# Studies on the Etiology of Leprosis in Citrus

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THE CAUSE of leprosis [Florida scaly bark, nailhead rust, lepra explosiva (10)] remains doubtful despite 70 years of investigation. Numerous theories regarding etiology have been advanced; of these, the most tenable are that leprosis is caused by a fungus, a mite, or a virus. The author reviews the evidence for each and presents pertinent findings of his own.

## *The Fungus Theory*

In the first comprehensive study of leprosis, Fawcett in 1911 (4) attributed the disease to a fungus. He summarized his pathogenicity studies as follows: "1) Pure cultures of the fungus were obtained from the diseased areas. 2) Young sweet orange trees in the greenhouse were inoculated from the pure cultures by spraying on the spores, and spots were seen to develop upon the inoculated trees in from 40 to 60 days; while trees not so treated were free from such spots. 3) These spots proved later to be identical with those started by bringing diseased pieces of wood affected with scaly bark into contact with healthy bark of trees in the greenhouse. 4) Pure cultures of a fungus, identical with those from which the inoculations were made, were isolated from these diseased areas." The fungus was named *Cladosporium herbarum* var. *citricolum* Farlow (6).

In Paraguay, Spegazzini in 1920 (17) associated lepra explosiva with the fungus *Amylorosa aurantiarum* Speg. Although infection experiments were never carried out, Spegazzini's attribution was accepted by investigators in Argentina until 1940.

The extensive spray trials carried out by Fawcett (4) were interpreted as demonstrating once again the efficacy of copper in combatting cladosporiaceous fungi. He recommended that bordeaux mixture be used for the control of leprosis.

Evidence contrary to the fungus hypothesis resulted from spray trials by Knorr and Thompson (11) in Florida in 1950. They found that copper fungicides actually increased leprosis over levels in the unsprayed controls. Percentages of fruits developing lesions after applications of copper were 15.1, 16.3, and 22.7, whereas percentages in the unsprayed checks were 9.2 and 13.8. Bitancourt (1) mentioned similar increases in Brazil in 1955, following use of copper in two of seven spray trials.

It seems likely that Fawcett's control of leprosis resulted not from the use of copper, but inadvertently from whale-oil soap, a known miticide, which he employed for combatting scale insects.

### *The Mite Theory*

This theory holds that mites produce leprosis by injecting a toxin into host tissue.

In Argentina, Frezzi (7) observed false spider mites in leprosis lesions of twigs. When transferred to healthy seedlings under bell jars, these mites reproduced typical leprosis lesions on leaves and twigs. The association of false spider mites with leprosis was confirmed by Vergani (18). Mites used by Frezzi and Vergani in their experiments were identified by Blanchard (2) as *Tenuipalpus pseudocuneatus* Blanchard, since synonymized by Pritchard and Baker (15) with *Brevipalpus obovatus* Donnadieu.

In Florida, the writer wrapped adhesive tape around very young shoots of a leprosis-affected sweet orange tree to provide alternate areas of covered and uncovered bark. When shoots were unwrapped 6 months later, leprosis lesions were found to be present only in areas left untaped (Fig. 1). Leprosis lesions were also produced by placing affected fruits, twigs, and leaves inside cages containing sweet orange seedlings, and by transferring suspect mites to caged seedlings. The identity of mites that led to leprosis proved to be *B. californicus* (Banks), syn. *B. australis* Baker (8, 9).

In Brazil, Rossetti *et al.* (16) associated leprosis with the mite *B. phoenicis* (Geijskes). This species is world-wide in distribution; the author has collected it on citrus from Florida, Trinidad, Venezuela, Argentina, Egypt, Syria, and Arabia. Only in Florida and Venezuela has *B. phoenicis* been found on trees affected by leprosis. However, monthly sampling in a leprosis-affected grove in Florida showed that this species often occurs mixed with *B. californicus* and at other times displaces the latter species completely. Repeated attempts over the past 15 years to reproduce leprosis with *B. phoenicis* have failed consistently. A diffuse laminar chlorosis and marginal necrosis of leaves (designated "phoenicis blotch") develops (12), but true leprosis spotting or stem cankering is never present. As many as a thousand individuals of *B. phoenicis* may occur on a single fruit in Florida, yet trees infested solely with this species have never shown leprosis.

Morphological characteristics of *B. phoenicis* and *B. californicus* are so alike that separation requires microscopic examination under oil im-

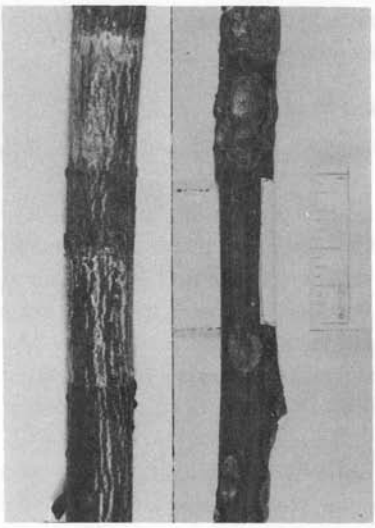

FIGURE 1. *Leprosis on sweet orange sprouts. Note absence of lesions in areas previously taped to prevent inoculation under field conditions.*

mersion. Mites identified in this manner cannot be used, of course, in subsequent pathogenicity experiments. Therefore, in trials reported below, progenies derived from single eggs were used and species were identified at the conclusion of pathogenicity tests.

METHODS.—The single-egg technique begins with the collection of *Brevipalpus* eggs from leprosis-affected leaves, twigs, and fruit. Twelve eggs are placed in a circle on filter paper in a petri dish kept moist by a wick attached to the inner surface of the lid. Eggs are incubated at room temperature, and petri dishes are examined twice daily for hatched nymphs. Newly-hatched nymphs are transferred to sweet orange seedlings [*Citrus sinensis* (L.) Osbeck cult. Pineapple] growing in sterilized soil in glass mason jars. Each jar with its single nymph is screened with 3 thicknesses of cheesecloth and placed in a tank containing several inches of water to prevent migration of mites from jar to jar. Nymphs, apparently all females, reproduce parthenogenetically and lead eventually to the development of sizeable colonies. When starting with newly hatched nymphs, 1 per jar, an incubation period of from 6 to 9 months is usually required for *B. californicus* to produce leprosis and for *B. phoenicis* to produce phoenicis blotch.

Using the foregoing technique, answers were sought to the following questions. 1) Will the rearing of *B. californicus* in the absence of leprosis lesions prevent the transmission of leprosis? 2) Can *B. obovatus*, which

in Florida has been found only on nonrutaceous hosts, produce leprosis in Florida as it does in South America? 3) Are present species concepts in *Brevipalpus* adequate for separating mites that are pathogenic from those that are nonpathogenic?

RESULTS.—With the foregoing technique, an attempt was made to determine whether *B. californicus*, reared in the absence of leprosis lesions, can transmit leprosis. Twenty Mason-jar cultures were infested with nymphs (presumably *B. californicus*), one mite being placed on the seedling in each jar. Development of leprosis on leaves and twigs of caged sweet orange seedlings (vars. Pineapple and Hamlin) was recorded at

TABLE 1. OCCURRENCE OF LEPROSIS ON CAGED SWEET ORANGE SEEDLINGS (*C. sinensis* CULTS. PINEAPPLE AND HAMLIN) INFESTED WITH PETRI-PLATE-HATCHED NYMPHS AND WITH ADULTS OF *Brevipalpus californicus* (BANKS) FROM LEPROSIS-AFFECTED CITRUS

| Treatment | Total number of plants | Number of plants showing[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | No symptoms | | Phoenicis blotch | | Leprosis | |
| | | Mites present | Mites absent | Mites present | Mites absent | Mites present | Mites absent |
| Single nymph,[b] per plant | 20 | 0 | 8 | 0 | 0 | 12 | 0 |
| Eggs,[c] 12-16 per plant | 13 | 0 | 3 | 2 | 0 | 8 | 0 |
| Adults,[c] 2-32 per plant (check A) | 16 | 3 | 2 | 4 | 0 | 7 | 0 |
| Uninfested controls (check B) | 3 | 0 | 3 | 0 | 0 | 0 | 0 |

a. Number of plants on which mites or their casts were present or absent at conclusion of experiment.
b. From eggs hatched in petri plates.
c. From leprosis-affected fruit and twigs.

9 and 16 months after infestation. There were two checks: one (A) consisted of 16 jars infested with 2 to 32 adults taken directly from leprosis-affected fruit and twigs; the other (B) consisted of 3 jars left uninfested. On conclusion of the experiment, living mites were identified; all proved to be *B. californicus*. Results are given in Table 1.

The same type of experiment was used to demonstrate that *B. phoenicis* does not produce leprosis under Florida conditions (Table 2).

The correlation between numbers of mites on a plant and numbers of lesions produced is usually low, both under controlled conditions and in the field. Apparently, not all mites of pathogenic species cause leprosis. In California, populations of *B. californicus* are at times so high as to require spraying, yet leprosis is unknown.

The question of whether Florida strains of *B. obovatus* can cause leprosis was answered by the same type of experiment. The results, summarized in Table 3, show that in Florida, *B. obovatus* is capable of producing leprosis, at least under greenhouse conditions. This species has not been found on citrus in Florida. The strain used in this experiment was obtained from Spanish needle (*Bidens pilosa* L.) plants growing under leprosis-free citrus trees at Lake Alfred. This species is responsible for the decline of orange groves in the Province of Corrientes, Argentina.

These results raised the question of whether present taxonomic criteria are valid for separating species of *Brevipalpus*. Single-egg progenies of

TABLE 2. OCCURRENCE OF LEPROSIS ON CAGED SWEET ORANGE SEEDLINGS (*C. sinensis* CULTS. PINEAPPLE AND HAMLIN) INFESTED WITH PETRI-PLATE-HATCHED NYMPHS AND WITH FIELD-COLLECTED ADULTS OF *Brevipalpus phoenicis* (GEIJSKES)

| Treatment | Total number of plants | Number of plants showing[a] No symptoms | | Phoenicis blotch | | Leprosis | |
|---|---|---|---|---|---|---|---|
| | | Mites present | Mites absent | Mites present | Mites absent | Mites present | Mites absent |
| Single nymph,[b] per plant | 14 | 5 | 5 | 4 | 0 | 0 | 0 |
| Adults,[c] 30 per plant (check A) | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Uninfested controls (check B) | 4 | 0 | 4 | 0 | 0 | 0 | 0 |

a. Number of plants on which mites or their casts were present or absent at conclusion of experiment.
b. Derived from eggs hatched in petri plates.
c. Derived from fruit and twigs.

*B. phoenicis* reared by the writer were examined by Pritchard and Baker (14) who concluded: "*B. yothersi* and *B. mcbridei* were originally named as separate entities because of differences noted in the lateral setae of the nymphs. . . . L. C. Knorr of the Citrus Experiment Station, Lake Alfred, Florida, has reared a series of nymphs from a single female on citrus. This material shows the comparative development of these setae to be a variation. Nymphs of *B. papayensis* resemble those of the *yothersi* type in having the humeral as long as the third dorsal propodosomal, but the first dorsal propodosomal and the first dorsolateral hysterosomal are somewhat larger than in *yothersi*. The comparative length of these setae is also shown to be variable in the Knorr series."

Consequently, Pritchard and Baker synonymized *B. yothersi*, *B. mcbridei*, and *B. papayensis* with *B. phoenicis*. Baker later examined progenies of *B. californicus* reared from single eggs and reported no

morphological differences that might account for the fact that certain individuals produce leprosis and others do not.

In South Africa, Dippenaar (3), working with concentric ring blotch of citrus, wrote: "that the role of an eriophyid mite as the cause of nail-head rust or lepra explosiva might have been overlooked, and that lepra explosiva and ring blotch are one and the same (type of) disease."

This hypothesis is open to question on the basis that *Brevipalpus* mites, because of their small size, require the same degree of magnification for examination as *Calacarus* mites, that no eriophyids, except the

TABLE 3. OCCURRENCE OF LEPROSIS ON CAGED SWEET ORANGE SEEDLINGS (*C. sinensis* CULTS. PINEAPPLE AND HAMLIN) INFESTED WITH PETRI-PLATE-HATCHED NYMPHS AND WITH ADULTS OF *Brevipalpus obovatus* DONNADIEU FROM *Bidens pilosa*

| Treatment | Total number of plants | Number of plants showing[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | No symptoms | | Phoenicis blotch | | Leprosis | |
| | | Mites present | Mites absent | Mites present | Mites absent | Mites present | Mites absent |
| Single nymph,[b] per plant | 29 | 4 | 11 | 6 | 0 | 8 | 0 |
| Eggs,[c] 15 per plant | 20 | 3 | 5 | 4 | 0 | 8 | 0 |
| Adults,[c] 3-20 per plant (check A) | 28 | 3 | 4 | 7 | 0 | 14 | 0 |
| Uninfested controls (check B) | 4 | 0 | 4 | 0 | 0 | 0 | 0 |

a. Number of plants on which mites or their casts were present or absent at conclusion of experiment.

b. Derived from eggs hatched in petri plates.

c. Derived from *Bidens pilosa*.

rust mite, were encountered in the Florida work with *Brevipalpus*, and that neither the eriophyid mite, *Calacarus citrifolii* Keifer, nor the concentric ring blotch that it causes is known in Florida.

### *The Virus Theory*

When it became clear that *Brevipalpus* mites and not fungi were involved in leprosis, Fawcett (5), Frezzi (7), and Marchionatto (13) assumed that the primary agent was a virus. However, no evidence for this assumption was presented.

Vergani (18) attempted the transmission of a hypothetical virus by budding, but in 19 trials obtained no infections, either locally or sytemically. The writer repeated these experiments, grafting patches of leprotic bark into the main stems of susceptible varieties of sweet and sour

orange seedlings. After 1 year, 13 patches were still alive, but no symptoms appeared that would indicate the existence of a localized or systemic virus.

Since leprosis lesions often enlarge for years, it seemed that if a virus were involved, the causal agent could be made to diffuse into healthy tissue. Accordingly, patches of diseased green bark were cut from miti-

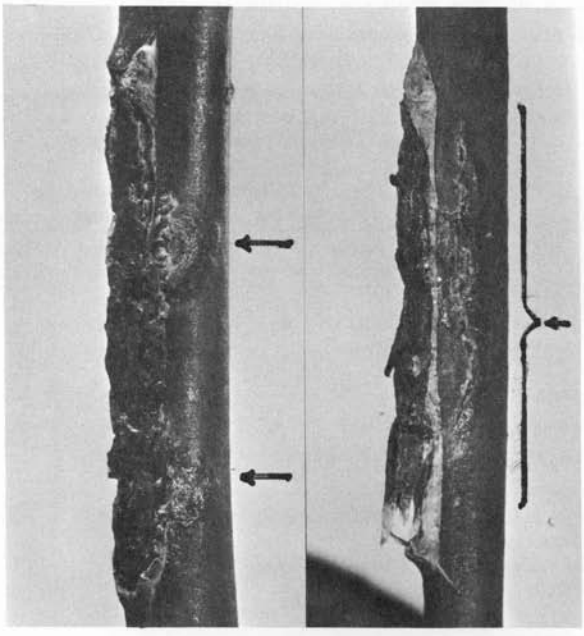

FIGURE 2. *Transmission of leprosis from affected donor patch graft to receptor tissue. This degree of involvement occurred four months after grafting with miticide-treated budwood.*

cide-treated leprosis-affected shoots so that each patch contained a section of a lesion. These patches were inserted into healthy shoots from which similar-sized patches of cortical tissue had been removed. Bark patches were wrapped with plastic budding tape and the test seedlings were placed in the greenhouse. After 3 weeks, wraps were removed. Of 193 grafted patches, 57 were alive at time of unwrapping; 11 of the latter led eventually to the spread and development of leprosis in bark of the seedlings (Fig. 2). Spread took place only when leprosis-affected patches were inserted into immature green shoots; no spread occurred when

patches were grafted into aging wood that was striated or covered with cork. Affected areas of inoculated plants continued to expand with time.

### *Discussion*

Three approaches were taken in investigating the etiology of leprosis. The first sought to determine whether leprosis-inducing mites need a source of inoculum in order to acquire virulence. As shown in Table 1, nymphs reared from eggs in a petri dish (i.e., in the absence of leprosis lesions) were fully as capable of producing leprosis on caged seedlings as were mites taken directly from leprosis-affected trees.

The second approach involved *B. obovatus*, a species that causes leprosis in South America, but does not occur on citrus in Florida. Table 2 shows that petri-dish-reared nymphs and adults taken directly from *Bidens pilosa* are capable of producing leprosis.

The third approach was intended to test the validity of certain taxonomic criteria for separating species of the genus *Brevipalpus*. Clonal lines of various species of mites derived from single eggs were submitted for determination. Inspection showed that specific characteristics once regarded as valid have become untenable because of the wide variations among progenies of single eggs.

Although results presented in Table 1 show that mites not fed previously on a leprosis-affected tree have the ability to induce leprosis, the possibility remains that a theoretical virus may be transmitted through the egg. The virus hypothesis is also supported by the finding that leprosis can be transmitted by grafting. On the other hand, it is difficult to implicate a virus when *B. obovatus*, collected from *Bidens pilosa* and from an area where leprosis is unknown, will produce the disease. The third line of evidence regarding taxonomic criteria suggests that conflicting reports of pathogenicity may have as their explanation the unsettled state of knowledge concerning the mites of this group.

At present, because of inadequate evidence, we are unable to conclude whether leprosis is caused by a virus or a toxin. The findings presented can be explained either way. Instances are known of both agents being transmitted through grafting and through eggs and of both resulting in localized lesions.

To discriminate between these two alternatives will apparently require a methodology other than that of biological assay. The electron microscope may offer a means of detecting virus-like particles in lesioned tissue and, conversely, no virus-like particles in unaffected tissue. Additional support for the virus hypothesis would come from the finding of these

same particles in leprosis-inducing strains of *B. californicus* and *B. obovatus*. On the other hand, a cytologic examination of morbific tissue might disclose the causal agent to be acting in the manner of a phytotoxin having mutagenic effects similar to colchicine or the gall-inducing metabolites of *Agrobacterium tumefaciens*.

Future taxonomic studies may resolve other matters that are still enigmatic. For instance, why does *B. californicus* produce leprosis in Florida but not in California where populations of the same species are sometimes so high as to require spraying? Why is the damage associated with *B. californicus* in Spain and South Africa limited to a fine speckling of the peel? Why does *B. phoenicis* produce leprosis in Brazil and not in other parts of the world? To the extent that these questions arise from nomenclatorial uncertainties, they may be resolved by further taxonomic studies.

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