# Apparent Lack of Transmission of Citrus Blight Using Bark Patch Inoculations

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ABSTRACT. Several experiments were established to determine if citrus blight could be transmitted to healthy receptor trees using bark patches (about 1.5 X 2.5 cm) collected above and below the budunion from blight-affected trees and grafted with one patch each above and below the bud union on the receptor tree. In South Africa two plots were established in 1985, one in the Northern Transvaal and one in Natal. These plots also included trees inoculated with root pieces with or without oxytetracycline treatment. In Sao Paulo State, Brazil, one plot was established in 1986. In Florida, two plots were established in 1986, one in the Central Ridge area, the second in the Southwest Flatwoods. All experiments have been evaluated yearly for visible symptoms of blight, and recent evaluations have been made using the diagnostic tests developed for blight: zinc analysis, water uptake tests and/or serological assays for the blight-related 12 kDa protein. In South Africa, no blight has developed in self-grafted control trees self grafted or in trees grafted with bark patches collected from blighted trees, but some of the trees grafted with root pieces collected from blighted trees have developed blight. In Brazil, no visible blight symptoms have developed, but an equal number of grafted and self-grafted control trees have reacted positively in the blight serological assay. In the two Florida experiments, blight has developed with about equal incidence in trees graft-inoculated with bark patches collected from blighted trees and in trees which were self-grafted with their own bark patches. These results suggests that blight is not transmitted by the bark patch grafting method evaluated in this study.

*Index words*. Declinio, serology, root grafting.

Citrus blight is a serious disease of unknown etiology which limits citrus production in Florida. The disease was first described in Florida in 1896 by Swingle and Webber (14). An estimated one half million trees are removed from production annually in Florida due to blight (17). A similar disease in Brazil, called Declinio, was first reported in 1968 in Sao Paulo State (11). This disease removes from production an estimated 10 million trees per year in Brazil (2). In South Africa, a blight-like decline was first reported in 1979 (4). Later, it was confirmed that this disease had the characteristics of blight and a survey was done to better determine its distribution in South Africa (7). Blight is a serious production problem in the warmer areas of the Transvaal and while present in Natal, it does not severely limit citrus production in that region (7).

While blight remains a disease with unknown etiology, a number of diagnostic markers have been identified to differentiate blight from other citrus tree declines. These markers are: accumulation of zinc in the trunk wood (18), lack of water uptake into the trunk wood (8), presence of amorphous plugs in the xylem (3), and presence of blight related proteins (5). By these four diagnostic markers, blight from Florida, Brazil and South Africa are closely related diseases and probably the same disease (6, 7, 10).

In 1983 blight was graft transmitted by the use of approach root grafts to healthy receptor trees, and all the symptoms and diagnostic markers of blight were reproduced in the receptor trees (16). Since that initial indication that blight may be caused by a graft transmissible infectious agent, blight has been graft transmitted by approach root graft, and use of root piece inoculations repeatedly in Florida (15), Brazil (12), and South Africa (9).

We now report on a series of experiments established in Florida, Brazil and South Africa to determine if citrus blight could be transmitted by the use of bark patches (phloem inoculum) collected above and below the budunion on the trunk of blight-affected trees.

## MATERIALS AND METHODS

**Diagnosis for citrus blight.** Canopy symptoms were rated on a scale of 0 - 3; 0 = healthy; 1 = mild symptoms (small leaves with blotchy mottle patterns and zinc deficiency symptoms, but no thinning of the foliage), 2 = moderate (leaves small, often flaccid, with blotchy mottle patterns and zinc deficiency symptoms, small fruits, delayed blossom and sparse canopy), 3 = severe (canopy sparse, substantial twig dieback, small fruits, delayed blossom, wilt and frequent trunk sprouting. Zinc concentration in trunk wood was determined by the method of Wutscher *et al.* (18). Water uptake was measured by the syringe injection method of Lee *et al.* (8). Serological assays were run as previously described by Derrick *et al.* (5) using the antiserum made against the 12 kDa blight-related protein (5).

**Grove selection.** In the Natal, South Africa, a grove of 9-yr-old Valencia orange on rough lemon rootstock was selected in an area where blight did not occur in the immediate vicinity. The grove had been planted on land previously cropped with sugarcane, this was the first time it had been planted to citrus. The experiment was initiated in September, 1985. In the Northern Transvaal, South Africa, a grove of 4-yr-old Valencia orange on rough lemon rootstock was selected. There was no blight in the immediate vicinity of the trees selected, but blight does occur at a low incidence in the area (7). The experiment was initiated in October, 1985. At both South African locations, the experiment had four treatments with six trees in each treatment: self grafted controls (control), graft-inoculation with bark patches collected from a blighted tree (bark patch), graft-inoculation with root pieces from a blighted tree (root piece), and graft-inoculation with root pieces collected from a blighted tree after treatment with oxytetracycline (root piece + PMT).

In Brazil, a 3-yr-old grove of Valencia orange on Rangpur lime rootstock was selected at Sete Lagoas farm, Sao Paulo State. The land had previously been planted to sugarcane. There was no blight in the grove, although blight occurs at a high incidence in surrounding groves of trees on Rangpur lime rootstocks. The experiment was initiated in May, 1986. Twenty trees were selected for each of two treatments: graft-inoculation with bark patches collected from a blighted tree (bark patch) and self-inoculation with their own bark patches (control).

In Florida, two grove locations were selected. In the Central Ridge area where deep sandy soils occur, a 4-yr-old grove of Hamlin orange on Volkamer lemon rootstock was chosen. This land had been cleared of the previous citrus (Valencia orange on rough lemon rootstock) due to a high incidence of blight and replanted. Blight occurs in the surrounding area at a high incidence. The experiment was initiated in July, 1986. The second location was in the Southwest Flatwoods with bedded groves in an area having a high water table. The grove selected was 4-yr-old Valencia orange on Volkamer lemon rootstock and had been planted on land which was previously in pasture. The experiment was initiated in September, 1986. At both Florida locations two treatments were used: 12 trees were graft-inoculated with bark patches collected from a blighted tree (bark patch) and 12 trees were self-inoculated with their own bark patches (control).

**Grafting procedures.** At all locations, a blight-affected tree was selected from the general area (about 1 km away), which had all the diagnostic symptoms of blight. This tree was used as the donor for the bark patches which were to be grafted onto the selected receptor trees. In the South African locations, this selected blighted tree was also used as the source for roots used as inoculum for root grafts.

A grafting tool (Forestry Supply Inc.) which cuts a rectangular patch about 1.5 by 2.5 cm was used to obtain bark patches. A hammer was used to tap on the handle of the template to cut through the bark, then the bark pieces were carefully removed and placed on a damp paper towel on ice until used. Care was taken to replace the patches in the proper vertical orientation when they were used for grafting. Patches were cut above and below the budunion on the blight source tree, and these were grafted to the receptor trees at about 20 cm above the budunion and 5 cm below the budunion, respectively, after using the tool to remove a bark patch from the receptor tree. Control trees were self-grafted by cutting the bark patches from their trunk above and below the bud union, removing the bark patch from the tool, then regrafting to the same trees. All grafts were tightly wrapped with heavy plastic strips about 5 cm wide by 1 m long. The survival of the bark patches was observed after three weeks, and trees regrafted if necessary.

In South Africa, grafting with root pieces collected from the blighted tree, with and without treatment with oxytetracycline, was also done. Roots were collected from the blighted source tree and roots having no water flow were selected (15). Five root pieces, each about 10-15 cm long, were grafted to the roots of each of six receptor trees. For another six receptor trees, the roots collected from the blighted tree were immersed in a 300 ppm ai oxytetracycline solution made in water, a vacuum pulled on the solution using a bell jar, after 3 min the vacuum was released, the antibiotic solution replenished, and the roots were again submerged and vacuum infiltrated for 3 min. The roots were then grafted onto six receptor trees, using five roots per tree. Survival of the grafted roots was checked about 1 month after grafting, at least four of the five roots were still alive at that time.

TABLE 1
SUMMARY OF THE BLIGHT DIAGNOSTIC MARKERS OF RECEPTOR TREES GRAFT-INOCULATED WITH BARK PATCHES, ROOT PIECES, OR ROOT PIECES TREATED WITH OXYTETRACYCLINE (PMT) FROM BLIGHTED TREES IN 1985 AND THE SELF-GRAFTED CONTROL TREES IN SOUTH AFRICA

| Treatment | Canopy rating[z] | Water uptake (ml/sec) | Wood zinc (ppm) | Diagnosis |
|---|---|---|---|---|
| *Northern Transvaal* | | | | |
| Control 1 | 0 | 0.8 | 3.0 | Healthy |
| Control 2 | 0 | 1.1 | 2.0 | Healthy |
| Control 3 | 0 | 1.0 | 1.0 | Healthy |
| Control 4 | 0 | 1.1 | 2.0 | Healthy |
| Control 5 | 0 | 0.7 | 1.0 | Healthy |
| Control 6 | 0 | 1.0 | 1.0 | Healthy |
| Bark patch 1 | 0 | 0.7 | 1.0 | Healthy |
| Bark patch 2 | 0 | 1.0 | 1.0 | Healthy |
| Bark patch 3 | 0 | 1.3 | 1.0 | Healthy |
| Bark patch 4 | 0 | 0.9 | 1.0 | Healthy |
| Bark patch 5 | 0 | 1.3 | 1.0 | Healthy |
| Bark patch 6 | 0 | 0.9 | 1.0 | Healthy |
| Root pieces 1 | 1.5 | 1.1 | 2.0 | Healthy? |
| Root pieces 2 | 1.5 | 0.0 | 33.0 | Blight |
| Root pieces 3 | 0 | 0.9 | 1.0 | Healthy |
| Root pieces 4 | 0 | 1.0 | 1.0 | Healthy |
| Root pieces 5 | 0 | 1.1 | 1.0 | Healthy |
| Root pieces 6 | 2 | 0.0 | 39.0 | Blight |
| Root pieces + PMT 1 | 0 | 0.9 | 1.0 | Healthy |
| Root pieces + PMT 2 | 0 | 1.4 | 3.0 | Healthy |
| Root pieces + PMT 3 | 0 | 0.7 | 1.0 | Healthy |
| Root pieces + PMT 4 | 0 | 1.0 | 1.0 | Healthy |
| Root pieces + PMT 5 | 0 | 1.1 | 1.0 | Healthy |
| Root pieces + PMT 6 | 0 | 1.3 | 1.0 | Healthy |

TABLE 1 (CONTINUED)
SUMMARY OF THE BLIGHT DIAGNOSTIC MARKERS OF RECEPTOR TREES GRAFT-INOCULATED WITH BARK PATCHES, ROOT PIECES, OR ROOT PIECES TREATED WITH OXYTETRACYCLINE (PMT) FROM BLIGHTED TREES IN 1985 AND THE SELF-GRAFTED CONTROL TREES IN SOUTH AFRICA

| Treatment | Canopy rating[z] | Water uptake (ml/sec) | Wood zinc (ppm) | Diagnosis |
|---|---|---|---|---|
| *Natal location* | | | | |
| Control 1 | 0 | 0.7 | 4.0 | Healthy |
| Control 2 | 0 | 1.1 | 3.0 | Healthy |
| Control 3 | 0 | 0.7 | 9.0 | Healthy |
| Control 4 | 0 | 0.7 | 4.0 | Healthy |
| Control 5 | 0 | 0.7 | 3.0 | Healthy |
| Control 6 | 0 | 0.8 | 2.0 | Healthy |
| Bark patch 1 | 0 | 0.8 | 6.0 | Healthy |
| Bark patch 2 | 0 | 0.5 | 12.0 | Healthy |
| Bark patch 3 | 0 | 0.7 | 5.0 | Healthy |
| Bark patch 4 | 0 | 0.7 | 1.0 | Healthy |
| Bark patch 5 | 0 | 0.7 | 6.0 | Healthy |
| Bark patch 6 | 0 | 1.0 | 2.0 | Healthy |
| Root pieces 1 | 0 | 0.8 | 12.0 | Healthy |
| Root pieces 2 | 0 | 0.8 | 8.0 | Healthy |
| Root pieces 3 | 0 | 0.8 | 13.0 | Healthy |
| Root pieces 4 | 0 | 0.5 | 2.0 | Healthy |
| Root pieces 5 | 0 | 0.5 | 2.0 | Healthy |
| Root pieces 6 | 3 | 0.0 | 16.0 | Blight |
| Root pieces + PMT 1 | 0 | 0.8 | 27.0 | Healthy |
| Root pieces + PMT 2 | 0 | 0.7 | 12.0 | Healthy |
| Root pieces + PMT 3 | 0 | 1.0 | 10.0 | Healthy |
| Root pieces + PMT 4 | 3 | 0.0 | 18.0 | Blight |
| Root pieces + PMT 5 | 0 | 0.7 | 26.0 | Healthy |
| Root pieces + PMT 6 | 0 | 0.8 | 1.0 | Healthy |

[z]Canopy symptoms were rated on a 0 - 3 visual scale in which 0 = healthy and 3 = severe blight affected as described in Materials and Methods.

## RESULTS

The zinc concentration and water uptake, respectively, for the blighted trees used as source on inoculum blight bark patches/roots were: Northern Transvaal, South Africa, 38.0 ppm, 0 ml/sec water uptake; Natal, South Africa, 20.0 ppm, 0 ml/sec water uptake; Sete Lagoas, Brazil, 7.5 ppm, 0 ml/sec water uptake; Central Florida, 9.0 ppm, 0 ml/sec water uptake; and Southwest Flatwoods, Florida, 2.5 ppm, 0 ml/sec water uptake. Amorphous plugs were present in all xylem vessels of the trunk wood from all donor trees.

At the time of inoculation, the average zinc concentration and water uptake, respectively, for the receptor trees were: Northern Transvaal, South Africa, 2.4 ppm, > 1.0 ml/sec water uptake; Natal, South Africa, 6.4 ppm, 0.5 ml/sec water uptake; Sete Lagoas, Brazil, 2.9 ppm, 0.65 ml/sec water uptake; Central Florida, 0.7 ppm, 0.73 ml/sec water uptake; and Southwest Flatwoods, Florida, 1.8 ppm, 0.85 ml/sec water uptake. The zinc concentration of the receptor trees in the South Africa trees was not determined at the time of inoculation due to the small size of the trees.

The trees in South Africa were last evaluated for blight in September, 1992. The results are summarized in Table 1. In the Northern Transvaal, none of the trees which were self grafted (control) or graft-inoculated with bark pieces from blighted trees (bark patches) developed blight, three of six trees graft-inoculated with root pieces from blighted trees (root piece) developed blight, and none of the trees graft-inoculated with root pieces which had been treated with oxytetracycline antibiotic (root piece + PMT) developed blight. At Natal, there was no blight in any of the control trees or trees graft-inoculated with bark patches, and one tree out of six developed blight in the treatments, root pieces and root pieces + PMT. Additionally there were one, one, two and four trees in the treatments: control, bark patch, root piece, and root piece + PMT, respectively, which had high zinc content but appeared healthy and had good water uptake. These trees might be developing blight.

In Brazil, the trees were evaluated by water uptake and zinc analysis of the trunk wood in January 1991. At that time, there was no indication of reduced water uptake or zinc accumulation in any of the trees (data not shown). In January 1992, the trees were visually rated for blight, and samples collected for serological assay. No blight symptoms were present in any of the trees. Seven of the 20 trees graft-inoculated using bark patches from blighted trees and seven of the 20 trees which were self-inoculated with their own bark patches reacted positively in the blight serological assay, suggesting these trees might soon develop blight symptoms.

Trees at the Central Ridge location in Florida were evaluated in June, 1992. The results are summarized in Table 2. Four of 12 trees inoculated with bark patches had developed blight, and

TABLE 2
SUMMARY OF THE BLIGHT DIAGNOSTIC MARKERS OF RECEPTOR TREES GRAFT-INOCULATED WITH BARK PATCHES FROM A BLIGHT-AFFECTED OR SELF-GRAFTED CONTROL TREES AT THE CENTRAL RIDGE LOCATION, FLORIDA. TREES WERE GRAFT-INOCULATED IN SEPTEMBER 1986 AND EVALUATED IN JUNE 1992

| Treatment | Canopy Rating[z] | Water uptake (ml/sec) | Wood zinc (ppm) | Serology | Diagnosis |
|---|---|---|---|---|---|
| Bark patch 1 | | (previously removed due to blight) | | | Blight |
| Bark patch 2 | | (previously removed due to blight) | | | Blight |
| Bark patch 3 | 0 | 0.0 | 5.3 | – | Healthy |
| Bark patch 4 | 0 | 0.1 | 1.0 | – | Healthy |
| Bark patch 5 | 0 | 0.1 | 1.1 | – | Healthy |
| Bark patch 6 | 0 | 0.1 | 1.4 | – | Healthy |
| Bark patch 7 | 0 | 0.1 | 1.1 | – | Healthy |
| Bark patch 8 | 2 | 0.0 | 3.9 | + | Blight |
| Bark patch 9 | 2 | 0.0 | 1.8 | + | Blight |
| Bark patch 10 | 0 | 0.0 | 1.8 | – | Healthy |
| Bark patch 11 | 0 | 0.1 | 1.4 | – | Healthy |
| Bark patch 12 | 0 | 0.0 | 2.3 | – | Healthy |
| Control 1 | 0 | 0.1 | 1.5 | – | Healthy |
| Control 2 | 0 | 0.1 | 1.5 | – | Healthy |
| Control 3 | 0.5 | 0.1 | 8.1 | + | Blight |
| Control 4 | 1 | 0.0 | 1.9 | + | Blight |
| Control 5 | 0 | 0.1 | 1.1 | – | Healthy |
| Control 6 | 0 | 0.1 | 1.1 | – | Healthy |
| Control 7 | 0 | 0.1 | 1.1 | – | Healthy |
| Control 8 | 0 | 0.0 | 1.8 | – | Healthy |
| Control 9 | | (previously removed due to blight) | | | Blight |
| Control 10 | 0 | 0.1 | 0.9 | – | Healthy |
| Control 11 | 0 | 0.2 | 5.1 | – | Healthy |
| Control 12 | 0 | 0.1 | 1.8 | – | Healthy |

[z]Canopy symptoms were rated on a 0 - 3 visual scale in which 0 = healthy and 3 = severe blight affected as described in Materials and Methods.

one tree had high zinc content, but none of the other diagnostic indications of blight. Four of 12 control trees had developed blight. Trees surrounding the experiment were developing blight with about 30% incidence. Trees at the Southwest Flatwoods were evaluated in April, 1992, and the results are summarized in Table 3. Four of 12 control trees had developed blight, and one had high zinc concentration, but none of the other diagnostic markers of blight. Three of 12 trees which were graft-inoculated with bark patches from a blighted tree had developed blight, and two more had high zinc level, but did not have typical blight symptoms. Most of the trees at the Southwest Flatwoods location have poor water uptake, and the trees were being adversely affected by recurring high water levels. Blight also is naturally spreading in this location and the plantings surrounding this experiment were having about 25% incidence of infected trees.

## DISCUSSION

The trees and locations used for this series of experiments to determine if citrus blight could be graft-transmitted by using bark patches (phloem tissue) were carefully chosen. The grove locations were selected in areas where blight was not occurring at the initiation of the experiment, and several plots were established in areas which had not been planted to citrus previously. Where possible, young groves were selected. The Florida plot locations have reached the point where blight is naturally occurring at 25-30% incidence in the area, and blight is beginning to occur in the other geographic locations. It will be increasingly difficult from now on to determine if trees are developing blight because of

TABLE 3

SUMMARY OF THE BLIGHT DIAGNOSTIC MARKERS OF RECEPTOR TREES GRAFT-INOCULATED WITH BARK PATCHES FROM A BLIGHT-AFFECTED OR SELF-GRAFTED CONTROL TREES AT THE SOUTHWEST FLATWOODS LOCATION, FLORIDA. TREES WERE GRAFT-INOCULATED IN JULY 1986 AND EVALUATED IN APRIL 1992

| Treatment | Canopy Rating[z] | Water uptake (ml/sec) | Wood zinc (ppm) | Serology | Diagnosis |
|---|---|---|---|---|---|
| Bark patch 1 | 0 | 0.2 | 3.1 | – | Healthy |
| Bark patch 2 | 0 | 0.2 | 2.1 | – | Healthy |
| Bark patch 3 | 0 | 0.1 | 7.4 | – | Healthy? |
| Bark patch 4 | 1 | 0.1 | 6.6 | – | Healthy? |
| Bark patch 5 | 0 | 0.0 | 15.3 | – | Healthy? |
| Bark patch 6 | 0 | 0.0 | 3.8 | – | Healthy |
| Bark patch 7 | 1.5 | 0.0 | 15.3 | + | Blight |
| Bark patch 8 | | (previously removed due to blight) | | | Blight |
| Bark patch 9 | 0 | 0.2 | 2.1 | – | Healthy |
| Bark patch 10 | 0 | 0.1 | 10.3 | – | Healthy? |
| Bark patch 11 | | (previously removed due to blight) | | | Blight |
| Bark patch 12 | 0 | 0.8 | 3.0 | – | Healthy |
| Control 1 | 0 | 0.1 | 14.1 | – | Healthy? |
| Control 2 | 0 | 0.1 | 2.4 | – | Healthy |
| Control 3 | 0 | 0.1 | 2.8 | – | Healthy |
| Control 4 | 0.5 | 0.1 | 22.3 | + | Blight |
| Control 5 | 0 | 0.1 | 3.1 | – | Healthy |
| Control 6 | 0 | 0.3 | 2.3 | – | Healthy |
| Control 7 | | (previously removed due to blight) | | | Blight |
| Control 8 | 0 | 0.0 | 4.5 | – | Healthy |
| Control 9 | 0 | 0.4 | 4.1 | – | Healthy |
| Control 10 | 0.5 | 0.1 | 8.9 | + | Blight |
| Control 11 | 0 | 0.2 | 3.1 | – | Healthy |
| Control 12 | 0.5 | 0.1 | 16.3 | + | Blight |

[z]Canopy symptoms were rated on a 0 - 3 visual scale in which 0 = healthy and 3 = severe blight affected as described in Materials and Methods.

the graft transmission of the blight agent, or through natural disease spread. For these reasons, we decided to evaluate and summarize the present status of these experiments.

In all locations, there was little difference in the number of trees developing blight between the control trees and trees inoculated with bark patches. In both Florida locations, the incidence of blight in both treatments was essentially the same. In Brazil, the first trees in the grove were beginning to develop blight. While none of the experimental trees showed visual symptoms of blight, there was an equal number (7) of trees reacting positively to the blight serological assay in both control and bark patch treatments. In the South African locations, where blight had not yet moved into the groves, none of the control or bark patch inoculated trees had developed blight, whereas three out of six trees at the Northern Transvaal and one of six at the Natal location did develop blight when root pieces from a blighted tree were used as the inoculum. If the trees with high zinc levels, but none of the other diagnostic markers of blight, eventually develop blight, this would make transmission rates of one of six, one of six, three of six, and five of six for the treatments: control, bark patch, root piece, and root piece + PMT, respectively. In all instances, there is no indication that the bark patch inoculations as described in this paper transmitted blight.

Albrigo *et al.* (1) have reported the failure to transmit citrus blight by continuous limb grafts, and Rossetti *et al.* (13) have reported the lack of transmission by use of above ground plant parts. Earlier experiments indicated the lack of transmission of blight by the use of budwood inoculum (17, 19). The transmission of blight by the use of root pieces grafted onto roots of receptor trees in the South African locations further demonstrates the infectious nature of the blight agent, indicates the presence of the infectious agent in the roots, and serves to contrast blight transmission by root grafting but not by bark patch inoculum.

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