

Cohen (2). Wood samples were taken on both sides of the trunk of 18 declining and 9 healthy trees and analyzed for Zn, K, and water-soluble phenolics (6, 7). The trees carried a mild strain of tristeza (G. D. Bridges, personal communication); therefore, the roots of 4 declining and 4 healthy trees were tested for starch with the iodine method (dipping in 2% KI — 0.2% I₂ solution). The F-test for a completely randomized design was used to determine statistically significant differences.

Results and Discussion

The trees grew rapidly after planting and produced well when they were still young. Yields increased from 21.4 tons/ha (218 90-lb boxes/acre) and 1251 kg solids/ha (1147 lb solids/acre) when the trees were 5 years old to 46.6 tons/ha (475 90-lb boxes/acre) and 2553 kg solids/ha (2340 lb/acre) in 1979 when the trees were 9 years old. These yields were higher than those of trees on rough lemon rootstock in adjacent blocks. Periodic Mg deficiency symptoms were the only abnormality observed in the 4 beds on *C. macrophylla* rootstock until they were 8 years old. In 1979, 2, 6, 5 and 6% of the trees on the 4 beds showed visual symptoms of blight.

Tests for blight in May 1980 showed that healthy trees absorbed more than 10 times as much water as declining trees (Table 1) during 24-hr trunk injections; these absorption rates were somewhat higher than those reported by Cohen (2). This result is probably attributable to the larger water-injection holes we used. The zinc content of the outer trunk wood of declining trees was 3 times greater than that of healthy trees (Table 1). The water-soluble phenolics in the wood of declining trees were 43% higher than in healthy trees (Table 1). With all 3 blight tests positive, there can be little doubt that the decline of the 18 trees checked was due to blight. Potassium was 59% higher in the wood of declining trees than in the wood of healthy trees, and was also an indication of blight (7).

Trees on *C. macrophylla* are tristeza susceptible (5), but the visual appearance of the trees and their performance show that the virus strain present in the trees used in this experiment probably causes little damage. Iodine tests showed large amounts of starch present in the roots of the declining and healthy trees, indicating that tristeza had little effect on the trees.

Table 1. Water absorption and levels of zinc, potassium, and water-soluble phenolics in the wood of declining and healthy 9-year-old 'Pineapple' orange/*C. macrophylla* trees.

| | Water absorption ^a (ml/24 hr) | Zn (ppm) ^a | K (%) ^a | Water-soluble phenolics (mg/g) ^a |
|-----------|---|--------------------------|-----------------------|--|
| Declining | 77 | 13 | .316 | 5.4 |
| Healthy | 959 | 3 | .199 | 3.8 |

^aMeans of 10 declining and 5 healthy trees. Difference significant at the 1% probability level.

^aMeans of 18 declining and 9 healthy trees. Differences significant at the 1% probability level.

The cause of the tree decline was blight. *C. macrophylla* should therefore be considered blight susceptible. The number of blight-affected trees found in the blocks observed indicates that this rootstock is quite susceptible to blight, although the disorder appeared later than in trees on rough lemon at this location. However, 24 15-year-old 'Marsh' grapefruit (*C. paradisi* Macf.) trees on *C. macrophylla* rootstock at another location in the same area show no signs of blight (Mortimer Cohen, personal communication). Discrepancies like this are common with blight and cannot be explained until the cause of the disorder is found.

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SIMILARITIES BETWEEN CITRUS BLIGHT AND PIERCE'S DISEASE OF GRAPEVINE¹

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Abstract. Citrus blight and Pierce's disease of grapevine are both wilt- and dieback-type diseases that have many

symptoms in common. The common symptoms include leaf mottle, delayed spring growth flush, wilting, decline of vigor, twig dieback, and plugs in xylem vessels with a concomitant reduction in water flow rate. In both diseases, symptoms usually appear first in only 1 branch of the vine or tree. Pierce's disease is found in wild grapevines throughout the citrus-growing areas of Florida, and the leafhopper vectors of Pierce's disease commonly feed in great numbers on citrus. Using the leafhopper vector, the Pierce's disease bacterium was obtained from citrus with blight and was transmitted to grape. In greenhouse inoculations, the Pierce's disease bacterium produced dieback-type symptoms on rough lemon citrus plants.

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Citrus blight, also known as young tree decline (YTD) or sand hill decline, causes extensive losses in citrus groves and currently is the most serious disease affecting Florida citrus. The etiology of blight has not been determined although there have been many attempts to determine the causal agent (4, 8, 10, 12). Blight has been a problem in citrus groves since the 1870's and may be endemic to Florida (14).

Pierce's disease (PD) of grapevines is the principal factor limiting bunch grape production in Florida and the Southeast. The causal agent of Pierce's disease was considered to be a virus until 1970, but is now known to be caused by a small fastidious bacterium referred to as rickettsialike (3, 9). Pierce's disease is endemic to Florida.

Common Characteristics of Citrus Blight and Pierce's Disease of Grapevine

Both of these diseases produce wilt-type symptoms which suggest that disruption of the water-conducting system is the major mechanism of pathogenesis. Wilting, delayed spring growth flush, decline of vigor, and dieback are symptoms common to both diseases. Symptoms often develop first on only one branch or sector of a blighted tree or PD-infected vine. Wilting of PD-infected grapevines is not a usual symptom, but in some cases a vine or branch will wilt suddenly and die with no obvious prior symptoms. Dwarfing, as short internodes and smaller leaves, occurs in both grape and citrus; in citrus the smaller leaves often are dull green and upright in growth habit. Foliage is thin on blighted trees, and leaves develop marginal necrosis and drop from grapevines with PD, sometimes defoliating the vines. Dieback of twigs and branches can be severe in both cases but generally is more rapid in grapevine. Death of the root system usually follows the death of the top in grapevine with PD. The crown of the grapevine is the last part to die, with new roots and suckers often arising from the crown; blighted citrus also tends to produce water sprouts from the trunk of the tree.

Chlorotic leaf mottling occurs with both blight and PD. In citrus, the mottling is a zinc deficiency pattern. In grapevine, the symptom is somewhat similar to a manganese deficiency pattern. In both disease, fruit production declines and fruit size diminishes. Grapes may wither on the vine.

Internal symptoms of these two diseases include the occlusion of xylem vessels. The occlusions in PD-infected grapevines include bacterial aggregates, gum plugs, and tyloses; in blighted citrus, gum and lipid plugs have been reported (11, 16). With PD, vascular occlusions are found to be most numerous in leaves and petioles; whereas, with blight, occlusions are more numerous in the older wood.

Restricted water movement and zinc accumulation in the wood of diseased trees are the major physiological symptoms of blight (2, 5, 13). We have found in antibiotic studies and in vacuum extraction attempts that reduced water uptake and movement also occur with PD-infected grapevine.

Stress factors appear to play a role in symptom development with both diseases. Observations indicate that disking, irrigation practices, hedging, and fruit production may influence the occurrence of blight symptoms. Symptoms usually don't develop until trees are 8 or more years of age and in full production. The European-type bunch grape (*Vitis vinifera* L.) is completely susceptible to PD and dies under most conditions, but the Florida bunch grapes and muscadines are more tolerant to PD and react more like

citrus does to blight. With muscadines, we observed that cutting roots by disking increased symptom development; and with some cultivars, initiation or aggravation of symptoms often occurred under the stress of heavy fruit yields.

Tetracycline antibiotics, which are effective against rickettsialike bacteria, have provided some remission of symptoms of citrus blight and of PD. Tucker et al. (15) obtained some improvement in 3 of 5 trees with blight which were treated with soil drenches of oxytetracycline-HCl. Soil drenching with tetracycline antibiotics provided symptom remission in a low percentage (<30%) of mature, infested grapevines (7). Preventive drench and foliar spray treatments were more effective.

Two of the principal leafhopper vectors of PD also commonly feed on citrus in Florida. Wild grapes in Florida are good sources of the PD bacterium and these infected wild grapes are often found around citrus groves. The PD bacterium also has a wide host range that includes many grasses and weeds that occur in Florida. Leafhopper vectors commonly feed on these wild grapes, weed hosts of PD, and citrus—certainly exposing the citrus trees to the bacterium (1).

The association of infected wild grapes with blighted groves is especially intriguing. In a few groves we have observed locations at the periphery where blight symptoms occurred in a half-circle pattern in the grove, and infected wild grapevines were found adjacent to these locations. These observations and the similarities between citrus blight and PD prompted us to perform the following experiments.

Recovery of PD Bacterium from Blighted Citrus

Oncometopia nigricans (Walker) leafhoppers, an important vector of PD of grape in Florida, were field-collected from a single location and shown not to be naturally infective with Pierce's disease. These leafhoppers were used in attempts to recover the PD bacterium from blighted citrus and transmit it to grapevine (8).

Trees on rough lemon (*Citrus jambhiri* Lush.) root stocks and with obvious symptoms of blight were selected for the transmission studies. The trees used had high zinc content in the wood and very poor water flow rates.

Generally 10-20 leafhoppers were caged on a branch of the blighted trees for 4 days. The surviving leafhoppers, usually 5-10, from each source tree were caged on a Carignane indicator grapevine for 2 weeks. The indicators were observed for the next 4 months for symptom development. When PD symptoms developed in the indicator, the bacterium was cultured from the grapevine using techniques and medium of Davis, et al. (3).

The PD bacterium was recovered from 2 of the blighted trees by leafhopper vectors and transmitted by them to the indicator grapevines, producing typical PD symptoms (Table 1). One of the transmissions, on May 23, was from a tree with severe blight symptoms and the other, on June 9, was from a tree with moderate symptoms. A bacterium was isolated from both these indicator grapevines on the JD-3 medium. Agglutination tests indicated that it was the PD bacterium, or very closely related to it serologically (8).

Since the bacterium was obtained and transmitted from citrus to grape with leafhoppers, these studies demonstrated that the PD bacterium can be present in blighted citrus trees. The PD bacterium is known to have a wide host range that includes members of at least 28 families of monocotyledonous and dicotyledonous plants and many of the hosts are symptomless (6). Involvement of the PD bacterium in the etiology of blight was not proven in these

Table 1. Transmission of the Pierce's disease (PD) bacterium from blighted citrus trees to grapevine in 1977.

| Blight tree ^z | PD symptoms in indicator grapevine ^y | | |
|--------------------------|---|-------|-------|
| | 5/9 | 5/23 | 6/13 |
| 1 — severe | — (6) | + (5) | NT |
| 2 — moderate | NT | NT | + (4) |
| 3 — mild | — (8) | — (9) | — (5) |
| 4 — severe | — (5) | — (3) | NT |
| 5 — moderate | — (5) | — (6) | NT |
| 6 — mild | NT | NT | — (5) |

^zSevere, moderate, and mild refer to severity of blight symptoms on citrus.

^yThe date insects were first placed on the blight tree, where they remained for 4 days. The surviving leafhoppers, number in parenthesis, were caged on Carignane grapevines for 2 weeks in the greenhouse. The + or — refers to the presence or absence of PD symptoms in the Carignane grapevine. NT indicates not tested.

studies, and citrus could be merely another symptomless host.

Numerous attempts to culture the PD bacterium directly from blighted citrus have failed. Other workers have used vacuum extractions of vascular fluid to demonstrate the presence of bacteria with rickettsia-like morphology in citrus (4). Lee, et al. (10) used immunofluorescence techniques to demonstrate the presence of rickettsia-like bacteria in vacuum filtrates collected from rough lemon roots of trees showing blight symptoms.

Inoculation of Rough Lemon Citrus with the PD Bacterium

To investigate the possible involvement of the PD bacterium in blight, we inoculated 6 plants of rough lemon with each of 3 PD bacterium isolates. The isolates used were the two recovered from blighted citrus (8) and one from grape. The plants were inoculated in December 1977 by making an L-shaped cut, halfway through the stem and extending 1 inch longitudinally in the stem. The spur resulting from the cut was submerged in a bacterial suspension ($>10^8$ cells/ml) in a vial. The suspension was taken up by the spur. The plants were reinoculated during the winter of 1978-79 by pin pricking the branches through drops of bacterial suspension.

Dieback-type symptoms developed in 5 of 7 inoculated rough lemon plants maintained in the greenhouse (Table 2). Symptoms were first observed in August 1978. Symptoms included curling and tipburn of younger leaves, leaf drop, and dieback of young branches from the tip. The repeated production of new shoots that died back resulted in a bushy-type plant. Immunofluorescence was used to detect the PD bacterium in 4 of the 5 rough lemon plants that showed dieback symptoms.

Table 2. Greenhouse inoculations of rough lemon citrus with the Pierce's disease bacterium.

| Treatment | Bacteria detected | |
|--------------|-------------------|-----------------------|
| | Dieback symptoms | by immunofluorescence |
| Inoculated | 5/7 | 4/7 |
| Uninoculated | 0/7 | 0/7 |

Both the isolates from citrus and a grape isolate produced the symptoms in rough lemon. Although the dieback-type symptoms produced resemble those of blight, this does not prove that blight is caused by the PD

bacterium. We have not yet been able to produce symptoms in sweet orange on rough lemon rootstock, as occurs in the field. However, this is the first study in which the PD bacterium has been shown to produce disease symptoms in citrus.

Conclusions

Pierce's disease of grapevine and citrus blight have many symptoms, both external and internal, in common. Tetracycline antibiotics have suppressed the symptoms of both diseases. The two diseases are closely associated in the field in Florida and the leafhopper vectors of Pierce's disease feed on citrus in large numbers. The PD bacterium is present in some trees that have blight. The PD bacterium is pathogenic to rough lemon citrus producing die-back symptoms similar to blight, and rough lemon is known from field observations to be the rootstock most susceptible to blight.

Blight symptoms have not been produced on mature sweet orange citrus on a susceptible rootstock in the grove, as blight occurs naturally. Therefore, proof that the PD bacterium is a causal agent of blight has not been obtained. We believe that the results presented here support the hypothesis that the PD bacterium, or a closely related bacterium, may be involved in citrus blight. Thus, we are performing additional laboratory and field experiments to examine this possibility.

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