Incidence and Effect of *Citrus tristeza virus* on Commercial Orchards of Tarocco O.L. Sweet Orange in Italy

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ABSTRACT. In 2001, many foci of *Citrus tristeza virus* (CTV) were found in different areas of eastern Sicily, Italy, in particularly in Catania and Syracuse provinces. Monitoring of two CTV-infected blocks of 297 trees located in the area of Belpasso, Catania province, where the virus was first detected on Tarocco O.L. sweet orange grafted on sour orange was initiated the same year. The study aims were: (i) to monitor the diffusion and spread of the infection in this area, (ii) to analyze the aphid vector population; (iii) to assess tree decline in the two blocks with regard to yield and fruit quality. Diffusion of CTV was monitored by direct tissue blot immunoenzymatic assay (DTBIA) tests using a mixture of two monoclonal antibodies. Most of the aphid population present in the two blocks was composed of cotton and melon aphids (*Aphis gossypii* (Glover) and the study results showed that this species is the main vector of the virus in the different citrus areas of Sicily. Many infected trees were symptomless giving yield comparable to CTV-free plants, while infected plants exhibiting decline showed reduced yield and fruit size.

Index words. Aphid population, yield, fruit quality.

Citrus tristeza virus (CTV) was first reported in Italy at the beginning of 2001 in nurseries on numerous trees imported illegally from abroad and also sporadically on isolated trees in the field (3). Italy was therefore one of the few citrus growing areas in the world where CTV had not caused severe damage. In June 2001 there were reports of rapid decline of many trees in orchards in different areas of eastern Sicily, at Belpasso in Catania province and Cassibile in Syracuse province.

In Belpasso the focus of CTV was on 25-yr-old Tarocco O.L. sweet orange trees grafted on sour orange rootstock, with this rootstock being the most commonly used in the Italian citrus groves (3). The isolates of CTV detected previously had been described as mild strains that had long been present in different areas of the Mediterranean Basin, whereas the most recent ones resembled Asiatic or American strains (5) and are transmissible by aphids (4).

When vectors are present, this virus poses a great threat and has caused severe damage to commercial orchards (8). Therefore, systematic monitoring of two blocks of 297 trees was initiated to assess diffusion and spread of CTV infection in this area, and the effects of aphid vector population and tree decline were assessed for their effect on yield and fruit quality.

MATERIALS AND METHODS

Two blocks (A and B) of 297 trees each of Tarocco O.L. sweet orange grafted on sour orange, were selected for a 4-yr-trial in Belpasso (CT), Italy, where the focus of CTV was identified. Each tree of the two blocks was periodically tested for CTV infection by direct tissue blot immunoenzymatic assay (DTBIA) using a mixture of two monoclonal antibodies, 3DF1+3CA5 (Plant-Print, Valencia, Spain) (1).

In the field printing on cellulose membrane was performed with petioles of four leaves removed from the four cardinal points, and in springtime additional tests were performed with peduncles of closed flowers (7) when temperatures were favourable (minimum 17°C-21°C, maximum 24°C-28°C) (9).

The CTV incidence was calculated, for each block, as the percent of the total number of trees which were CTV positive during the test period. The progression (temporal incidence) was analyzed by linear regression analysis (WinStat program) using as independent variable the time and as dependent variable the percentage of trees infected.

Each spring, 20 bouquets of flush colonized by aphids were removed from both blocks when aphid colonization was at its height and observed with dissecting binocular microscope to assess the percentage of the different species of aphids present in the field.

Four tests (1, 2, 3 and 4) were replicated 40 times (20 in block A and 20 in block B) in a randomized block design with single tree plot. All tests were CTV infected except test 4, which was a CTV-free control. The trees of each test showing different symptoms were selected to analyze the effect of CTV disease on yield and fruit quality. In particular, all plants selected in test 1 showed severe decline, all trees in test 2 showed initial symptoms of decline, all plants in test 3 were symptom free with good vegetative growth while the trees in test 4 showed an excellent vegetative growth and were used as a CTV-free control (Fig. 1).

Every year, fruits from four tests were collected to determine the mean yield per plant (Kg/plant).

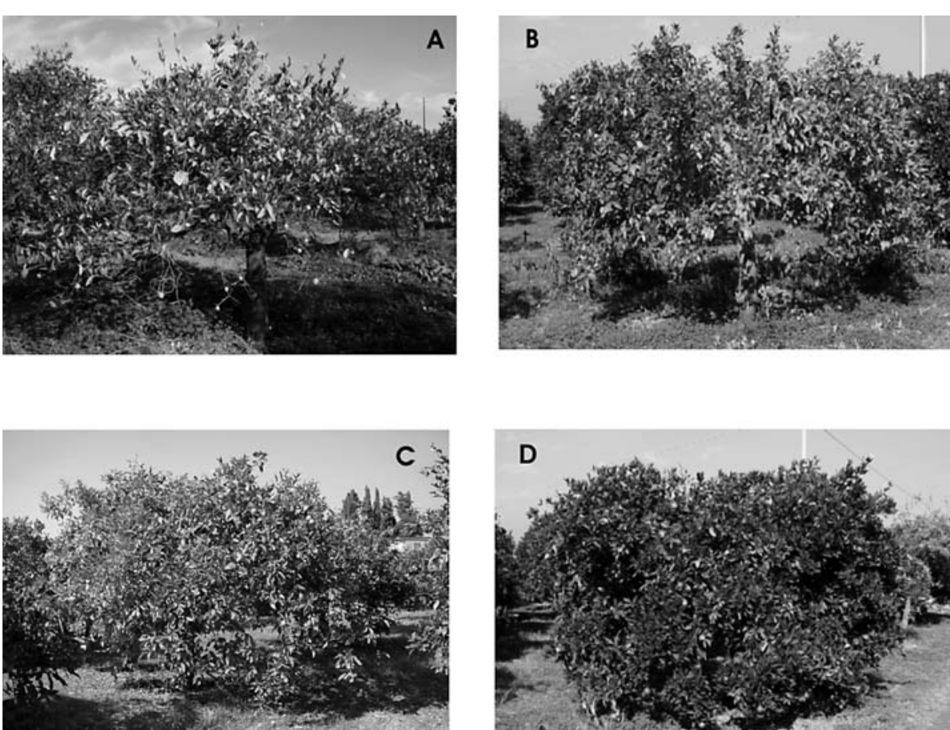

Fig. 1. Trees of 'Tarocco O.L.' sweet orange grafted on sour orange. A) Test 1, CTV infected tree showing severe decline. B) Test 2, CTV infected tree showing initial symptoms of decline. C) Test , CTV infected tree with good vegetative growth. D) Test , CTV free tree.

From the four tests samples of 20 fruits were collected every year from each plant over the three year study interval to analyze fruit quality (average fruit weight (g), percentage total soluble solids (TSS), percentage total acidity (TA) and ripening ratio (TSS/TA)).

In test 4 observations were made only on the trees DTBIA tests showed to be free from CTV at the end of the trial.

Simple variance analysis and Tukey's test were performed on yield and fruit quality parameters every year.

RESULTS

Tests performed in October 2001 revealed that the percentage of CTV-infected trees was 19.4% and 19.2% in blocks A and B, respectively (Fig. 2). Tests carried out in September 2004 revealed the percentages had risen to 36.7% and 35.6% in blocks A and B respectively (Fig. 2). The percentage of infected trees in these blocks increased during the testing period. The increases were 1.9% and 1.5% between tests in October 2001 and May 2002 and were 5.5% and 5.3% between May 2004 and September 2004.

Tests carried out in September 2002 and in October 2003 showed a lower percentage increase compared

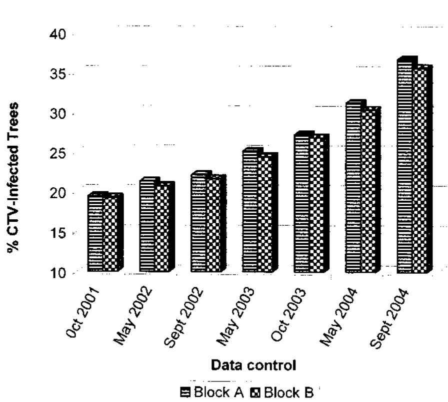

Fig. 2. CTV incidence over time in blocks A and B.

to May 2003 (+3% in block A, +2.8% in block B). Unlike the previous years, the maximum increase in year 2004 was observed between spring and autumn. This was due possibly to the climatic conditions as year 2004 was characterized by a very mild summer, and hence favorable conditions for increase in aphid populations. In contrast, summer temperature maximums had been higher in trial years 2002 and 2003.

The data from each block analyzed using a linear regression model (Fig. 3) showed a high correlation between the time and the percentage of incidence, with a corresponding coefficient of correlation (r) of 0.96 for two blocks being highly significant ($p \leq 0.05$). The statistical analysis was comparable for the two blocks and the results were confirmed also by the incident straight line for CTV (Fig. 3), which similarly showed the rapid increase of virus infection during four years of the trial.

Observation of the aphid populations revealed homogeneous, stable aphid colonies of mainly *Aphis gossypii* Glover (90%), bean aphid (*A. fabae* (Scop.)) (5%) and green potato aphid (*Macrosiphum euphorbiae* (Thomas)) (5%) (6).

Comparison of the three yearly yields (Fig. 4) revealed significant differences between the four tests investigated. As expected, yield was lower in tests 1 and 2 every year, with fruit size smaller than standard and often elongated (Fig. 5). Almost all infected trees flowered profusely in winter (Fig. 6). Statistically significant, albeit less marked, differences were observed between yields between tests 3 and 4. As shown in Fig. 4, there is a perfect correlation between degree of infection and yield during the trial period when comparing the four tests.

The average fruit weight (Table 1) had a significant and constant trend during three years for the four tests. The lowest value was obtained in test 1 while the highest value was found in test 3.

Block	Intercept	Regr. Coef. (b)	Std. Err.	Correl. Coef. (r)	Determ. Coef. (R^2)	p
A	14.90	2.81	0.31	0.969	0.940	0.00030
B	14.90	2.59	0.30	0.967	0.935	0.00037

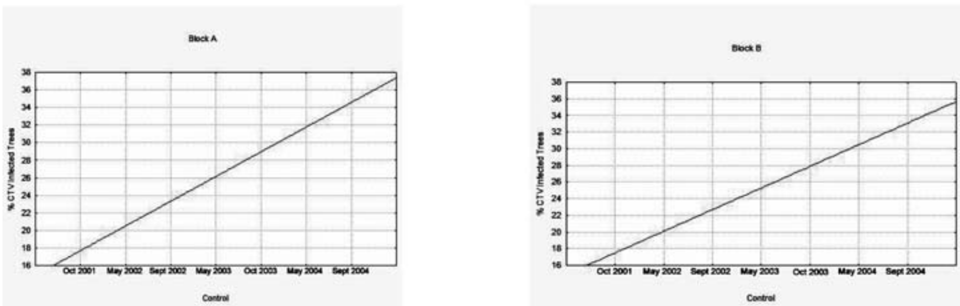

Fig. 3. Linear regression analysis of the incidence of CTV sampled at seven time points.

Total solids (Table 1) did not differ greatly and did not show a constant significant trend during the trial.

The differences in the acidity levels were highly significance between the tests. The highest values were in tests 3 and 4, while the lowest values were observed in tests 1 and 2, which had trees affected by severe and mild decline. This directly influenced the ripening ratio which was highest and statistically significant between tests 1 and 2, while there were no statistically significant differences between tests 3 and 4.

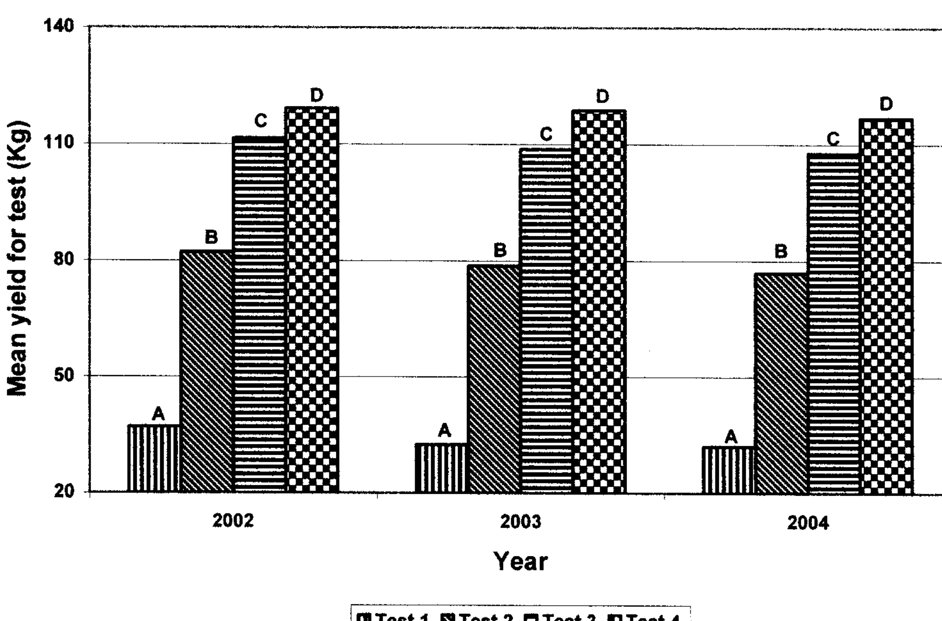

Fig. 4. Mean yield of four different tests over 3 years. Figures followed by the same letter do not differ significantly. Capital letters apply to p = 0.01.

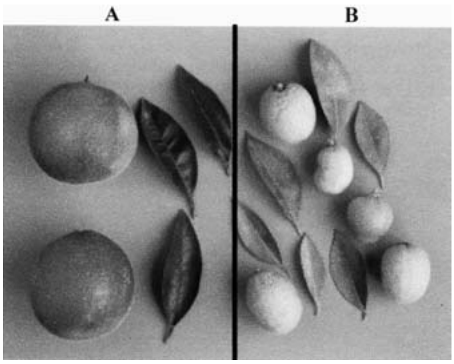

Fig. 5. Different sized fruit. A) Fruits from healthy trees. B) Fruits smaller and elongated from CTV infected trees.

DISCUSSION

Tests carried out over a four year period showed that incidence of CTV varied depending on the season and aphid populations. The percentage of infected trees almost doubled during the trial, rising from 19.4% to 36.7% and from 19.2% to 35.6% in blocks A and B, respectively.

The increase in CTV incidence during the 4 yr of trials showed an almost perfect statistical correlation with the sampling time and the controls. The calculated incident strait line for both blocks showed a comparable trend. All this demonstrates that the aphid populations are able to spread the infection particularly well during spring season, except during mild summers when conditions were optimal for the aphid reproduction.

Fig. 6. CTV infected 'Tarocco O.L.' tree shown flowering profusely in winter (December).

As in other citrus growing areas, *A. gossypii* (also the demonstrated CTV vector in Sicily) constituted 90% of the aphid colonies present in commercial orchards. Even if they are less efficient in transmitting CTV than the brown citrus aphids (*Toxoptera citricida*), *A. gossypii* is nonetheless able to spread CTV effectively in the field as shown by previous studies (2).

Our results agree with data reported from other citrus growing areas where CTV has spread and where *A. gossypii* is the main vector and where *T. citricida* is not present. In fact, the infection incidence during the time is comparable to that found by other researchers (8).

Every year, when compared to CTV-free controls, yield was reduced by about 8% in all symptom free infected trees, by about 30% in trees showing initial symptoms of decline and by over 70% in trees affected by severe decline.

The average fruit weight was lower in tests 1 and 2, which showed evident decline symptoms. Fruit weight was higher in test 3 when compared to controls because the trees, although infected, were symptom free with good vegetative growth and producing fewer fruits (data not shown).

Significant differences in fruit quality parameters were observed for acidity and consequently for ripening ratios in trees with severe and mild symptoms. In fact the fruits of these plants have accelerated ripening as compared to healthy and symptomless infected trees as reported for other citrus diseases.

CTV reduced yield and fruit quality led to commercial losses. Moreover, the symptoms worsened and a high percentage of initially symptomless trees later presented more or less evident symptoms of decline, and trees with severe decline died

TABLE 1
MEASUREMENTS OF FRUIT QUALITY FROM DIFFERENT PLOTS

Plot	Fruit weight (g)	TSS (%)	TSS/TA (%)	TA (%)
1	119 d	11.1 a	8.5 d	1.29 d
2	157 c	10.9 b	9.2 c	1.17 b
3	192 a	11.3 a	10.8 a	1.03 c
4	178 b	10.9 b	10.2 b	1.06 c

Values in a column which share the same letter are significantly different.

during the trial. The CTV diffusion caused by *A. gossypii* in Sicily (which holds the more important Italian citrus groves) represents a serious threat to Italian citriculture. The situation could further worsen with the arrival in Italy of *T. citricida*, which is already present in some areas of Portugal and Spain.

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