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## Further Research on Impietratura Disease of Citrus

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RUGGIERI (2, 3, 4) was the first to describe impietratura disease and to show that it is caused by a bud transmissible virus although the symptoms had been known for many years in Sicily. His untimely death left much experimental work incomplete and unreported. This paper presents new information on impietratura and reports certain results from both Ruggieri's and our experiments.

IMPIETRATURA SYMPTOMS ON LEMON.—Symptoms on lemon have not been reported previously, but in 1964, trees of the Femminello di Sira-

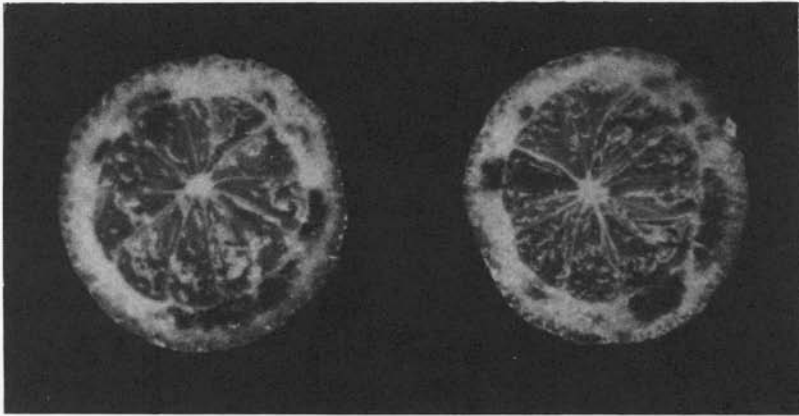


FIGURE 1. Fruits of Femminello di Siracusa lemon showing typical gumming in the albedo.

cusa variety of lemon [*Citrus limon* (L.) Burm. f.] in the Avola district were observed with fruits showing symptoms similar to those of impietratura-affected oranges (Fig. 1). These trees suffered severe fruit drop in July-August, a time when fruits were normally the size of a nut. Many of the remaining fruits exhibited malformed rind, small size (one-half to three-fourths of normal), early yellowing, and gum impregnated vascular bundles, especially in the columella. When ripe, these fruits fell easily from the trees. Over a two-year period, these symptoms reappeared with more or less severity. The winter crop (from March-April bloom) is the principal one affected. The "bianchetti" crop (from May-June bloom) and the "verdelli" fruits (from August-September bloom) show no gum impregnation although they dropped heavily, out of season.

A similar situation occurs in sweet orange [*C. sinensis* (L.) Osb.], especially in the Ovale variety, in which normal bloom fruits develop impietratura symptoms and late bloom fruits do not.

IMPIETRATURA SYMPTOMS NOT PREVIOUSLY REPORTED.—In the course of our inoculation experiments on grapefruit (*C. paradisi* Macf.), certain symptoms appeared that are different from those described previously (Fig. 2). Certain areas of the rind appear pale green to nearly yellow-colored while the surrounding areas remain dark green. Dark-green-colored areas are slightly prominent, but gum is not present in the lower albedo. In recent years, similar symptoms have been observed on the fruit of certain varieties of sweet orange (Fig. 3).

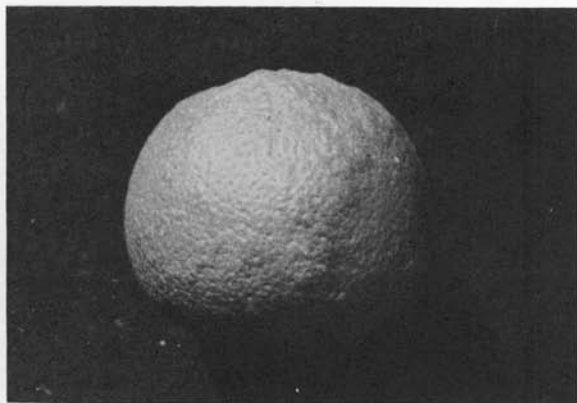


FIGURE 2. Fruit of inoculated grapefruit showing the described veining.

#### *Materials and Methods*

Ruggieri's experiments were designed to answer certain questions regarding impietratura, as follows: 1) Is Rough lemon (*C. jambhiri* L.) tolerant to impietratura? This question was raised because grapefruit selections on Rough lemon rootstock imported from Florida showed no symptoms of impietratura in Sicily or in Cyprus. On the other hand, buds from these trees propagated on sour orange (*C. aurantium* L.) produced typical impietratura-affected fruit. 2) Is the Santa Teresa strain of the Femminello variety of lemon tolerant to impietratura? Buds of this variety propagated on impietratura-affected sweet orange trees failed to produce any symptoms on the fruit. 3) Does inarching of affected trees with Rough lemon or Volkameriana lemon (*C. volkameriana* Pasq.) mask the disease?

**INDEXING METHODS.**—In our experience the following indexing procedure, developed by Ruggieri (3), gives the best results and is the method used unless otherwise specified. Inoculations are made during the regular blooming period when flower buds are nearly full grown (partially opened). Nucellar grapefruit is used as a test plant because it reacts more quickly and with more pronounced symptoms than do other citrus species. Buds or bark patches from the candidate tree are worked on the test plant not more than 10-15 cm from the flower buds. Symptoms usually appear on the young fruits 3-4 months after inoculation. Fruiting, but uninoculated twigs on the same plant serve as controls.

**TEST OF IMPIETRATURA INFECTION OF GRAPEFRUIT TREES FROM FLORIDA.**—In 1962, 3-4 buds per tree were collected from 89 grapefruit trees



FIGURE 3. Fruits of *Ovale* orange showing symptoms similar to those in Figure 1.

on Rough lemon imported from Florida and growing in two orchards in the Lentini area. These buds were worked on sour orange seedlings at the Experiment Station field plot in Palazzelli. A plot of 240 test plants was established. As a control, 3 Rough lemon seedlings, 3 years old, were budded with impietratura-infected grapefruit buds.

To obtain information on the reaction of Rough lemon as a top, buds were top worked on impietratura-infected sweet orange trees on sour orange rootstock, as follows: 2 buds on 2 four-year-old Sanguinello trees, 1 bud on a 5-year-old *Ovale*, and 1 bud on a 5-year-old Valencia. Thus, these trees each had a sweet orange interstock infected with impietratura virus.

**TEST OF SUSCEPTIBILITY OF FEMMINELLO LEMON.**—In 1962, 11 trees of impietratura-infected Sanguinello orange on sour orange rootstock

were top worked with the Santa Teresa strain of Femminello lemon. At the same time, Marsh grapefruit, and Moro, Tarocco, and Washington navel sweet orange buds free of impietratura were worked on 20 impietratura-infected Sanguinello trees on sour orange rootstock.

**INARCHING IMPIETRATURA-INFECTED TREES.**—In 1963, 5 five-year-old impietratura-infected trees of Marsh grapefruit on sour orange rootstock were inarched with Volkameriana lemon.

**INDEXING FEMMINELLO DI SIRACUSA LEMON TREES.**—In May, 1965, several buds from Femminello lemon trees suspected of impietratura infection in the Avola district were indexed by the method described.

TABLE 1. OCCURRENCE OF IMPIETRATURA VIRUS IN GRAPEFRUIT TREES FROM FLORIDA AND MANIFESTATION OF SYMPTOMS BY TEST PLANTS

Trees from Florida <sup>a</sup>	Occurrence of symptoms in bud propagations <sup>b</sup>							
	1965		1966					
No. 8	+	+	+	+	-	+	-	+
11	+	-	-	-	-	-	-	-
25	-	-	-	-	-	+	-	-
45	+	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	+
30	-	+	-	-	-	-	-	-

a. Trees 8, 11, 25, and 45 in Matarazzo orchard and trees 28 and 30 in S. Lio orchard. Only these trees were found infected.

b. All fruits produced were examined both years.

Budwood was taken from twigs bearing affected fruits and worked on 1 tree of Marsh grapefruit and 1 of Moro sweet orange, both on sour orange rootstock. At the same time, buds from the same source were worked on 2 trees of Santa Teresa lemon.

### *Results and Discussion*

The test plants from the index test of Florida grapefruit trees were examined in 1965 and 1966 (Table 1). Of the 240 test plants only 6 produced fruit with impietratura symptoms. All 4 propagations from tree No. 8 produced fruit with symptoms in 1965, but only 2 of them produced symptoms in 1966. Of the 3 propagations from tree No. 11, one produced symptoms in 1965, but none of the 3 produced symptoms in 1966. This pattern was repeated in propagations from the other 4 Florida trees. On the other hand, the control plants, 3 propagations of impietratura-infected local grapefruit on Rough lemon, all produced fruit with symptoms in 1965 and 1966. Also, Rough lemon tops worked on impietratura-infected sweet orange seedlings produced fruits with symptoms in both 1965 and 1966.

Because symptoms of impietratura disease appeared in propagations from 6 of the grapefruit trees from Florida, it is obvious that some of them are infected. Whether they were infected before or after arrival in Sicily is unknown. In any case, it is a fact that the original plants carried very mild symptoms or none at all. At first, the absence of symptoms on the trees from Florida was attributed to their being on Rough lemon rootstock. However, the failure of Rough lemon to inhibit symptoms, and the development of symptoms on Rough lemon fruits indicates that Rough lemon has no ameliorating effect on impietratura disease.

The trees of the Santa Teresa strain of Femminello lemon, top worked on impietratura-infected sweet orange of several varieties in 1962, developed several fruits, but none with symptoms in 1965 and 1966. These results suggest that this variety is tolerant to the impietratura virus.

The grapefruit trees inarched with Volkameriana seedlings in 1963 all developed fruit with typical impietratura symptoms in 1965 and 1966. This indicates that Volkameriana, like Rough lemon, had no ameliorating effect on the disease.

The Marsh grapefruit and Moro sweet orange inoculated with buds of Femminello lemon (Avola District) both developed typical impietratura symptoms in 1965 and 1966. However, the two Santa Teresa lemon trees inoculated from the same source failed to develop fruit symptoms. Thus, it is evident that the Femminello di Siracusa trees tested were infected with impietratura virus. However, the failure of the inoculated Santa Teresa lemon trees to develop symptoms further confirms the tolerance of this variety to impietratura.

### *Conclusions*

Budding Rough lemon and inarching Volkameriana lemon on impietratura-infected sweet orange and grapefruit trees, respectively, failed to prevent or decrease the impietratura symptoms in infected trees.

The absence of symptoms in the grapefruit trees on Rough lemon rootstock from Florida, except for one mild case, is still not explained satisfactorily. Our results show that at least six trees are infected with impietratura virus as evidenced by the appearance of symptoms in propagations from these trees. The original trees may have been free of impietratura when received and some of them may have become infected subsequent to planting in Sicily.

The Santa Teresa strain of Femminello lemon has proved tolerant to impietratura virus, or resistant to the disease, in two experiments involv-

ing 20 trees. However, the Femminello di Siracusa variety of lemon develops moderate symptoms of impietratura.

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