FURTHER STUDIES ON THE OVERWINTERING OF PSEUDOMONAS CITRI[1]

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INTRODUCTION

An opportunity was afforded to study certain phases of the overwintering of *Pseudomonas citri* Hasse in connection with the investigations on the susceptibility and resistance of Citrus plants to canker, in the isolation field in southern Alabama, during the years of 1917 to 1921. To a large extent these studies were confined to the overwintering of the organism under natural conditions within the tissues of the host plant, as this is by far the most important, if not the only, way that overwintering of *P. citri* occurs.

Owing to the many types of plants growing in the field, their individual behavior to environmental conditions could be closely noted and correlated with their susceptibility during the growing season and the overwintering of the canker organism. The winters of 1919–20 and 1920–21 were normal ones in most respects for southern Alabama. The first was characterized by freezing temperatures the fore part of March; the second by killing frosts early in November. In both instances temperatures low enough and of sufficient duration occurred to kill the young leaves of the Citrus plants in the spring of 1920, and caused or hastened the defoliation of the mature leaves in November of the same year. Lower temperatures were recorded during the season of 1918–19, while the winter of 1917–18 was rather severe. The variety of these winter seasons encountered in the field made possible a rather comprehensive study of the overwintering of *Pseudomonas citri.*

MEANS OF THE OVERWINTERING OF THE ORGANISM

The Citrus canker organism may possibly overwinter in one of the following four ways:

(1) In the soil.

(2) In spots on defoliated leaves.

(3) In old diseased spots on the leaves attached to the trees, together with cankers on the twigs and larger limbs.

(4) By an extended or arrested incubation period.

IN THE SOIL

Numerous attempts to isolate *Pseudomonas citri* directly from orchard soil failed. The senior writer spent the greater part of two seasons making isolations from soil collected under many and widely

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divergent conditions in the field with negative results. D. C. Neal and Julius Matz, both formerly connected with the work, and the junior author of this paper continued these efforts to isolate the organism from the soil. All attempts yielded negative results, except in one instance when Mr. Neal was able to isolate the organism from soil taken from under a badly infected grapefruit tree directly after a heavy rain.

Our negative results can be readily explained in the light of the work of Fulton [2] and Lee.[3] Both investigators found that *Pseudomonas citri* underwent a rapid and continuous decline in numbers in all the types of soil which they tested, and that the vanishing point was reached in about two weeks. The writers in their present study were attempting to recover the organism from soil on which infected trees had been destroyed a month to three years previously. From their failure to recover the organism from the soil after infected trees were removed, and from the fact that both Fulton and Lee found that it disappears in soil after an interval of two weeks, the writers conclude that *Pseudomonas citri* can not survive for any length of time in the soil, and that the soil does not afford a means for the organism to live over winter.

IN THE SPOTS ON DEFOLIATED LEAVES

The possibility of *Pseudomonas citri* passing the winter in canker spots on defoliated leaves was pointed out earlier by Wolf,[4] who stated:

> Old leaves on the ground may possibly harbor the organism and there it may remain viable for a long time. It is believed, moreover, that the organism survives the winter in fallen leaves and that these fallen leaves constitute a very important source of infection in the following spring.

He further records unsuccessful attempts to isolate the organism from leaves kept in the laboratory from September to May and from twig cankers kept from March to October.

The importance of such a manner of overwintering is readily seen when it is considered that during the winter of 1917–18, and to a less extent in 1918–19, complete defoliation of both infected and uninfected leaves of the two important Citrus fruits grown in Alabama—grapefruit (*Citrus grandis* (L.) Osbeck) and Satsuma (*Citrus nobilis var. unshiu* Swingle)—occurred. *Poncirus trifoliata* (L.) Raf., being of deciduous nature, sheds all of its leaves normally with the approach of cold weather. The winter 1919–20 was normal for this locality and less defoliation occurred; in fact, a large number of infected leaves remained on grapefruit, Satsuma, and trifoliate orange hybrids until after the first growth matured. Thus, weather conditions determined to a great extent the severity of defoliation, not only of healthy but also of infected leaves.

To determine just how long the Citrus canker organisms were able to survive in spots on leaves that had defoliated and fallen to the ground, the following experiments were carried out during the winter of 1919–20. Defoliated and infected leaves were collected from be-

[2] FULTON, H. R. DECLINE OF PSEUDOMONAS CITRI IN THE SOIL. Jour. Agr. Research 19: 207–223. 1920.
[3] LEE, H. A. BEHAVIOR OF THE CITRUS-CANKER ORGANISM IN THE SOIL. Jour. Agr. Research 19: 189–206, illus. 1920.
[4] WOLF, F. A. CITRUS CANKER. Jour. Agr. Research 6: 69–100, illus. 1916.

neath badly diseased grapefruit and citrange plants and each group was divided into three sets—one of which was exposed on the surface of the soil, the second buried 3 to 6 inches in the soil, and the third kept in the laboratory. Isolations were made at weekly intervals from a number of representative spots from each set. Positive results were obtained from three grapefruit spots after two weeks exposure buried in the soil, and from one spot after one week in the laboratory. From citrange leaves *Pseudomonas citri* was isolated from one spot after one week's exposure on the surface of the soil. In no case was the organism recovered after three weeks exposure under any conditions. It is interesting to note in this connection that a rapid disintegration of the buried leaves took place, so that by the end of the third week the identity of the leaf was almost lost. Thus, from these results it may safely be concluded that the life of *P. citri* in defoliated leaves is not of sufficient duration to carry the organism through the winter.

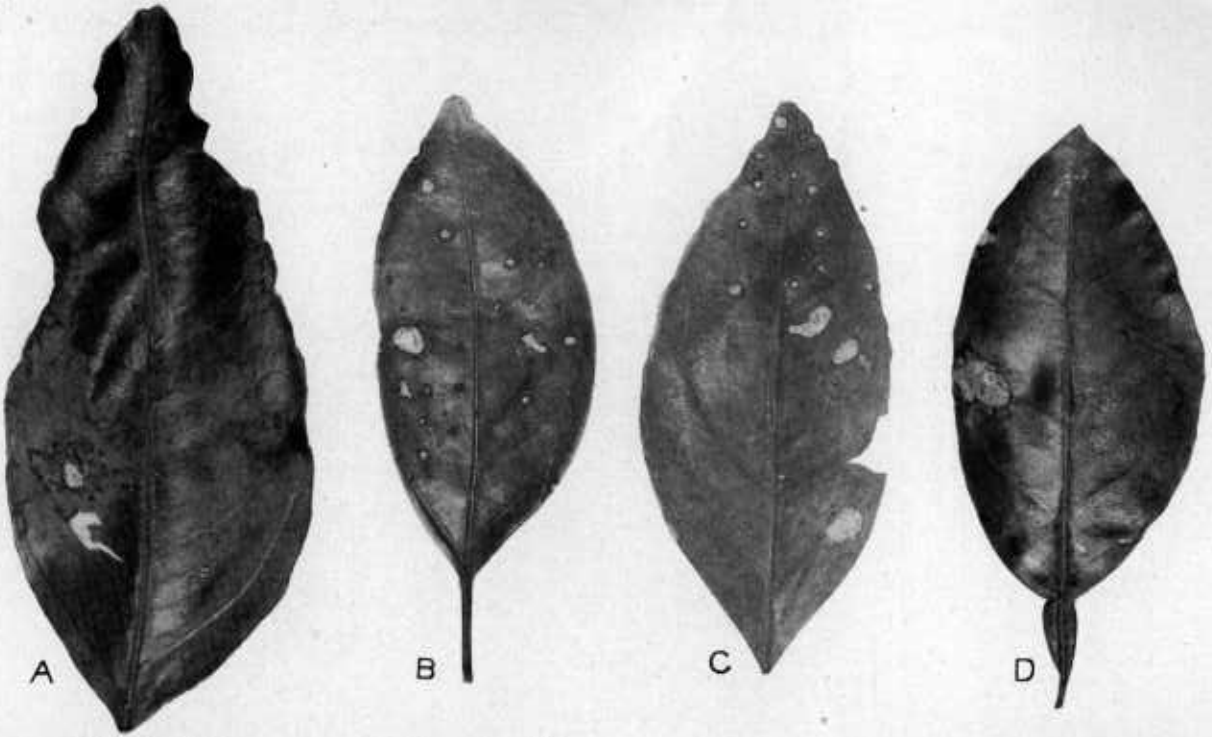
IN OLD SPOTS ON THE LEAVES ATTACHED TO THE TREES, TOGETHER WITH CANKERS ON THE TWIGS AND LARGER LIMBS

The number of canker spots on the leaves which go through the winter depends primarily on the amount of defoliation. During some winters the percentage of defoliation of the infected leaves is very high. Counts made during two winter seasons showed that the percentage of infected leaves which drop was much higher than the percentage of noninfected leaves which drop.

That viable bacteria are present in some of the old spots on the leaves which go through the winter attached to the trees has been shown by repeated isolations of *Pseudomonas citri* made during the winter and spring. Further, around the margin of these old spots many small fresh spots are formed in the spring. A number of such spots are shown in Plate 1 on a number of leaves of different Citrus plants. In the many isolations made, the largest numbers of viable spots were on those leaves which were formed late in the summer or fall.

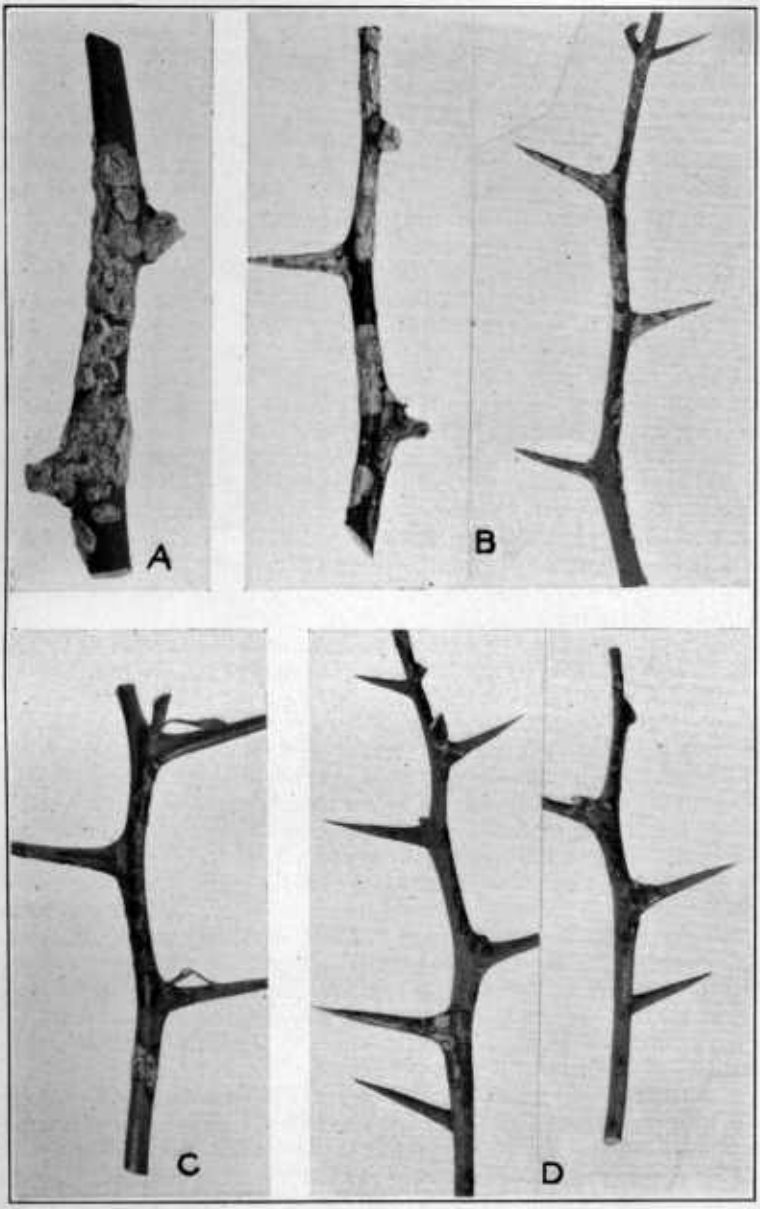
The large number of twig and stem cankers present in the isolation field afforded abundant material for observations and isolations throughout the winter of 1919–20. These cankers were classified according to age, condition, and location on the tree. Taking these in their chronological order, the first are those formed only on citranges early in the spring of 1918 following inoculations made in September of the preceding year. These areas of canker tissue are characterized by their location, usually near the base of the original trunk, by their extremely loose, corky construction, and further by the fact that their margin lacks a definite oily outline. The striking differences between these areas and the cankers formed later in the same season are clearly shown in Plate 2, A, B. Those shown in Plate 2, A, are the result of artificial inoculation, and such as these are dealt with in the overwintering paper by Peltier and Neal.[5] The cankers formed later in 1918 resulted from natural infection, and are more limited in area. These two types of cankers are quite similar.

[5] PELTIER, G. L., and NEAL, D. C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. Jour. Agr. Research 14: 523–524, illus. 1918.



A.—Satsuma (Ikeda) leaf B.—Satsuma (Owari) leaf C.—Satsumelo leaf D.—Grapefruit (Duncan) leaf

(Photographs taken May 1, 1920, to show the breaking out of many small canker spots at the margins of old spots formed the preceding season. About natural size)



A.—Citrandarin. Cankers formed in the spring of 1918, as a result of inoculations made in September, 1917

B.—*Poncirus trifoliata.* Cankers formed during the summer of 1918 as result of natural infection. Isolations made in the spring of 1920 from cankers, similar to those shown in A and B, were all negative

C.—*Poncirus trifoliata.* Cankers formed during the spring and summer of 1919 as a result of natural infection. A small percentage of spots similar to these were viable in the spring of 1920

D.—*Poncirus trifoliata.* Cankers formed in the fall of 1919 as a result of natural infection. A high percentage of spots of a similar nature were viable in the spring of 1920

(Photographs taken May 1, 1920. All about natural size)

Cankers formed during the growing season of 1919 were quite numerous. For convenience they are classified under two heads—those formed during the spring and summer, and those formed in the fall. The first can easily be distinguished from cankers of the preceding year, by their position on the tree, by their construction, and by general appearance. They are found, in practically all cases, on the wood of the last season's growth which has either rounded or shows some sign of bark formation. These spots are generally ruptured and corky, depending somewhat on the host and time produced, and they range in color from light yellow to brown. A large number of them showed a more or less distinct oily margin late in the fall. Cankers such as these are shown on Plate 2, C. However, with mild weather in the fall, an entirely different type of spot was produced on the twigs of the trifoliate orange and its hybrids and grapefruit; these cankers formed on the last growth of the season ranged in diameter from 1 to several millimeters, were extremely oily (with a reddish-brown color), and remained unruptured during the winter. (This type of canker is clearly shown in pl. 2, D.) An exception must be made to this statement in the case of grapefruit. Owing to the quick response of grapefruit to temperature changes, cankers of this type on angular wood which was not killed by the first low temperature were able to develop from time to time as the host plant became active, and so did not go through the winter in unruptured condition.

Thus, four types (as to age) of twig and stem cankers were present in the fall of 1919. The results of numerous isolations late in the winter of 1919–20 and early in the spring from the above-described cankers showed that practically 95 per cent or more of the cankers with viable bacteria belonged under the type formed late in the summer of 1919. Cankers formed earlier in 1919, although they showed all signs of being active when they went into the winter, gave negative isolations in the majority of cases. Isolations from canker formed the two preceding years were negative in all instances.

The results from the large number of isolations from twig and stem cankers belonging to the various types just described clearly indicate that the life of the bacteria in a canker is more or less limited, and that only some of the cankers formed the preceding season serve as a source for new infections in the spring. In fact, there seems to be a close correlation between the type of wood (angular and round) and spring viability of stem and twig cankers. With possibly only one or two exceptions, spots which went into the winter upon round wood which showed definite bark formation have in all cases yielded negative results upon isolation. The cankers classified as having been formed in 1918 were all dead, while a large per cent of those formed in the early spring and summer of 1919 were no longer viable. Only the spots formed late in the fall of 1919, on angular wood, gave a high percentage of positive results upon isolation throughout the winter and spring.

An interesting observation was made in connection with the cankers of various ages in the spring of 1920. Upon carefully removing the corky tissue from the cankers formed in 1918, it was noted that they were gradually being delimited from the healthy tissue by a pronounced layer of new green bark. The viable spots formed late in 1919, however, extended down through the cambium layer, often discoloring the wood.

BY EXTENDED OR ARRESTED INCUBATION PERIOD

Pure-culture inoculations on citrange, citrange hybrids, and trifoliate orange, grapefruit, and Satsuma were made September 16, 1917. During the remainder of the season only a few canker spots developed on the leaves of the trifoliate orange and grapefruit as the immediate result of these inoculations. Owing to the severe winter, all the infected leaves fell off and no spots of any description were visible on these plants in the spring. However, with the resumption of active growth of the plants in the spring, cankers developed over extensive areas[6] on the stems of the trifoliate oranges and some of its hybrids. Apparently the bacteria were able to gain entrance into the plant tissues, possibly through the lenticels, and by means of an incubation period was carried over until the following spring.

With these results in mind, a careful examination of the plants was made during the following years in order to determine whether anything of this sort occurred under natural conditions. During the winter of 1919–20 and again the following year this method of overwintering was observed to take place on the leaves of Satsuma. In April of 1920 a number of characteristic spots were observed on Satsuma leaves of the last growth of the preceding year. These leaves did not show any signs of spots prior to and during the winter. When they first developed on the leaves they resembled melanose in some respects, and it was only by isolations that the identity of the causal organism was definitely determined. Apparently initial infection took place shortly before the plants went into dormancy, and incubation of the organism was arrested until active growth of the plants was resumed in the spring. As a rule the spots were bun ched, small, very oily, slightly raised on both sides of the leaf, and remained unruptured for some time (pl. 3, A). That these spots were not due to early spring infection was determined by the fact that the first canker spots appeared on Satsuma leaves of the first spring growth May 10, one month later than the others were observed on the mature leaves. All attempts to inoculate leaves formed the preceding fall had failed.

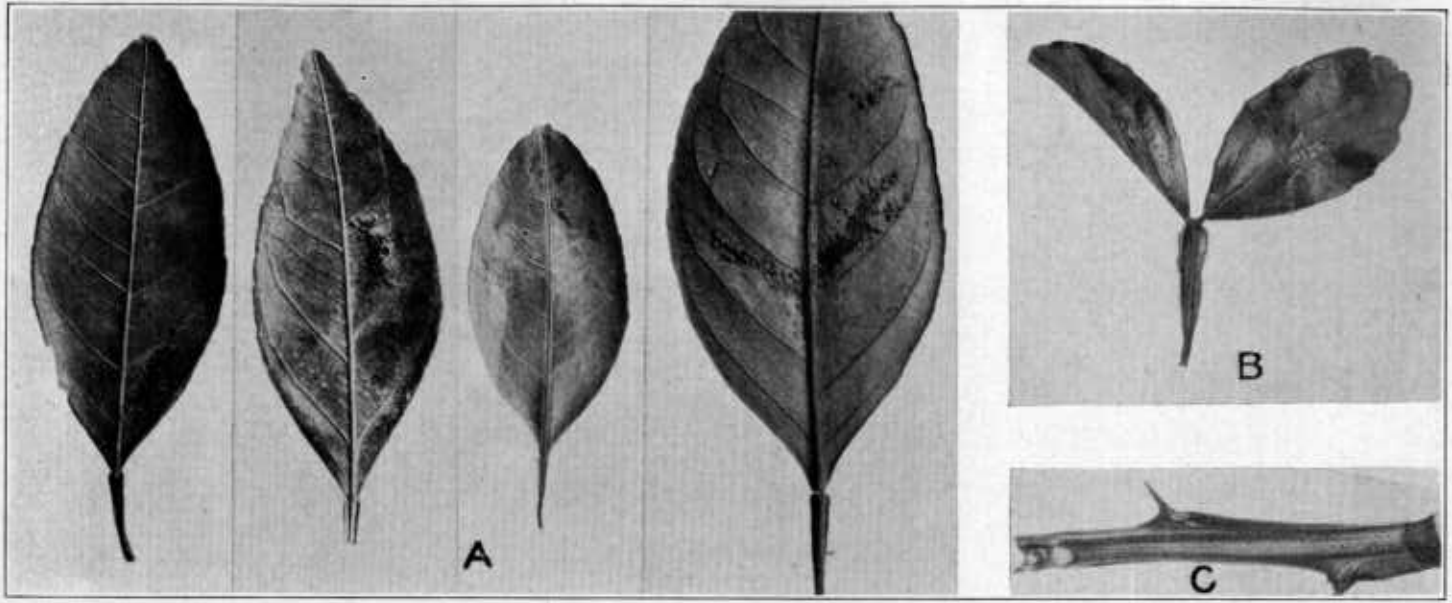
Similar observations were made during the following winter season on Satsuma. Whether such delayed infections occur on other Citrus plants could not be determined, in spite of the many observations made.

SOURCES OF EARLY SPRING INFECTION

The sources of early spring infection varies from season to season. Since this paper is primarily concerned with the overwintering of *Pseudomonas citri* within the tissues of the host, a general statement of the condition of the plants, the amount of infection present, and the weather conditions during the winter are necessary.

The winter of 1917–18 was the most severe to which the plantings in the isolation field were subjected. A minimum temperature of 15.5° F. was recorded in January, and only the more hardy plants survived, while complete defoliation of all plants resulted. With

[6] PELTIER, G. L., and NEAL, D. C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. Jour. Agr. Research 14: 523–524, pl. 58. 1918.



A.—Typical specimens of Satsuma leaves, showing the nature of the arrested incubation period. These leaves were formed late in 1919, infected naturally by *Pseudomonas citri* before they matured. Owing to adverse environmental conditions, the organism failed to develop, but remained quiescent until the following April, when characteristic canker spots were formed

B.—Citrandarin leaf, showing new spots developing at margin of spot formed the preceding season

C.—Grapefruit twig, showing the renewed activity of *Pseudomonas citri* at the base of the leaf scars

(Photographs taken May 1, 1920. All about natural size)

the exception of a few leaf spots on grapefruit and trifoliate orange, none of the other plants showed any visible lesions of citrus canker when they went into dormancy. Consequently, the only viable organisms were those which produced cankers in the early spring in the bark tissues of the trifoliate orange and some of its hybrids.[7] These organisms overwintered within the tissues by extended or arrested incubation period.

During the summer of 1919, all the plants were in an excellent condition, having made a good and abundant growth. Consequently, the more susceptible plants were heavily infected. Leaf, twig, and stem cankers were numerous. The autumn was unusually mild and active growth of grapefruit and Satsuma plants continued until well into November. Thus, the cankers were able to continue development and also new infections occurred.

After a normal winter, such as that of 1919–20, two distinct sources of spring infection were recognized—viable spots on the leaves and twigs formed the previous year, and through the extended incubation period of the organism on leaves infected late in the fall.

The winter of 1918–19, while not as severe as that of the preceding year, caused considerable defoliation. The weather records of the four years previous showed that the winter of 1918–19 could be classed as intermediate between the severe winter of 1917–18 and the normal winter of 1919–20. In fact, the monthly mean temperature, and the number and severity of frosts for the winter of 1918–19 and 1919–20 varied but little. However, a minimum temperature of 17° F. was recorded in January, 1919, which was low enough to cause complete defoliation of practically all of the plants in the field. The lowest temperature reached during th winter of 1919–20 was 25° F. A heavy infection on the plants was present in the fall of 1918. However, owing to the extensive defoliation, very little overwintering of viable bacteria occurred in the old canker spots on the leaves, so most of overwintering must have taken place in the cankers on the twigs and stems of the grapefruit, Satsuma, and trifoliate orange plants.

As deciduous Citrus plants such as the trifoliate orange shed their leaves each fall, infection in the spring must come only from the viable spots on the twigs or from outside sources. In Plate 2, D, is shown the early spring development of active canker tissue immediately adjacent to and surrounding the typical cankers formed on the last growth of the preceding year. The darker area represents the oily, unruptured viable spots. The lighter zones surrounding them are the result of the spring activities of the organism. The lighter areas become evident soon after growth of the plants begins. These cankers develop rapidly and soon rupture. Between 50 and 75 per cent of the cankers of this type are viable in the spring.

Many of the trifoliate hybrids retain their foliage during the winter, so overwintering of the organism may occur in the spots formed in late summer as well as in the cankers which develop on the twigs.

[7] PELTIER, G. L., and NEAL, D. C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. Jour. Agr. Research 14: 523–524, illus. 1918.

Grapefruit and allied plants which react differently to environmental influences presented a somewhat different problem. As has been noted, twig cankers are produced late in the summer on angular wood. However, owing to the rather unstable dormancy of this plant, the organism can and does develop from time to time as the host responds to periods of favorable temperatures for growth. Consequently, the viable cankers do not go through the winter in unruptured condition. Such spots as these may extend down on the angular wood for some distance. During the winter 1919–20 these ruptured spots remained viable.

When defoliation is not complete, the organism is also able to overwinter in some of the spots on the leaves, and with the renewed activity in the spring many small new spots develop around the margin of the old cankers (pl. 1, D). These in turn serve as a source of infection for the new leaves which are developing. Another quite common source of infection present on grapefruit plants was found on or near the leaf scars; each spring many small viable cankers are found near the base of the leaf scars and these develop very much as those described on the twigs (pl. 3, C).

On Satsuma and plants of similar nature, the organism is overwintered, with but few exceptions, in the viable cankers on the leaves. The most common means by which the organism overwinters is in the spots formed late in summer on the last growth (pl. 1, A. B, C). The second means of overwintering is through delayed incubation, which has already been described (pl. 3, A). The same type of overwintering was again observed to be common during the season of 1920–21.

DISCUSSION

It has been shown that *Pseudomonas citri* can not live over the winter, except when in the host tissue, and then only in cankers developed the previous season. Even cankers formed during the early growing season are usually not viable. The cankers formed on the last growth during the season, either on leaves or twigs, are usually the ones which are the sources of the early spring infections. The activity of these old spots begins at the same time growth of the hosts starts, so by the time the new leaves are unfolding the cankers have ruptured and the disease spreads.

The method of overwintering through an extended incubation period which has been found to occur on Satsuma are merely late season infections which are formed on the last growth, but which do not develop sufficiently to produce a typical spot because of adverse environmental conditions. That they do spread somewhat internally during the dormant season can be shown by the fact that when they develop during the spring they seem to cover part of the leaves.

The citrus canker organism was not recovered from any of the cankers one year old or older, so it may safely be assumed that only some of the canker spots formed during the current year on leaves and angular wood on the trees are viable the following season.

SUMMARY

In southern Alabama, *Pseudomonas citri* Hasse is overwintered as follows:

(1) In old spots on the leaves which remain on the tree.

(2) In spots on the twigs formed near the end of the growing season, principally upon angular wood.

(3) By means of an extended or arrested incubation period of the organism on leaves and twigs of the last growth of the season. This method of overwintering is likely to occur only during seasons when frost is long delayed.

The number of viable spots which winter over is influenced by the severity of the winter. A severe winter causes almost complete shedding of the infected leaves, and kills back the angular wood.

The viability of an individual canker spot is more or less limited, and all spots on an infected tree do not serve to carry the organism through the winter.

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