# Performance of 'Comune' Clementine Infected with *Citrus exocortis viroid* (CEVd) on Seven Rootstocks

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ABSTRACT. The performance of Comune Clementine trees infected with an isolate of *Citrus exocortis viroid* was evaluated on seven different rootstocks (BA-300, Carrizo and Troyer citranges, Swingle citrumelo, sour orange, Flying Dragon and 'Rubidoux' trifoliate oranges) growing in an experimental field of Bertolami farm located in Lamezia Terme-Catanzaro, Italy. Tree growth, fruit quality, yield and symptoms were evaluated annually to determine the effects of viroid infection. Bark-scaling was observed in Flying Dragon and Rubidoux trifoliate oranges two years after planting. Trees grafted on Flying Dragon trifoliate orange showed severe decline and died. No symptoms were observed on the other rootstocks. The lack of symptoms and the tree size and yield data suggest that BA-300 citrange could be a useful rootstock in high density plantings especially for old-line citrus varieties infected with the exocortis viroid.

*Index words.* Exocortis, tree growth, fruit quality, yield, symptoms.

Sour orange rootstock is the most frequently used rootstock in Italy, with an incidence of 98%. Rootstocks suitable for replant sites and tolerance to *Citrus tristeza virus* (CTV) are required. Incidence of CTV was sporadic but is now widespread in Italian citrus growing areas (4, 5). Therefore, a number of assays have been initiated to evaluate the performance of the most common species and varieties of citrus grown in Italy on other rootstocks (7). Rootstocks commonly used overseas and others selected in Italy have been investigated to assess their behaviour when affected by common virus and virus-like diseases present in Italy. The present study focussed on the effects of CEVd on diverse rootstocks, some of which are known to be very susceptible, others known to be tolerant and some new Italian rootstocks of unknown sensitivity (8). This pathogen was selected because it is the most widespread in old plantings and it is easily transmitted by pruning tools to new orchards in which healthy propagation material had been grafted on different rootstocks susceptible to CEVd.

## MATERIALS AND METHODS

The trial was carried out in Calabria, Italy on the A. Bertolami nursery farm in the Lamezia Terme plain (CZ). The rootstocks evaluated were: S. Marina sour orange (S.O.), BA-300 (BA.C) (8), Carrizo citrange C.R.C. 2863 (C.C.) and Troyer citrange C.R.C. 1459 (T.C.), Rubidoux C.R.C. 838 trifoliate orange (R.T.O.), Flying Dragon trifoliate orange (F.D.T.O.) and Swingle citrumelo (S.C.).

The CEVd isolate used (isolate ISA1-CT-I, GenBank accession number AY523582) had been collected from a Monachello lemon tree. Biological indexing on Etrog 861 citron seedlings showed leaf epinasty characteristic of CEVd infection whereas it was negative for *Citrus variegation virus*. Bioassay on Parsons' Special mandarin, Navelina ISA 315 and Madame Vinous sweet oranges was negative. The isolate also tested negative for CTV by DAS-ELISA.

In spring 1989, two years after rootstock seeds were sown, the seedlings were grafted with a virus-free Comune Clementine clone, and in

spring 1990 were graft-inoculated with three bark chips collected from CEVd-infected Etrog 861 citron seedlings. CEVd infection was confirmed by bidirectional polyacrylamide gel electrophoresis (dPAGE) of nucleic acid extracts from green bark samples (5-10 g) according to the method of Albanese et al. (1).

In spring 1992 the infected trees were planted in sandy soil in a $6 \times 4$ m spacing and allocated in random block design. The blocks were replicated five times and each block was made up of three plants per rootstock. The trees in the field were periodically monitored for onset of symptoms. Every year, canopy height and circumference were measured in order to evaluate canopy volume and yield. At fruit maturity, samples of twenty fruits per tree were harvested and fruit quality parameters such as, fruit weight, peel-thickness, juice (% by weight), total soluble solids (%) TSS, total acidity (%) TA and TSS/TA ratio, were determined. Simple variance analysis was performed on the data and the means were compared using Tukey's test. Yield data were compared on a yearly basis starting in 1996.

## RESULTS

Bark scaling was recorded on Rubidoux C.R.C. 838 and Flying Dragon trifoliate oranges 2 yr after planting, with symptoms more pronounced on Flying Dragon trifoliate orange (Fig. 1) than on Rubidoux C.R.C. 838 trifoliate orange (Fig. 2). After about 9 yr, CEVd had killed the trees on Flying Dragon trifoliate orange. Fifteen years after inoculation, none of the other rootstocks showed exocortis bark scaling symptoms, even though dPAGE analysis showed that were all infected.

Tree growth was compared by analysis of canopy volume data at the end of the trial. Figure 3 shows major growth of trees grafted on Swingle citrumelo, followed by trees grafted on S. Marina sour orange, Carrizo C.R.C. 2863 and Troyer C.R.C. 1459 citranges that were significantly smaller than those on Swingle citrumelo. The smaller growth was recorded for trees grafted on BA-300 citrange, and Rubidoux C.R.C. 838 and Flying Dragon trifoliate oranges.

**Fig. 1. Bark-scaling on Flying Dragon trifoliate orange grafted with Comune Clementine infected with CEVd.**

All trees began to yield fruit in 1994 (Table 1) but a remarkable production was only observed from 1996, 4 yr after planting. In 1996 the highest yield was achieved in trees grafted on Swingle citrumelo but the difference was statistically

**Fig. 2. Bark-scaling on Rubiduox trifoliate orange grafted with Comune Clementine infected with CEVd.**

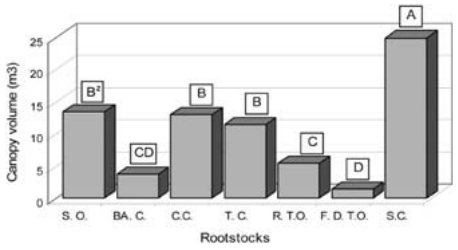


**Fig. 3. Canopy volume ($m^3$) of CEVd infected Comune Clementine grafted on seven rootstocks: S. Marina sour orange (S.O.), BA-300 citrange (BA.C), Carrizo citrange C.R.C. 2863 (C.C.) and Troyer citrange C.R.C. 1459 (T.C.), Rubidoux C.R.C. 838 trifoliate orange (R.T.O.), Flying Dragon trifoliate orange (F.D.T.O.) and Swingle citrumelo (S.C.). [z]Bars with the same letter do not differ significantly (P = 0.01)**

significant only when compared with trees grafted on BA-300 and Carrizo C.R.C. 2863 citranges, and Flying Dragon trifoliate orange. This trend became more and more evident in the following years, and in the last 2 yr of the trial, the yield of trees grafted on Swingle citrumelo was significantly higher than those of trees grafted on the other rootstocks. Trees grafted on Marina sour orange and Carrizo C.R.C. 2863 and Troyer C.R.C. 1459 citranges gave intermediate yields, whereas trees on BA-300 citrange and Rubidoux C.R.C. 838 trifoliate orange rootstocks gave the lowest yields. Over the last 2 yr of the trial, trees grafted on Flying Dragon trifoliate orange were affected by severe decline and did not produce any fruit. Differences in yield were confirmed by cumulative yield results (Fig. 4) showing similar statistically significant differences.

Fruit quality parameters were similar regardless of the rootstock (Table 2). Nevertheless, fruit weight was heaviest in trees grafted on BA-300 citrange, peel-thickness was thickest on Flying Dragon trifoliate orange rootstocks, percentages of juice and total soluble solids were highest on Rubidoux C.R.C. 838 trifoliate orange rootstocks, while none of the rootstocks investigated were significantly different in total acidity and the TSS/TA ratio.

## DISCUSSION

The CEVd isolate used in the trial caused bark scaling and stunting, and thus minor yield, in the two trifoliate orange selections. Symptoms were more evident on Flying Dragon trifoliate orange. The trees on this rootstock were very stunted and yield very low from the start of the trial.

TABLE 1
YIELD (KG) OF CEVD INFECTED COMUNE CLEMENTINE GRAFTED ON SEVEN ROOTSTOCKS

| | Year | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rootstocks | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 |
| S. Marina sour orange | 0.7 | 1.1 | 6.5 AB[z] | 41.9 BC | 27.0 AB | 54.9 AB | 67.0 B | 73.0 B |
| BA-300 citrange | — | — | 0.8 B | 15.9 CD | 14.0 B | 27.0 B | 35.0 C | 44.0 C |
| Carrizo citrange C.R.C. 2863 | 1.6 | 1.7 | 6.4 AB | 66.0 AB | 39.0 A | 58.2 AB | 62.0 B | 68.0 B |
| Troyer citrange C.R.C. 1459 | 2.2 | 1.8 | 1.3 B | 63.8 AB | 34.5 AB | 60.0 A | 65.0 B | 70.0 B |
| Rubidoux trifoliate orange C.R.C. 838 | 4.8 | 6.7 | 13.0 AB | 26.3 C | 18.5 B | 30.0 B | 25.3 C | 20.7 C |
| Flying Dragon trifoliate orange | 1.3 | 1.5 | 1.2 B | 7.7 D | 7.7 B | 9.0 C | — | — |
| Swingle citrumelo | 7.2 | 4.2 | 21.9 A | 83.0 A | 65.4 A | 73.0 A | 91.3 A | 87.6 A |

[z]Figures in each column followed by the same letter do not differ significantly (P = 0.01).

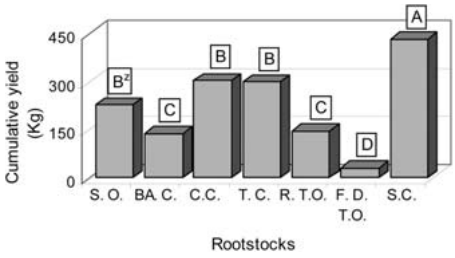


**Fig. 4. Cumulative yield of CEVd infected Comune Clementine grafted on seven rootstocks: S. Marina sour orange (S.O.), BA-300 citrange (BA.C), Carrizo citrange C.R.C. 2863 (C.C.) and Troyer citrange C.R.C. 1459 (T.C.), Rubidoux C.R.C. 838 trifoliate orange (R.T.O.), Flying Dragon trifoliate orange (F.D.T.O.) and Swingle citrumelo (S.C.). [z]Bars with the some letter do not differ significantly (P = 0.01).**

**Fig. 5. BA300 citrange grafted with Comune Clementine infected with CEVd.**

Trees grafted on Rubidoux C.R.C. 838 trifoliate orange showed exocortis symptoms, and they were stunted and gave poor yield 15 yr after inoculation. BA-300 citrange did not show any bark-scaling 15 yr after inoculation, demonstrating its tolerance to the exocortis viroid (6). The CEVd isolate used in the trial which is widely spread in many different old-line citrus caused no exocortis symptoms in any of the citrange rootstocks under our environmental conditions. Observations on some farms and on other fields in Sicily showed that Troyer and Carrizo citranges affected by CEVd have good growth and productivity and are symptomless (Davino, unpublished data). Since trees on this

TABLE 2
FRUIT QUALITY OF CEVD INFECTED 'COMUNE' CLEMENTINE GRAFTED ON SEVEN ROOTSTOCKS

| Rootstocks | Fruit weight (g) | Peel thickness (mm) | Juice (%) | TSS (%) | TA (%) | TSS/TA |
|---|---|---|---|---|---|---|
| S. Marina sour orange | 95.54 AB[z] | 3.34 AB | 37.92 ab | 9.86 AB | 0.76 ns[y] | 13.23 ns |
| BA-300 citrange | 105.31 A | 3.48 AB | 35.30 b | 9.40 AB | 0.76 ns | 12.50 ns |
| Carrizo citrange C.R.C. 2863 | 97.25 AB | 3.11 B | 37.25 ab | 9.36 B | 0.78 ns | 12.40 ns |
| Troyer citrange C.R.C. 1459 | 97.98 AB | 3.14 B | 39.35 ab | 9.82 AB | 0.74 ns | 13.49 ns |
| Rubidoux C.R.C. 838 trifoliate orange | 95.62 AB | 3.28 AB | 39.99 a | 10.18 A | 0.82 ns | 12.28 ns |
| Flying Dragon trifoliate orange | 98.28 AB | 3.71 A | 36.08 ab | 9.90 AB | 0.80 ns | 12.56 ns |
| Swingle citrumelo | 90.32 B | 3.34 AB | 38.52 ab | 9.98 AB | 0.79 ns | 12.73 ns |

[z]Figures in each column followed by the same letter do not differ significantly. Small and capital letters apply to P = 0.05 and P = 0.01, respectively.
[y]ns = non significant.

rootstock have a smaller canopy, the lower productivity could be overcome by growing higher numbers of trees in high density plantings (8). Similar results have been obtained in other studies (6). Because of its tolerance to CEVd this rootstock can grafted with propagation material from old-line citrus trees infected with CEVd.

Our results are in contradiction with other researches (2). As also reported by Cohen et al., (3), these observations indicate that the classification of rootstocks into 'sensitive' and 'not sensitive' to CEVd is artificial and their response may vary in different geographic areas.

## ACKNOWLEDGMENTS

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