Historical review of Citrus tristeza virus in Portugal

Nolasco G.

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CTV and its vectors in the Mediterranean basin

Historical review of *Citrus tristeza virus* in Portugal

Nolasco G.

Centre for Biodiversity, Functional and Integrative Genomics, Universidade do Algarve
Campus de Gambelas, Faro, Portugal

Abstract. Most of the citrus production in Portugal is located in the Algarve region, in the south, which accounts for almost 70% of the national production. A boom of citrus planting occurred in the late 1980s with the introduction of modern varieties. This was accompanied by the introduction of CTV in the plant propagation material. Surveys for the virus and vectors started also at that time accompanied by the eradication of several *foci* of the disease. In parallel, works aiming at the molecular characterization of the virus were pursued. Up to the year 2000 most of the virus variants found in the orchards were similar to the Spanish predominant variant. Later the appearance of different variants became frequent. In 2005 new *foci* were detected in the north western part of the country in places where *T. citricidus* was present. With these new virus variants, the complete set of strains existing at worldwide level is now present in Portugal mainland.

Keywords. Citrus – Portugal – Rootstocks – Tristeza – Virus.

Aperçu historique du virus de la tristeza des agrumes au Portugal

Résumé. *Au Portugal, la production des agrumes est essentiellement concentrée dans la région de l'Algarve, dans le sud du pays, qui représente presque 70 % du total national. Au cours des années 80, le verger agrumicole a connu un nouvel essor à la suite de l'introduction des variétés modernes. Parallèlement, le CTV a été introduit à travers le matériel de multiplication. Des enquêtes ont donc été entreprises pour la détection du virus et de ses vecteurs, accompagnées de l'éradication de plusieurs foyers de la maladie. En même temps, la caractérisation moléculaire du virus a été complétée. Jusqu'à l'an 2000, la grande partie des variantes du virus décelées dans les vergers étaient similaires à la variante prédominante en Espagne. Successivement, diverses autres variantes ont été observées. En 2005, de nouveaux foyers ont été identifiés dans le nord-ouest du pays, dans des régions où le T. citricidus était présent. Vu la mise en évidence de ces nouveaux variantes, il est possible de conclure que la gamme complète des souches de CTV connues à l'échelle mondiale est actuellement représentée au Portugal.*

Mots-clés. *Agrumes – Portugal – Porte greffes – Tristeza – Virus.*

I – Introduction

Citrus trees are grown all over mainland Portugal, covering an area of 26,200 Ha (Anonymous, 2007). The Southern region of Portugal, Algarve, has the most dynamic and intensive citriculture and accounts for 17,860 Ha. In the remaining areas, citrus is grown in small scattered orchards or as backyard trees. The Algarve region accounts for 60% of the national sweet orange production, 85% of mandarin production and 40% of lemon production.

The first boom of citrus planting in Algarve occurred in the period 1960-1970 as a result of the implementation of new irrigation schemes. The citrus area raised from 1560 Ha in 1950 to 5500 ha in 1970. At that time most of the production was based on sweet orange (Washington navel and local varieties with reduced commercial value) grafted onto sour orange rootstock. Symptoms attributable to graft-transmissible diseases could be easily observed in the field e.g. conspicuous symptoms of concave gum and psorosis.

A second boom of citrus planting occurred in the late 1980s as a consequence of the income of specific funds from the EU for the development of Portuguese agriculture and of the increasing demand for citrus fruits. In 1989 the citriculture in Algarve reached about 15,000 Ha. Since then the curve of the citrus-growing area has been flattening. This boom was accompanied by a strong desire of the farmers to obtain modern varieties with high market value. A significant influx of illegally introduced budwood occurred in that period in parallel with authorized limited importations of certified plants from Spain. Starting at that time, most of the new plantations were grafted on tolerant rootstocks, namely Troyer and Carrizo, which now account for 70% of the plantations. The introduced varieties that are nowadays the most representative are for the sweet oranges: Newhall, Navelina, Lanelate and Frost Valencia; for the clementines and hybrids: Fina, Nules, Marisol, Hernandina, Encore, Ortanique, Nova and Fortuna.

II – Historical review of CTV in Portugal

Before the late 1980s boom of citriculture in Algarve, no systematic survey for CTV had been done. Declining trees could be sporadically observed in the orchards but its cause was usually attributed to root damages by rats.

1986. In a collaborative action between the University of Algarve and the Regional Services of Agriculture, Nolasco (unpublished results) performed a prospective ELISA survey using the 3DF1 monoclonal antibodies provided by IVIA, targeting old declining trees. No positive results were found.

1988. A first systematic survey was done in part of Algarve based on aerial photography maps (scale 1/15000) by Nolasco and Faustino (Faustino, 1989). The survey was done by ELISA (Ingenasa Mca, 3DF1) on 1/1000 of the trees, corresponding to 236 Ha. No virus was detected in the field.

In an effort to modernise the citriculture sector, a special authorization for the limited importation of citrus plants from certified sources was issued. The material was imported from two certified citrus nurseries from Valencia region in Spain and re-tested in Portugal by ELISA. Surprisingly, some batches were infected, reaching 6% incidence. Confirmation of the positive results was done by ISEM by Dr. Diamantina Louro at the Central Plant Protection Services in Lisbon. Two of these isolates were preserved and later their coat protein genes sequenced (Sequeira and Nolasco, 2002) and deposited in the GenBank (haplotypes 19-121 and 25-120, accessions AF184114 and AF184114, respectively).

1989 – 1994. The prohibition to further import new varieties led several farmers to illegally import budwood from Spain for top-working old orchards. This situation pushed the governmental services to start surveys in nurseries and in suspicious top-worked orchards. CTV was detected occasionally in locations all over the country, being in principle, eradicated. This enabled Portugal to be considered as a Protected Zone for CTV during this period and subsequent years. However, the definite destruction of the infected material occurred sometimes several months after the first detection. Some old citrus introductions present in the germplasm collections were also surveyed. CTV was detected in an infected line of symptomless Genoa lemon in a germplasm collection of a deactivated citrus research station in Setubal (near Lisbon). Later molecular characterization of this isolate showed that it was made up of a single haplotype, 28C, which was deposited in the GenBank (accession number AF184118), (Nolasco, unpublished results). Surveys in the surroundings of infected foci always produced negative results, suggesting the absence of natural aphid vectoring.

During this period the first legislation envisaging citrus certification was issued, (Decreto lei 277/91 and Portaria 416/94), but except for CAC material, it was not mandatory.

1995 - 2000. Two types of ELISA surveys were designed: targeted sampling (nurseries, top-worked orchards and their surroundings) in which 5% to 10% of the plants were tested and random sampling of orchards at a rate of 1 plant per 1.5 Ha (approximately 0.1% to 0.24% of the plants) was done. However, due to logistic limitations, in the random sampling survey only a small part of Algarve region could be tested every year, averaging 775 ha per year. The targeted survey detected 3 foci in nurseries and 15 in top-worked orchards with illegally introduced material. This resulted in the destruction of about 31 thousand trees. No infected plants were detected in the surroundings of hot spots. In the random sampling assay 3 foci were detected which corresponded also to top-worked orchards. The results obtained during these years characterize a situation in which CTV is starting to become endemic and which is mainly disseminated by low quality agricultural practice.

Collaboration of the University of Algarve with National and Regional Plant Protection Services has allowed the molecular characterization of the infected material detected since 1989. Most of the coat protein gene sequences obtained from these isolates were very closed related (less than 1% difference at nucleotide level) to the sequence of the haplotype 25-120 (introduced from Spain in 1988) and to the mild T30 isolate from Florida. Partial biological characterization on Mexican lime, Madam Vinous and Sour Orange as well as the absence of reaction with monoclonal antibody MCA13, showed that these isolates were of mild type (Nolasco, 2000). On the contrary, the isolate obtained in Setubal had a haplotype which differed by less than 2% from the VT haplotype obtained from a severe isolate from Israel. The biological and serological characterization confirmed that this is a severe isolate although not inducing stem pitting on sweet orange. Other samples collected in the central part of Portugal depicted a coat protein gene sequence very similar to the haplotype 28C obtained in Setubal.

Surveys for CTV vectors were done almost every year. The most abundant species captured in Moericke traps was *Aphis spiraecola*, followed by *A. gossypii* and *Toxoptera aurantii*. The prevalence of these two was variable from place to place. *A. craccivora* was rare and *T. citricidus* was not found (Ramos *et al.*, 2000). Except for *T. citricidus,* the ability of these aphids to acquire the virus from infected plants was demonstrated in the laboratory by RT-PCR (Reis *et al.*, 2000).

2001 - 2002. These years mark, probably, the starting of the natural transmission of CTV in the Algarve region. Eighteen new foci were found, including old orchards in the surroundings of newly top-worked orchards. Additionally, an orchard which was previously found negative became infected. In this period 13,800 trees were marked for eradication. It should be noticed that since the start of the random sampling systematic surveys from 1995 to 2002 only 7,000 Ha , i.e,. only 40% of the total citrus area has been surveyed in Algarve.

2003 - 2005. Due to economic constraints, the governmental services decreased significantly the intensity of the systematic surveys. The molecular characterization of novel isolates based on the coat protein gene was carried out. Besides haplotypes molecularly close to the 25-120 (Spanish origin), new haplotypes appeared which were never found before in Portugal. These new types were geographically found close to the region of Silves, Algarve, and had a close relationship with haplotypes found in very severe CTV isolates e.g, SY568 from the USA (GenBank Accession AF001623) or 13C from Madeira Island (GenBank Accession AF184118). These findings suggest a new source of CTV, whose origin can be traced back to the budwood illegally introduced from Spain, which is usually molecularly close to the 25-120 haplotypes.

During this period *T. citricidus* was found in the north western part of Portugal (Ilharco and Sousa-Silva, Pers com.). A few scattered small orchards in that region were surveyed for CTV in the fall of 2005. CTV was found in infested trees. Molecular characterization of CTV from 2 trees about 30 Km apart showed that these harbored a mixture of haplotypes which include some newer ones in Portugal (close to T36 from Florida, T3 from Florida and B249 from Venezuela). The structure of the CTV population found in both trees was not significantly different (Nolasco *et al.*, 2007).

These findings suggest another new source of introduction and the existence of natural spreading of CTV by *T. citricidus* in the region.

Taking into account all the coat protein gene haplotypes that have been detected, Portugal has now all the haplotypes that can be found elsewhere in the world.

III – Conclusions

This history illustrates a few critical points on the introduction of CTV in a country. To become competitive, the farmers feel the pressure to obtain new varieties. In the absence of an efficient national programme for the development of citriculture, the illegal introduction of budwood starts. In practice the Plant Protection Services can not control all the budwood movement. Hence, an efficient survey is of paramount importance to be able to evaluate the evolution of the situation in the field. This needs a sampling rate much higher than that applied in Portugal. A few calculations show that with the sampling rate used, if the disease incidence is low (e.g. less than 1%) the probability of finding an infected tree is also lower than 1%, which is useless! The building up of inoculum in the field may be overlooked by the farmers and agricultural services until natural transmission by aphids starts, even in the absence of *T.citricidus.*

The increase in vector-mediated transmission implies a change in the short-term control strategy. Surveys should be much more efficient to be able to follow the situation. Molecular typing of strains is now of utmost importance to concentrate the eradication efforts in the elimination of strains that are able to deteriorate trees grafted onto tolerant rootstocks (e.g stem pitting strains). Besides this selective eradication, the objective of general eradication should be to maintain the inoculum level at a low, economically acceptable, level.

References

Anonymous, 2007. Citricultura Algarvia. Published on-line by the regional services of agriculture of Algarve. http://www.draalg.min-agricultura.pt.

Faustino J.J., 1989. Prospecção do virus da tristeza dos citrinos no Algarve pela técnica ELISA. Relatório de estágio de fim de curso de Engª Agrícola. Universidade de Évora, Portugal.

Nolasco G., 2000. Desenvolvimento de bases de protecção contra o virus da tristeza dos citrinos e vector Toxoptera citricidus. Relatório final do Projecto Praxis XXI 3/3.2/Hort/2159/95.

Nolasco G., Fonseca F., Silva G., 2007. Occurrence of genetic bottlenecks during Citrus tristeza virus acquisition by Toxoptera citricidus in field conditions. *Archives of Virology*. Published online. DOI DOI 10.1007/s00705-007-1089-8.

Ramos N., Fernandes J.E., Reis J., Nolasco G., 2000. Afídeos vectores do vírus-da-tristeza-dos-citrinos (CTV) no Algarve. Livro de Actas do Congresso Nacional de Citricultura, Faro, Nov. 2000, pp. 167-183.

Reis J., Ramos N., Fernandes J.E., Mendes C., Santos C., Nolasco G., 2000. Estudo da transmissibilidade do vírus da tristeza por afídeos vectores. Livro de Actas do Congresso Nacional de Citricultura, Faro, Nov. 2000.

Sequeira Z., Nolasco G., 2002. Bacterial expressed coat protein: development of a single antiserum for routine detection of Citrus Tristeza Virus. *Phytopathologia Mediterranea*. 41: 55-62.