Management of a High Density Clementine Orchard Inoculated with Pathogenic and Non-Pathogenic Viroids

S. Rizza[1,2], G. Nobile[1,2], M. Tessitori[1], G. Albanese[3], R. La Rosa[1], and A. Catara[1,2]

[1]*Department of Phytosanitary Sciences and Technologies, University of Catania, Italy*
[2]*Laboratory of Phytosanitary Diagnosis and Biotechnologies, Science and Technology Park of Sicily, Catania, Italy*
[3]*Department of Management of Agricultural and Forest Systems, Mediterranean University of Reggio Calabria, Italy*

ABSTRACT. Over 4,000 Clementine trees grafted on trifoliate orange, spaced at 2 x 3 m, were inoculated 1 yr after planting with four different isolates combinations of *Citrus exocortis viroid* (CEVd), *Citrus viroid III* (CVd-III) and *Hop stunt viroid* (HSVd). As expected, 12 yr after inoculation, all the trees containing CEVd showed bark cracking and/or scaling, whereas those with only CVd-III were healthy in appearance. The trees inoculated with CVd-III alone had the largest average circumference of both scion and rootstock, fruit quality reached high standards and yields were more than twice that of a conventional orchard. Those inoculated with CVd-III+HSVd, with CEVd+CVd-III, and CVd-III+HSVd+CEVd were smaller. The phenotypic response of host plant was not related with the titer of CVd-III.

Citrus trees are often naturally infected with various combinations of viroids inducing different phenotypic reactions (4). Some variants of the *Citrus viroid III* have been studied for many years as potentially useful, due to their capability of reducing the canopy of citrus grafted on trifoliate orange without any detrimental effects. In fact. There are reports of enhanced yield per canopy volume (3). Therefore, the name of *Citrus dwarfing viroid* (CDVd) has been proposed to replace CVd-III, since it is more descriptive of the host phenotype induced by the infection (5).

High density citrus plots, obtained through the inoculation of citrus viroids in different combinations have been established in Italy for several years with variable results, depending on the scion-rootstock combination, the viroids and the pedoclimatic conditions (1). In order to obtain some clarity, a large orchard of over 4,000 Comune Clementine grafted on trifoliate orange was established in the south of Italy close to Lamezia (Calabria region, Catanzaro province), GPS coordinates N 38°08' 33,9"/ E 016°09' 01,6".

In spring 1992, about 4,500 seedlings of trifoliate orange were grafted in a commercial nursery with buds of certified Clementine SRA, and after 2 yr they were planted at a spacing of 3 x 2 m (1,666 trees /ha). The trees were inoculated 1 yr after planting by grafting onto the each scion two pieces of bark taken from source viroid-infected plants. There were four different isolate combinations of *Citrus exocortis viroid* (CEVd), *Citrus viroid III* (CVd-III) and *Hop stunt viroid* (HSVd).

The plants were maintained standard agricultural practices and periodically checked for symptoms, fruit quality and growth. Normal fertilizer and pesticide applications for the area were performed, and irrigation was applied by a sprinkler irrigation system. From the fourth year, the trees were pruned regularly in order to allow good lighting and fruiting and to reduce pest control sprays. In the first 5 yr, pruning tools were disinfected with sodium hypochlorite solution between trees to prevent cross transmission of viroids.

During the summer 2005, 40 randomly selected trees of Comune Clementine, showing different phenotypes, were monitored for symptoms, growth, yield and viroid content by RT-PCR. CVd-

III titre was determined using RT sybr green real-time PCR (2).

Bark symptoms (scaling and cracking) were evaluated yearly for 5 yr and then again from the eighth to the tenth year. Symptoms under the bark, in the wood and at the bud union line were recorded since 2005. Trunk circumferences of both the scion and the rootstock (10 cm above and below the bud union line) and fruit yield were measured for the last 3 yr (2005-2007).

Bark scaling was observed at the soil level 4 yr after inoculation in a small percentage of trees and increased over time only on trees orange infected by the inoculum mixture containing CEVd. Ten years after inoculation, the expression of symptoms varied from severe to very severe. A poor volume of the canopy and severe stunting were associated with the scaling. Small cracks were also observed in some trees until they were masked by the intense scaling.

Nevertheless, the cumulative yield per hectare between 1996 and 2006 was 313 tons/ha, whereas each single tree gave a cumulative yield of 188 kg (an average of 17 kg/year). The maximum yield was reached in 2003 with 38 kg/tree.

Groups of trees, randomly selected and tested for the three viroids, showed a relationship between phenotypic response and viroid infection. As shown by rootstock and scion circumferences, viroid infection affected vegetative growth of trees in variable measures. The trees inoculated with CVd-III alone had the largest average circumference of both scion and rootstock (Table 1), fruit quality reached high standards and yield was more than twice that of a conventional orchard. Those inoculated with CVd-III+HSVd, with CEVd+CVd-III, and CVd-III+HSVd+CEVd were smaller. The results are in agreement with the potential use of CVd-III as “dwarfing factor”.

Regardless of the viroid combination, the CVd-IIIb titre was almost the same (from 3.03^{1} to 2.08^{4} copies/mg and an overall median value of 9.53^{2}), suggesting the phenotypic response of host plant is not related with the titer of the “dwarfing viroid”, but is mediated by the pathway undergone after the infection.

TABLE 1
EFFECT OF VIROID INFECTION ON GROWTH PARAMETERS OF COMUNE CLEMENTINE GRAFTED ON *PONCIRUS TRIFOLIATA* INOCULATED WITH DIFFERENT VIROIDS

Viroid detected	Average circumference (cm)		Internode length (cm)
	scion	rootstock	
CVd-III	34.7	55.8	2.07
CVd-III+HSVd	34.2	54.6	1.94
CVd-III+CEVd	31.8	44.6	1.90
CVd-III+HSVd+CEVd	29.6	45.1	1.73

LITERATURE CITED

1. Albanese, G., R. La Rosa, M. Tessitori, E. Fuggetta, and A. Catara
1996. Long-term effect of CVd-III of plants on citrange, trifoliate and sour orange. In: *Proc. 13th Conf. IOCV*, 367–369. IOCV, Riverside, CA.
2. Rizza, S., G. Nobile, M. Tessitori, A. Catara, and E. Conte
2009. A real time RT-PCR assay for quantitative detection of *Citrus viroid III* in plant tissues. Plant Pathol. 58: 181-185.
3. Semancik, J. S., A. G. Rakowski, J. A. Bash, and D. J. Gumpf
1997. Application of selected viroids for dwarfing and enhancement of production of "Valencia" orange. J. Hort. Sci. 72: 563–570.
4. Vernière, C., L. Botella, A. Dubois, C. Chabrier, and N. Duran-Vila
2002. Properties of citrus viroids: symptom expression and dwarfing. In: *Proc. 15th Conf. IOCV*, 240–248. IOCV, Riverside, CA.
5. Vernière, C., X. Perrier, C. Dubois, A. Dubois, L. Botella, C. Chabrier, J. M. Bové, and N. Duran-Vila
2004. Citrus viroids: symptom expression and effect on vegetative growth and yield of clementine trees grafted on trifoliate orange. Plant Dis. 88: 1189-1197.