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### Authors

Catara, A.  
Azzaro, A.  
Davino, M.  
et al.

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# Yellow Vein Clearing of Lemon in Pakistan

A. Catara, A. Azzaro, M. Davino and G. Polizzi

**ABSTRACT.** In different citrus areas of Pakistan, lemon and sour orange trees show vein clearing frequently associated with leaf crinkling. Bark inoculation of different citrus species and varieties reproduced the symptoms on sour orange and Femminello Siracusano lemon, whereas Navelina ISA 315, Hamlin and Pineapple sweet orange showed only mild mottling and rings. Rough lemon, Ciaculli and Parson's Special mandarin, Marsh Seedless grapefruit, Mexican lime, alemow, Etrog Arizona 861-S1 citron and Dweet tangor were symptomless. Mechanical inoculation with scalpel blade failed to transmit the disease. DAS-ELISA tests for citrus variegation virus and citrus tristeza virus and electron microscopic observations for viral particles were negative. On the basis of symptoms and host range, it is suggested that yellow vein clearing might be a new graft transmissible disease.

During an investigation on the sanitary state of citrus growing in different areas of Pakistan within the framework of a cooperative project between the Italian and Pakistani governments, widespread infectious variegation and ring-spot-like symptoms were observed on lemon and sour orange. Since ELISA for citrus variegation virus (CVV) was negative in these trees, it was suspected to be a new disease (4). Later Bové (2) reported the same symptoms and called the disease "yellow vein clearing" (YVC).

We report here on a preliminary investigation on YVC.

## MATERIALS AND METHODS

**Field survey.** The investigation was conducted between 1987 and 1991 in four different citrus growing areas of Pakistan; Islamabad, Renala Kurd, Sahiwal and Sihala, noting the disease symptoms and the citrus species affected. Careful inspection of a collection of 15 lemon lines at the National Agricultural Research Council (NARC), Islamabad, was undertaken.

**Transmission trials.** Two means of transmission were investigated, tissue grafting, and mechanical inoculation. The first trial was performed on ten Femminello Siracusano lemon trees obtained by shoot-tip-grafting, which were bud-inoculated in December 1989 with Eureka Cascade lemon field source and grown in the glasshouse at 18-32 C. For mechanical transmission, ten sour

orange seedlings were inoculated in the spring of 1992 by making about 20 cuts in the bark using a scalpel that had repeatedly cut a young symptomatic twig of Femminello Siracusano lemon, and kept in the glasshouse at 18-32 C.

**Host range.** Bark patches of Femminello Siracusano lemon were inserted in the trunks of eight one-yr old budlings of each of the following: Femminello Siracusano lemon, alemow, Ciaculli and Parson's Special mandarin, Marsh seedless grapefruit, rough lemon, Mexican lime, Etrog Arizona 861-S1 citron, Dweet tangor, Hamlin, Pineapple and Navelina ISA 315 sweet orange. Eight sour orange seedlings were also inoculated. Field sources of CVV and citrus psorosis were used as controls. Plants were kept in the glasshouse at 18-35 C.

**Electron microscopy (EM) and ELISA tests.** Small pieces of lateral veins from symptomatic leaves of Femminello Siracusano lemon were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8 for 6 hr at room temperature followed by post fixation in 2% osmium tetroxide in the same buffer for 4 hr at room temperature. The material was then dehydrated in an ethanol series and embedded in Epon araldite. Ultrathin sections were cut and stained with uranyl acetate, and examined in a (Zeiss EM 109) transmission electron microscope.

Leaf samples were assayed by the DAS-ELISA technique using a specific antisera for CVV (5) and CTV (1). The

tests were repeated at three different times of the year (April, June and September).

## RESULTS

The disease was detected in almost all the lemon trees in all the field locations investigated. Symptoms were also observed on sour orange trees and on suckers of this species used as rootstock of symptomatic lemon trees. The disease was not observed on sweet orange (Mosambi and Blood-red), mandarin (Kinnow and Nagpur), Jaffa grapefruit, Palestine sweet lime or Kagzi lime. All these species are widely cultivated in the surveyed area. No symptoms were detected on rough lemon trees or on suckers of this species where it had been used as the rootstock for symptomatic trees.

The leaves of affected trees showed vein clearing, which was more visible on young leaves using transmitted light. The vein clearing mainly involved the lateral veins which appeared as yellow flecks of varying length. This symptom was frequently associated with leaf crinkling, which made the leaves appear warped, ring-spot-like symptoms with round and/or elongated areas and browning of the veins on the underside of the leaves.

The disease was more severe on spring and autumn leaves, whereas summer leaves were mostly free of symptoms. The symptoms were constantly present on mature leaves. The fruits were normal. The diverse symptomatology observed in the lemon varieties collection plot at NARC (Islamabad) is shown in Table 1. During a nursery inspection, young sour orange seedlings of infected plants were found to be asymptomatic.

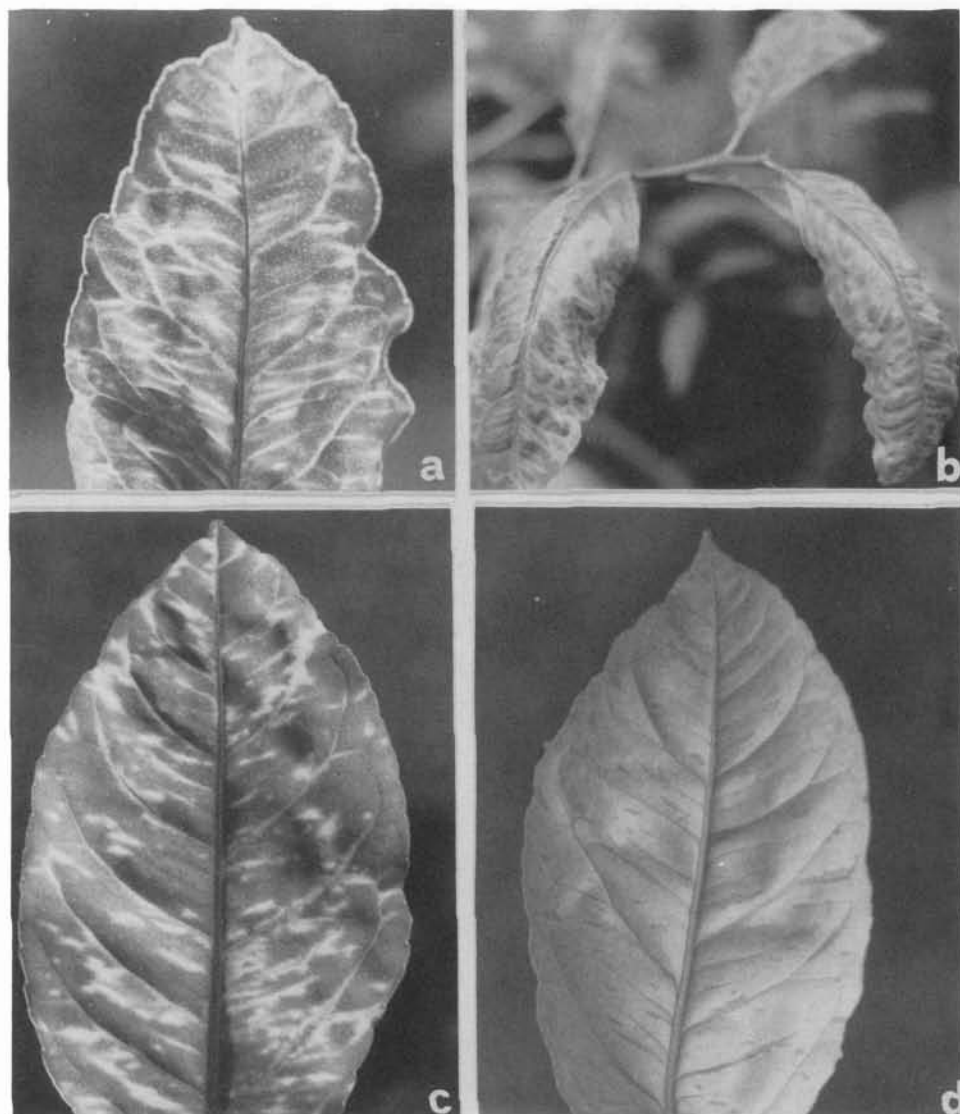
The transmission trials clearly showed that yellow vein clearing is a graft transmissible disease. Symptoms were obtained on Femminello Siracusano lemon and sour orange inoculated by bark grafting. All the symptoms observed in the field were reproduced on lemon and sour orange (Fig.1). The first symptoms were seen in few plants about 20 days after inoculation and by 60 days all the inoculated plants had symptoms.

In the host range study, only slight mottling and ring-spot-like symptoms were observed on Hamlin, Navelina and Pineapple. None of the other inoculated species and varieties presented symptoms. Psorosis-inoculated controls showed clearcut flecking of young leaves and oak-leaf patterns. None of the trees mechanically inoculated developed symptoms.

TABLE 1  
LEMON VARIETIES SHOWING SYMPTOMS OF YELLOW VEIN CLEARING IN A COLLECTION PLOT AT NARC (ISLAMABAD)

| Variety                  | No. of affected trees/total | Symptoms <sup>z</sup> |     |     |
|--------------------------|-----------------------------|-----------------------|-----|-----|
|                          |                             | CL                    | YVC | RSL |
| Eureka Cook              | 3/3                         | ++                    | ++  | +   |
| Eureka Allen             | 3/3                         | +++                   | +++ | +   |
| Eureka Cascade           | 3/3                         | +++                   | +++ | +   |
| Eureka Frost             | 1/3                         | +++                   | ++  | +   |
| Eureka Corona            | 3/3                         | +                     | +   | -   |
| Interdonato              | 0/2                         | -                     | -   | -   |
| Lisbon Calier's old line | 3/3                         | +                     | ++  | +   |
| Lisbon Calier's          | 1/3                         | +                     | +   | -   |
| Lisbon Frost             | 0/3                         | -                     | -   | -   |
| Lisbon Prior             | 3/3                         | ++                    | ++  | +   |
| Molla Mehmet             | 1/2                         | +                     | +   | +   |
| Monachello               | 1/3                         | ++                    | ++  | -   |
| Santa Teresa             | 3/3                         | +                     | +   | -   |
| Unknown                  | 0/1                         | -                     | -   | -   |

<sup>z</sup>Symptoms were rated as follows: - = none; + = intermediate; +++ = severe; CL = Crinkling leaf; YVC = Yellow vein clearing; RSL = Ringspot-like.



**Fig. 1.** Symptoms on a Femminello Siracusano lemon leaves associated with yellow vein clearing from glasshouse inoculated plants: a) yellow vein clearing; b) crinkling and bright yellow veins; c) round and elongated yellow areas; d) browning of veins on the underside leaf.

EM observations of symptomatic tissues and neighbouring areas failed to reveal any viral particles, and DAS-ELISA for CVV and CTV were negative.

#### DISCUSSION

Our results show that YVC is a graft transmissible disease. Although the symptoms bear some resemblance to psorosis, ringspot, crinkly leaf (CLV), and yellow vein, the host range

study indicates that it is a different agent. CVV and CLV cause symptoms in all species tested (6), psorosis induces symptoms in Dweet tangor, citron and Mexican lime (7), and yellow vein produces symptoms in citron (3).

ELISA and EM confirmed that there was no relationship between the causal agent of YVC and CVV-CLV.

Although mechanical inoculation was unsuccessful in our trial the high percentage of symptomatic lemon

trees found in commercial fields, the presence of YVC in almost all the lemon varieties in the collection fields at NARC (Islamabad), and the random

detection of symptoms on sour orange seedlings requires the need for further investigation of mechanical, or possibly insect vector transmission.

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