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Historical review of Citrus Tristeza in Morocco

Zemzami M.¹, Benali D.², Moukhtari A.², Soulaymani A.²

¹ DDA-UCP, Domaine Maamora, Salé, Morocco

² Université Ibn Tofaïl, Dpt de Biologie, Kénitra, Morocco

Abstract. This review outlines important facts and dates which marked the history of the long and laborious struggle to fight against citrus tristeza virus in Morocco. CTV is widespread worldwide. It has been detected in Morocco several times in various varieties of germplasm introduced to Morocco during the second quarter of the 20th century. Meyer lemon is the only infected variety planted to a large extent. This variety was tracked and eradicated everywhere in the country. Some recent importations of new citrus varieties were also CTV-infected. However, each time CTV was found, immediate eradication actions were taken. Eradication has, up to now, been quite effective. Legislation has been enforced to backup CTV control strategies by mandatory eradication, limitation of citrus importations, compulsory quarantine, and finally instauration of a certification program. Significant efforts were devoted by public and private institutions to develop appropriate means and facilities for efficient CTV control. MAbs were produced in Morocco and as well, new diagnostic techniques were acquired. These means were used for extensive tristeza surveys and eradication in the country. Awareness towards the danger of CTV and its efficient vector is growing, but more is still needed to secure the future of citrus industry in Morocco.

Keywords. CTV – Eradication – Morocco – Tristeza.

Revue historique de la tristeza des agrumes au Maroc

Résumé. Cette revue retrace les faits et dates importants qui ont marqué l'histoire de la lutte laborieuse contre le virus de la tristeza des agrumes (CTV) au Maroc. Le CTV est répandu à travers le monde. Il a été décelé au Maroc à plusieurs reprises dans diverses variétés de collection introduites au Maroc durant le second quart du 20ème siècle. Le citronnier Meyer est la seule variété atteinte qui a été cultivée à large échelle. Il a été traqué et éradiqué partout dans le pays. Certaines introductions récentes de nouvelles variétés ont aussi été infectées de CTV. Cependant, chaque fois que le CTV est mis en évidence, des actions immédiates pour son éradication ont été prises. L'éradication a jusqu'à présent été très efficace. La législation a été renforcée pour renforcer la stratégie de lutte contre le CTV, en instaurant l'obligation d'éradiquer le CTV, la limitation des introductions des citruses de l'étranger, la quarantaine obligatoire, et enfin, l'instauration du règlement de la certification. Des efforts louables ont été dédiés par les institutions publiques et privées au développement de moyens et d'infrastructures pour une lutte efficace contre le CTV. Ainsi, des anticorps monoclonaux ont été développés au Maroc, de même des nouvelles techniques de diagnostic ont été acquises. Ces moyens ont servi pour des prospections étendues pour l'éradication de la tristeza dans le pays. La prise de conscience vis-à-vis du danger du CTV et de son vecteur le plus efficace « *Toxoptera citricidus* » est grandissante. Cependant, beaucoup reste à faire pour sécuriser l'avenir de l'agrumiculture au Maroc.

Mots-clés. CTV – Éradication – Maroc – Tristeza.

I – Introduction

Citrus trees are a major fruit crop in Morocco. They cover about 80 thousand ha with an average yearly production of 1.5 million metric tons of which about 50% is exported as fresh fruits. Commercial varieties and cultivars include early and late clementines, Navels, Valencias, blood oranges and very little of lemon and grapefruit. The main rootstock by far is sour orange. Nevertheless, other rootstocks are now commonly used in new plantings, in particular Citranges (Carrizo, Troyer, C-35), Volkameriana and Macrophylla.

II – Tristeza historical events in Morocco

CTV was probably introduced in Morocco early in the 1930's with citrus culture development in the country. Furthermore, commercial germplasm repositories were established before 1951, with material introduced from abroad, when the aetiology of tristeza disease was still unknown. Hence, there is no realistic reason to preclude the introduction of CTV-infected material with the massive number of citrus varieties existing in germplasm blocks, most of which are of foreign origin (Nadori and Zemzami, 1987). Irrefutable proof for that is the commercial planting of Meyer lemon, known to harbour CTV (Wallace and Drake, 1955) to a large extent. This variety was later confirmed in Morocco to harbour CTV (Chapot and Cassin, 1961).

1930 - 1950. Establishment of Souihla Germplasm block with the supervision of Chapot, which contains 368 varieties and selections imported mainly from California, Florida, South Africa, Australia, Argentina, Brazil, Spain and other neighbouring countries (Nadori and Zemzami, 1987).

1951. Issuing of a decree to regulate importation of plant material of *Rutaceae* to Morocco (Anonymous, 1951).

1955. Rebour reported the results obtained by Wallace and Drake which proved infection of the original Meyer lemon budwood source by CTV in the USA. He alerted all Mediterranean countries which imported Meyer lemon. This alert was reiterated for Morocco by Mendel in 1956.

1959 - 1961. Cassin conducted CTV indexing on Mexican lime (Wallace, 1959) of Meyer lemon and some other varieties of foreign origin. Meyer lemon and 8 other accessions were found infected with CTV. They were eradicated from germplasm blocks (Cassin, 1963).

1964. Chapot and Delucchi (1964) reported infection of some exotic varieties with CTV, including all Meyer lemons in germplasm repositories as well as in commercial plantings, various Satsumas and Kumquats in Morocco, Sicily, Israel and Egypt.

1967. Bové confirmed the introduction of tristeza into Morocco in budwood of exotic varieties including Owari Satsuma and King Mandarin imported from Florida between 1945 and 1948. Preservation of that material was done by grafting sour orange rootstocks established in the field. It happened to graft more than one variety on a single rootstock and to re-graft sour orange plants with other varieties when the first grafting failed. He adverted against the risks of having CTV-infected Meyer lemon trees planted to a large extent in the Marrakech valley.

1968. The Citrus Committee of the Franc Zone (CAZF) stressed out the urgent need to eradicate all known sources of CTV in the country. A governmental decree was issued for mandatory eradication of Meyer lemons in the region of Marrakech. Professionals and farmers cooperated fully with the authorities to launch an eradication campaign of Meyer lemon that included 18 farms. Subsequent surveys and indexing of neighbouring orchards indicated absence of any signs of natural propagation of tristeza. This action may be questionable as whether it was fully successful. However, the awareness raised among Moroccan vis-à-vis the danger of tristeza that has stood strong since then.

1969. The False Alert of Tristeza: in an indexing block of budwood from nucellar varieties introduced in 1964/65 from the USA, a Hamlin orange grafted onto Mexican lime with typical CTV symptoms was discovered by Bourge and Nhami at El Menzeh Experimental Citrus Research Station.

Since budwood from Hamlin orange was largely distributed, its potential infection with CTV was an issue of concern. The first expertise survey made an alarming assessment, indicating that tristeza was largely present in various orchards in Beni Mellal area. This same year, a decree was issued to regulate circulation of budwood and nursery plants of citrus and citrus relatives between different regions in the country (Anonymous, 1969).

1970. The CTV alert triggered a general mobilisation among officials and the professionals (INRA, SODEA OCE, ASPAM, SASMA). The CTV alert enigma was further investigated by other worldwide experts. The outcome of the surveys and study tours conducted by these experts indicted unanimously that the Hamlin/Mexican lime tree had unequivocal CTV symptoms. However, none of the Hamlin trees produced from budwood of USA origin was infected with CTV. Hamlin material collected from the field in Morocco was indexed in France and two Hamlin trees in California. All results were negative. It was shown that the initial expertise confused severe Stubborn symptoms with those of tristeza. Therefore, the alert of tristeza being widespread in Beni Mellal region was a false one. Experts revealed that they found during their surveys some former Meyer lemon trees topworked with other varieties, which is a real danger for the dissemination of CTV. They requested immediate eradication. Again, no signs of natural spread of CTV were noticed (Calavan, 1969). The false Tristeza alert raised awareness among officials and professionals for the need to set a strong CTV control strategy by devoting adequate means (fund, trained human resources, appropriate equipment and infrastructure) for this mission.

1978. Nhami, from Société de Développement Agricole (SODEA), conducted a survey for the sanitation of SODEA farms, with special consideration of Meyer lemon. Seven Meyer lemon trees were found in 3 farms. Their eradication was swift (Nhami, 1981).

1980. Fourteen Meyer lemon trees were again found in 3 citrus orchards in Marrakech area. They were eradicated as well (Nhami, 1981).

1983. Setting up of a certification program for citrus nursery plants (Anonymous, 1984). A scheme was inspired by the Californian and the Spanish certification programs, requiring systematic indexing of CTV for initial and pre-basic material and screening by ELISA and molecular techniques of increase blocks. No CTV positives were ever revealed during the subsequent decade (Nhami *et al.*, 1993).

1984. The first quick declining tree was found in the citrus germplasm block at INRA-Station of El Menzeh. A tree of Pan American mandarin declined suddenly exhibiting typical quick decline symptoms (Fig. 1). A systematic testing of the entire germplasm block was ordered.

The remaining trees of Pan American mandarin were found infected with CTV. However, the survey could not be completed due to the lack of means (Nadori, personal communication).



Figure 1. Quick decline symptoms observed in the CTV infected trees.

1987. A thorough survey was undertaken to test the citrus germplasm block of INRA-Souihla, near Marrakech (Nadori and Zemzami, 1987). A total of 1749 trees were tested; 67 trees of 19 varieties were positive. After the preservation of plant material for research purposes and sanitation of infected varieties, eradication of all CTV-infected trees was completed meticulously (Fig. 2).

A similar survey was carried out in El Menzeh germplasm block in the Gharb valley near Kénitra. A total of 2130 trees were tested; 21 trees of 6 varieties were positive. They were also immediately eradicated (Nadori and Zemzami, 1992).

Similar surveys were accomplished to control the other INRA-germplasm of citrus: Aïn Chaïb and Melk-Zhar in the Souss valley near Agadir and Aïn-Taoujdate in the Saïss valley near Fès. Mainly varieties duplicated from Souihla germplasm were in these blocks; 9 positives were found all among duplicate trees of Pan American at Aïn-Chaïb and Aïn-Toujdate (Zemzami and Nadori, 1989).



Figure 2. Eradication steps of CTV infected trees.

1988. The first massive importation of certified citrus nursery plants from Spain to Morocco was made. It concerned 60 thousand plants of Fortuna, Nova and Marisol varieties. The plants had the label of the Spanish certification authority; nevertheless, a systematic testing by ELISA was imposed by the Plant Protection Services of Morocco. Huge efforts were deployed by the importer to fulfil the required tests. Plants were maintained for quarantine under an insect-proof screenhouse in an isolated locality far from citrus orchards (Fig. 3).

All plants were tested systematically by ELISA in autumn 1988. Several hundreds of positive cases were found, representing percentages varying from 5 to 10% depending on batches and varieties (unpublished data). All the infected material was eradicated (Fig. 3).

Released plants were tested again the following spring before undergoing further increase in the nurseries.



Figure 3. Overview of the imported certified plants maintained in screenhouse and the following eradication of some CTV infected plants.

1990. Domaines Agricoles created a facility (Unité de Contrôle des Plants UCP) devoted to the development of disease diagnostics means and sanitary surveillance of citrus and other crops in their farms. Work on the development of monoclonal antibodies for CTV detection was initiated. Immuno-reagents of high quality were produced (Zemzami *et al.*, 1993; Zemzami, 1995) and it became possible to undertake large surveys for CTV wherever suspected declines are reported. A total of 400 thousand ELISA tests were done from 1994 to 1998.

Impressive efforts were made to raise awareness for an open dialogue about tristeza through seminars and workshops animated by sound international CTV experts. Unfortunately, the subject of tristeza remained a taboo and every suspected CTV case is eradicated in silence (Lbida *et al.*, 2005).

1995. Domaines Agricoles extended the activities of UCP to conduct a certification program for their citrus nurseries. Indexing and sanitation facilities as well as molecular and serological laboratories were set up (Fig. 4). Hundreds of local selections were regenerated by thermotherapy/shoot-tip-grafting.

A sound certification program was operative. Tactics for Tristeza control strategies based on meticulous sanitary control and substitution of sour orange with tolerant rootstocks were fully integrated. Thorough indexing as well as serological and molecular testing were adopted for detection of viruses, and CTV tolerant root-stocks including Carrizo, Troyer and C-35 citranges, Citrumelo sacaton, and Volkameriana lemon were forced in the plantings with outstanding results.

Joined efforts were deployed by UCP and Plant Protection Services to provide Quarantine service for imported new varieties to other Citrus producers. Several Clementine varieties were released to the farmers through this collaboration.



Figure 4. Indexing facilities at the UCP Domaines agricoles.

1997 to date. UCP engaged in regional networks cooperating in coordinated actions to control CTV in the Mediterranean region. UCP participated actively in a CFC project for the control of CTV by new efficient RT-PCR techniques and the Mediterranean Network for Certification of Citrus. Characterization of Moroccan isolates of CTV was carried out. It showed presence of highly severe strains capable of causing disastrous ravages if they become efficiently disseminated (Zemzami *et al.*, 2002).

1998. Interception of CTV in citrus material introduced legally from Spain to Morocco by Plant Protection Quarantine Service (Lbida *et al.*, 2005).

1999. The Plant Protection Service was informed by SODEA technicians about abnormal dwarfing of 12 trees of Nova mandarin and Lane Late navel in a topworked orchard in the Souss valley. Presence of CTV in samples collected from suspected trees was confirmed by ELISA at both SODEA and Plant Protection laboratories. The owner of the orchard cooperated fully with the authorities and eradicated the whole plot composed of 900 trees. The origin of the material used for topworking could not be traced back to any local source. Tests conducted in the neighbouring

plots the next spring showed no positive cases, indicating again that no natural spread had occurred (Plant Protection Services, CTV-Team report, 1999).

2000. Again, interception of CTV in citrus material introduced legally from Spain to Morocco by the Plant Protection Quarantine Service (Lbida *et al.*, 2004).

2004. Isolates of CTV (P1 and P2) intercepted in citrus material imported from Spain by Quarantine Service in 1998 and 2000 were characterized. They showed genomic variability (Lbida *et al.*, 2004) as well as biological and serological differences (Lbida *et al.*, 2005).

2005. The General Council of the Ministry of Agriculture organized a meeting for national experts for an open debate about CTV control strategy to be adopted in Morocco in the light of the appearance of the Brown Citrus Aphid in Spain and Portugal. The input of Mr Nadori and Mr Nhami was highly enriching, both men have devoted much of their outstanding careers to the development of Moroccan citrus culture and its preservation from a disaster by *tristeza*. It was clear that CTV was found and eradicated to a higher extent than we knew. Fortunately, from both experiences, we drew 2 important conclusions:

- CTV foci found were all limited to some varieties and their distribution patterns do not reflect the presence of any natural propagation. This was reported on many occasions by various experts who surveyed Morocco.
- Accomplished eradications were always very successful. Further testing up to 2 springs after eradication never revealed any positive cases. This is of significant importance because many question if CTV eradication is ever possible!

Two other meetings were organized at the Plant Protection Headquarters in Rabat and at Domaines Agricoles in Casablanca. Both were open to a large audience including the Growers' Association ASPAM and major stakeholders of the citrus industry. The general consensus was that in the Mediterranean Basin we are all facing a serious threat as reflected by the widespread of CTV foci in all Mediterranean countries, the presence of severe strains and the arrival of *T. citricidus* in the region. Enthusiasm for implementing immediate CTV control actions was everyone's desire. However, until now the follow-up is weak.

III – Conclusion

Citrus *tristeza* virus was introduced into Morocco early in the last century in budwood introduced from abroad. CTV infected Meyer lemon was propagated largely in homogeneous plantings which were maintained until 1967 (Bové, 1995). It didn't fortunately find favourable conditions to become endemic. Infected trees remained isolated foci until their eradication. The numerous eradications that were carried out helped certainly very much contain the disease and prevent its diffusion. However, it would be naïve to think that Morocco is undoubtedly free of CTV. Characterization studies showed that some of the strains found are highly severe. They could cause extensive damage to our citrus culture if they get disseminated efficiently. Remarkable efforts have been deployed to develop adequate means for quick and efficient detection at affordable costs. A dependable certification program has been established since 1984. Substitution of the CTV-sensitive sour orange rootstock is underway. In spite of these achievements, we still have to remain alert and double our efforts at all levels of action:

- raising awareness of the growers and the public;
- engaging in germplasm exchange to make it safely accessible to the growers;
- intensifying efforts in quarantine, surveys and eradication of the virus;
- enforcing surveillance at the borders;

- stimulating substitution of sour orange and implementation of additional rootstock trials;
- strengthening of common harmonized regional actions with neighbouring countries throughout the Mediterranean basin.

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