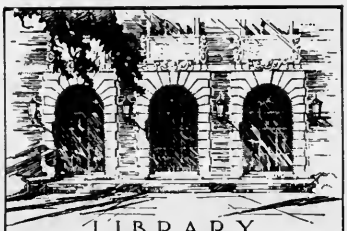

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Bacterial Spot of Stone Fruit

With special reference to epiphytotics and dissemination of the causal organism

By HOWARD W. LARSH
and H. W. ANDERSON

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CONTENTS

The authors gratefully acknowledge the assistance of H. H. Thornberry of this Station in the direction of the field and laboratory work reported herein. They also acknowledge their indebtedness to him for the use of data from his unpublished experiments and observations — some made over a period of years prior to and some during the course of this investigation.

Most of the material in this publication was originally presented in 1941 by the senior author as a thesis in partial fulfillment for the degree of Doctor of Philosophy in the Department of Botany, University of Illinois. Its preparation as a bulletin of the Agricultural Experiment Station as delayed by priority of other work during the war period.

Urbana, Illinois November, 1948

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BACTERIAL SPOT OF STONE FRUIT:

With Special Reference to Epiphytotics and Dissemination of the Causal Organism

By Howard W. Larsh and H. W. Anderson[a]

BACTERIAL SPOT of stone fruits, caused by *Xanthomonas pruni* (E. F. S.) Dowson (formerly *Phytomonas pruni* (E. F. Smith) Bergey *et al.*), was first described as a serious disease of Japanese plum in Michigan. Since the original description was published, this pathogen has also been reported as causing disease of apricots, nectarines, and peaches. From the standpoint of fruit growers and pathologists this organism is of importance: first, because of its serious effects on the trees; second, because no successful means of control has been found.

The work reported in this bulletin was undertaken primarily to fill in gaps in the life history of *Xanthomonas pruni,* especially the period from invasion of the organism in the shoots during late summer and fall to the time when spring cankers are evident in the spring. Another object was to find the cause of the sudden extensive multiplication and spread of the organism in the tissues of the shoots from the initial focus of infection in the spring cankers. A third purpose was to study in more detail the dissemination of the organism in order to ascertain the correctness of the observations of previous investigators that most of the infection in recently established orchards arises from neighboring infected orchards or, rarely, from a few spring cankers that were present on nursery trees.

PART I
EPIPHYTOLOGY AND DISSEMINATION

Review of Literature

The literature on bacterial spot of stone fruits has been adequately reviewed by Rolfs,[11*] Roberts,[10*] and Dunegan.[3*] Consequently only a brief résumé of the history of the disease, with special reference to the overwintering and the dissemination of the causal organism, is in-

[a] Howard W. Larsh, formerly Assistant in Botany; and H. W. Anderson, Professor of Plant Pathology Research.

* These numbers, thruout this bulletin, refer to literature citations on page 55.

cluded, together with a review of the literature that has appeared since the above papers were published.

The bacterial spot disease was first described on Japanese plum from Michigan by E. F. Smith[13*] in 1903. He described the first visible symptoms as numerous, small, water-soaked spots on the leaves and fruits. He stated that the bacteria entered thru the stomata and in the earliest stage of the disease are confined to the substomatal chambers. Between 1903 and 1908 numerous reports appeared on premature defoliation of peach trees, with characteristic "shot hole" symptoms of the leaves. In 1909 Rorer[12*] described a disease occurring on the leaves, twigs, and fruit of peach in Georgia. That the disease was caused by a bacterium was proved by Rorer. From young leaf spots he isolated a motile, yellow organism and reproduced the disease on peach leaves the following year. His attempts to isolate the organism from fruit spots proved unsuccessful. Sections of the diseased fruits, however, revealed masses of bacteria present within the tissue. E. F. Smith[14*] in 1908 obtained a yellow organism from black spots on the Japanese plum with which he successfully inoculated the foliage of peach. He suggested to Rorer that the two diseases were caused by the same organism. Rorer,[12*] upon culturing under comparable laboratory conditions organisms taken from the plum and the peach, found that they had the same cultural characteristics. This fact and the previously mentioned pathogenicity experiments were considered by Rorer sufficient proof that the two diseases were caused by the same organism.

Rolfs,[11*] in 1915, in a monographic treatment of the disease, reported many successful cross inoculations that confirmed the previous investigations of Smith and Rorer. He presented the first detailed description of the growth of the organism on various media and added apricot and nectarine to the known susceptible species of plum and peach. The first attempt to explain the seasonal life history was presented by Rolfs. Dunegan,[3*] in his treatise of the disease and the causal organism, agreed with the earlier investigations of Rolfs and others.

Since the discovery and description of the causal organism, several theories as to where and how the bacteria spend the winter have been postulated. Rolfs[11*] stated that they overwinter in cankers on twigs and limbs and that these are the principal source of infection in the spring. He also implied that the tissue of the buds may become invaded, and in some cases bacteria so harbored may originate early spring infection. Roberts[10*] stated that the causal organism passes the winter within the twig lesions. Higgins[4*] also stated that the bacteria live overwinter in twig cankers but under certain conditions survive in the fallen leaves.

He agreed with Rolfs that the most important source of spring infection seems to lie in the twig cankers. Dunegan[3*] presented evidence to show that the initial outbreak could be correlated with the presence of overwintered twig cankers. Dissemination of the bacteria, according to his orchard surveys, is thru the agencies of wind, rain, and dew.

The possibility of the causal organism overwintering in leaves was mentioned by Higgins.[4*] He stated that under certain conditions the bacteria could survive in old fallen leaves. Anderson[1*] was able to isolate bacteria from dead leaves in early spring and thought that they might overwinter in this manner.

In 1930 a new type of twig canker now designated as "spring canker" was discovered. This type of lesion, first described by Thornberry and Anderson[15*] in 1933, suggested the mode of overwintering of the bacteria on peach in Illinois. Spring cankers develop on young succulent twigs of the past season's growth. These cankers form about the time the first leaves develop, whereas typical summer cankers develop later in the summer on current year's growth, usually after foliage infection has become well established. These workers were convinced that most, if not all, primary foliage infection originated from such cankers.

Overwintering of the bacteria in diseased buds was mentioned as a possibility by Rolfs.[11*] Recent reports intimate that the practice of budding young trees with buds secured from bearing orchards is a factor in the dissemination of the bacteria in young orchards. Manns *et al.*[6*] suggest that the infection found in young orchards originates from budding of trees with infected buds. In their studies they report, "Cultures of peach buds on nutrient agar readily showed that *Xanthomonas pruni* was well established in the buds in early August, and that nurseries had used infected buds not only for their August budding but also for their earlier June budded stock." Hopperstead and Manns[5*] have recently indicated that the terminal buds of peach harbor the organism from one season to the next. They were unable to show conclusively that the lateral buds were a factor in the overwintering of the bacteria. The percentage of terminal buds from which positive isolations could be obtained was high during the early part of the dormant season, but decreased rapidly as the season progressed. Histological work showed the presence of bacterial masses in the intercellular spaces of the terminal buds.[a]

[a] More detailed information on these points is included in a publication released after the above was written: Hopperstead, S. L., and Manns, T. F. Bacterial spot of peach and its behavior in Delaware. Del. Agr. Exp. Sta. Bul. 258, pages 5-24. 1945.

Development and Spread of the Disease

The bacteria have been reported as overwintering in a dormant condition within the buds or somewhere within the twigs.[5*] With the advent of warm spring rains, conditions again favor the rapid reproduction of the bacteria. Accompanying this renewed activity, dissemination and the infection of the leaves can be expected, provided climatic conditions are favorable.

E. F. Smith[13*] as early as 1902 proved that the mode of infection was by the entrance of the bacteria thru stomata. Under favorable conditions of humidity and temperature bacteria which have entered thru the stomata undergo rapid reproduction within the stomatal chambers. Later, bacteria may be found sunken in the tissue as a result of an enzymatic reaction which dissolves the walls of adjacent cells. According to Rolfs,[11*] the bacteria are able to enter the host thru lenticels and stomata on twigs as well as thru stomata on green fruit.

The time during which the disease remains invisible varies considerably with climatic conditions. Many investigations have revealed that temperature as well as moisture plays an important part in determining the time needed for the visible development of the disease. During warm weather the incubation period has been reported to be from 7 to 15 days. However, it appears from unpublished work of Anderson and Thornberry to be rather prolonged in cool weather and may be from 20 to 25 days. These data were obtained from leaves and fruit that had been inoculated by atomizing with a suspension of the causal bacteria. Dunegan[3*] and Rolfs[11*] agree on the time required for incubation of the disease when suspensions of the organism are introduced into the tissue of the host whether by puncture or with a hypodermic needle. The period usually varies from 4 to 12 days for the first symptoms to appear, the shorter period naturally occurring under the most favorable environmental conditions. The experiments of the writers agree with the above in regard to the incubation period when the organism is artificially injected or atomized into the host. During the three years 1938-1940 this variation in incubation period was found by the writers to occur when the pathogenicity of new cultures obtained from cankers and leaf infections was being determined.

Primary infection. The writers, following the work of Anderson;[2*] have made a study of buds, both terminal and lateral, for possible solution of the problem of the overwintering of the organism in Illinois. In these observations microscopical examination, as well as cultures of the buds, were made without successful isolation of the causal organism. Occasionally yellow bacteria were isolated from buds

during early winter. However, in no instance has it been possible to isolate *X. pruni* from buds after January.

In so far as the disease is concerned in this region, the primary infection originates from spring cankers. Thornberry and Anderson[15*] have adequately described these so-called "spring cankers." They arise on young succulent twigs of the past summer's growth and may appear at the time of bud swelling. They are rather scarce at this time, however, and are not usually detected until the first leaves appear.

The bacteria, which were in a dormant condition thruout the winter, reproduce rapidly at the time of renewed growth in the spring and as a result cause swelling within the bark. Eventually longitudinal splitting of the bark along the invaded area permits the oozing of the bacteria. This phenomenon of spring canker development precedes actual cork cambial activity. Thus no walling off of the invaded area occurs, as happens later when summer cankers are formed.

Secondary infection. Early secondary infection is usually indistinguishable from primary infection. However, primary infection, as stated above, is closely correlated with spring cankers on the twigs.

Secondary infection may occur thruout the entire growing season under favorable climatic conditions. Perhaps the most striking phase of this infection is that of the development of summer cankers. These cankers have their origin on the current year's shoots. It is this type of canker which earlier investigators suspected of harboring the bacteria thruout the winter.

Secondary infection accounts for the major spread of the disease from tree to tree and from orchard to orchard. It is also the cause of much injury to the host plant by severe defoliation, with subsequent devitalization, and serious spotting of fruit. Bacteria which overwinter are introduced into the host late in the season by these secondary infections.

Climatic conditions favoring development and dissemination of the disease. Bacterial spot of stone fruits is sporadic in its development, as are most bacterial diseases. The variation in severity from year to year is governed in no small degree by climatic conditions. Of these climatic conditions the writers have observed that rainfall particularly plays an important part in the dissemination of the bacteria and development of the disease. A period of prolonged precipitation is not necessary for the spread of the disease, altho lengthy periods of rain increase its severity. After longer periods of rainfall the development of the disease is, as a rule, accelerated. Water-soaked spots were observed under these conditions and probably account for the abundant infections.

Temperature is an important factor in the development of the disease. It influences the time of the initial appearance of spring cankers, as well as the subsequent progress of the disease. As the temperature rises in the spring, the bacteria renew their activity and by their rapid reproduction soon produce the inoculum necessary for dissemination. Subsequent development is also influenced by temperature because the organism grows most vigorously at temperatures varying between 24° and 28° C. The maximum temperature at which the organism multiplies is 37° C., and the thermal death time is 10 minutes at 51° C.

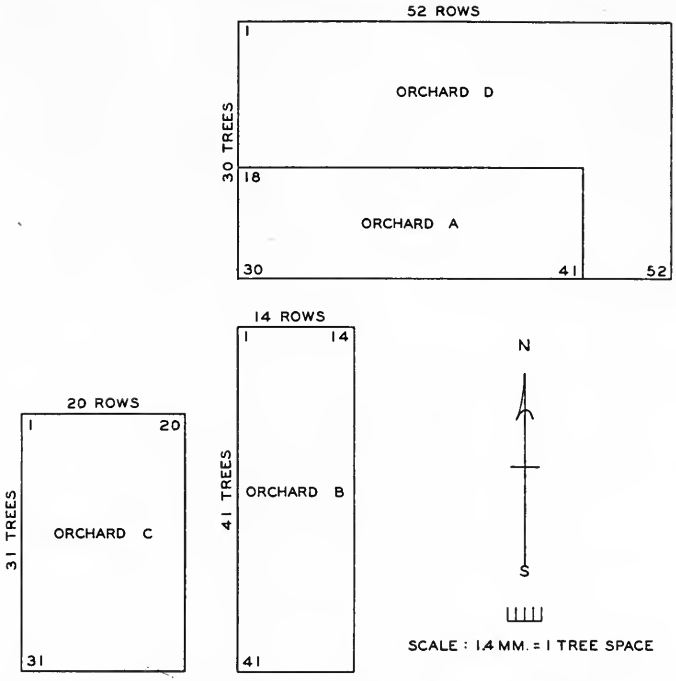
Field observations indicate that wind, as well as moisture, is necessary for the dissemination of the organism. Rain and droplets of dew carrying bacteria from the initial infection may be blown to other parts of the tree and to adjacent trees. When other factors are favorable for infection, spread within a tree or from tree to tree can be directly correlated with the direction and velocity of the wind. This fact is substantiated by detailed data presented later.

Epiphytotic Studies

Studies concerning the spread of *X. pruni* and bacterial spot were carried out from 1938 to 1941 in Orchards A, B, and C located at Irvington, Illinois. These orchards were selected because of their favorable arrangement (see Fig. 1). Orchard A was planted in 1935 and afforded a good supply of inoculum. Orchards B and C, planted in the winter and spring of 1938-39, afforded disease-free trees (assuming the disease is not brought in on young trees from the nursery).

Each orchard was examined periodically for spring cankers, primary foliage infection, and secondary foliage infection. A study was also made on the spread of the causal organism within a tree, from tree to tree, and from orchard to orchard. The purpose of these studies was to secure information on the factors favoring development of the disease and the spread of this organism under orchard conditions.

That insects may play a role in the dissemination of the organism was assumed by Rolfs,[11*] but Dunegan[3*] found no evidence of insect transmission during his studies of the disease. The writers collected a number of insects found visiting peach trees. These insects were cultured to determine the possibility of their harboring or spreading *X. pruni.* With the exception of one ant which was caught immediately after visiting an oozing canker, negative results were obtained. Flies have frequently been seen visiting cankers and they may account for



Location of orchards at Irvington, Illinois. Orchard A is 130 feet from Orchard B. Orchard B is 85 feet from Orchard C. (Fig. 1)

sporadic foliage infection some distance from the original source of inoculum.

Observations during 1939. Bacterial spot was moderate in its severity during 1939 but, nevertheless, every tree in Orchard A showed some bacterial-spot infection on July 18, 1939. Spring cankers were found, but no attempt was made to determine the spread of the organism within this orchard. However, the spread of the organism from Orchard A to the young plantings in Orchards B and C was carefully studied.

Orchard B consists of 569 trees planted in the fall of 1938. In the spring of 1939 a search for spring cankers in this orchard proved unsuccessful. Nevertheless, foliage infection was found on 68 trees at the end of the growing season. The position and relative severity of the disease in Orchard B clearly suggested that the inoculum originated in Orchard A.

Orchard C included 578 trees which were also planted in the fall of 1938. One spring canker was found in this orchard. The relation

of the foliage infection to the canker corresponds with the description given by Dunegan[8*] for the foliage infection occurring immediately below overwintered cankers. Dunegan stated, "The most striking feature was that the canker, when once located, was seen to be at the apex of a conical region of infection in the tree. The bacteria had been washed to the limbs below and produced the primary infection on the leaves and fruit. No infected fruit or leaves were found above cankers." This description characterizes the foliage infection in relation to the spring canker found in Orchard C except that foliage infection was found immediately above the spring canker. On October 13, 1939, just previous to leaf fall, foliage infection was found on only three trees in this orchard.

Observations during 1940. During 1940 the orchards were examined at more frequent intervals than during 1939. Observations were made at weekly intervals as the new growth began to develop and at biweekly intervals late in the growing season. In addition to the orchards surveyed during the 1939 season, Orchard D (Fig. 1) was added to the studies in 1940. The results of these studies are incorporated in Tables 1 thru 6.

Tables 1 and 2 give a summary of the development of the disease

Table 1. — Orchard B: Prevalence and Position of Spring Cankers of Bacterial Spot on Twigs, 1940

(Elberta variety)

Date	Tree location	Number of cankers per tree	Position of cankers on tree
April 22	.....	None	.....
May 6	Row 1, Tree 5	1	Terminal node
	Row 4, Tree 5	2	Terminal nodes
	Row 7, Tree 13	1	Third node
	Row 8, Tree 2	2	Terminal nodes
	Row 8, Tree 4	1	Below terminal dieback
	Row 8, Tree 5	1	Between terminal and second nodes
	Row 8, Tree 8	1	Terminal node
	Row 8, Tree 30	1	Terminal node
	Row 12, Tree 32	1	Sixth node
	Row 13, Tree 32	1	Terminal node
May 13	Row 7, Tree 12	1	Terminal node
May 23	Row 5, Tree 1	1	Seventh node
June 8	Row 6, Tree 8	2	Terminal node and below terminal dieback
	Row 8, Tree 9	3	Terminal dieback, fifth and sixth nodes
	Row 8, Tree 32	1	Terminal node
	Row 9, Tree 3	1	Terminal node
	Row 11, Tree 1	1	Fourth node
	Row 12, Tree 1	4	Terminal, second, third, and fourth nodes
	Row 13, Tree 1	1	Terminal node
	Row 14, Tree 1	1	Terminal node
	Row 14, Tree 19	3	Terminal, second, and fourth nodes

and spread of the organism in Orchard B. A total of 31 spring cankers were observed on 21 trees. Fifteen of the 21 diseased trees had been severely infected with bacterial spot during the previous season. The spread of the organism from the initial infection to adjacent trees was rather closely correlated with the direction of the wind. At the time of leaf fall every tree in this orchard showed bacterial spot in varying degrees of severity. In every case where a spring canker was present, the foliage below was found to be infected, and this infection invariably developed as a cone with the canker at the apex.

Tables 3 and 4 summarize the spread of the disease in Orchard C. Spring cankers were observed on three trees, two of which had been severely infected during the 1939 season. In 1940, 17 spring cankers were found on these three trees. On one tree 15 cankers were located (this was the only tree in this orchard upon which a spring canker was

Table 2. — Orchard B: Relation of Inoculum in Spring Cankers of Bacterial Spot to Origin of Foliage Infection, 1940

(Elberta variety)

Date	Number of trees with—		Location of foliage foci in relation to cankers
	Spring cankers	Foliage infection	
April 22	None	None	.....
May 6	10	9	8 near cankers 1 near terminal dieback
May 13	11	12	11 near cankers 1 near terminal dieback
May 23	12	13	12 near cankers 1 near terminal dieback
June 8	21	28	21 near cankers 2 near terminal diebacks 5 not near cankers
June 28	21	109	21 near cankers 2 near terminal diebacks 86 not near cankers
July 13	21	159	21 near cankers 2 near terminal diebacks 136 not near cankers
July 26	21	251	21 near cankers 2 near terminal diebacks 228 not near cankers
August 10	21	395	21 near cankers 2 near terminal diebacks 372 not near cankers
August 24	21	529	21 near cankers 2 near terminal diebacks 506 not near cankers
September 6	21	559	21 near cankers 2 near terminal diebacks 536 not near cankers
September 26	21	569	21 near cankers 2 near terminal diebacks 546 not near cankers

Table 3. — Orchard C: Prevalence and Position of Spring Cankers of Bacterial Spot on Twigs, 1940

(Elberta variety)

Date	Tree location	Number of cankers per tree	Position of cankers on tree
April 22	.....	None	.....
May 6	Row 17, Tree 2 Row 20, Tree 19[a]	1 1	Below terminal dieback Terminal node
May 13	Row 20, Tree 19[a]	9	3 on terminal nodes 2 on second nodes 1 on third node 1 on fourth node 1 on fifth node 1 on seventh node
May 23	Row 4, Tree 10 Row 20, Tree 19[a]	1 5	Terminal node 4 on terminal nodes 1 on second node
June 8	No additional trees	No additional cankers	

[a] Additional cankers reported on same tree.

Table 4. — Orchard C: Relation of Inoculum in Spring Cankers of Bacterial Spot to Origin of Foliage Infection, 1940

(Elberta variety)

Date	Number of trees with—		Location of foliage foci in relation to cankers
	Spring cankers	Foliage infection	
April 22	None	None	.....
May 6	2	1	1 near canker
May 13	2	1	1 near canker
May 23	3	2	2 near cankers
June 8	3	2	2 near cankers
June 28	3	5	3 near cankers 2 not near cankers
July 13	3	18	3 near cankers 15 not near cankers
July 26	3	35	3 near cankers 32 not near cankers
August 10	3	103	3 near cankers 100 not near cankers
August 24	3	238	3 near cankers 235 not near cankers
September 6	3	378	3 near cankers 375 not near cankers
September 26	3	387	3 near cankers 384 not near cankers

present during the 1939 season). This case was unusual, since the largest number of spring cankers previously found on a single tree was four, and as a rule only a single spring canker occurred on a tree. At

the time of the last examination of the trees, foliage infection was present on 387 of the 578 trees.

In Tables 5 and 6 a summary of the development and spread of the organism in Orchard D is given. Here 23 spring cankers were found on 19 trees. Foliage infection was present on 1,169 trees. The addition of Orchard D permitted a study of the spread of the organism to the north and east of the old orchard (A).

The injury caused by bacterial spot was not severe during the 1940 season. This was true even tho climatic conditions favorable to the development of the disease prevailed early and late in the season.

Observations during 1941. During 1941 the orchards were checked at frequent intervals in order to estimate whether any deviations occurred in comparison to 1939 and 1940. The results were similar to those obtained in the previous years.

Discussion of results of epiphytotic studies. The value of epiphytotic studies is apparent. Thru careful studies of this type one is able to follow with considerable certainty the development and dissemination of an organism and to determine the conditions favorable for an outbreak of a given disease. Field observations give evidence of the actual progress in the development of the disease without the influence of artificial or controlled conditions. Because bacterial spot of stone

Table 5. — Orchard D: Prevalence and Position of Spring Cankers of Bacterial Spot on Twigs, 1940

(Elberta, Hale, and Gage varieties)

Date	Tree location	Number of cankers per tree	Position of cankers on tree
April 22	.....	None	.....
May 6	Row 9, Tree 11	1	Terminal node
	Row 9, Tree 17	1	Terminal node
	Row 21, Tree 12	1	Third node
	Row 43, Tree 2	1	Fifth node
May 13	Row 11, Tree 13	1	Third node
	Row 29, Tree 15	1	Second node
	Row 32, Tree 8	1	Terminal node
	Row 46, Tree 29	2	Terminal node and below terminal dieback
	Row 48, Tree 4	1	Seventh node
May 23	Row 11, Tree 16	1	Terminal node
	Row 22, Tree 13	1	Fourth node
	Row 32, Tree 17	1	Sixth node
	Row 35, Tree 5	1	Terminal node
	Row 42, Tree 27	2	Terminal nodes
	Row 45, Tree 26	2	Terminal node and third node
	Row 48, Tree 5	1	Second node
	Row 50, Tree 21	1	Terminal node
	Row 51, Tree 17	1	Third node
	Row 52, Tree 8	2	Third node and terminal node
June 8	No additional trees	No additional cankers	

Table 6. — Orchard D: Relation of Inoculum of Spring Cankers of Bacterial Spot to Origin of Foliage Infection, 1940

(Elberta, Hale, and Gage varieties)

Date	Number of trees with—		Location of foliage foci in relation to cankers
	Spring cankers	Foliage infection	
April 22	None	None	.....
May 6	4	4	4 near cankers
May 13	9	13	9 near cankers 2 near terminal diebacks 2 not near cankers
May 23	19	28	19 near cankers 2 near terminal diebacks 7 not near cankers
June 8	19	46	19 near cankers 3 near terminal diebacks 24 not near cankers
July 14	19	238	19 near cankers 3 near terminal diebacks 216 not near cankers
July 27	19	421	19 near cankers 3 near terminal diebacks 399 not near cankers
August 25	19	1032	19 near cankers 3 near terminal diebacks 1010 not near cankers
September 26	19	1169	19 near cankers 3 near terminal diebacks 1147 not near cankers

fruits causes serious losses to the orchardists, the ultimate purpose of a study of this kind is to find a means of controlling the disease. It is therefore highly desirable to study the organism under natural conditions.

Since the discovery and the description of the organism by E. F. Smith, it has been noted that the disease may vary in severity from year to year. For example, least injury occurs during hot dry weather, which is more injurious to the bacteria than is extremely cold weather. Due to this fluctuation, epiphytotic studies over a long period are necessary for a clear understanding of the development and dissemination of *X. pruni* under all types of weather conditions.

Research carried on at the University of Illinois indicated that bacterial spot was negligible during the years of 1933-1937.[2*] In 1938 especially favorable climatic conditions brought about a serious outbreak of bacterial spot, the worst outbreak observed by the writers over a period of three years. In 1939 the disease was only moderately severe, and in 1940 the least amount of injury was observed that was noted in the three years of this study. At the beginning of the 1940 season primary inoculum was abundant, as evidenced by the number

of spring cankers that developed, yet the disease was not severe. While a large number of trees were infected, most of them were only slightly injured. The severity of the disease varied from a single diseased leaf on a tree to trees with one-fourth of all the leaves infected. A small percentage of the trees showed heavy defoliation.

Overwintering and primary infection. The source of the inoculum which causes primary infection has been a matter of dispute. Various investigators have suggested such sources for this inoculum as overwintered summer cankers, diseased buds, fallen leaves, and spring cankers. Whether the inoculum is present on the tree internally or externally during the dormant season, or whether it is to be found elsewhere, has not been ascertained.

Dunegan,[3*] as well as others, has postulated the possibility of *X. pruni* overwintering in cankers on the twigs of peach. These summer cankers which develop on the current year's growth have been subjected to critical study in Illinois. In no instance has it been found that *X. pruni* lives over in these cankerous areas. During the present studies many summer cankers were marked during their development and later collected and cultured at various times thruout the winter to determine whether or not the pathogen could survive the winter in these lesions. Since none of these cankers yielded *X. pruni* after December, it seems reasonable to assume that the organism is unable to survive in such cankers in Illinois.

Several hundred terminal and lateral buds were cultured during the winters of 1938-39 and 1939-40 to determine whether or not they harbored the bacteria during the winter. These buds were taken from trees heavily infected during the summer. The bud scales were first removed and cultured separately to determine if the pathogen was internal or external. In a few instances during early winter yellow bacteria were obtained from bud scales. In no instance, however, was *X. pruni* isolated from the buds after January. As a result of these Illinois studies it can be definitely stated that *X. pruni* does not spend the winter in the bud proper in this region. This conclusion is further supported by the data obtained by Anderson,[2*] who reported attempts to isolate the organism from several hundred buds.

There may be a possibility of the bacteria overwintering in fallen leaves, especially during mild winters, but the amount of inoculum which is present in fallen leaves during early spring is usually negligible. The only satisfactory explanation of the manner in which the bacteria may reach the host is that they are splashed on during periods of rain. This, however, is not enough to explain the serious outbreaks of the disease.

The most logical explanation of the origin of the inoculum for primary foliage infection in Illinois is the spring cankers which are found on young succulent tissue of the past year's growth. But no satisfactory explanation has yet been given as to where the bacteria are located prior to the formation of these cankers.

During the winter of 1940-41 evidence was found which indicates that the primary inoculum is present within the twig. With the hope of revealing the mode of overwintering of the bacteria, the writers collected twigs from trees of known susceptibility to the disease and brought them to the laboratory in order to break their dormancy. Efforts to break the dormancy of the twigs collected during the early winter proved unsuccessful, perhaps because of the mild weather. However, twigs collected at Olney, Illinois, on January 17, 1941, began to break dormancy when placed in a humid culture chamber and subjected to artificial illumination. Other twigs were collected at weekly intervals beginning with February 6, 1941. Twigs which had been placed in a moist chamber in a jar of warm water and subjected to artificial illumination presented ideal conditions for the rapid reproduction of any bacteria present within the twigs. Observations were made periodically to determine whether or not spring cankers developed on these twigs. Positive results were first obtained on February 12, 1941, at which time incipient cankers were found on three twigs from the collection made February 6, 1941. A photograph showing the developing cankers is presented in Fig. 2.

Since placing the twigs in a moist chamber and subjecting them to artificial illumination proved successful in producing activity of the bacteria and the eventual development of cankers, this procedure was followed on twigs collected subsequently. A total of 16 cankers developed on 183 twigs up to April 1, 1941.

Sources of inoculum. It has been reported in the literature that a frequent source of infection of young trees in the nurseries is from infected buds inserted in budding.[6*] With this in mind, the writers examined several hundred thousand trees in a number of nurseries thruout the state. In no instance was bacterial spot found where the practice was followed of using bud wood secured from young nursery trees which were isolated from other stone fruits harboring the disease. In a few cases, however, the disease was found in nurseries where the bud wood was taken from mature orchard trees. In one nursery there were bearing orchards in close proximity to the diseased nursery trees.

Probably the most important source of the inoculum in nurseries is from species of stone fruits that harbor the disease from one year to the next. It was proved by the junior author and H. H. Thornberry

Development of spring cankers on forced twigs of Chinese peach seedlings in the laboratory. (Fig. 2)

that the bacteria are able to survive from year to year in cankers on apricots and plums (*unpublished data*). In one particular nursery where the practice is followed of planting apricot trees in the same plot with peach trees, bacterial spot was found on the leaves of a few peach trees. The buds used in budding these peach trees were obtained from nonbearing trees in the nursery, and examination of the diseased peach trees disclosed no spring cankers. Bacterial spot was well developed on the leaves of the young apricot trees and likewise on the leaves of a few peach trees in adjacent rows but not at a great distance from the apricot trees. These observations and the fact that the nearest bearing peach orchard was at least a half mile from the nursery lead to the conclusion that the inoculum causing the bacterial spot on the young peach trees had its origin in the adjacent apricot trees. Successful isolations were made from overwintering cankers on the apricot.

PART II

RELATION OF CHEMICAL CONSTITUENTS OF BARK TISSUES TO INITIAL INFECTION

Succulent tissues have generally been believed by most investigators to present a more favorable situation for the invasion and development of bacterial pathogens than tissues of less vigorous growth. Since the degree of succulency is generally related to the amount of nitrogen present within the plant, it has been thought that susceptibility of tissue might be correlated with the total or relative amounts of nitrogen present. McNew and Spencer[7]* reported that the addition of nitrogen to sweetcorn seedlings increased the severity of the wilting induced by *Phytomonas stewarti*. Other workers had stressed the concept that the availability of carbohydrates was the limiting factor governing the severity of bacterial attacks. It was not known to what extent the availability or concentration of carbohydrates was influenced by the amount of nitrogen within a plant.

Observations by the writers during a three-year period had revealed that bacterial spot developed at an earlier date on Chinese peach seedlings[a] than on standard varieties of peach. Likewise, spring cankers were more numerous and the disease was considerably more severe on these Chinese peach seedlings than on the Elberta variety. Because of the luxuriant twig growth produced yearly by the Chinese seedlings, it was assumed that this succulent tissue rendered the trees more susceptible to bacterial invasion.

The purpose of these investigations was to determine whether the carbohydrate and nitrogen content of the bark of peach twigs might be correlated with the development of spring cankers.

Materials and Methods

Materials used for the chemical analyses consisted of the bark tissue from the current twigs of Elberta and Chinese peach seedlings.[a] Representative trees of these varieties were selected from the University orchard at Olney, Illinois.

For each of the analyses ten to fifteen twigs were collected from the selected trees at frequent intervals from April 22, 1940, to April 14, 1941 (see Tables 7 and 8 for dates). As a rule, each collection was

[a] These Chinese seedlings are described as white freestone and white honey peach in Ill. Agr. Exp. Sta. Ann. Rpt. 50 (1936-37), page 269, Illus. Nos. 4 and 5 in Table 72.

made at a comparable time. The shoots were cut into uniform lengths, and the twigs were stripped of their leaves and wrapped carefully to eliminate possible drying while in transit to Urbana. Immediately upon arrival of the twigs the bark was stripped from them, cut into fine pieces, mixed thoroly, weighed, and stored in pint Mason jars. In no instance were the twigs removed from trees more than three hours before the tissues were put into the preservative.

Triplicate samples were taken for both carbohydrate and nitrogen determinations. Twenty-five-gram samples were used in most cases. Samples for determining the carbohydrates were killed by dropping them into simmering 95-percent alcohol, and those for determining nitrogen were killed and stored in ether. After the samples were cooled, the jars were sealed and the samples stored until analyzed. The samples used in the determinations for sugars and nitrogen were run in duplicate.

Analytical Procedure

For the determination of reducing and total sugars, modification of the method of Munson and Walker was employed.[8*] The alcohol and minced tissue were poured from each pint jar into an alundum thimble, the alcohol being caught in a beaker. The jar was rinsed thoroly with 80-percent alcohol, and each washing was poured on the tissue in the thimble. The thimble was placed in a large Soxhlet extractor and the sample was extracted with 80-percent alcohol for 20 hours. The extract was transferred to the 400-ml. beaker containing the original extract and washings and the alcohol evaporated. To prevent complete evaporation, water was added when necessary. When the odor of alcohol had disappeared from the sample, it was cleared by adding 10 ml. of saturated neutral lead acetate solution, which was allowed to precipitate, then filtered.

To delead the samples, 14 ml. of a saturated solution of Na_2HPO_4 was added. After the precipitate settled, it was removed by filtering, and the contents of the beaker were transferred to a 500-ml. volumetric flask and brought to volume with distilled water. Exactly 250 ml. of this solution was pipetted into a dry volumetric flask of that capacity. Reducing sugars were determined on an aliquot of this solution, usually 25 ml., as described later. Ten ml. of concentrated hydrochloric acid were added to the 250-ml. solution in the 500-ml. volumetric flask to hydrolyze the nonreducing sugars present in the sample. This was allowed to stand for 24 hours, then nearly neutralized with a concentrated sodium hydroxide solution, and made up to the original volume

with distilled water. Total sugars were determined on a 10-ml. aliquot of this solution, as described later.

Determination of sugars as dextrose equivalent was made. Fifty ml. of Fehling solution, the aliquot of sugar solution, and enough distilled water to make a total volume of 100 ml. were placed in a 400-ml. beaker, which was covered with a watch glass, brought to boil within four minutes, and boiled altogether for two minutes. The hot solution was immediately filtered thru a Gooch crucible containing a specially prepared asbestos mat for collecting the precipitated cuprous oxide and washed four times with hot distilled water. The crucible was removed and the outside rinsed with hot distilled water. The asbestos mat was transferred into the original beaker and thoroly broken up into about 25 ml. of distilled water, after which the crucible was placed in the beaker. Twenty-five ml. of ferric sulfate was added to the beaker by means of a pipette. The beaker was heated on the steam bath for 30 minutes (or longer) to effect the complete oxidation of the copper. The total volume in the beaker was increased to about 175 ml. by adding distilled water, and the ferrous ion was titrated against (.04 to .05 N) ceric sulfate solution which had been standardized against pure dextrose, two to three drops of orthophenanthroline indicator being used.

Determination of total, water-soluble, and water-insoluble nitrogen was made. *Total nitrogen* (including that from nitrates) was determined on a portion of the dried bark by the official Kjeldahl method modified to include the nitrogen of nitrates.[8**] *Water-soluble nitrogen* was determined by the iron-powder reduction method of Pucher, Leavenworth, and Vickery.[9**] *Water-insoluble nitrogen* was determined by the Kjeldahl-Gunning-Arnold method[8*] on a portion of the bark residue after the water was extracted.

After cutting and thoroly mixing the original sample, the moisture was determined by drying a portion at 100° to 101° C. in an electric oven.

Results of Analyses

Seasonal rise and fall of sugars and nitrogen. The data in Tables 7 and 8 represent analyses of bark tissue for two seasons during which spring cankers developed.

The analyses of 1939 bark (Fig. 3) indicated that the concentration of sugars in the two varieties of peaches differed at various periods during the growing and dormant season, as expected. In the Chinese peach seedlings the percentage of reducing sugars and total sugar was

Table 7. — Nitrogen Content of Bark From 1939 Twigs of Chinese Peach Seedlings and Elberta Peach Trees

(Expressed in percentage on dry basis)

Date collected	Chinese peach seedlings			Elberta trees		
	Total N	H_2O soluble N	H_2O insoluble N	Total N	H_2O soluble N	H_2O insoluble N
1940						
April 22	1.62	.340	.647	1.56	.425	.565
May 6	1.54	.296	.687	1.48	.340	.648
May 13	1.44	.268	.722	1.39	.326	.669
May 23	1.36	.266	.728	1.37	.297	.690
June 8	1.35	.266	.730	1.32	.287	.708
June 17	1.34	.264	.724	1.30	.271	.709
June 29	1.20	.233	.757	1.15	.262	.728
July 14	1.15	.198	.794	1.05	.211	.779
July 27	1.005	.197	.813	1.12	.166	.828
August 9	1.15	.230	.756	1.15	.231	.754
August 25	1.15	.260	.728	1.25	.243	.741
September 7	1.20	.277	.716	1.36	.324	.668
September 27	1.35	.390	.602	1.36	.337	.652

high at the time of the first sampling, April 22, 1940. A considerable decrease in total sugar and a less marked decline in reducing sugars occurred during the following two weeks. This reduction probably resulted from the increased rate of metabolism at the time of flowering and early growth of leaves. The concentration of total sugars was lowest during May and increased gradually until September 7, when there was a marked increase. Reducing sugars showed a continuous increase from May until late July. From August 9 until the last collection of September 27, there was a marked decrease in reducing sugars which was paralleled by a marked increase in total sugars.

The Elberta variety followed somewhat the same course as the Chinese seedlings (Fig. 3). Reducing sugars showed a decided increase beginning with the sample of May 23 and including sample of June 17; after which a gradual but continuous decline in percentage of reducing sugars occurred up to the final sampling of the 1939 bark on September 27, 1940. The total sugar showed a marked increase from May 23 to June 29, 1940, and then remained almost constant until August 25, at which time a definite increase again occurred.

Results of the nitrogen determinations reveal very little difference between varieties in total nitrogen and in water-soluble and water-insoluble nitrogen (Table 7). The percentage of water-soluble nitrogen was highest during April and decreased as the growing season progressed. Water-soluble nitrogen was lowest on July 27, 1940, and increased gradually to the last collection.

The analysis of 1940 bark was begun on November 1, 1940, while the leaves were still on the trees. Both varieties showed a marked increase in total sugar in the second sampling made December 10, 1940 (Fig. 4). The highest concentration in both varieties was found in the collection made on March 3, 1941. In the Elberta variety a decrease occurred from March 3 to March 22, 1941. Chinese peach seedlings differed from the Elberta in that the decline in total sugar was gradual up to March 29, 1941. The collection made on April 5 revealed a 2.1-percent decrease in total sugar compared with the collection of the previous week.

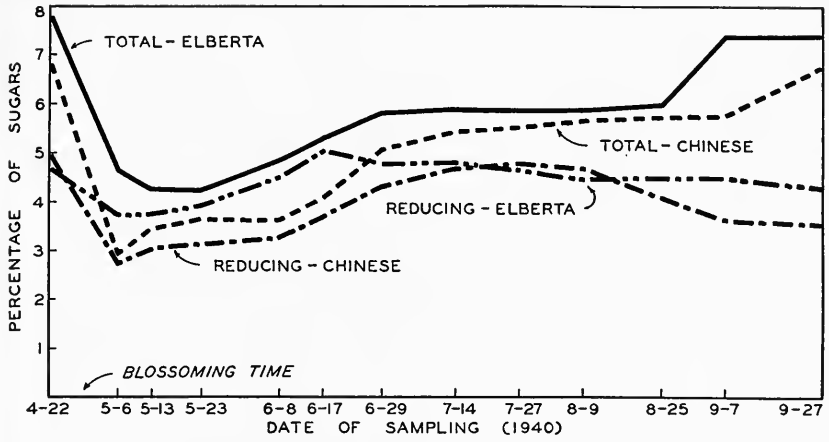
Reducing sugars in the two varieties showed different trends. In Elberta the highest percentage of reducing sugars was found in the collection taken on February 6, 1941; after which there was a gradual decline in reducing sugars up to the time of the last collection on April 14, 1941. In the Chinese peach seedlings a gradual but steady increase in reducing sugars occurred from November 1, 1940, until March 29, 1941. The last collections of April 5 and April 14 showed a sharp decrease in reducing sugars. Even with this reduction the amount of reducing sugars in the Chinese peach seedlings was 20 percent greater than that in Elberta at this time.

The two varieties showed very little difference in amount of total and water-soluble and water-insoluble nitrogen at any period (Table 8).

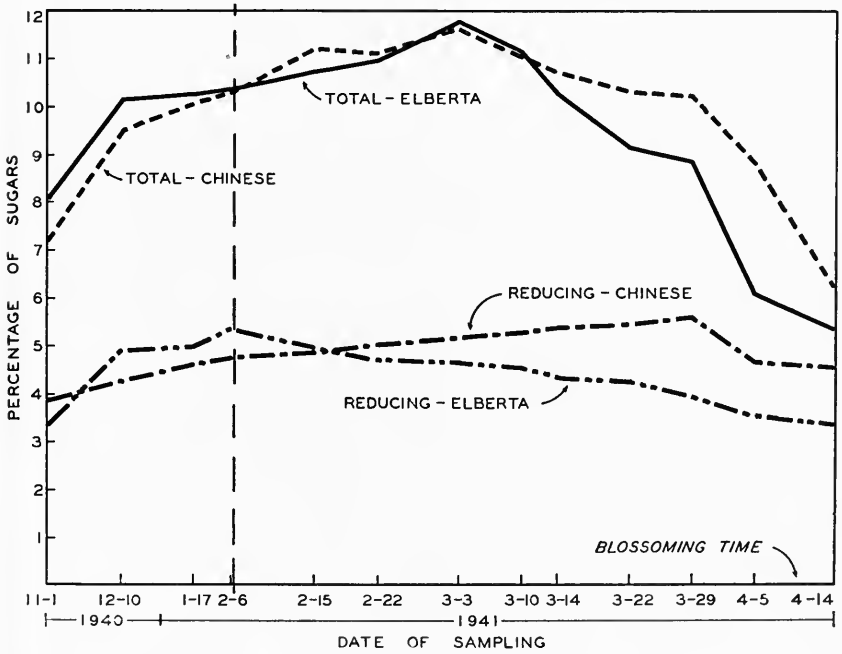
Table 8. — Nitrogen Content of Bark From 1940 Twigs of Chinese Peach Seedlings and Elberta Peach Trees

(Expressed in percentage on dry basis)

Date collected	Chinese peach seedlings			Elberta trees		
	Total N	H_2O soluble N	H_2O insoluble N	Total N	H_2O soluble N	H_2O insoluble N
1940						
November 1	1.065	.34	.638	1.05	.316	.674
December 10	1.675	.28	.709	1.635	.263	.729
January 17	1.735	.18	.808	1.74	.18	.808
1941						
February 6	1.74	.196	.798	1.75	.173	.819
February 15	1.825	.21	.776	1.77	.21	.874
February 22	1.795	.224	.762	1.665	.236	.750
March 3	1.735	.23	.748	1.615	.253	.738
March 10	1.725	.245	.742	1.595	.26	.726
March 13	1.685	.26	.724	1.59	.26	.712
March 22	1.667	.29	.688	1.575	.295	.705
March 29	1.615	.34	.636	1.49	.326	.652
April 5	1.49	.39	.592	1.47	.38	.607
April 14	1.445	.32	.667	1.44	.309	.684



Total and reducing sugars in the bark of Chinese seedlings and in Elberta during the growing season (April 22 to September 27, 1940). (Fig. 3)



Total and reducing sugars in the bark of Chinese seedlings and Elberta during the dormant and early spring seasons (November 1, 1940, to April 14, 1941). (Fig. 4)

Water-soluble nitrogen increased at the time of leaf fall but decreased during the early winter, when the water-soluble nitrogen increased. Beginning in early February water-soluble nitrogen increased until April 5, 1941, but showed a decrease in the last collection of April 14, 1941. The increase of water-soluble nitrogen was accompanied by a decrease in water-insoluble nitrogen.

Correlation of spring canker development with analyses. From the results of the analyses of the bark of the two peach varieties, a correlation was evident between the increase in percentage of reducing sugars and the development of the disease. It might be that the availability of reducing sugars to the bacterium was one of the determining factors in the development of spring cankers. At the time of the first collection of the 1939 twigs on April 22, 1940, the bark of Elberta contained 4.7 percent reducing sugars (Fig. 3). During the next two weeks the percentage of reducing sugars dropped to 3.7. The following two weeks the percentage remained about constant. On May 23, 1940, spring cankers and foliage infection were first observed. At this time 92 percent of the total sugar present was reducing sugars. In Chinese seedlings the concentration of reducing sugars on April 22, 1940, was about 5 percent. During the next two weeks reducing sugars dropped to 2.75 percent. This was a more rapid drop than that observed in Elberta during the same period. On May 6, 1940, 93 percent of the total sugar was reducing sugars. It was at this period that spring cankers and foliage infection were first observed in the bark of the 1939 twigs of this variety.

In the bark from the 1940 twigs the percentage of sugar in the two varieties differed greatly thru the winter months until the time when spring cankers occurred (Fig. 4). The reducing sugars in Elberta showed a marked increase from the time of the first sampling on November 1, 1940, until February 6, 1941. But from this time up to and including the last collection of April 14, 1941, a continuous decrease in the reducing sugars occurred in Elberta. In the Chinese peach seedlings the reducing sugars followed a different curve, showing a gradual but continuous increase from November 1, 1940, until March 29, 1941. On the 1940 twigs the first spring cankers were found in the Chinese peach seedling twigs collected April 14, 1941. A search for spring cankers on Elberta at this time proved unsuccessful. It is significant that at this date there was 34 percent more reducing sugars in the Chinese peach seedlings than in Elberta. The lower percentage of reducing sugars in the Elberta might account for the absence of spring cankers at this time in this variety. It can be assumed that the con-

tinuous increase in reducing sugars in the Chinese peach seedlings, combined with the advent of warm spring rains, induced rapid reproduction by the bacteria present within the twigs, thus producing cankers.

Total nitrogen in the bark of both varieties was comparable in the 1939-40 twigs (Table 7). Water-soluble nitrogen showed variations during the growing and dormant seasons. In early spring in 1940 just previous to full blossoming, Elberta contained more water-soluble nitrogen than did the Chinese peach seedlings. On May 23, 1940, the time at which spring cankers were first found on twigs of Elberta, 30 percent of the total nitrogen was water-soluble. Thus at the time spring cankers were first found on Chinese peach seedlings there was 25 percent less water-soluble nitrogen present than in Elberta, on which no cankers were observed. During full blossoming in 1941 both varieties contained approximately the same percent of water-soluble nitrogen (Table 8). Spring cankers were observed only on the Chinese peach seedlings. The increase of water-soluble nitrogen was accompanied each year by a decrease in water-insoluble nitrogen.

Spring cankers developed at an earlier date and in greater numbers on Chinese peach seedlings than on the Elberta trees. The chlorophyl of the twigs of Chinese peach seedlings began to show a marked increase in the early part of February. No definite quantitative analysis was made of this increase, but a macroscopic examination of the various samples prepared for subsequent carbohydrate and nitrogen determinations revealed a continuous increase in the density of the pigment. This increase in chlorophyl content suggests that metabolic processes began very early and may partially account for the earlier appearance of spring cankers on Chinese peach seedlings.

Temperature and moisture, along with other factors, must also be considered as having an influence on the development of spring cankers. Since both varieties were growing in the same orchard under similar environmental conditions, one is justified in assuming that while these factors play an important part, they are not apparently the controlling factors in determining the development of spring cankers. Since the bacteria are unable to grow, regardless of availability of nutrients, until sufficiently warm weather appears with spring, the concentration of reducing sugars in the twigs when optimal temperatures occur would seem to be the most important factor for spring canker development. This assumption is greatly strengthened by the correlation of high reducing-sugar content and extreme susceptibility for spring cankers in the Chinese seedlings.

SUMMARY AND CONCLUSIONS

The object of this study was to trace the life history of *Xanthomonas pruni* under Illinois conditions and to gather further information about the various factors that influence its seasonal development.

Bacterial spot of stone fruit is of economic importance because of its serious effects on infected trees and the fact that no successful means of control has been found. The rapid spread of this disease in Illinois is probably due to the rapid expansion of the fruit industry and the planting of peach trees in many sections of the state where conditions for the development of the disease are favorable.

The data presented here show without question that primary foliage infection has its origin in spring cankers or terminal diebacks caused by terminal invasion by the pathogen.

Epiphytological studies indicate that *X. pruni* is disseminated by wind, rain, and dew. The actual spread of the organism and development of the disease within a tree or from tree to tree was ascertained by field observations in orchards whose histories were clearly known. The extent to which the disease spreads depends largely upon environmental conditions. Fluctuations in these conditions account for the severity or mildness of epiphytotics. Sufficient evidence was obtained to show that the organism overwinters within the twigs. Observations on the development of spring cankers on twigs brought into the laboratory before their dormancy was broken outside prove beyond question that these cankers must arise from infections of the previous season.

Inspection of nurseries in various sections of Illinois has shown that it is possible for young trees to become diseased while in the nursery. Especially is this true where the nurseryman uses bud wood from infected bearing orchards for budding stock. Nursery trees may also become infected if the nursery rows are too close to bearing orchards infected with bacterial spot or if other stone fruits that harbor the bacteria from year to year are planted in the same plot with the young peach trees.

Chemical analyses of the bark of the two peach varieties suggest the possibility that reducing sugars in the bark tissues have more influence than nonreducing sugars and nitrogen on the development of spring cankers.

There is a gradual increase in reducing sugars during the winter months, especially in the highly susceptible Chinese seedlings, the maximum content being reached about the time spring-canker development appears to start. It would therefore seem that the increase in

reducing sugars may account, in part, for the extremely rapid bacterial invasion of the bark in early spring, the resulting development of extensive cankered areas, and the splitting of the bark along these areas. As the developing foliage draws some of the supply of reducing sugars from the bark, there is a cessation of bacterial invasion in the bark.

Since nitrogen determinations of the bark were similar in the two test varieties, we must assume that nitrogen is not the determining factor in the development of this organism.

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