Association of a Filamentous Virus with Yellow Vein Clearing of Lemon

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ABSTRACT. Young spring leaves of Femminello Siracusano lemon and sour orange, showing yellow vein clearing were examined by transmission electron microscopy. Crude extracts and clarified viral concentrates revealed filamentous virus particles having a diameter of 13-14 nm and an apparent length ranging from 530 nm to 1,800 nm, with a modal value of 670-700 nm. In addition, ultrathin sections of lateral veins revealed low numbers of aggregates of a filamentous virus in phloem cells. This is the first report of virus particles associated with yellow vein clearing.

A new citrus virus disease was reported to infect almost all the lemon trees and varieties in Pakistan (2) which was later named yellow vein clearing (YVC) (3). The disease was demonstrated to be graft-transmissible and other inoculated citrus species, such as sour orange, develop typical YVC symptoms (2, 3). An earlier attempt to detect virus particles by electron microscopy was unsuccessful (3). Therefore, a new electron microscope investigation was undertaken to see if any virus particles are associated with the disease.

In this investigation, 1-yr old Femminello Siracusano lemon budlings and sour orange seedlings were inoculated by bark patches of Femminello Siracusano lemon previously inoculated with Eureka Cascade lemon samples collected in Pakistan and maintained at 18 to 35°C in a glasshouse at the University of Catania, Italy (3). Two years later, the inoculated plants were pruned to force new growth for electron microscopy analysis.

Leaf dip analysis was performed by grinding 0.5 cm^2 of Femminello Siracusano and sour orange leaves in a few drops of 0.1 M phosphate buffer (pH 7.5) containing 2% PVP (MW 10,000) and 1:5000 NaN_3. To prepare clarified viral concentrates (4), a 1 g-leaf sample was triturated in liquid nitrogen with a mortar and pestle and homogenized with 2 ml of extraction buffer (0.1 M potassium phosphate buffer, pH 7.4 containing 1% sodium sulfite and 0.02 M EDTA). The virus was precipitated with 6% PEG (MW 6,000) and 0.125 M NaCl and the final pellet was resuspended in 200 µl of extraction buffer. Samples were stained on the grids with 2% uranyl acetate and examined in a transmission electron microscope (Zeiss EM109). Symptomatic leaves were processed for ultrathin sectioning as previously described (3).

Five to six weeks after the pruning, inoculated plants developed the whole range of typical YVC symptoms, namely vein clearing of the leaves, leaf crinkling, and round and elongated ringspot-like areas. Leaf tissues along the adaxial veinlets became water soaked and later turned brown. Crude extracts contained a few filamentous virus particles having the closterovirus-like morphology, a diameter of 13-14 nm and an apparent length ranging from 530 nm to 1,800 nm, with a modal length of about 670-700 nm (Fig. 1). Particles of about 1,800 nm were observed only in grids prepared with the simple leaf dip method. Clarified and concentrated preparations contained a higher concentration of virus particles. Aggregates of the filamentous virus particles, which were rarely found in single phloem cells, were observed in ultrathin sections of lateral veins

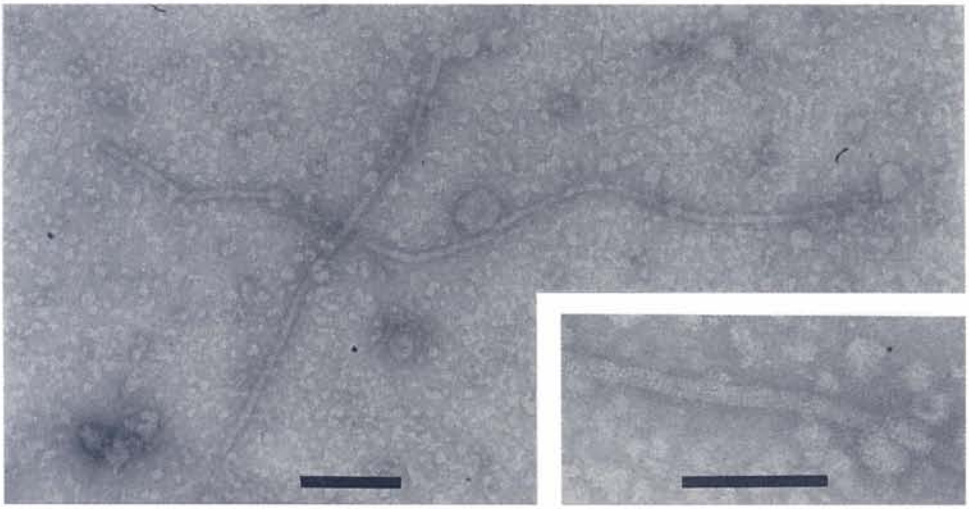

Fig. 1. Closterovirus-like particles associated with yellow vein clearing of citrus observed in clarified viral concentrate (Bar = 100 nm). Higher magnification in the insert (Bar = 100 nm).

(Fig. 2). A few isolated necrotic phloem cells were observed near infected cells.

Positive results in virus detection were obtained by testing symptomatic spring leaves, 3 to 5 cm long, collected from both the hosts 5 to 6 weeks after plants were pruned when temperature did not exceed 25°C. All the tests performed on symptomatic leaves of glasshouse plants collected at other times during the year were always unsuccessful in detecting virus particles.

Results reported here indicate that plants showing YVC symptoms harbor a filamentous virus in the phloem. This is the first report of a virus associated with YVC of lemon and seems to be in agreement with the prediction that YVC, on the basis of the symptom expression of the

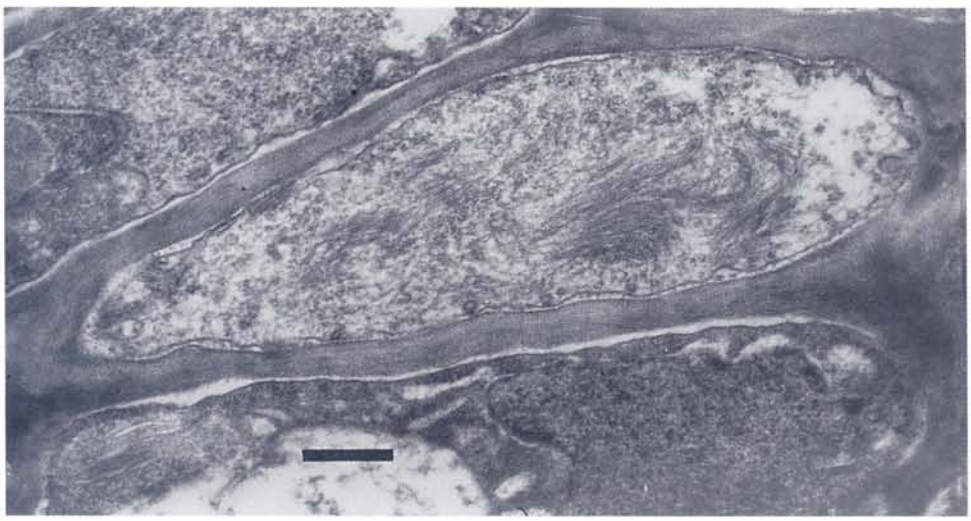

Fig. 2. Ultrathin section of YVC-affected lemon leaf showing a phloem cell densely packed with closterovirus-like particles (Bar = 500 nm).

affected plants, should be a phloem disease. Because of their morphology the filamentous particles are thought to represent a closterovirus-like virus. The particles were visualized in YVC-inoculated plants that were free of any known citrus virus (3) and cannot be identified with CTV (7, 8), the rod-shaped particles associated with a latent virus of citrus (5, 6), or citrus tatter leaf. A morphologically similar filamentous virus was associated with citrus ringspot in India (CRSV-I) (1), but the symptomatology and host range of these viruses are different. Further characterization of the virus associated with YVC of lemon is advisable.

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