Spread of *Citrus tristeza virus* in an Endemic Area in Argentina

M. I. Plata and C. M. Anderson

ABSTRACT. Natural spread of *Citrus tristeza virus* (CTV) in a heavily infested citrus area in Concordia, Entre Ríos, Argentina has been monitored since 1993 in different citrus receptor hosts under field conditions. The plot, planted in 1990 using virus-free budwood, included navel and late orange, tangerine, and grapefruit varieties, all on trifoliate orange rootstock. Three young shoots, about 10-15 cm long, were collected from each tree. Diagnosis for CTV was done initially by ELISA-DAS and, since 1998, by direct immunoprinting-ELISA. In 1993, 43% of the grapefruit and 100% of the late orange trees indexed CTV-positive and by 1999 all grapefruit trees were infected. Seventy-six percent of the navel orange trees were CTV positive in 1993 and 100% in 1997. After 9 yr of field exposure, 100% of the grapefruit, late orange and navel orange trees indexed CTV-positive, whereas 86% of the tangerine trees were infected. This is an indication that tangerine may be less susceptible than grapefruit, late and navel oranges under Concordia conditions.

Citrus tristeza virus (CTV) has been present in Argentina since 1930 and was the cause of death of more than 18 million citrus trees budded on sour orange rootstock (4). At present, tristeza is endemic, and therefore tolerant rootstocks are used (1). No data on the actual rate of virus spread under field conditions is available.

Since 1993, the spread of CTV in a heavily infested citrus area in Concordia, Entre Ríos, has been monitored in a plot established to evaluate virus-free cultivars performance in the field. Natural spread of CTV in different citrus receptor hosts was studied under field conditions. The plot, planted in 1990, included, 38 navel and 20 late orange trees, 25 tangerine and 15 grapefruit trees, all of them budded on trifoliate orange rootstock.

Tristeza spread was monitored yearly by indexing all trees, initially using ELISA-DAS and, since 1998, by direct-immunoprinting-ELISA. Each tree was sampled collecting three young shoots about 10-15 cm long, from around the canopy at a height of approximately 1.5 m. For ELISA-DAS, shoots were trimmed, placed into plastic tubes and homogenized in an extraction buffer in a ratio 1:5 (w/v), using a tissue homogenizer. Extracts were tested by a standard ELISA double antibody sandwich procedure (5) using CTV antisera 512 (kindly provided by Guillermo Marcó). Direct-immunoprinting-ELISA sample imprinting was done on 0.45 µ nitrocellulose membranes (Millipore), followed by blocking and addition of CTV-specific antibodies (3DF1+3CA5 monoclonal antibodies) alkaline phosphatase conjugate and substrate addition and reading, according to Cambra et al. (3).

Variation of CTV incidence with time is summarized in Table 1. All late orange trees were infected by tristeza 3 yr after being planted in the field and more than 97% of the navel trees were already infected in the fourth year. In contrast, less than 43% of the grapefruit and 50% of the tangerine trees were infected in the third year. Figure 1 shows the disease progress in grapefruit, tangerine and orange trees planted in the plot (data from Table 1). Figure 2 shows tristeza increase using the logistic transformation of the per unit incidence (8, 10).

The data can be reasonably well fitted to a straight line with the logistic transformation Ln(x/1-x), where x = proportion of infected trees. Correlation coefficients were 0.88 for grapefruit, 0.85 for tangerine and 0.73 for navel orange. CTV incidence in the late orange trees

TABLE 1
RATE OF *CITRUS TRISTEZA VIRUS* SPREAD IN CONCORDIA, ENTRE RIOS

Year	Grapefruit[z] (% infected)[y]	Tangerine[z] (% infected)[y]	Late orange[z] (% infected)[y]	Navel orange[z] (% infected)[y]
1990	0	0	0	0
1993	42.8	50	100	76.5
1994	71.4	50	—	97.1
1995	71.4	50	—	97.1
1996	78.6	68.2	—	97.1
1997	92.9	72.7	—	100
1998	92.9	86.4	—	100
1999	100	86.4	—	—
2000	—	86.4	—	—

[z]Group of different citrus varieties.
[y]As determined by ELISA or Direct-immunoprinting-ELISA.

was too high from the beginning as to make any mathematical consideration about the rate of disease increase in the following years (8).

Thus, natural spread of tristeza in grapefruit under Concordia conditions may be explained by the exponential or compound interest model (9, 10). The different rates of CTV progress measured and given by the slopes of the regression equations (Fig. 2), suggest that late and navel orange trees are much more susceptible than tangerine and grapefruit trees, at least under the present study conditions.

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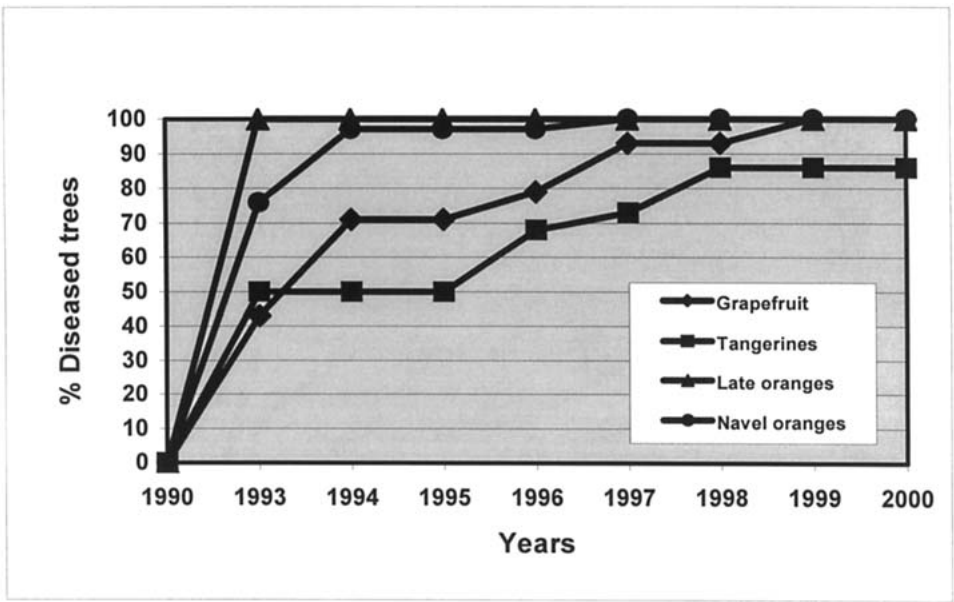

Fig. 1. *Citrus tristeza virus* disease progress in different citrus species in a plot in Concordia, Argentina.

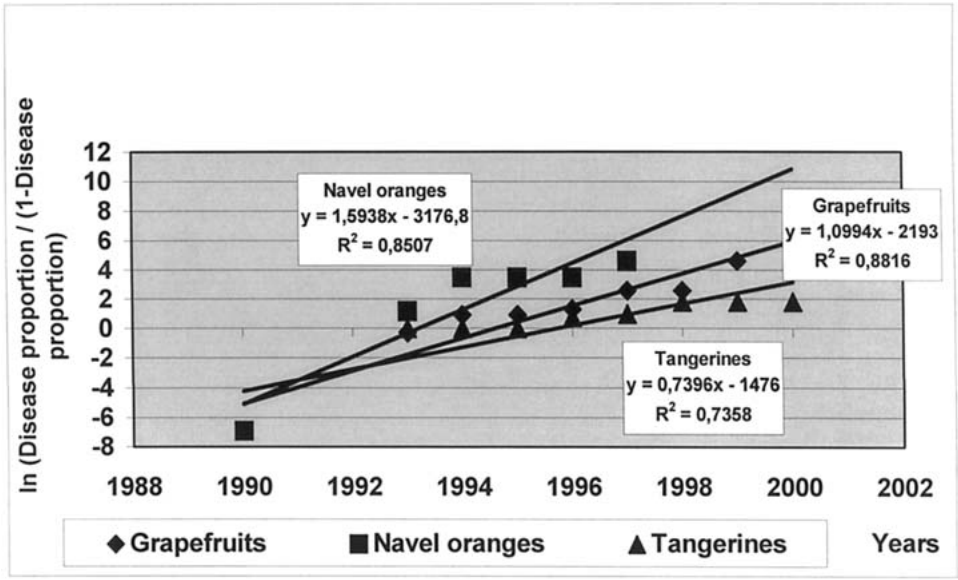

Fig. 2. *Citrus tristeza virus* disease progress in a plot in Concordia, Argentina, using the logistic transformation.

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