordeaux mixture. We have likewise observed injury to young walnut leaves in varying degrees of severity on numerous occasions during the past 10 years (*53, 54, 55, 56, 57, 59, 60, 61*).

Bordeaux injury was of two general types, a marginal necrosis, Figure 21, A, and an interveinal spot necrosis, Figure 21, B. Severe marginal spray injury frequently causes growing leaflets to be distorted in shape when mature due to a checking of the growth in the affected areas (Figure 22, A).

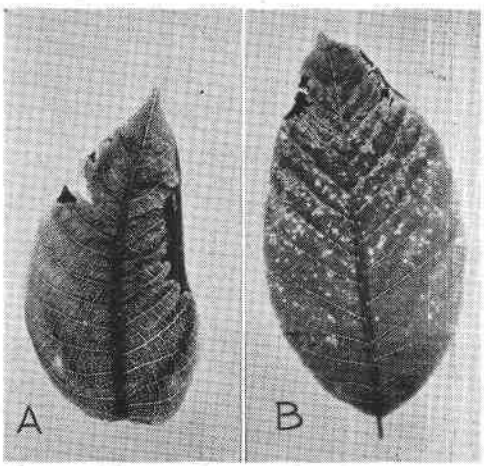

Figure 21. Young walnut leaflets injured by spraying with bordeaux mixture: A, marginal necrosis; B, interveinal spot necrosis.

In some cases the interveinal type of spray injury resulted in a perforated or "shot-hole" appearance due to the "corking-out" and later the falling out of the injured areas (Figure 22, B). The injured leaves typically persisted on the trees and continued to grow and function though occasionally severe injury caused them to drop prematurely.

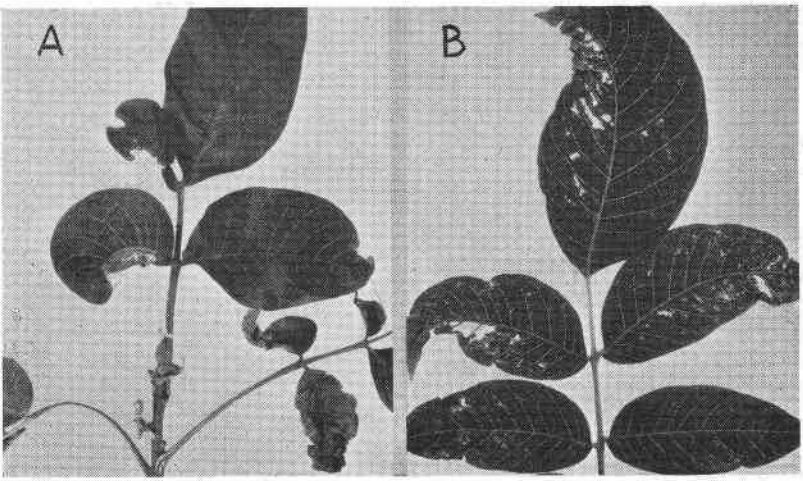

Figure 22. Walnut leaves showing the results of bordeaux spray injury: A, dwarfing and distortion of the leaflets from the marginal spray injury; B, "shot-hole" or perforated appearance due to the abscission and falling out of the interveinal injured areas.

Defoliation from spray injury as it occurs in peaches and other sensitive plants did not take place in walnuts. The new growth that developed during the latter part of the season generally obscured much of the damage so that by late summer the injury could not be readily detected unless attention was called to it.

While no effects from bordeaux spray injury have been noted as regards tree growth or yield, it is recognized that there may be deleterious cumulative effects. In consideration of this possibility, a study of spray injury has been made directed towards finding: (a) the important factors concerned in its development, and (b) practical methods of controlling or reducing it.

Studies of Some Factors Concerned in the Development of Bordeaux Spray Injury

The relation of concentration of the spray mixture. The results of numerous tests carried on over a period of 14 years conclusively show that the more concentrated the bordeaux mixture the more severe the foliage injury. Thus, in one typical experiment carried on in 1936, bordeaux mixture 2-2-100 caused only a trace of foliage injury, bordeaux 4-4-100 caused a moderate amount, while bordeaux 8-8-100 caused severe injury. It seems advisable, therefore, not to use any stronger concentration of bordeaux mixture than is necessary to control the disease satisfactorily.

The relation of the age of the foliage. Our studies show that walnut leaves pass through a stage of maximal susceptibility to spray injury into a period of increased resistance. The leaves were found most susceptible to injury when they were very young. At this stage the leaves were purplish red. As the leaves grew older they became progressively more resistant to injury. Fully developed, dark green leaves were found highly resistant to spray injury and tolerated repeated applications of a relatively strong concentration of bordeaux mixture with little or no detectable detrimental effect.

Table 21. THE RELATION OF ENVIRONMENT TO THE SEVERITY OF WALNUT FOLIAGE SPRAY INJURY; SCHOLLS, OREGON, 1936

Plot, material, and concentration	Environmental conditions prevailing at time of application				Relative amount of foliage injury
	Kind of weather	Time of day applied	Temperature	Relative humidity	
			Degrees F.	*Per cent*	
1 Bordeaux 4-4-100	Hazy	9:15 a.m.	57	58	Slight
2 Bordeaux 4-4-100	Clear	1:15 p.m.	68	41	Moderate
3 Bordeaux 4-4-100	Clear	3:30 p.m.	75	26	Severe

Relation of the environment. The temperature prevailing at the time the spray is applied was found to be important in determining the severity of injury. In general, the higher the temperature, the more severe was the injury.

Typical examples of the data showing this relationship are presented in Table 21.

Rainfall was found to be concerned also in the development of bordeaux spray injury. A form of interveinal spot necrosis has been known to follow prolonged rainy periods which occurred after spraying. However, rainfall is obviously not the most important environmental factor as severe marginal injury has, in numerous instances, developed from 24 to 48 hours after spraying in the absence of rainfall.

Studies of Possible Methods of Controlling Spray Injury

Studies were made to control bordeaux spray injury (a) by the addition of certain supplements to the spray mixture, and (b) by varying the lime content. An extensive search was made also for a substitute material for bordeaux mixture that would be equally as efficacious and yet be noninjurious to the foliage.

The effect of adding certain supplements to bordeaux mixture on the severity of injury. The following accessory materials were added to bordeaux mixture during the period covered by these investigations in an effort to decrease its injuriousness: casein-lime, skim milk powder, lignin pitch, rosin-fish oil, mineral oils and oil emulsions of a wide range of viscosities and purities, salmon oil, herring oil, raw linseed oil, blood albumen, higher sulphonated alcohols, soy bean flour, ferrous sulphate, and zinc arsenite. Typical examples of the data obtained are presented in Table 14. It is evident from a study of these data that the addition of small quantities of a number of oils and oil emulsions reduced the severity of foliage injury under most conditions though they did not entirely eliminate the damage. Mineral, fish, and vegetable oils were about equally effective. None of the other supplements used had any effect in this respect.

It is concluded from the results of these experiments that the use of either mineral, fish, or some form of vegetable oil or oil emulsion with all preblossom applications of bordeaux mixture at the rate of 1 pint of oil or oil emulsion to 100 gallons may be expected to reduce the severity of bordeaux foliage injury under most conditions, though their use will not entirely prevent it. From a practical standpoint, a mineral oil or an oil emulsion made from an oil having

a viscosity of from 60 to 75 seconds Saybolt and a sulphonation or purity test of between 70 and 85 would be one of the most economical oils to use, being considerably cheaper than either fish or vegetable oil and more readily obtainable.

If the spray machine does not have an efficient agitator, the use of an oil emulsion instead of oil is recommended. Not more than ½ gallon of oil or oil emulsion in 100 gallons of bordeaux mixture should be used as injury may follow. It is not necessary to use oil with postblossom applications of bordeaux mixture as the leaves are highly resistant to spray injury by that time.

Table 22. THE EFFECT OF VARYING THE HYDRATED LIME CONTENT IN BORDEAUX MIXTURE ON THE SEVERITY OF WALNUT FOLIAGE SPRAY INJURY; WESTERN OREGON, 1936-1940

Year and orchard—plot number, formula of bordeaux mixture, and stages of development when applied	Relative amount of foliage injury
1936 (Brown and McClure)	
1 Bordeaux 4-4-100: LP, EPB[1]	Moderate
2 Bordeaux 4-1-100: LP, EPB	Slight
1936 (Leonard)	
3 Bordeaux 4-4-100: LP, EPB	Moderate
4 Bordeaux 4-1-100: LP, EPB	Slight
1937 (Brown and McClure)	
5 Bordeaux 4-4-100: EP, LP, EPB	Moderate
6 Bordeaux 4-2-100: EP, LP, EPB	Slight
1937 (Brown)	
7 Bordeaux 4-4-100: LP, EPB	Severe
8 Bordeaux 4-2-100: LP, EPB	Slight
9 Bordeaux 4-1-100: LP, EPB	Trace
1937 (Woodford)	
10 Bordeaux 4-4-100: LP, EPB	Moderate
11 Bordeaux 4-1-100: LP, EPB	Trace
1937 (Neibert)	
12 Bordeaux 8-8-100: EP, LP, EPB	Severe
13 Bordeaux 4-4-100: EP, LP, EPB	Moderate
14 Bordeaux 4-1-100: EP, LP, EPB	Slight
1937 (Wolf)	
15 Bordeaux 8-8-100: EP, LP, EPB	Severe
16 Bordeaux 4-4-100: EP, LP, EPB	Moderate
17 Bordeaux 4-2-100: EP, LP, EPB	Slight
1938 (Brown and McClure)	
18 Bordeaux 8-8-100: EP, LP, EPB	Severe
19 Bordeaux 8-2-100: EP, LP, EPB	Slight
1938 (Brown)	
20 Bordeaux 4-4-100: EP, LP, EPB	Moderate
21 Bordeaux 4-1-100: EP, LP, EPB	Trace
1938 (Wolf)	
22 Bordeaux 8-8-100: EP, LP, EPB	Moderate
23 Bordeaux 8-2-100: EP, LP, EPB	Trace
1939 (Brown and McClure)	
24 Bordeaux 6-6-100: EP, LP	Severe
25 Bordeaux 6-1.5-100: EP, LP	Slight
1939 (Wolf)	
26 Bordeaux 6-6-100: EP, LP, EPB	Moderate
27 Bordeaux 6-2-100: EP, LP, EPB	Slight
1939 (Brown)	
28 Bordeaux 4-4-100: EP, LP	Moderate
29 Bordeaux 4-1-100: EP, LP	Trace
1940 (Brown and McClure)	
30 Bordeaux 6-6-100: EP, LP	Severe
31 Bordeaux 6-1.5-100: EP, LP	Slight

[1]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; LP = late preblossom; EPB = early postblossom.

Effect of varying the lime content in bordeaux mixture. Studies on the effects of varying the amount of hydrated lime in relation to the copper content of bordeaux mixture were made under field conditions and the relative injuriousness of the various mixtures to walnut foliage determined. Typical examples of the data obtained from these studies are given in Table 22.

As shown by the data given in Table 22 the higher the hydrated lime content in relation to the copper sulphate, the greater was the injury. Walnuts are apparently subject to injury by alkaline solutions which increases in severity in direct proportion to the alkalinity of the mixture.

The phytocidal effect of some "low-soluble" copper materials. In an attempt to find a suitable noninjurious substitute for bordeaux mixture, a large number of "low-soluble" copper materials were tested under field conditions. The phytocidal effect and comparative efficacy of the materials tested are given in Table 11. These data show that practically all of the low-soluble copper compounds tested caused little or no foliage injury, but the majority of them were inferior in efficacy to bordeaux mixture. However, two of the materials tested, namely copper oxalate and yellow cuprous oxide, approximated bordeaux mixture in effectiveness, giving practically as good control under normal conditions though they were not quite so efficacious when conditions for control were difficult. While leaf injury can be almost eliminated by the use of these and a number of the other low-soluble copper materials, their use would increase the cost of spraying and one would run the risk of poorer control in a bad "blight" season.

Studies on the Effect of Bordeaux Foliage Injury on the Size, Weight, and Quality of the Nuts

Rudolph (*94*) reports that, under California conditions, bordeaux mixture caused injury that affected the size and quality of the nuts adversely. However, in recent studies carried on in that state, Ark and Scott (*11*) report that the reduction in the size and quality of nuts sprayed with bordeaux mixture was not due to the spray *per se,* but to the increased aphid infestation which is much greater in bordeaux-sprayed than on nonsprayed trees. In consideration of these divergent reports it was thought necessary to determine what, if any, effect bordeaux mixture has on the size and quality of the nuts under Pacific Northwest conditions. Accordingly, representative samples of nuts chosen at random from bordeaux-sprayed and nonsprayed plots were harvested and the size, weight, and degree of filling of the nuts determined. The data obtained are presented in Tables 23 to 25 inclusive.

It does not appear from the data given in Tables 23 to 25 that the size, weight, or degree of filling of the nuts was affected to any significant extent by injury to the foliage from bordeaux mixture. To check upon the possibility of error from the random sampling methods used in these studies, additional investigations were carried on in 1940 and 1941 under more closely controlled sampling conditions. In these studies, a number of nuts from a representative number of sprayed and untreated trees were harvested while they were still on the trees from (a) known shoots which had been badly injured by bordeaux and (b) uninjured nonsprayed shoots. The nuts were grouped in certain classes according to treatment and to the length of the shoots from which they came and the average size, weight, and degree of filling of the nuts in each group determined. These data are given in Table 26.

The data shown in Table 26 confirm the results of other investigations. It is concluded, therefore, that leaf injury of the type that occurs in the Pacific

Table 23. The Effect of Bordeaux Leaf Injury on the Size and Weight of Walnuts, Western Oregon, 1931-1940

Year and orchard—plot number, material, and stages of development when applied	Relative amount of leaf injury	Dry weight of sample	Nuts in sample	Average dry weight per nut	Nuts per pound	Average dry weight of kernels per nut	Average volume per nut as determined by cubic centimeters of water displaced
		Grams	*Number*	*Grams*	*Number*	*Grams*	
1931 (Edes)							
1 None	None	410.0	50	8.2	55.5	3.85	19.7
2 Bordeaux 8-8-100: LP, EPB[1]	Severe	430.5	50	8.6	53.1	4.04	19.5
1933 (Wolf)							
3 None	None	18,143.6	2,036	8.9	50.0	.....	.....
4 Bordeaux 6-6-100: LP, EPB, MPB	Severe	18,143.6	1,694	10.7	42.3	.....	.....
1936 (Brown and McClure)							
5 Bordeaux 4-4-100: LP, EPB	Slight	3,159.7	356	8.87	51.1	4.35	.....
6 Bordeaux 8-8-100: LP, EPB	Severe	3,250.8	365	8.90	50.9	4.29	.....
1938 (Brown and McClure)							
7 None	None	22,888	2,171	10.5	44.2	4.09	18.1
8 Bordeaux 6-6-100: EP, LP, EPB	Severe	22,888	2,136	10.7	43.7	4.22	17.9
1939 (Brown and McClure)							
9 None	None	19,525	1,865	10.46	43.3	4.06	17.62
10 Bordeaux 6-6-100: EP, LP	Severe	19,525	1,868	10.45	43.4	4.23	17.52
1940 (Brown and McClure)							
11 None	None	1,911.3	189	10.11	44.8	4.34	17.06
12 Bordeaux 6-6-100: EP, LP	Severe	1,907.0	188	10.77	44.7	4.33	17.42
1941 (Brown and McClure)							
13 None	None	10,291.05	1,102	9.3	48.7	3.99	16.4
14 Bordeaux 6-6-100: EP, LP	Severe	10,291.05	1,053	9.7	46.7	4.14	16.6

[1]The stages of pistillate flower development are referred to by letter as follows: EP = Early preblossom; LP = late preblossom; EPB = early postblossom.

Table 24. THE EFFECT OF BORDEAUX LEAF SPRAY INJURY ON THE SIZE OF WALNUTS; SCHOLLS, OREGON, 1937-1941

Year, plot, material, and stages of development when applied	Relative amount of leaf injury	Number of nuts in sample	Distribution of sizes of nuts in samples							
			Babies[1]		Medium[2]		Fancy[3]		Large[4]	
			Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
1937										
1 Bordeaux 4-1-100: EP, LP, EPB[5]	Trace	1,001	2	1.9	119	11.8	405	40.4	475	47.4
2 Bordeaux 4-4-100: EP, LP, EPB	Considerable	1,001	1	.9	128	12.7	380	37.9	493	49.2
1938										
3 None	None	2,171	6	0.2	203	9.3	625	28.7	1,337	61.5
4 Bordeaux 6-6-100: EP, LP, EPB	Moderate	2,136	4	0.1	165	7.7	658	30.8	1,309	61.2
1939										
5 None	None	1,865	4	0.2	258	13.8	505	27.0	1,098	58.8
6 Bordeaux 6-6-100: EP, LP	Severe	1,868	1	0.05	234	12.5	571	30.5	1,062	56.8
1940										
7 None	None	189	30	15.8	74	39.1	34	17.9	51	26.9
8 Bordeaux 6-6-100: EP, LP	Severe	188	18	9.5	69	36.7	30	15.9	71	37.7
1941										
9 None	None	1,126	0	0	152	13.4	478	42.4	496	44.0
10 Bordeaux 6-6-100: EP, LP	Severe	1,058	2	0.1	73	6.8	362	34.2	621	58.6

[1]Cross sectional diameter: 60/64 inch or less.
[2]Cross sectional diameter: 61/64 to 74/64 inches inclusive.
[3]Cross sectional diameter: 75/64 to 79/64 inches inclusive.
[4]Cross sectional diameter: 80/64 inches and over.
[5]Stages of pistillate flower development are referred to by letter as follows: EP = early preblossom; LP = late preblossom; EPB = early postblossom.

Table 25. THE EFFECT OF BORDEAUX LEAF INJURY ON THE DEGREE OF FILLING OF THE NUTS; SCHOLLS, OREGON, 1939-1941

Year, plot, material, and stages of development when the applications were made	Relative amount of leaf injury	Number of nuts in sample	Nuts with well filled kernels		Nuts with partially to completely shriveled kernels									
					Up to one-fourth shriveled		Approximately one-half shriveled		About three-fourths shriveled		Completely shriveled		Total	
			Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
1936														
1 Bordeaux 4-4-100: EP, LP EPB[1]	Slight	356	118	33.1	0	0	80	22.4	64	17.9	94	26.4	238	66.8
2 Bordeaux 8-8-100: EP, LP, EPB	Moderate	365	150	41.0	0	0	70	19.1	59	16.1	86	23.5	215	58.9
1937														
3 Bordeaux 4-1-100: EP, LP, EPB	Trace	1,001	783	78.2	93	9.2	64	6.3	28	2.7	33	3.2	218	21.7
4 Bordeaux 4-4-100: EP, LP, EPB	Considerable	1,001	700	70.0	137	13.6	89	8.8	38	3.7	37	3.6	301	30.0
1938														
5 None	None	2,171	1,717	79.0	277	12.7	105	4.8	37	1.7	35	1.6	454	20.9
6 Bordeaux 6-6-100: EP, LP, EPB	Moderate	2,136	1,811	84.7	173	8.0	57	2.6	17	0.7	78	3.6	325	15.2
1939														
7 None	None	1,865	1,783	95.6	27	1.4	19	1.0	10	0.5	26	1.3	82	4.3
8 Bordeaux 6-6-100: EP, LP	Severe	1,868	1,788	95.7	46	2.4	11	0.5	5	0.2	18	0.9	80	4.2
1940														
9 None	None	189	129	68.2	36	19.0	19	10.0	4	2.1	1	0.5	60	31.7
10 Bordeaux 6-6-100: EP, LP	Severe	188	127	67.5	38	20.2	18	9.5	3	1.5	2	1.0	61	32.4
1941														
11 None	None	1,102	1,003	91.0	34	3.0	12	1.0	2	0.1	51	4.6	99	8.9
12 Bordeaux 6-6-100: EP, LP	Severe	1,026	980	95.5	15	1.4	5	0.4	1	0.09	25	2.4	46	4.4

[1]EP = early prebloom; LP = late prebloom; EPB = early postbloom.

Table 26. The Relation of Bordeaux Leaf Spray Injury to the Size, Weight, and Degree of Filling of Walnuts; Scholls, Oregon, 1940-1941

Year, group, material, and concentration	Relative amount of leaf injury	Shoots		Total number of nuts on specified number of shoots	Average diameter per nut	Average volume per nut as determined by cubic centimeters of water displaced	Average dry weight per nut	Average dry weight of kernels per nut	Percentage of nut well-filled
		Number	Length						
			Millimeters		*Millimeters*		*Grams*	*Grams*	
1940									
1, a None	None	11	30-36	14	29.8	17.4	9.8	4.64	86.5
b Bordeaux 6-6-100	Severe	14	"	16	31.7	12.2	10.5	4.56	85.3
2, a None	None	10	37-42	14	30.8	16.7	10.8	4.53	87.5
b Bordeaux 6-6-100	Severe	20	"	21	31.9	18.0	10.6	4.93	89.2
3, a None	None	19	43-48	26	30.8	17.9	10.1	4.98	98.0
b Bordeaux 6-6-100	Severe	12	"	17	31.4	17.9	10.7	4.71	88.2
4, a None	None	13	49-54	20	30.9	17.9	11.2	5.00	93.7
b Bordeaux 6-6-100	Severe	11	"	14	31.0	18.2	10.9	4.65	87.5
5, a None	None	9	55-60	13	30.0	17.6	9.7	4.28	98.0
b Bordeaux 6-6-100	Severe	10	"	12	31.8	19.0	11.3	5.07	83.3
6, a None	None	2	61-66	2	31.5	18.0	10.7	4.25	75.0
b Bordeaux 6-6-100	Severe	7	"	11	30.2	17.2	10.5	4.80	93.1
7, a None	None	8	67-72	14	31.5	18.1	11.3	4.93	96.1
b Bordeaux 6-6-100	Severe	5	"	9	32.5	19.7	11.3	5.26	94.4
8, a None	None	5	75-80	9	30.6	16.4	9.6	4.35	88.8
b Bordeaux 6-6-100	Severe	3	"	4	31.5	18.0	11.3	5.40	87.5
1940									
9, a None	None	1	81-86	2	32.0	18.0	11.8	5.15	100.0
b Bordeaux 6-6-100	Severe	5	"	7	31.8	20.0	11.9	5.72	96.4
10, a None	None	2	87-93	3	31.3	17.3	10.7	4.46	83.3
b Bordeaux 6-6-100	Severe	3	"	4	32.5	19.0	11.9	5.62	100.0
1941									
11, a None	None	7	53-63	4	31.0	18.2	9.2	4.68	100.0
b Bordeaux 6-6-100	Severe	6	"	4	30.6	18.6	9.6	5.11	100.0
12, a None	None	19	64-70	10	31.4	18.7	9.6	4.94	94.7
b Bordeaux 6-6-100	Severe	19	"	9	30.3	17.7	8.9	4.53	92.1
13, a None	None	16	71-76	8	31.0	17.8	9.2	4.68	96.8
b Bordeaux 6-6-100	Severe	22	"	12	31.3	18.5	9.6	5.17	98.8
14, a None	None	15	77-83	9	31.4	18.8	9.8	5.01	93.3
b Bordeaux 6-6-100	Severe	24	"	15	31.3	19.4	9.6	4.99	92.7
15, a None	None	16	84-93	10	31.1	18.7	9.2	4.56	89.0
b Bordeaux 6-6-100	Severe	18	"	11	32.0	19.7	9.9	4.95	91.6
16, a None	None	6	94-105	3	30.5	17.6	9.0	4.73	100.0
b Bordeaux 6-6-100	Severe	6	"	3	31.0	19.6	9.2	4.28	83.3

Northwest from the use of relatively weak concentrations of bordeaux mixture has no significant effect on the size, weight, or degree of filling of the current crop of nuts. This is probably due to the translocation of food materials from neighboring uninjured leaves which compensates in a large measure for the localized reduction in leaf area from spray injury.

RECOMMENDATIONS FOR THE CONTROL OF WALNUT BACTERIOSIS IN THE PACIFIC NORTHWEST

Walnut 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 special local needs or exceptional seasonal conditions.

The Recommended Spray Materials

Bordeaux mixture is recommended for general use for the control of walnut bacteriosis in the Pacific Northwest as it is the cheapest effective material and has never failed to give satisfactory control where a sufficient number of thoroughly applied and properly timed applications were made.

As possible substitutes, yellow cuprous oxide or copper oxalate may be used where convenience and freedom from injury are more important considerations than cost.

The Spray Formula

Bordeaux mixture. The 4-1-100 concentration (4 pounds of copper sulphate, 1 pound of caustic (quick) lime or 2 pounds of hydrated lime, and 100 gallons of water) is advised for general use. This formula has given consistently good control without serious injury when a sufficient number of properly timed and thorough applications were made. The addition of a light mineral oil emulsion at the rate of 1 pint to 100 gallons of bordeaux mixture is advised with all preblossom applications to reduce foliage injury.

In preparing bordeaux mixture it is important that at least one of the ingredients be highly diluted before the other is added. While there are a number of acceptable methods of preparing bordeaux mixture, the "two-package" or so-called "instantaneous" method of making bordeaux mixture has a number of advantages not possessed by the stock solution and other methods. These are: (1) no extra equipment needed, (2) very economical in the use of labor, and (3) time is saved. In preparing bordeaux mixture by this method, the spray tank is filled about half full of water and the requisite amount of hydrated lime added with the agitator in motion. Then slowly add the powdered copper sulphate, pouring it into a stream of water flowing from the source of supply into the spray tank via a trough. The agitator should be kept running while completing the filling of the tank with water.

When oil is used with bordeaux mixture to reduce foliage injury it should always be added *last* after the bordeaux mixture has been prepared. The requisite amount of oil emulsion should be diluted with a small quantity of water in a bucket and added to the bordeaux mixture with the agitator in motion.

Yellow cuprous oxide. It is recommended that this material be used at the rate of 1 pound in 100 gallons of water.

Copper oxalate. This material should be applied at the rate of 4 pounds in 100 gallons of water if the "extended" form containing 20 per cent, by weight, of metallic copper is used, or at the rate of 3 pounds in 100 gallons if the pure form, containing 40 per cent of metallic copper, is employed.

Number and Timing of Applications

Three properly timed applications are needed under epidemic conditions for the control of the disease in the Pacific Northwest. Under less severe conditions, 2 applications or even 1 may give satisfactory control. Since it is impossible to predict at the beginning of the season the extent of rainfall during the infection period, the wisest course for the average grower to pursue is to apply the full number of applications which should be made at the following stages of pistillate flower development: (a) early preblossom, (b) late preblossom, and (c) early postblossom. If, for some reason, it is necessary to eliminate one application, the early postbloom one may be omitted with the least danger, as this application is important only in seasons having prolonged rainfall after bloom. However, the omission of one or more applications from the spray program involved the risk of unsatisfactory control and is not recommended.

A detailed description of the various stages of pistillate flower development at which spray applications should be made follows:

The early preblossom application. This application should be made when approximately 50 per cent of the terminal buds have broken open and the longitudinally folded leaves are beginning to separate from one another (Figure 23, A). At this stage the pistillate flowers are only rudimentary protruberances, not exceeding 1/16 of an inch in diameter, located at the apex of the central stem axis. To see them plainly it may be, and often is, necessary forcibly to part the terminal folded leaflets.

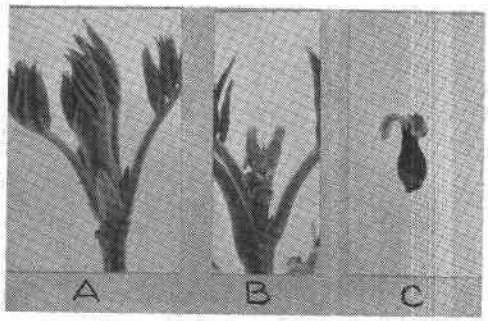

Figure 23. Stages of pistillate flower development in relation to control: A, the early preblossom stage; B, the late preblossom stage; C, the early postblossom stage.

The late preblossom application. This application should be made just before the majority of the pistillate flowers come into full blossom. At this stage the apical end (stigmas) of most of the pistillate flowers will be pink in color; in only an occasional flower will the stigma be fully expanded. The pistils will average about $\frac{1}{8}$ of an inch in diameter at their widest point, or will be roughly about the size of a grain of wheat (Figure 23, B).

The early postblossom application. This treatment should be applied when about 50 per cent of the pistillate flowers contain tiny brown spots in the expanded stigmas but before they turn completely brown. At this time the young nuts will average about 3/16 of an inch in diameter, or will be about the size of a common vetch seed (Figure 23, C).

Each spray application should be completed just as soon as possible—within a three-day period at the longest—to forestall the possibility of the intervention of a rainy period which may affect the control adversely should it come before the application can be completed.

Supplementary applications. If the development of the pistillate flower-bearing buds is very uneven and the rains before bloom exceptionally frequent, a supplementary spray application made in the middle preblossom stage may be needed to control the disease satisfactorily. This application, if needed, should be made about a week after the early preblossom application.

Variation in the Spray Program for Seedling Orchards

The disease in seedling orchards is much more difficult to control than in orchards of uniform variety from grafted stock. This is because of the great variation in the blooming periods of individual trees which makes it very difficult to time the applications correctly. To control the disease satisfactorily in seedling orchards it is necessary to spray the individual trees as they reach the proper stage of development. This means keeping every tree in the orchard under observation and when it reaches the proper stage spraying without delay. Since only certain trees will be found to be in the proper stage of development at any one time, as many as four trips through the orchard may be necessary to complete any one spray application, the exact number depending on the extent to which the trees vary in time of flowering. This procedure is practicable only in small orchards. In large orchards it is too time-consuming and too costly to follow. The only practical method of treating large acreages of seedlings is to spray every tree in the orchard when a majority of them are in the proper stage with the expectancy that some loss from blight will doubtless occur on certain trees that were not in the proper stage of development when sprayed. The application of 3 instead of 2 preblossom treatments will often enhance the degree of control in seedling orchards, particularly in very rainy seasons. The additional preblossom application should be made in the middle preblossom stage.

The Dust Program

While a single liquid spray application is apparently more effective and lasts longer than a single dust treatment, the application of a sufficient number of properly spaced and correctly timed dust treatments will give commercial control of walnut blight under Pacific Northwest conditions. The use of dusts is particularly warranted in instances where the available supply of labor is insufficient for spraying, or where a lack of spraying equipment makes spraying an impossibility, or where there is not enough water for spraying. Dusts can also be employed with benefit as a supplement to the general spray program when it is obvious that an impending rainy period will intervene before the entire orchard can be sprayed.

The dusts to use. A dust composed of 20 per cent of monohydrated copper sulphate, 40 per cent of hydrated lime, 10 per cent of 325-mesh dusting sulphur, 18.5 per cent of talc, 10 per cent of diatomaceous earth and 1.5 per cent of light mineral oil is recommended for general use.

A dust composed of 8 per cent of yellow cuprous oxide, 15 per cent of dusting sulphur, 10 per cent of diatomaceous earth and 67 per cent of talc may be used as an alternative.

Number and timing of applications. A minimum of 4 dust applications made at 7-day intervals beginning in the early preblossom stage is needed in a normal Pacific Northwest season to give commercial control of the disease. The indications are that a still greater number of applications are necessary in an exceptionally wet season to control the disease satisfactorily.

VII. SUMMARY AND CONCLUSIONS

Walnut bacteriosis, caused by *Xanthomonas juglandis* (Pierce) Dowson, is one of the limiting factors in the production of Persian (English) walnuts in the Pacific Northwest, the economic control of which is a primary requisite to profitable walnut culture.

The disease is widely distributed, the range of its occurrence closely paralleling that of walnut culture throughout the world. It has been reported as being present in the following countries: United States, New Zealand, Russia, Canada, Tasmania, Mexico, Australia, Chile, South Africa, Italy, Holland, Switzerland, England, Rumania, West Indies, and France. In the United States, the disease has been reported from 18 different states, which embrace practically all states in which the Persian walnut is grown.

The disease attacks the leaves, nuts, buds, catkins, and succulent stems of young shoots of current growth. Twigs one year of age or older are not susceptible to infection.

Hosts reported infected under natural conditions are *Juglans regia* L., *J. hindsii* Jepson, *J. cordiformis* Maxim var., *ailantifolia* (Carr.) Rehd, (*J. sieboldiana* Maxim.), and Paradox hybrids. The disease has also been artificially induced on *J. nigra* L., *J. californica* S. Wats., *J. cinerea* L., *J. cordiformis* Maxim., and Royal hybrids.

The causal organism is a capsulated rod, 1.3 to 2.8 μ by 0.5 to 0.66 μ, averaging 1.86 by 0.58 μ; motile by one polar flagellum; Gram-negative; non-acid-fast; staining readily with gentian violet and carbol fuchsin; forming abundant, glistening, pale yellow lemon-yellow to coppery-yellow colonies on potato dextrose agar at 22° C.; growth in nutrient dextrose broth at 22° C. is abundant, forming a ring at the surface after 2 to 5 days; slowly liquefying gelatine; milk peptonized; starch hydrolized; producing ammonia in peptone-containing broth; aerobic; reducing selenium dioxide, and litmus in litmus milk, but not nitrates; does not form gas nor produce indole or hydrogen sulphide; at 28° C. producing acid from dextrose, levulose, galactose, lactose, sucrose, maltose, xylose, raffinose, mannitol, glycerol, and starch; alkali produced from citrate, lactate, malate, and succinate; utilizing (in order of availability) peptone, aspartic acid, alamine, leucine, sodium ammonium phosphate, allantoin, tyrosine, uric acid, and brucine; glutamic acid, hippuric acid, and sodium nitrite are not utilized; actively lipolytic when studied by spirit-blue cottonseed oil agar technique; diastase, rennet, proteolase, and pectinase produced; growth temperature range from 1° C. to 37° C., optimum about 28° C.; thermal death point 53° C. to 55° C.; pH range for growth 5.4 to 10.5, optimum pH 6.8 to 7.2; growth not affected by illumination or lack of it; relatively long-lived in culture media but short-lived when air dried at room temperatures.

Walnut bacteriosis was found to be a disease of the parencymatous tissues primarily, 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. Enzymic action, osmosis, internal pressures, and perhaps asphyxiation appear to be re-

sponsible for the death and disintegration of the cellular structure.

The pathogen overwinters under Pacific Northwest conditions primarily in diseased buds and, to a lesser extent, in lesions on stems of the previous year's growth.

Both the primary and secondary inocula are spread by rain-drip.

The pathogen gains access to the host tissues through stomata. Mechanical injuries may also serve as infection courts.

The presence of moisture on the host parts 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 parts, the extent of water-soaking of the tissues, and the degree of opening of the stomata. Infection of the nuts and leaves occurred after only 5 to 15 minutes, respectively, of wetting when they were very young, the stomata wide open, and the tissues more or less watersoaked. While only relatively short minimal periods of wetting were necessary to infect the young leaves and nuts, an increase in the incidence and extent of infection accompanied prolongation of the moist period to the limits reached in these investigations. Subjecting the plants to a preinoculation moist treatment increased the incidence and extent of infection.

Infection took place over a relatively wide range of temperatures, ranging from 4° C. to 30° C. for the leaves and from 5° C. to 27° C. for the nuts. More lesions developed in a shorter period of time on the leaves following inoculation at temperatures of 20° C. to 30° C. than occurred at lower temperatures. In the case of the nuts, temperature was without effect except to hasten the appearance of the lesions.

The period of incubation as determined by inoculation experiments varied from 5 to 34 days depending on the age of the host parts and environmental conditions. In general, the younger the host organs and the higher the temperature, the shorter was the incubation period. The incubation period under field conditions ordinarily ranged from 10 to 15 days.

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

Diseased buds were found to be the most important source of primary inoculum under Pacific Northwest conditions. Lesions on twigs of the preceding year's growth and "latent" infections at the margins of young leaves coming from diseased buds of the previous year's origin were found to be less important sources of primary inoculum. The greater the number of sources of primary inoculum and the more frequent and abundant rainfall during the forepart of the critical period for infection, the greater was the occurrence of primary infection. Bacteria from primary infections were found to be the source of secondary infection. The occurrence of secondary infection was, therefore, governed by (a) the relative abundance of primary infections and (b) the frequency and amount of rainfall during the latter part of the infection period. Secondary infection occurred in successive "waves" traceable to specific rainy periods.

Rainfall was found to be of special significance in the epidemiology of walnut bacteriosis, being the most important, if not the sole factor, concerned in determining the incidence of the disease under Pacific Northwest conditions.

The most critical period for the development and control of epidemics of the disease was found to extend from the time the buds open sufficiently to allow the bacteria to gain access to the interior and come in contact with the rudimentary pistillate flowers, until the nuts are about half-grown. The fore-

part of this period, specifically the preblossom and blossoming stages, was found to be the most critical in relation to control. At this time the host organs are most susceptible to infection, and natural resistance of the tissues to invasion by the bacteria is at its lowest level. Moreover, the host parts are expanding most rapidly during this period which necessitates frequent spraying in order to keep them adequately protected. For efficient control of the disease, it has been found necessary to adjust the control program to this critical period for infection.

Control experiments performed during the years 1930 to 1945 inclusive are reported and discussed. It is concluded therefrom that the only practical method of controlling this disease is by spraying or dusting with protectant bactericides. Of 30 different spray materials tested, home-made bordeaux mixture consistently gave the most effective control. Copper oxalate and yellow cuprous oxide sprays approximated bordeaux mixture in effectiveness under normal conditions, but they were not quite so efficacious when conditions for control were difficult. Under severe epidemic conditions, three spray applications made in (1) the early preblossom stage, (2) the late preblossom stage, and (3) the early postblossom stage were needed to control the disease satisfactorily. Under less severe conditions fewer applications sufficed.

The 4-1-100 concentration of bordeaux mixture in full program gave as good control as stronger concentrations and caused proportionately less injury. Concentrations weaker than 4-1-100 did not always give satisfactory control, although they were less injurious.

No significant increase in the efficacy of bordeaux mixture resulted from the addition of the following supplements: casein-lime, milk powder, lignin pitch, resin-fish oil, salmon oil, herring oil, raw linseed oil, blood albumen, soy bean flour, sulphonated alcohol, ferrous sulphate, zinc arsenite, and mineral oils and oil emulsions of different viscosities and purities.

The injuries associated with spraying walnuts with bordeaux mixture and the factors governing their development are described and discussed. Spraying walnuts with bordeaux mixture at the recommended stages did not interfere with the set of nuts. It was found that the more highly concentrated the spray mixture the more severe the injury. The leaves are most susceptible to injury when they are very young. Fully developed, dark green leaves tolerated repeated applications of a highly concentrated bordeaux mixture with little, or no, injury. In general, the higher the temperature and the lower the humidity at the time the spray material was applied the more severe was the injury. Bordeaux spray injury was accentuated by increasing the hydrated lime content in proportion to the copper sulphate above a ratio of 1 to 4. The severity of foliage spray injury was reduced but not entirely eliminated by the addition of 1 pint of mineral, fish, or vegetable oil or of oil emulsions in 100 gallons of low-lime bordeaux mixture. The size, weight and degree of filling of the nuts produced was found to be unaffected by the amount of leaf injury that occurred.

The following "low-soluble" copper spray materials were found to be relatively noninjurious to Persian walnuts under Pacific Northwest conditions at the concentrations employed: basic copper sulphate, copper carbonate, zinc-ammonical copper silicate, copper ammonium silicate, copper phosphate, copper oxalate, cuprous oxide, copper acetonate, copper oxychloride, copper zeolite, copper hydroxide, manganese bordeaux, and copper resinate. However, only two of these fungicides—copper oxalate and yellow cuprous oxide—consistently approximated bordeaux mixture in effectiveness. Several others gave fair to good control under sparse to moderate disease conditions, but were not so ef-

fective as bordeaux mixture or copper oxalate or yellow cuprous oxide under epidemic conditions.

Under epidemic conditions dusts were not generally so effective as bordeaux mixture though some of them gave good control under moderate disease conditions. Of the dusts tested, a copper + lime + sulphur + oil (20-40-10-2) dust and a yellow cuprous oxide + sulphur (8-15) dust gave the best control.

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