Long-Term Effect of CVd-III of Plants on Citrange, Trifoliate and Sour Orange

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ABSTRACT. The effects of CVd-IIIb on Troyer citrange, trifoliate orange and sour orange seedlings, or budded with Moro sweet orange or SRA-63 clementine as scions, were evaluated in the field. After 11 yr, all inoculated plants contained CVd-IIIb, one of them had been contaminated with CEVd, and a multiple viroid contamination had occurred in a non-inoculated control plant. Troyer citrange and trifoliate orange seedlings or scion-budded plants inoculated with CVd-IIIb, showed a growth reduction rate of 16 to 32% as compared with uninoculated controls. Other negative effects were not observed. CVd-IIIb inoculation did not significantly affect sour orange plants. These results suggest potential application of CVd-IIIb to reduce tree size in high density plantings on Troyer citrange or trifoliate orange rootstock.

Several graft transmissible dwarfing sources have been tested to reduce tree size in high density plantings of some scion/rootstock combinations. Later, most of these sources were found to contain several viroids singly or in various combinations (1, 3). Recent studies have shown the specific effect of different viroids on tree size and fruit yield of infected plants (3, 4, 5, 6, 9). In this study, the long-term effect of CVd-IIIb viroid on three different rootstocks was determined.

The inoculum source plant, named CMC, was selected from a group of 18 Clementine trees grafted on alemow. Seventeen of these plants died from a severe cachexia syndrome; whereas CMC, the sole survivor, showed only mild stunting. Graft-inoculation of CMC on Etrog citron induced a mild epinasty typical of CVd-III.

Troyer citrange, trifoliate orange and sour orange seedlings, and plants of Moro sweet orange and SRA-63 Clementine both propagated on the above rootstock species were planted in 1983 on a 1 m × 1 m spacing. After 1 yr, half of the plants of each species and combination were graft-inoculated with two bark chips from CMC; while the other half were left as uninoculated controls. In 1991, the planting was cleared by removing 75% of the trees, and in 1995 (11 yr after inoculation) 18 inoculated plants and 18 uninoculated controls (two per each treatment and combination) were measured and analyzed for viroid content.

Green bark from the above field trees was collected in summer 1994 and nucleic acids extracted by the procedure of Flores and Llácer (2). Sequential polyacrylamide gel electrophoresis (sPAGE) analysis (8) of these extracts showed that all plants inoculated with CMC contained a viroid that migrated as CVd-IIIb (7) (~293 nucleotides). One of them also contained CEVd. Uninoculated plants were viroid-free except one that was co-infected with CEVd, CVd-IIa and CVd-IIIb. These unexpected infections probably resulted from accidental mechanical inoculation. Each of the accidentally contaminated plants had a canopy size similar to the other plants within the same treatment, which suggests that contamination occurred at a late stage.

Reverse transcription (RT)-PCR was performed as described in a companion paper (10) using primers specific for CEVd, CEVd-II, and CVd-III. This analysis confirmed the identity of the viroids detected by sPAGE. No amplification product was obtained from healthy plant extracts.

The effect of CVd-IIIb on tree growth was evaluated by measuring canopy volume and trunk circumference 30 cm above the soil level. Inoculation with CMC caused a 20% canopy volume reduction in Troyer citrange seedlings, a 26% reduction in trifoliate orange seedlings and no detectable effect on sour orange seedlings. Symptoms other than tree reduction were not observed. Moro sweet orange plants inoculated with CMC showed a canopy volume reduction of 30%, 32% and 0.3% when grafted on Troyer citrange, trifoliate orange and sour orange, respectively. Canopy volume reductions observed on CMC-inoculated SRA-63 Clementine trees were 16%, 25% and 3%, respectively. The reduction rates in trunk circumference paralleled those of the canopy volume. Symptoms of finger imprints and horizontal line striations in the trunk reported by other authors (9) were not observed in CVd-IIIb-infected plants on trifoliate orange rootstock.

Size reduction caused by CVd-IIIb on Troyer citrange and trifoliate orange in our conditions was relatively small compared with that observed by other authors. This might be due to the growing conditions of our plants during the first 8 yr (high planting density), that probably limited the growth of both inoculated and uninoculated plants.

The effect of viroid inoculation on fruit yield, size and quality were evaluated 8 years after planting (6). No difference was observed between healthy and CMC-inoculated clementine plants on Troyer citrange or trifoliate orange; whereas inoculation of Moro sweet orange plants on these rootstocks showed early fruiting and a significant yield increase in comparison with uninoculated controls.

The long-term effect on tree size observed in this experiment suggests that CMC is a potential dwarfing agent that could be used for high density planting of trees on Troyer citrange or trifoliate orange rootstocks. Nevertheless, the putative risk of accidental contamination with other viroids should be carefully assessed before extensive application of this dwarfing technique.

ACKNOWLEDGMENTS

We thank Annamaria Mondio and Giancarlo Usignuolo for technical assistance. This research was supported by Consorzio Produttori Pompelmo Italiani (COPPI) within the project Programma Operativo Multiregionale, Sottoprogramma 2, Misura 2.

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