The project proposal of the CIHEAMMediterranean Research Network on Certification of Citrus (MNCC): the regional programme for the mandatory control of Citrus tristeza virus and its major vector Toxoptera citricidus in the Mediterranean region

D'Onghia A.M.

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The project proposal of the CIHEAM-Mediterranean Research Network on Certification of Citrus (MNCC): the regional programme for the mandatory control of *Citrus tristeza virus* and its major vector *Toxoptera citricidus* in the Mediterranean region

D'Onghia A.M.

Coordinator of the CIHEAM-Mediterranean Research Network on Certification of Citrus
CIHEAM – Mediterranean Agronomic Institute, Valenzano (Ba), Italy

Abstract. The Mediterranean citrus industry is faced with a major threat: citrus tristeza, a devastating virus disease whose very efficient vector, "*Toxoptera citricidus*", has suddenly appeared in Northern Portugal and Spain. A very large proportion of the Mediterranean citrus orchards are still on sour orange; a root-stock highly sensitive to tristeza. With CTV inoculum present in almost all citrus producing countries, invasion of the Mediterranean region by *T. citricidus* will cause a rapid dissemination of CTV that will devastate all trees grafted on sour orange in a few years. A sudden collapse of the majority of the Mediterranean citrus industry will occur with unpredictable dramatic socio-economic consequences.

The present project proposal aims at preventing such a disaster through a joint effort of all countries in the region for the implementation of urgent preventive and control measures against both the virus and its vector. The main steps of the work plan include:

- enhancement of harmonized phytosanitary control measures and regulations (quarantine and certification);
- CTV monitoring and characterization;
- elimination of CTV-infected plants;
- monitoring aphid population and implementing efficient control measures;
- raising awareness;
- strengthening of harmonized research and know-how;
- technology transfer and information exchange.

Keywords. Citrus – Citrus tristeza virus – Control – Mediterranean – Project – Toxoptera citricidus.

Proposition du projet CIHEAM-Réseau Méditerranéen de recherche sur la certification des agrumes (MNCC) : Contrôle obligatoire du virus de la tristeza des agrumes (CTV) et de son principal vecteur Toxoptera citricidus dans la région Méditerranéenne

Résumé. *L'Industrie des agrumes dans le bassin Méditerranéen est confrontée à une très grave menace : la tristeza des agrumes, une maladie virale dévastatrice dont le vecteur le plus efficace, Toxoptera citricidus a été récemment rapporté au Nord du Portugal et de l'Espagne.*

Une très grande proportion de vergers d'agrumes au niveau du bassin Méditerranéen sont encore greffés sur bigaradier, un porte-greffe très sensible à la tristeza. Avec le CTV comme inoculum présent dans presque tous les pays producteurs d'agrumes, l'invasion de la région méditerranéenne par T. citricidus provoquera une diffusion rapide du CTV, induisant en quelques années un effet dévastateur sur tous les arbres greffés sur bigaradier. Un effondrement rapide de la majeure partie de l'industrie des agrumes du bassin Méditerranéen, portant à des conséquences socio économiques dramatiques. La présente proposition de projet a pour objectif de prévenir une telle catastrophe, à travers un effort conjoint de tous les pays de la région, afin de mettre en œuvre des mesures préventives d'urgence et des mesures de lutte contre le virus et son vecteur.

Les principales étapes de la stratégie comprennent:
- *Renforcement des mesures de contrôle harmonisées et réglementations phytosanitaires (quarantaine et certification);*

- *le suivi du CTV et l'éradication des arbres infectés par le CTV*
- *le suivi des populations de pucerons et la mise en œuvre des mesures de lutte efficaces;*
- *la sensibilisation;*
- *le renforcement de la recherche harmonisée et du savoir-faire;*
- *le transfert de la technologie et l'échange d'expériences et d'informations.*

***Mots-clés.**. Agrumes – Virus de la tristeza – Contrôle – Méditerranée – Proget –Toxoptera citricidus.*

I – Introduction

1. Importance of the Mediterranean citriculture

Citrus is one of the most important fruit crops in the Mediterranean Basin which contributes over 20% of the overall citrus production in the world and about 60% of fresh citrus world trade (CLAM, 2007). This area is the homeland of easy peelers (primarily Clementine and mandarin etc.) accounting for 75% of the worldwide exportation of these species; a worldwide increase of easy peelers was observed over the last two decades at the expense of fresh oranges, due to the evolution of consumer preferences (CLAM, 2007). However, the region produces special quality fresh fruits of orange, lemon and lime; the latter is of great economic importance in the Near-East countries. Grapefruit production is restricted to Israel which is the fourth world producing country. Hence, Citrus is a major segment in the Mediterranean agricultural industry; developing countries account for almost 40% of the production and citriculture represents a major source of income to a significant number of farmers. A number of citrus processing industries have developed in some Mediterranean countries, mainly for refreshing juice, jams, candies, flavors and other by-products destined to pharmaceutical and cosmetic industries.

Citriculture is therefore of strategic importance for the Mediterranean region for its socio-economic role as a source of income, of employment opportunities at various levels of the chain (production, processing trade and farming consumable suppliers) and for its contribution to the well being of people and the political stability of the countries.

Considering that the Mediterranean has been an important diversification zone for oranges, mandarins and lemons, they also represent a valuable reservoir of genetic resources which are still represented by old aged trees in most of the countries (i.e. Syria, Malta). The improvement of local citrus genotypes is therefore a great market opportunity, as for the Italian red oranges 'Tarocco' from Sicily, the mandarin 'Nadorcot/Afourer' in Morocco and 'Clementine' in Corsica, but also an important action for the preservation of the Mediterranean citrus biodiversity.

2. Citrus tristeza virus

Citrus Tristeza is the most devastating virus disease, caused by *Citrus tristeza virus* (CTV), which has destroyed millions of citrus trees throughout the world, mainly where sour orange was the rootstock: i.e. Brasil, Peru, Florida, South Africa etc. (Bar-Joseph *et al.,* 1989; Roistacher, 1995). It is spread by the movement of contaminated budwood and disseminated in the field by different aphid species (Raccah *et al.,* 1989) among which *Toxoptera citricidus*, the brown citrus aphid (BrCA), the most efficient vector, followed by *Aphis gossypii*; the latter was significantly active in CTV dissemination in Spain (Hermoso de Mendosa *et al.,* 1984; Moreno *et al.,* 2008) and Israel (Raccah *et al.,* 1976), where the virus is now endemic. The virus could not be eliminated, it was widespread and could no longer be contained by the elimination of the CTV-infected foci (Loebenstein, 1993).

The Tristeza status and its economic impact on citrus in any location depend on four main factors:

i. presence of the sour orange as susceptible rootstock;

ii. severity of CTV strains;

iii. ability of local aphid species to virus dissemination;

iv. presence of *T. citricidus*, which is the most efficient virus vector, also able to disseminate selectively the severe CTV strains.

When severe CTV strains are introduced into a citrus area where *T. citricidus* is present, Tristeza disease will spread rapidly and damage all citrus trees grafted onto any rootstock (Rocha-Pena *et al.,*1995).

3. State of the art of CTV infections in the Mediterranean Region

Tristeza poses a tremendous threat to the Mediterranean citrus industry, where areas are extensively grown onto the susceptible sour orange rootstock. Apparently, in all cases tristeza disease in the Mediterranean can be traced back to the introduction of infected budwood from abroad; all countries which introduced the Meyer lemon (e.g. Algeria, Cyprus, Israel, Italy, Morocco and Tunisia) have introduced tristeza as well (Bové, 1966).

Tristeza has caused significant damage to citrus in Spain in 1957 (Moreno et al., 2008) and Israel in 1970 (Bar-Joseph *et al.*, 1983) when *A. gossypii* developed a specific efficiency to disseminate local CTV strains. These two countries were forced to cope definitely with Tristeza and launched a total conversion of their citrus industry using Tristeza tolerant rootstocks (Bar-Joseph *et al.* 1989).

CTV infections are widely distributed in all Mediterranean countries, mostly as isolated foci and without showing clear-cut tristeza symptoms (Bové, 1995; Djelouah and D'Onghia, 2001). Luckily the virus incidence is still bellow critical levels although disease outbreaks have also been reported in Cyprus (Kyriakou *et al.,* 1996) and, recently, in Italy (Davino *et al*., 2003).

The CTV strains found in the Mediterranean region range from mild to highly severe capable of inducing the most devastating symptoms on citrus, when grafted onto the sour orange, such as quick decline; however, exotic severe virus strains, CTV-seedling yellows and CTV-stem pitting, were also detected in few countries (Ballester-Olmos et *al*. 1993, Zemzami *et al.* 1999) and they can be easily selected and disseminated through BrCA transmission.

Although the threat of Tristeza is a great concern, Mediterranean countries that have escaped disease outbreaks still rely heavily on the use of sour orange as a rootstock for its good adaptation and tolerance to saline water, calcareous soils and *Phytophthora* gummosis. Unfortunately sour orange replacement is still a major constraint because no other CTV tolerant/resistant rootstock has such broad spectrum characteristics.

4. New development on CTV vector status

The most efficient CTV vector is the brown citrus aphid (BrCA), *T. citricidus*, which can also colonize other plant species, i.e. *Rhododendron* sp., *Acerola* sp., *Malpighia punccifolia*, and *Eugenia uniflora* (Yokomi *et al.,* 1994), *Passiflora, Calodendron, Mangifera* and *Anacardium* genera (Roistacher, 1991). BrCA was found in Madeira (Aguiar *et al.,* 1994) where it causes the spreading of the severe CTV-stem pitting strain. Recently, it has been identified in Northern Portugal and Spain (Ilharco *et al.,* 2005) and apparently is already beyond eradicable stage. It is now only a matter of time before it reaches the citrus regions where favorable climatic conditions will enable its rapid invasion of the whole Mediterranean Basin.

The melon or cotton aphid, *A. gossypii*, is so far the most active CTV vector in the Mediterranean basin (Bar-Joseph *et al.,* 1983; Yokomi 1992; Cambra *et al.,* 2000). However, in some citrus areas *A. spiraecola* builds up larger populations than *A. gossypii* and its role in CTV dispersal could be important (Hermoso de Mendoza *et al.,* 1984).

A. gossypii is a polyphagous and migratory aphid, whereas *T. citricidus* feeds mainly on citrus and is a colonizer, which means that it attacks citrus trees and could reach heavily dramatic population levels. When present, *T. citricidus*, is able to pick and disseminate with 100% efficiency the severe strains of CTV, making it difficult to control the virus.

II – Objective

The overall goal of this proposal is to provide countries in the Mediterranean region with harmonized tools (useful information, technical protocols and regulations) for (i) controlling CTV and its vectors; (ii) preventing or delaying the introduction and dissemination of *T. citricidus*; (iii) assisting the citrus industry in maintaining its production in terms of quality and quantity, and preserving their export market share.

To achieve the overall goal the following specific objectives shall be pursued:

- the enhancement of harmonized Phytosanitary control measures and legislations at National and Regional levels (Quarantine and Certification of propagating materials);
- the application of harmonized technical protocols for CTV monitoring on a large scale, the characterization of CTV isolates and the eradication of infected trees;
- the application of harmonized technical protocols on aphid sampling, identification and vector efficiency;
- the raising of awareness;
- the strengthening of research and know-how for the control of CTV and *T. citricidus;*
- the technology transfer and information exchange.

III – Beneficiaries

The project will benefit all stakeholders in the citrus sector (Plant Protection, Quarantine and Horticulture services, nurserymen, citrus growers, citrus industry, packing houses, processing units, etc.).

IV – Justification

Tristeza situation in the Mediterranean region is a great concern. It is prone to evolve anytime now into a disastrous epidemic explosion. All factors are prevailing and remarkably favorable: the virus inoculum is widely distributed in all Mediterranean countries, severe virus strains are present, *T. citricidus* (the most efficient vector) has entered the region and the sour orange (the highly susceptible rootstock) is still widely cultivated as a major rootstock.

If no immediate coordinated actions are taken, the most severe forms of CTV will be rapidly spread by *T. citricidus*, and will inevitably lead to a sudden collapse of the citrus industry in many Mediterranean countries. The magnitude of socio-economic-environmental consequences to the region will be huge and devastating.

The aim of this project is to prevent such a disaster, by stopping and/or delaying its occurrence, and attenuating its effect where it is inescapable.

Within this context, the CIHEAM/Mediterranean Research Network on **C**ertification of **C**itrus (MNCC) has promoted a regional programme against CTV since 2004, during a network meeting

jointly organized with EPPO technical panels; after the first official finding of *T. citricidus* in Portugal, MNCC involved international organizations, as FAO and EPPO, to provide a serious support to this programme, which needs to involve all the Mediterranean citrus stakeholders. To this aim, three network meetings were organized in 2005 for raising awareness on this serious threat (Faro, Portugal; Cairo, Egypt; Adana, Turkey); posters and booklets in Arabic and English languages were widely distributed in different Mediterranean countries to support national extension services in disseminating information on tristeza and its vectors.

V – Coordination and partnership

The project could be executed by CIHEAM jointly with MNCC partners and with the collaboration of other organizations and donors. MNCC could play a decisive role in the project partnership at the regional level; the network is composed of a Mediterranean inter-institutional group which acts, on a continuous basis since more than 10 years, as a multidisciplinary working team of specialists from governmental, scientific institutions and private organizations actively involved in plant protection, quarantine, certification, citrus germplasm improvement and preservation, nursery and orchard management etc. Network activity, as reported in 4 CIHEAM publications and numerous papers published in scientific journals and proceedings, was mainly addressed to the acquisition of data on citrus pathogens and relative vectors in the Mediterranean and to the improvement and harmonization of protocols and procedures for the detection and control of citrus diseases. A Mediterranean certification scheme was developed by the network as an important proactive strategy for preventing the entrance and spread in the region of quarantine pests and pathogens affecting these species.

Surely the involvement of as many countries and institutions as possible is desirable, given the huge tasks ahead and the large amount of activities that are needed with particular reference to surveys and monitoring of production orchards, nurseries, new plantings etc..

VI – Workplan

Based on technical protocols set up by MNCC, the following activities will be carried out.

1. Enhancement of harmonized Phytosanitary control measures and regulations

Harmonized regulations on quarantine and certification should be soon implemented, issued (during the first year of the project) and promptly applied to assess virus/vector situation and implement immediate eradication of CTV foci, and to strengthen the establishment of a national certification programme.

Quarantine actions will focus on:

- the enforcement of phytosanitary controls at the critical points of entrance into the country and in virus/vector monitoring in the citrus growing areas;
- the setting up of regulations to prevent the entrance of *T. citricidus* through imported citrus plant materials and fruits;
- the setting up of a national virus/vector alert system by remote and proximal sensing for a rapid identification of virus/vector suspected sites to be immediately monitored;
- the development of a geo-database information system at national and regional levels regarding the progress of CTV infections, the distribution of the severe CTV strains and the presence of infestations by *T. citricidus*.

Certification actions will focus on:

- the development of an urgent and mandatory programme of CTV-tested citrus propagating material until a certification system is fully operational;
- the establishment or implementation of harmonized schemes, as set up by MNCC, for (i) the clonal and sanitary selection of citrus germplasm and for (ii) the certification and safe exchange of citrus propagating materials.

2. CTV monitoring and characterization

This activity will be carried out in partner countries where CTV monitoring is not routinely applied and the virus infections status is unknown or need to be updated. Technical protocols set up/ validated by MNCC and in this project will be applied.

Planning. Proximal and remote sensing techniques will support the monitoring planning to soon identify suspected infection sites. Information on the selected sites will be collected before field survey to quantify number of surveys and samples, quantity of materials and number of human resources.

Based on the results of processed satellite or aerial images, priority will be given to: (i) new plantings made of imported varieties, collection plots, budwood sources/mother trees and nurseries in the case of countries where *no information on CTV is available or need updating*; (ii) groves and nurseries in the infected sites in order to assess the infection spread where *CTV foci have already been reported* (with or without the eradication program) as isolated foci or at low infection spread; moreover, the activity will also include apparently CTV-free areas.

Virus monitoring procedures. Plants will be systematically monitored in the selected sites to assess virus presence, incidence and distribution using the hierarchic sampling as reported by Gottwald and Hughes (2000) and Direct Tissue Blot Immuno Assay (DTBIA) detection as reported by Garnsey *et al.* (1993); D'Onghia *et al.,* (2001). A preliminary testing with commercially available CTV kits/or antibodies will be annually conducted aimed at evaluating their performance with different CTV isolates, primarily the local ones.

Confirmation assays and communication of results. If a CTV infection is found in a new area, a confirmation test will be carried out by another institution using RT-PCR or Real time PCR. Results of virus infections should be officially communicated to the competent country authority.

Preparation of CTV infection maps. Based on the results, a map showing the monitored sites and, eventually, virus foci and incidence should be soon prepared in order to evaluate CTV infection progress in time and space.

Virus characterization. The characterization of the virus strain will be conducted in all monitored sites by biological, serological and molecular means in order to acquire information on their severity. In areas where CTV is already endemic the monitoring will concern only the severe and highly damaging virus strains.

3. Elimination of CTV-infected trees

Based on the results of virus monitoring, a program should be soon organized for the immediate elimination of the infected trees, if the infection is still at low levels. Virus monitoring and infected trees removal in the CTV foci should continue for at least three years to assess the complete elimination of the infection. The program will only concern the severe virus strains in areas where the infection is already endemic. The destruction of the infected trees or, if necessary (depending on the severity and incidence of the infection), of the whole orchard or nursery will consist in removing the tree (root apparatus included) which should be let to dry in safe places for final burning.

4. Monitoring aphid population and implementing efficient control measures

During virus monitoring, the identification of aphid populations will be carried out aimed at identifying the vector species, primarily *T. citricidus,* which is considered as a quarantine pest

Procedures for aphid monitoring are hereafter described.

Sampling. Aphid_sampling will be carried out using shoot sprays and/or traps, which will be periodically inspected in the laboratory for the identification of the aphid spp.

Aphid analysis for virus assessment. Identified aphid vectors collected in infected sites will be analyzed for virus infections by RT-PCR analysis.

Aphid transmission efficiency. Transmission trials with different aphid species will be carried out in climatic chambers to evaluate their capability in virus acquisition and transmission efficiency.

Results on aphid spp. Results on aphid vectors will be communicated to the national competent authorities in order to set up a control strategy, depending on their efficiency in virus transmission. In the case of natural virus transmission, isolation of nursery sites should be guaranteed or in areas where citrus trees are not grown or using insect proof facilities with an accurate aphid management. In the case of the presence of *T. citricidus* a dedicated pest control programme should be soon adopted to limit its spread.

5. Raising awareness

Awareness will be raised on the danger of Tristeza and other threatening diseases of citrus among the stakeholders (plant protection and quarantine personnel, extension services, nurserymen, citrus industry professionals/farmers and the general public) for preventing accidental introduction and dissemination of hazardous pests and pathogens of citrus. Information materials will be prepared for each audience: dedicated website, TV and radio spots, booklets, publications and technical reports in the mother tongue of the country. Particular emphasis will be given to the dangers of un-controlled introduction of citrus plant material and the benefits of the use of certified plants. Experience gained in countries which suffered from Tristeza disasters and where *T. citricidus* is the main vector will be presented to highlight the economic and social impacts of such threats on the citrus industry in our region and the need for setting up efficient harmonized control measures.

6. Strengthening of harmonized research and know-how

The project will promote a long-term research program by selecting equipped laboratories to conduct the following activity:

- molecular and biological tools for detection and identification of CTV strains will be improved and made available to technicians from participating countries; these methods will provide a quick identification of virus strains for the eradication of the severe ones and the collection of potential protective mild strains;
- virus characterization aimed at selecting local mild strains for cross protection studies;
- study of vector efficiency in virus transmission in the infected sites;
- study of *T. citricidus* behavior in various areas and identification of its natural enemies where already present; introduction and evaluation of exotic biocontrol agents under different Mediterranean conditions;
- evaluation trials for the selection of adequate alternative tolerant/resistant rootstocks to replace the sour orange suitable for the Mediterranean pedoclimatic and environmental conditions.

7. Technology transfer and information exchange

The project will aim at strengthening the technical know how of plant protection inspectors, quarantine officers, nurserymen and farmers for the monitoring of CTV and its vectors through the: (i) organization of dedicated training programs (short courses, scientific visits, study tours, farmers field schools...); (ii) exchange of researchers and technicians between partner countries (internships); (iii) organization of ringtests in selected laboratories for the standardization and validation of technical protocols and procedures; (iv) coordination of meetings, workshops and seminars for project information exchange and review of results which will be shared with all the citrus stakeholders at regional and national levels; (v collecting and updating the information on virus and vector in the region in the developed Mediterranean geo-database.

The project will strengthen collaboration and communication links among members of Mediterranean bodies and institutions dealing with phytosanitary issues in citriculture supplying Governments with an updated phytosanitary situation in order to soon adopt the most appropriate legislative protective measures.

VII – Facilities and equipment

The project will provide the partner countries with: technical assistance, training, basic equipment, some consumables for running activities. Moreover, the project will support the organization of meetings, visits, internships, etc. and the establishment and implementation of the geo-database. However, infrastructure and facilities with basic equipment and qualified personnel for running project activities (already available in most countries) should be provided by all project countries. Where such means are lacking, they need to be set up, as a contribution of the country under the project support, to a minimum level that should guarantee adequate implementation of essential tasks outlined in the work plan.

VIII – Result

With a well articulated framework and a good timetable, the project will permit to avoid the sudden collapse of Mediterranean citriculture by delaying the generalized spread of CTV and the entrance of *T. citricidus* in the rest of the region until a global protection strategy is operative. It will also contribute to raising awareness of the people about tristeza disease and its efficient BrCA vector, so that this threat may be equally considered throughout the Mediterranean countries, which have to join their efforts for the setting up of efficient control measures. Howevere, the project will not achieve adequate results without the full contribution of a large number of Mediterranean countries and the mobilization of their best human and technical resources.

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