

## Transmission Studies on Citrus yellow vein clearing virus

Nüket Önelge<sup>1</sup>, Serdar Satar<sup>1</sup>, Özer Elibüyük<sup>2</sup>, Orhan Bozan<sup>1</sup>, and Muharrem Kamberoğlu<sup>1</sup>

<sup>1</sup>Çukurova University, Agriculture Faculty, Plant Protection Department, Adana, Turkey

<sup>2</sup>Ankara University, Agriculture Faculty, Plant Protection Department, Ankara, Turkey

**ABSTRACT.** *Citrus yellow vein clearing virus* (CYVCV) was first observed in Experimental Research Orchard at Çukurova University in Turkey on citrus lemon and sour orange. Mechanical transmission of CYVCV was evaluated on nine different herbaceous plants from infected lemon plants. Only *Phaseolus vulgaris* var. Dermason, *Vigna unguiculata* (L.) and *Chenopodium quinoa* Willd. seedlings developed local lesions on inoculated leaves, but the infection became systemic in *P. vulgaris* and *V. unguiculata* (L.) with chlorosis, severe mosaic and necrosis symptoms. Young leaves of Kutdiken lemon and *P. vulgaris* showing yellow vein clearing were examined by transmission electron microscopy. Filamentous virus particles measuring 685x14 nm were observed in electron microscope. Adults and nymphs of *Aphis craccivora* Koch and *A. spiraecola* Patch were fed on infected lemon kidney bean seedlings for 24 h, transferred in groups of 10 to healthy beans and allowed to feed for a 24h. Systemic symptoms consisting of severe mosaic, blotching and necrosis developed in 3 weeks on bean plants exposed to both aphid species.

**Key words:** CYVCV, vector transmission, aphid, lemon

Yellow vein clearing (YVC) was first observed in Pakistan in 1988, and was thought to be a new disease (3). In 1996 Grimaldi and Catara (4) observed filamentous virus particles in leaf dip preparations associated with YVC. During the surveys in 1997, Ahlawat (1) observed YVC symptoms on lemon and sour orange in the Punjab state of India. In 2003, Alshami et al. (2) reported successfully purifying a filamentous virus associated with YVC and tentatively named it as Citrus yellow vein clearing virus (CYVCV).

YVC was first seen in the Çukurova region of Turkey in 2000 on lemon and sour orange trees (5). This disease causes a characteristic leaf vein clearing, crinkling and yellow flecks and is now spreading and causing adverse effects on lemon production reducing yield. The rapid spread of YVC on lemon trees in the area of Çukurova University where it was first observed in a small number of trees, suggests transmission by an insect vector. Lack of any control strategy for this disease and transmission by a vector makes the disease important, not only Turkey, but also for all citrus producing countries. CYVCV can infect most citrus

species and cultivars and some noncitrus hosts (2). Sensitive species that show symptoms include lemons and sour orange. The leaves of lemon trees infected with CYVCV show vein clearing which appears as yellow flecks of varying length on the lateral veins as well as leaf crinkling and distortion of young leaves. On the underside of the leaves, vein clearing symptoms appear as water soaked areas of the veins. Vein clearing symptoms are seen easily during spring and autumn flush, and the symptoms remain as the leaves mature.

CYVCV was mechanically inoculated onto seven different herbaceous plants with 0.1M phosphate buffer by stem slash method. CYVCV was transmitted successfully from lemon to *Chenopodium quinoa*, bean (*Phaseolus vulgaris* var. Dermason) and cowpea (*Vigna unguiculata* (L.)) plants. Local lesions on inoculated developed on all three, but systemic symptoms of severe mosaic and necrosis developed in both cowpea and kidney bean. None of the other herbaceous hosts tested developed symptoms.

Leaf dip samples stained with uranyl acetate were examined under

electron microscope (EM). Crude extracts of lemon plants contained filamentous virus particles with a diameter of 13-14 nm and length ranging from 550-800 nm with a model length of 685 nm (Fig. 1). DsRNA was extracted and purified from infected tissue of Kutdiken lemon and kidney bean (*P. vulgaris* cv. Dermason) by the procedure of Valverde (6) with modifications. The replicative form (RF) and several subgenomic RNAs with an estimated range of 800bp and 10kb were observed (Fig.2).

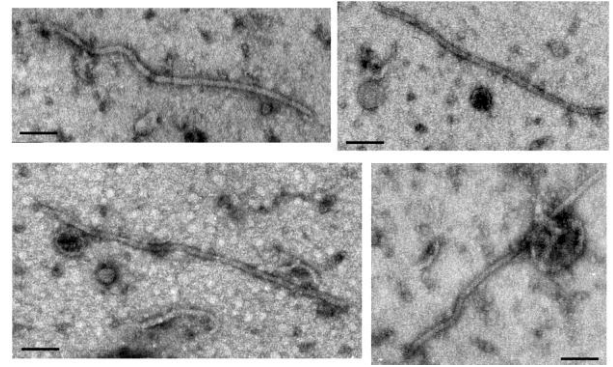


Fig. 1. Filamentous particles associated with Citrus yellow vein clearing virus (Bar= 100 nm).

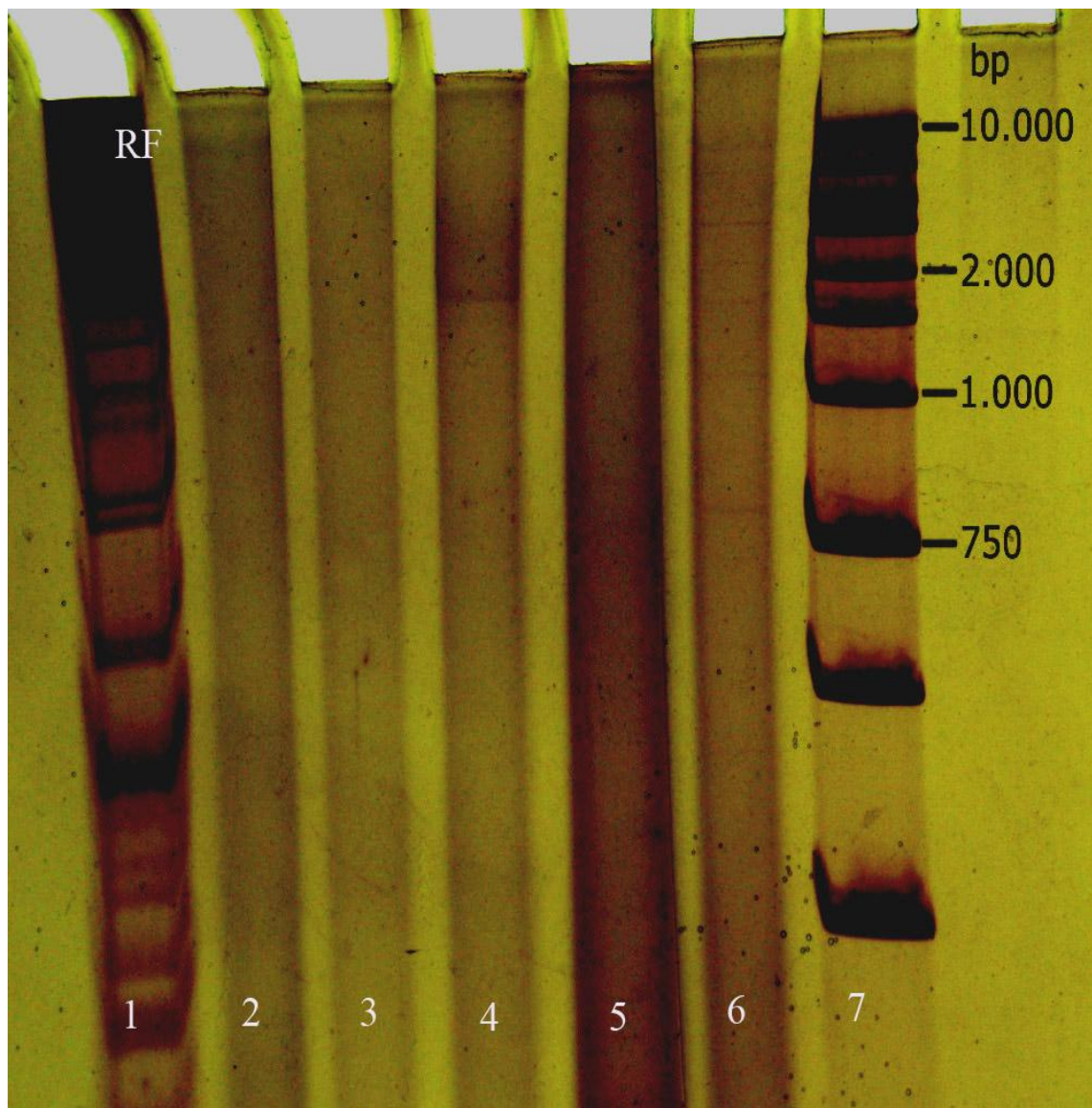


Fig. 2. DsRNA profiles obtained from CYVCV infected plants. Lanes 1 and 7 molecular markers. Lane 2 CYVCV infected bean, lane 3 healthy bean, lane 4 CMV, lane 5 healthy lemon, lane 6 CYVCV infected lemon.

To study natural spread and to identify the possible vectors of CYVC, adults and nymphs of *Aphis craccivora* Koch (Hemiptera; Aphididae) and *A. spiraeicola* Patch were allowed to feed for 24 h on infected Kidney bean and lemon in groups of 10 and then transferred to healthy kidney bean plants for 24 h. Systemic symptoms consisting of severe

mosaic and necrosis began to appear after 3 weeks. The transmission rate by *A. craccivora* from lemon to bean was 69% (62/90 plants), while transmission from beans to beans was only 52% (25/50). Transmission of CYVC by *A. spiraeicola* was 42% from infected bean to healthy bean, and 46% from infected lemon to healthy bean plants (Table 1).

TABLE 1  
APHID TRANSMISSION TESTS WITH CITRUS YELLOW VEIN CLEARING VIRUS

<i>Aphis</i> sp.	Acquisition plant	Receptor plant	Acquisition feed (h)	Transmission feed (h)	No. plants infected / No. inoculated (%)
<i>A. craccivora</i>	Kidney bean	Kidney bean	24	24	26/50 (52)
<i>A. craccivora</i>	Lemon	Kidney bean	24	24	62/90 (69)
<i>A. spiraeicola</i>	Kidney bean	Kidney bean	24	24	21/50 (42)
<i>A. spiraeicola</i>	Lemon	Kidney bean	24	24	23/50 (46)

Observations of transmission from infected lemon to healthy lemon seedlings with aphid populations were observed, but the symptoms were not initially typical of YVC.

#### ACKNOWLEDGMENTS

The authors are very grateful to Dr. M. Saponari and Angelo De Stradis for the electron microscopy studies.

#### LITERATURE CITED

- Ahlawat, Y. S.  
1997. Virus, greening bacterium and viroids associated with citrus (*Citrus* species) decline in India. *Indian J. Agric.Sci.* 67:51-57.
- Alshami, A. A. A., Y. S. Ahlawat, and R. P. Pant  
2003. A hitherto unreported yellow vein clearing disease of citrus in India and its viral etiology. *Indian Phytopath.* 56 :422-427.
- Catara, A., A. Azzora, M. Moghal, and D. A. Khan  
1988. Virus, viroid and prokaryotic diseases of citrus in Pakistan. In: *Proc. 6th Int. Citrus Congr.* 3:957- 962.
- Grimaldi, V. and A. Catara  
1996. Association of a filamentous virus with yellow vein clearing of lemon. In: *Proc. 13th. Conf. IOCV*, 343-345. IOCV, Riverside, CA.
- Önelge, N.  
2002. First report of yellow vein clearing of lemons in Turkey. *J. Turk. Phytopathol.* 32:53-55.
- Valverde, R. A.  
1990. Analysis of double-stranded RNA for plant virus diagnosis. *Plant Dis.* 74:255-258.