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Studies on Petri's Variegation of Sour Orange Leaves

In 1931, Petri (12) described in Sicily a disease of sour orange (*Citrus aurantium* L.) seedlings characterized by a pale-green, yellow or white variegation of the leaves of the spring shoots. The same author in cross sections through the discolored areas, observed several anomalies, such as a shortening of palisade cells, a hypertrophy of epidermal cells associated with a slight hyperplasia of the adjacent palisade. Furthermore, the cells of the latter tissue were small, irregular in shape, and often isodiametric.

Although the experimental reproduction of symptoms was not obtained either by sap or by graft transmission, Petri considered the variegation as the expression of an infectious disease, because of its ability to spread progressively. Moreover, because of the consistent association of the variegation with the presence of *Toxoptera aurantii* Boyer, he suspected that this insect might have been its vector.

Petri's paper is of historical interest because of the similarity of the symptoms he described and those reported in 1939 by Fawcett and Klotz (3) in California under the name "infectious variegation" and produced by a virus.

Petri's paper also antedates a report by Fawcett published in 1933 (1), in which the latter author produced evidence of the viral nature of citrus psorosis. It was itself antedated by the report of graft-transmissible infectious chlorosis of citrus by Trabut (9) in 1913.

Fawcett (2), in 1934, considered Petri's variegation as a disease distinct from psorosis, and very likely due to aphid damage. Later, Fawcett and Klotz (4), describing the infectious variegation of citrus in California, reported that the symptoms obtained on sour orange, by

grafting infected lemon [*C. limon* (L.) Burm. f.] buds, were similar to those described by Petri in Sicily.

In proposing a citrus virus nomenclature, Fawcett (4) finally distinguishes *Citrivir italicum*, the virus causing Petri's variegation, from *Citrivir psorosis*, the virus of citrus psorosis.

Klotz (8), during a visit to Italy in 1953, had the opportunity of seeing Petri's variegation on several orange (*C. sinensis* Osbeck) varieties. The field observations led him to conclude that the disease could be due to climatic factors or insects, rather than to a virus.

In 1960, Grant and Smith (6), and Grant and Corbett (5) ascribed to Petri the first description of citrus infectious variegation, considering the syndrome present in Sicily similar to that later reported by Fawcett and Klotz (3) in California.

In Corsica, a variegation of leaves of sour orange, Mexican lime [*C. aurantifolia* (Christm.) Swing.], sweet orange, and lemon seedlings, recalling that described by Petri, has been ascribed to cold injury by Vogel and Bové (14).

It seems useful to point out that Petri observed the variegation symptoms only on sour orange seedlings and not on sweet orange, lemon, or mandarin (*C. reticulata* Blanco) trees in spite of the fact that these last two species were mixed with sour orange seedlings in the same nursery.

In Italy, Gualaccini (7) observed and experimentally reproduced symptoms similar to those described by Petri and supposed that Petri's variegation was a peculiar "facies" of Californian infectious variegation. Likewise, Sibilia (13) regards Petri's variegation as analogous to crinkly leaf and infectious variegation of citrus.

Majorana and Wallace (10) have observed in Sicily a variegation of the leaves of sour orange seedlings in a nursery, which was very similar to that described by Petri, and have pointed out some contrasts between the behavior of infectious variegation in the United States and that of Petri's variegation.

In this paper are reported the results of investigations carried out in order to establish the etiology of the sour orange variegation in Sicily and the relationship of this disease to that studied by Petri and to the infectious variegation of citrus in the United States.

Experimental Part

Leaf variegations have been frequently observed on seedlings of sour orange in an experimental plot near the Institute of Plant Pathology,

University of Catania (Fig. 1,A). Such symptoms appeared on leaves of the spring flush of 2- to 3-year-old seedlings. They were still present on mature leaves in summer, although in a less severe form, and again they were more attenuated in autumn and winter.

In another section of the same experimental plot, about 80 per cent of a batch of sour orange seedlings 8 years old showed severe symptoms. On the contrary, it was not possible to observe any variegation on near-by growing lemon and mandarin trees grafted on sour orange, nor significantly on Mexican lime seedlings nor on sweet orange trees grafted on sour orange. Variegation symptoms were observed on one-two leaves of a very few trees of Mexican lime and sweet orange.

In the course of surveys made in several nurseries, the disease was frequently found on sour orange seedlings ready to be grafted.

The identity of the variegation that we have observed with the one described by Petri has been determined on the basis of the similarity of macroscopic symptoms and also on the basis of histological examinations.

Cross sections through the discolored areas of variegated leaves have been cut with a freezing microtome and have been stained with hematoxylin and safranin, or safranin and light green, or ruthenium red. They showed the same anomalies described by Petri. The thickness of the leaves in the whitish or yellowish areas was less than that in the green areas. Such a reduction is mostly due to a shortening of the palisade cells, which are also isodiametric and irregularly shaped. These

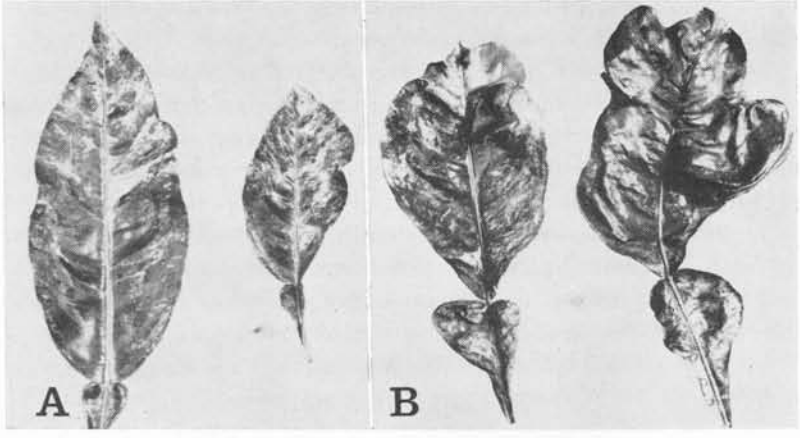

FIGURE 1. *A. Petri's variegation on spring flush leaves of sour orange. B. Infectious variegation on mature leaves of sour orange.*

cells rarely reach their normal size and their normal cylindrical shape except in the first layer just beneath the epidermis.

In order to establish the etiology of the disease, in May, 1962, buds were taken from variegated seedlings and were grafted onto symptomless sour orange seedlings growing in a greenhouse. Healthy, non-grafted seedlings were left as controls.

Previous trials gave good evidence that a nutritional deficiency of macroelements could not be the cause of the variegation. A balanced fertilization was supplied to a batch of 10 sour orange seedlings 8 years old, growing under field conditions and showing severe symptoms of variegation. In the next 2 years these plants developed variegation symptoms wholly the same as those showed by seedlings of the same age growing nearby and not fertilized. The grafted seedlings and the non-grafted controls were observed periodically, but they did not show any symptoms within one year after inoculation. They were then cut back and further observations were made on the new growth. In the 6 months after cutting back, there was no evidence of transmission of the variegation.

In a second trial, two lots of symptomless seedlings of sour orange and Mexican lime growing in a greenhouse at 20°-28°C were inoculated with buds taken from variegated sour orange seedlings and with buds of sour orange seedlings infected with a California strain of infectious variegation virus already used in mechanical transmission studies and considered a pure strain, not mixed with other viruses (11). Twenty days later, the seedlings inoculated with the infectious variegation virus showed symptoms typical of this disease on the young leaves, as yellowing of veins, subsequent yellow blotching of new shoots without shock effect, and one month later yellow spotting, variegation, and severe crinkling and distortion of the mature leaves (Fig. 1,B). The seedlings inoculated with the buds taken from the variegated sour orange seedlings did not show any symptoms within 6 months after budding.

At the same time, non-viruliferous individuals of *Toxoptera aurantii* Boyer, with or without a previous starvation period, were fed for different periods of time on variegated plants and then transferred to a lot of symptomless sour orange seedlings growing in a greenhouse. The aphids were killed at various intervals after transfer by means of a nicotine sulphate treatment. In no case did the seedlings show any symptoms during the following 6 months.

Another colony of aphids fed on non-variegated seedlings was transferred to other symptomless sour orange seedlings. These aphids were

maintained for 10 days on the seedlings, which developed slight crinkling of the leaves but no symptoms of variegation.

No further transmission tests have been attempted. To ascertain the relation between low temperatures and disease appearance, in May, 1962, a lot of 20 sour orange seedlings growing in clay pots was placed in a greenhouse at 20°-28°C and a similar lot of seedlings remained in a field under natural conditions. The next spring, variegation symptoms were observed only on the seedlings growing under field conditions.

Discussion and Conclusions

Comparative transmission tests by means of buds infected with the infectious variegation virus and by means of buds taken from variegated sour orange seedlings demonstrate that California infectious variegation is a different disease than Petri's variegation, which has been proved very likely to be the same as the variegation observed in Sicily.

The negative results of transmission tests, whether by grafting or by *Toxoptera aurantii*, suggest that Petri's variegation is not infectious. Neither does the disease appear to be caused by nutritional deficiencies of macroelements nor by insect toxicity.

The absence of symptoms of variegation on seedlings growing in a greenhouse at 20°-28°C seems to show that the disease could be due to a genetic sensibility of our local sour orange seedlings to winter cold.

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