TRISTEZA AND RELATED DISEASES

Present Situation of the Citrus Tristeza Virus in the Valencian Community

M. Cambra, J. Serra, D. Villalba, and P. Moreno

ABSTRACT. Tristeza disease of citrus (CTV) occurred heavily in the Ribera Alta areas of the Jucar Valley (Valencia) beginning in 1957. From that time an estimated 10 million trees grafted on sour orange have been killed. Most of them were in the Valencian Community (VC), which is composed of Alicante, Castellón, and Valencia, where about 160,000 ha (approximately 64 million trees) are planted. Only 20% of these trees are grafted on tolerant rootstocks. To determine the real incidence of CTV, an infection map was made by random sampling 0.1% of the trees in Alicante (15,705), Castellón (15,915) and Valencia (33,000). Diagnosis of CTV was done by ELISA-DAS using CTV-specific monoclonal antibodies. The selection of sampling fields was randomized and proportional to the citrus areas on aerial photographs. Three young shoots, about 10-15 cm long, were collected from each tree. The sample collection method, their transport to the laboratory, and analysis were standardized and the field data, together with the final diagnosis were included in a computerized databank. Preliminary data from 80% of the samples indicate that 8.5% of the hectarage does not appear to be diseased; that on 60.4% the incidence of infected trees is lower than 10%; that on 25.3% the incidence varies from 10% to 50%; and on 5.8% of the total hectarage in the VC, the incidence of infected trees is higher than 50%.

Index words. Disease surveys, ELISA, monoclonal antibodies, decline, sour orange, sampling methods.

Citrus tristeza virus (CTV) was probably introduced into Spain as early as 1930, but it was not reported until 1959 (15) after an outbreak in 1957. Since then more than 10 million trees, grafted on sour orange, have died, most of them in the Valencian Community (VC) which is composed of the provinces of Alicante, Castellón and Valencia.

About 160,000 ha of citrus (approximately 64 million trees) are grown in the VC. The majority (80%) are grafted on sour orange rootstock. Fifty per cent of the citrus hectarage of the VC is located in Valencia, about 25% in Alicante, and 25% in Castellón.

Tristeza disease incidence in the field varies among different citrus areas (13), and the available data on geographical distribution of the disease is based on visual symptoms in the field. These data are incomplete because infected trees frequently may be symptomless.

In 1986, a survey was started throughout the citrus areas of the VC to establish the geographical distribution of CTV. The survey was based on detection by ELISA (2, 6) using CTV-specific monoclonal antibodies (16). This information was needed to make specific recommendations to growers in different local areas, to prevent further spread of the disease and maintain, as long as possible, production from plantings grafted on sour orange.

In this paper we report data of the survey covering 80% of the citrus area of the VC. Surveys of CTV incidence have been done in other Spanish areas (4, 7, 12), Israel (3, 8, 9), U.S.A. (1, 9, 10), Jordan (11), Cuba (N. Matos, personal communication, 1981), and elsewhere (9).

MATERIALS AND METHODS

Number of samples and distribution. One in every thousand trees was sampled for the survey for a total of

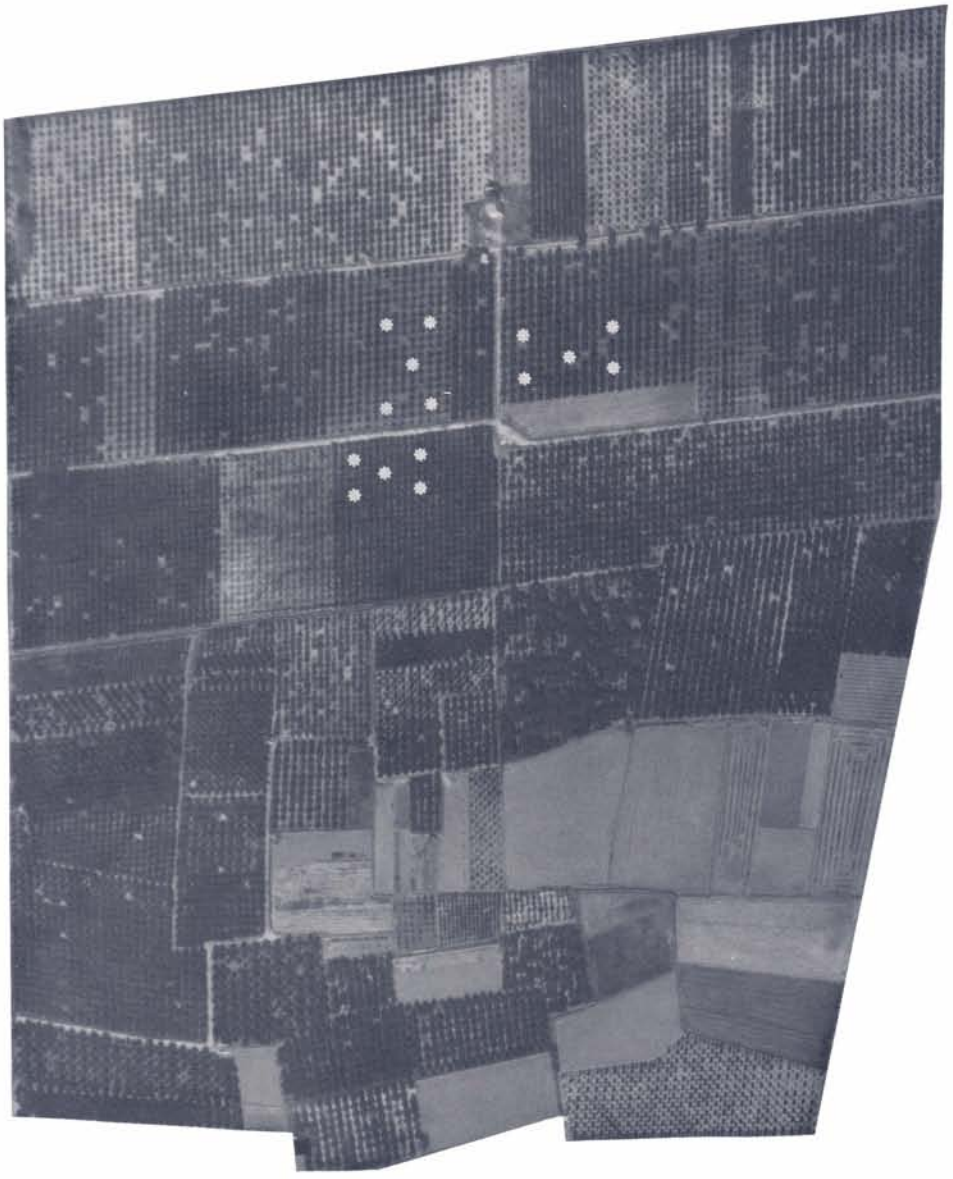

Fig. 1. Aerial photograph showing an area of the municipal district of Algemesí (Valencia) with about 25% CTV-infected trees. From the sampling area, 3 orchards have been selected and 5 trees located at least 15 m apart have been sampled in each one. (The trees are marked on the photo).

approximately 65,620 samples. Previous work in Andalucia (4, 7, 12) and the northern area of Castellón (Cambra *et al.*, unpublished) showed that sampling 0.1% of the trees, gave a very accurate indication of the real incidence of CTV infection.

The survey was done individually for each municipal district. The number of samples to be taken in each district was determined according to its total citrus hectarage. Then, the location of the sampling areas was selected randomly, using aerial photographs (scale 1:5,000). In each sampling area, three different orchards were selected and five trees located at least 15 m apart (8) were sampled

in each orchard. Grouping samples in sampling areas facilitated the work and it was statistically justified because of the small size and great variations in age and varieties of Spanish orchards. Figure 1 shows an aerial view of a sampling area.

Sample collection. Samples were collected by two people from the Agricultural Extension Service, using plastic bags, pruning scissors, marker, adhesive tape of various colours and aerial photos of the area to be sampled. Thirty-eight basic teams were involved in the survey. Three apical budsticks,10-15 cm long, of the youngest material existing at the moment of sampling were collected from different parts of each tree to be sampled. In the case of lemons, five budsticks were taken from each tree. In both instances, the number of budsticks taken per tree was related to the minimum number of budsticks needed from a CTV-infected tree to have 100% probability of getting an ELISA-positive reaction (5, Cambra, unpublished results).

The budsticks of the five trees of each orchard were individually fastened together in a bundle, with adhesive tape, as previously described (5) (fig. 2). Each bundle was labeled according to a preestablished code for easy location of each sample. This method allowed rapid sample collection and labeling and greatly facilitated laboratory determinations and tree identification.

Sample data and shipping. One record card was completed for each bundle at sample collection with the following data: sample code, variety and rootstock, age of planting, topworking information, location, presence of tristeza symptoms and other observations. The record card had a carbon copy.

Sets of 80 bundles were enclosed in a plastic bag and kept at 4 C until

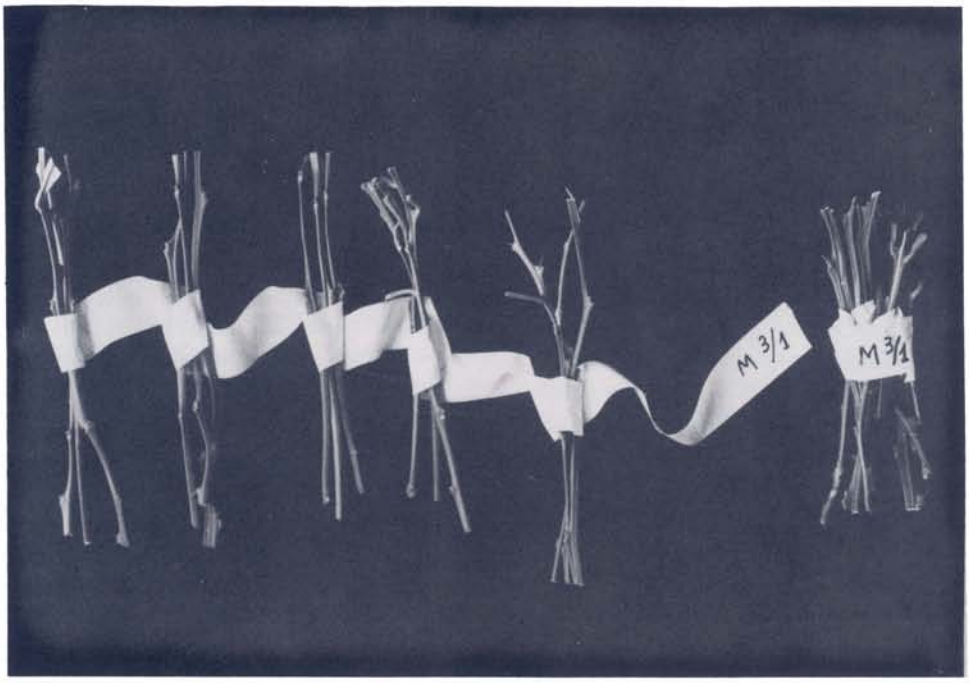

Fig. 2. On the left the method for placing the adhesive tape sets of 3 budsticks 10-15 cm long for each tree is shown. Five trees constitute one sample from one orchard. On the right, the bundle made up by putting together the sticks from 5 trees. This method affords considerable advantages when repeating ELISA tests, by separating trees in the case the bundle gives CTV ELISA positive. Code M 3/1 means: municipal district of Moncada, sampling area no. 3, orchard no. 1.

shipment to the testing laboratory. Shipping was made in standard boxes containing the samples and their record cards. Each sample box had a special label allowing quick processing through the post office and in the laboratory.

Sample analysis and databank. An ELISA laboratory was specially created at the Plant Protection Agency facilities to conduct this survey. The sample boxes were kept at 4 C in the laboratory until they were processed. Each box contained approximately the number of samples needed to fill up an ELISA microplate. The ELISA-DAS method was employed using CTV-specific monoclonal antibodies (16). Microplates were sensitized with 1.0 μg/ml of immunoglobulins. Sample preparation was performed by thinly slicing part of all budsticks of each bundle with pruning scissors. The remaining pieces of budsticks were kept at 4 C. Approximately 0.2-0.3 g plant material was homogenized with 2-3 ml of extraction buffer (PBS + 1% PVP-10, pH 7.2-7.4) using a homogenizer. The conjugate with alkaline phosphatase was used at 0.5 μg/ml (1/4,000 dilution). Reading of results was done after 1 and 2 h of incubation at room temperature, using an automatic ELISA reader at 405 nm. When a bundle of samples gave an ELISA-positive reaction, budsticks of individual trees of each bundle, which had remained individually fastened with the adhesive tape, were indexed again to determine which trees in the bundle were infected by CTV.

Optical densities at 1 and 2 h in positive samples were noted on the original record card, as well as the total number of diseased trees. One copy of the card was sent back to the Agricultural Extension Agency as information to the owners of the orchard. The data contained in the record card were entered in a computerized databank.

RESULTS AND DISCUSSION

Table 1 shows general data on CTV incidence in the provinces of Alicante, Castellón and Valencia. They include results of 80% of the total number of samples to be processed. Valencia was the province with the highest percentage of infection, followed by Alicante. The lowest percentage was in Castellón.

Eight and one-half percent of the area studied was found to be free of the disease. These areas fall within marginal or distant zones, far from points of maximum infection. In these zones the rate of topworking is lower, and so is the uncontrolled movement of plant material.

In 11.8% of citrus hectarage there was more than 25% infected trees. The highest rate of infection was found in 0.6% of the hectarage, where more than 75% of the trees were CTV infected. Trees heavily infected were located around the initial foci where the tristeza outbreak occurred in 1957. Tristeza has spread in these areas through movement of infected plant material and by aphid transmission.

In the larger part of the hectarage (55.9%) there was less than 5% of infection.

Only 60% of CTV-infected trees grafted on sour orange showed tristeza symptoms in the field. About 80% of these CTV-infected, symptomless trees were satsuma varieties. None of the CTV-infected trees grafted on tristeza-tolerant rootstocks showed any symptoms.

The largest relative percentage of infection occurred in sweet orange, followed by Clementines. The lowest percentage of infection was observed in grapefruit, sour orange and lemon. These results are similar to those obtained by other authors (14).

Fifteen percent of the samples tested came from trees grafted on tolerant rootstocks that were originally free of viruses. About 4% of them were CTV infected.

TABLE 1
INCIDENCE OF CITRUS TRISTEZA VIRUS (CTV) IN DIFFERENT CITRUS AREAS OF THE VALENCIAN COMMUNITY

	Percentage of trees infected															
	0		0-2		2-5		5-10		10-25		25-50		50-75		>75	
Province	Ha	Area %	Ha	Area %	Ha	Area %	Ha	Area %	Ha	Area %	Ha	Area %	Ha	Area %	Ha	Area %
ALICANTE	4,768	11.7	9,807	24.1	17,307	42.5	5,099	12.6	2,855	7.0	820	2.0	0	0.0	0	0.0
CASTELLON	595	2.0	1,595	5.3	13,822	45.6	4,398	14.5	6,590	21.8	0	0.0	0	0.0	0	0.0
VALENCIA	5,463	9.1	9,372	15.6	8,710	14.5	7,195	12.0	15,189	25.3	6,756	11.2	6,660	11.1	754	1.2
TOTAL	10,826	8.5	20,774	16.3	39,839	31.1	16,692	13.0	24,634	19.3	7,576	6.0	6,660	5.2	754	0.6

The results obtained in the survey show that there are scattered zones, basically in the north of Castellón, with very low rates of infection where it would be possible to consider an eradication program.

In most areas with a low rate of infection, it would be advisable for the growers to voluntarily remove infected trees to reduce inoculum density. With an adequate policy, it may be feasible to use sour orange in some soils of these areas where tristeza-tolerant rootstocks perform poorly. The risk will not be too high because CTV spread is slow in these areas.

In extremely contaminated areas topworking or bud collection should be avoided and if trees are grafted on sour orange, removal or interplanting with tolerant rootstocks should be started.

The final computer processing of all samples, in addition to the disease spread data which are being obtained from different citrus areas, will soon permit design of a general policy to control CTV in each citrus area of the VC.

Summarizing, the data of this survey will enable: a) selection of areas with very low incidence and spread of the disease, where eradication programs could be established; b) study of the possibility of using sour orange rootstocks in some soils of areas with low and very low CTV incidence, where tristeza-tolerant rootstocks perform poorly; and 3) location of areas with high CTV incidence, regardless of field symptomatology, where replantings on tristeza-tolerant rootstocks should be accelerated and movement of plant material entirely avoided.

ACKNOWLEDGMENTS

Survey for tristeza in the Valencian Community was possible thanks to the collaboration of a number of Departments of the Council of Agriculture and Fisheries of the Valencian Government; the Agricultural Extension Service (9 agencies in Alicante, 8 in Castellón, and 18 in Valencia), the Centre for Transfer of Technological Agriculture, and the Institute for Agricultural Research of Valencia (IVIA). A number of agencies from the local Agricultural Production Office have also cooperated. Our special thanks to about one hundred technicians collaborating in the preparation of the map, to J. Juárez for his photographs, and to A. Borràs for translations.

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