

## Investigations on Impietratura Disease

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ALTHOUGH IMPIETRATURA was known to citrus growers in Sicily for many years, Ruggieri was the first to describe the characteristic symptoms of small, malformed, stone-like fruits with gum deposits in the albedo. He reproduced the disease by sandwich or bud inoculations on healthy bearing trees of sweet orange [*Citrus sinensis* (L.) Osb.], grapefruit (*C. paradisi* Macf.), and Clementine mandarin (*C. reticulata* Blanco), but not on lemon [*C. limon* (L.) Burm. f.] (5, 6, 7, 8). In Cyprus, Papasolomontos (4) obtained positive results on young stems of grapefruit and Valencia orange, using infected buds as inoculum. He reported extreme variability in the incubation period of the disease.

More recently, Terranova and Scuderi (8) transmitted impietratura virus from Femminello di Siracusa lemon trees into Moro sweet orange and Marsh seedless grapefruit trees by inoculating young stems during the blooming period. The results of further transmission tests and field observations are reported here.

### *Methods and Results*

According to Ruggieri (5, 6), symptoms of impietratura were easily reproduced by top working apparently healthy Moro and Tarocco orange on the sour orange rootstock or on the scion of diseased trees.

TRANSMISSION TESTS.—Transmission of the virus by bark tissues or buds of different citrus varieties was attempted, using as inoculum old-line Ovale orange and Marsh grapefruit trees that showed impietratura symptoms on the fruits. Analyses of these trees showed that boron was present in adequate amounts. Previously, the inoculated trees and the inoculum source trees were indexed for psorosis and in some cases for exocortis, with positive results.

Transmission tests on 4- to 5-year-old branches of Femminello lemon, Avana mandarin (*C. reticulata* Blanco), and sour orange (*C. aurantium* L.) trees in 1961 gave negative results.

In the last 3 years and at different seasons, 10-15 each of young trees of Moro, Sanguinello, Ovale, Tarocco, Valencia orange, Marsh grapefruit, S. Teresa lemon, Avana mandarin, and Clementine and sour orange were inoculated on 1- to 2-year-old stems, using different inoculum sources. Two grapefruit trees and 1 Ovale sweet orange tree showed

symptoms (Fig. 1,B) 1 year after inoculation (Nov., 1965); 2 other sweet orange trees were inoculated in April, 1965, and reacted positively after 18 months.

The Tarocco and Valencia sweet orange, S. Teresa lemon, Avana mandarin, and Clementine and sour orange seedlings showed no symptoms of impietratura 1 year after inoculation. Some inoculations made in April, 1965, on Ovale sweet orange and Marsh grapefruit, using the same inoculum source, gave positive results only on the latter test plant. Most of the fruit on trees of other varieties tested were lost, unfortunately, and no symptoms have appeared on the few remaining fruit. Inoculations of gum impregnated albedo tissue from diseased fruit of Marsh grapefruit directly into the peel of Ovale orange fruits were negative.

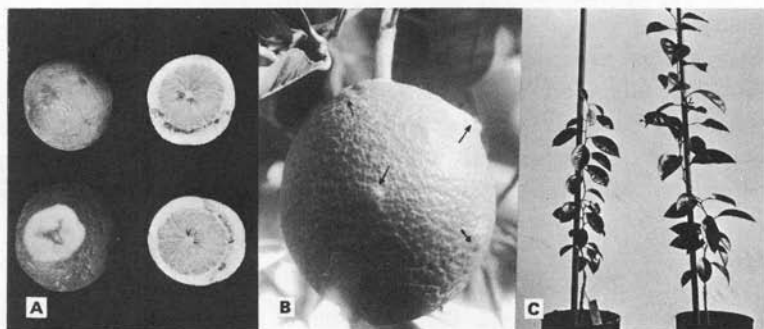


FIGURE 1. A. Symptoms of impietratura on fruits of Marsh seedless grapefruit. B. Impietratura symptoms on Ovale sweet orange fruit on an inoculated branch. C. Left: Reduction in growth of a Sanguinello seedling clone inoculated with impietratura. Right: Non-inoculated check.

Fruit arising on a sour orange sucker from the rootstock of an infected Ovale orange tree showed no symptoms, although symptoms were clear on fruit of the scion. Five Avana mandarin trees were top worked on 2 branches with buds from diseased Tarocco orange trees. After 2 years (2 crops), symptoms of impietratura were present on the Tarocco fruit, but not on the mandarin fruit. This suggests that sour orange and Avana mandarin are tolerant to the disease.

BEHAVIOR OF DIFFERENT CITRUS SPECIES AND VARIETIES INOCULATED WITH IMPIETRATURA.—Seedling clones of Sanguinello and Vaniglia orange, Eureka lemon, Joachimson grapefruit, Cleopatra mandarin (*C. reshni* Tanaka), and Orlando tangelo (*C. reticulata* x *C. paradisi*) were tested for their comparative reaction to impietratura. These varieties

were budded on sour orange seedlings previously inoculated with bark tissue or buds of infected trees.

The seedling clone of Sanguinello showed stunting 3 months after inoculation; 8 months after inoculation, growth was retarded by 20 cm in comparison with the check (Fig. 1,C). Other varieties showed no significant differences, and no other symptoms were observed.

That exocortis and stubborn viruses cause stunting is well known (1, 2, 3). Presumably, exocortis virus can be excluded in this case, because all the combinations used are tolerant to this virus and because only Sanguinello variety reacted to inoculation. Furthermore, symptoms of stubborn were never observed on the trees used as inoculum sources during the last 5 years, and no stunting was observed on Eureka lemon which is susceptible to stubborn (2, 3).

FIELD OBSERVATIONS.—An orchard of Sanguinello orange trees, 20 years old and showing symptoms of impietratura, was inspected periodically during the last 3 years to determine the spread of the disease. There are 360 trees in the orchard planted at a distance of 4 x 4 m. In 1964, 20 per cent of the trees showed symptoms of impietratura. In 1965 and 1966, 27 per cent and 31 per cent, respectively, showed symptoms. The increase in the number of trees infected with impietratura from 1964 to 1966, and their distribution, suggest that natural spread is occurring in this orchard, but the means is not clear. The appearance of symptoms in mature trees that have shown no symptoms previously and are located far from infected trees, strongly suggests transmission by insects.

Different citrus groves show considerable variability in the expression of symptoms. Even on surely infected trees, symptoms are not evident every year, or may be observed on fewer fruits. These results confirm those reported by Papasolomontos (4) in Cyprus. It appears also that the percentage of fruits with symptoms of impietratura is very low among late (summer) bloom fruit as compared with the percentage among normal spring bloom fruit. This situation is especially noticeable in Ovale orange which blooms 3 to 4 times per year. Therefore, it is possible that climatic factors may influence symptom expression of the disease.

### *Conclusions*

The results of transmission experiments reported here, confirm the virus nature of impietratura disease and emphasize that fruit symptoms and transmissibility of the disease are highly variable. Movement of the virus in the trees seems slow, but the incubation period is shorter when

inoculations are made near flowers. Natural spread of the disease occurs in the field, but the means is still not clear. In greenhouse tests, seedling clones of Sanguinello orange react very well to impietratura and show stunting. Sour orange and Avana mandarin appear tolerant in our experience.

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