

## Stubborn and Related Diseases

### A Review of Stubborn and Greening Diseases of Citrus

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TO DATE there has been a steady increase in the number of countries in which the presence of stubborn, greening, or similar diseases has been proved or is suspected. The stubborn-greening complex has been recognized as a major threat to citriculture, especially in countries where a vector is present.

Studies during recent years have led to a better understanding of the symptomatology of stubborn and greening in relation to temperature and have shown that both stubborn and greening pathogens exist in various strains.

#### *Symptomatology*

The symptoms of stubborn (1, 5) and greening (12) in many citrus species and varieties have been described. Both diseases have practically the same host range. In addition, the sequence of species from those with the mildest to those with the most severe symptoms is the same for both. Similarities and dif-

ferences in symptomatology of the two diseases in the sweet orange are consistent with those in other citrus species susceptible to greening and stubborn.

#### *Geographical Occurrence*

Recently, a greeninglike disease has been conclusively shown to be present in the Philippines (11, 13, 22) and in India (7, 14) as well as in various other parts of the world reported previously. In Argentina, material from Malvasio tangerine trees showing a decline (18) and material from trees with stubbornlike symptoms found in an orchard in the Concordia Province as well as in Brazil (20, 21) gave positive results when tested with the fluorescence test (unpublished). Although these results are evidence that stubborn-greening pathogens are the cause or part of the cause of the disorders, the proof must await indexing in indicator seedlings.

*Correlation between  
Temperature,  
Symptomatology,  
and Transmissibility*

Greening symptoms are more severe in the low-lying hot areas of South Africa than in the high-lying cool areas (23). In California and Arizona, on the other hand, the best symptoms of stubborn were found in the hottest areas. In glasshouses, greening shows up best under cool conditions (24), but stubborn symptoms are best at temperatures of 30–35°C (17). Schwarz and Green (30) found a significant positive correlation between heat index for certain areas in South Africa and the symptomatology of greening in these areas. Whereas an annual heat index in excess of 1,200 DH/30 (total degree hours above a threshold of 30°C) inhibits symptoms of greening totally, a value less than 300 DH/30 allows them to develop fully along latitude 25.5°S in the eastern Transvaal.

The best transmission of stubborn (6) was obtained in midsummer when the temperatures are high in California, whereas the best transmission of greening (24, 29) was in midwinter, which is the coolest time of year.

*Heat Inactivation*

Olson and Rogers (17) found that a treatment in budwood of 90–120 min at 51°C is necessary for heat inactivation of the stubborn pathogen in water-saturated hot air, but Rois-tacher and Calavan (19) reported

that the stubborn pathogen is usually inactivated in budwood at 50°C for 3–4 hours, although occasionally it survived this treatment. They also showed that material preconditioned, i.e., kept first for 28 weeks at temperatures between 30 and 42°C, showed a considerably higher percentage of graft survival than that not preconditioned. Schwarz and Green (30) showed that the greening pathogen can be heat-inactivated by a budwood treatment for 60 min at 51°C, 120 min at 49°C, and 240 min at 47°C, indicating again that the greening pathogen is more sensitive to heat than is the stubborn pathogen.

*Strains of Stubborn and  
Greening Agents*

Studies by Calavan and Christian-sen (4), Calavan (2), and Schwarz (24, 27) have shown that the agents of stubborn and greening exist in various strains. The stubborn agent strains were differentiated on the basis of severity of symptoms they produced in Madam Vinous seedlings. The reaction ranged from severe to mild. The severe strains produced stunting and severe leaf symptoms; the mild strains produced the typical symptoms only in a few leaves. In cross-protection studies, mild strains failed to protect against infection with severe strains.

The greening agent strains were differentiated on the basis of the symptoms induced in inoculated trees grown under fully controlled conditions and in inoculated seedlings (27). As an additional criterion

for infection, results of the bark fluorescence test were used. On the basis of external symptoms, the greening pathogen strains were rated as either severe or mild. The severe strains spread rapidly in the tree, and they induced severe stunting, severe leaf symptoms, and a severe and prolonged fruit drop. They are relatively easy to transmit by graft-inoculation. The mild strains, on the other hand, are difficult to transmit; they spread slowly in the tree and induce milder symptoms. All these strains stimulate the production of the gentisoyl glucoside marker in the bark of sweet orange. The marker is also found in seedlings that have been inoculated but do not show external symptoms under cool conditions. It is postulated that the latter phenomenon is an indication of a latent strain.

The pathogens of greening and stubborn, with all their strains, differ substantially in their sensitivity to heat. This difference in sensitivity may prove helpful as a criterion in distinguishing between strains of the stubborn group and those of the greening group, even though it is possible that the two groups are closely related.

Olson (16) grafted healthy fruit of various citrus species to stubborn-infected sweet orange and grapefruit seedlings and found that typical stubborn symptoms developed on the grafted fruit of sweet orange, mandarin, tangelo, and grapefruit. The method is useful in studying the effect of various stubborn and greening strains on the fruit of sensitive citrus species.

### *Indexing Techniques*

One of the major tasks of the phytopathologist is to develop reliable and rapid techniques for indexing virus and viruslike diseases. These techniques can then be applied on a commercial scale to index mother trees. Results of studies of the seasonal transmissibility of stubborn (6) and greening (24, 29), as well as those of studies on the correlation between temperature and symptom development of stubborn (17) and greening (25, 30), are of importance in indexing for the stubborn and greening pathogens by inoculation of indicator seedlings. From these studies it appears advisable that the indexing should preferably be carried out at a certain time of year—in the hot season for stubborn, and in the cool season for greening. In addition, the inoculated seedlings should be kept at certain temperatures—those with greening at 20–23°C, and those with stubborn at 30–35°C. The irregular distribution of greening and stubborn in infected trees makes it necessary to take budwood from various parts of trees that are to be indexed and to inoculate at least 10 seedlings from each tree.

The chromatographic albedo and bark fluorescence test (25) has been shown to be specific for greening in sweet orange (8, 9, 25) and other citrus species (26). The fluorescence marker was also shown to be present in citrus trees affected by leaf mottle, grapefruit dieback, likubin, and stubborn (8, 28). The marker substance specifically found in

greening-affected tissue was shown to be gentisoyl glucoside (9). Indexing with the bark fluorescence test makes it possible to detect a latent component of the greening complex that cannot be detected by other techniques. Recent studies by Feldman and Hanks (9) and Schwarz (29) have shown that the concentration of gentisoyl glucoside fluctuates seasonally in stubborn- and greening-affected sweet orange.

### *Causative Agent*

So far no virus particles have been found in stubborn- or greening-

infected citrus tissues. Nour-Eldin (15) found a plasmodiumlike organism in tissue cultures of material from trees affected by Safargali disease. The symptoms of this disease resemble closely those of stubborn.

Lafèche and Bové (10) found bodies of the mycoplasma type in sweet orange seedlings infected with the greening pathogen by the citrus psylla. Similar bodies were found in stubborn-diseased tissue (3). These observations indicate that a mycoplasma-like organism is the causative agent of greening and stubborn.

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