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Proposal of a scheme for the production, maintenance and utilization of citrus certified propagative material in the Mediterranean

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SUMMARY – A draft of a possible common Mediterranean certification scheme of citrus propagating material is given as the final product of three years of networking activity.
The basic of the protocol, taking into account the requirements characterizing other European certification schemes and the actual problems of citrus occurring in the Mediterranean, is described in all phases concerning the production and the utilization of healthy propagative

materials. Emphasis is given to the sanitary requirements and controls to be included in the protocol.

Key words: citrus, certification, graft-transmissible pathogens, diagnosis, Mediterranean

RESUME *– On propose un schéma de certification commun pour la région Méditerranéenne concernant le matériel de multiplication des agrumes qui représente le résultat final des trois années d'activités du réseau.*
Les grandes lignes de ce protocole sont présentés compte tenu des conditions requises par les schémas de certification Européens et des problémes qui affectent les agrumes dans le pourtour Méditerranéen.

Mots-clés: *agrumes, certification, pathogènes transmissibles par greffage, diagnostic, Méditerranéenne*

Introduction

Citrus is the most widespread fruit crop in the Mediterranean region, where favourable soil and climatic conditions occur.

Present-day production of all citrus types in the Mediterranean has been estimated to be 16,503 tons, representing 18% of the world production, 60% of which are oranges, 18% mandarins and mandarin-like, 13% lemons and limes and 4% grapefruit (Med Agri, 1996).

Citrus virus and virus-like diseases cause very important economic losses in most Mediterranean countries, such as decline, loss of vigour and shortening of the commercial life of the grove, low yields and poor fruit quality. These diseases also restrict the use of many rootstocks.

Differently from the other major fruit crops, citrus is affected by many destructive diseases, which can severely cripple the industry of any country. Witches' broom, greening, severe Capao Bonito or the Australian stem pitting strains of CTV, the new Turkish citrus chlorotic dwarf disease, citrus badnavirus mosaic in India, transmissible psorosis in Argentina and Uruguay, canker as occurring in the Maldive islands, exocortis in Belize are examples of such diseases. In addition, new diseases are popping up all of the time.

Most of these diseases are fortunately not present in the Mediterranean area the major threat for the citrus industry being "tristeza" whose agent (CTV) occurs in several countries (Albania, Cyprus, Egypt, Israel, Italy, Lybia, Lebanon, Morocco, Spain, Tunisia, Turkey) and the new Turkish citrus chlorotic dwarf disease, which is reported only from the country of origin.

The absence of certification and quarantine programmes in several Mediterranean countries has enabled these graft-transmissible pathogens to spread through propagating material.

Although several initiatives are at present underway in the Mediterranean region for the sanitary improvement of citrus propagative material, it is necessary to improve their effectiveness by supporting complementary actions. The MNCC activity falls within this context. MNCC is promoted

by CIHEAM-IAMB/EC-DG.1 with the collaboration of DPPM, University of Bari and aimed at establishing and/or harmonizing sanitary certification schemes.

During three years of activity (meetings, workshops, training, exchange of experts and material, surveys and research) useful information was collected by MNCC members on the sanitary status (especially concerning CTV) and certification of citrus in the Mediterranean countries and on the harmonization of Mediterranean certification protocols. Concerning the latter a specific study was carried out on the: (i) production of "healthy" citrus propagative material; (ii)definition of propagating material categories; (iii)minimum sanitary requirements of citrus propagative material; (iv)organization and controls of certification; (v)technical requirements for the definition of the different categories of plant material taken into the scheme.

Surveys for the presence of virus and virus-like diseases were conducted in some Mediterranean countries in the 80's under the FAO patronage. The same activity was carried out in other countries through the MNCC, contributing to the acquisition of exhaustive information on the sanitary conditions of the citrus industry in the Region.

The increased demand for high quality planting material, the future role of the Mediterranean region as a free trade area, the activation of MEDA programmes, which promote the Euro-Med partnership, has compelled MNCC members to implement measures aimed at producing propagating material with a healthy status compatible with the economic returns expected from this crop.

This implementation was achieved taking into account EC requirements for the minimal sanitary status of citrus trade material and the European schemes for citrus certification.

The scheme, reported in this paper, for the production, maintenance and use of citrus propagative material represents the final proposal approved by the members of MNCC and other networks (Mediterranean Network on Fruit Trees), by experts of different Mediterranean origin and by the representatives of Lebanese and Italian citrus producers during a round table on the "Setting up of national and regional strategies for the production of virus-free plant material" held in Beirut (30 November – 6 December, 1997). This meeting was organized under the auspices of CIHEAM/IAM-B, DGCS-Italian Ministry for Foreign Affairs and by the Italian Embassy in Lebanon in the framework of a Symposium on "Resources management facing agricultural challenges".

Protocol for the production, maintenance and utilization of certified citrus trees and rootstocks (*Citrus, Poncirus, Fortunella* and their hybrids)

The certification protocol was set up on the basis of the facilities available in most Mediterranean countries, of the experience acquired by different laboratories actively involved in these programmes and of the results obtained during the network activity.

The protocol was structured taking into account the sanitary requirements characterizing the European and Mediterranean Plant Protection Organization (Table I) and other certification schemes (Navarro,

1986; Navarro *et al.*, 1981; Savino *et al.*, 1998), providing healthy and true-to-type nursery plants and rootstocks to the growers with the same quality standards. This objective will initially meet the EC requirements for quality (CAC-*Conformitas Agraria Comunitatis)* with respect to the minimal sanitary status and trueness-to-type of citrus trade material (Table II).

Moreover, the application of this protocol will consider that (i) budwood of internationally-grown cultivars is generally available from other certification programmes, but healthy budwood of locally-grown clones is not; (ii) tristeza, vein enation and stubborn are the only diseases transmitted by vectors in the Mediterranean region.

Mainly local varieties will go through the selection and certification procedures while international varieties from other certification programmes will be submitted only to sanitary controls before entering the national programme. If international varieties have long been present in the country, it is advisable to include them in selection scheme.

Table I – Virus and virus-like diseases of citrus occurring in the EPPO region and covered by the scheme (OEPP/EPPO, 1995)

Disease	Indicator	Laboratory tests
Tristeza	Mexican lime *(C. aurantifolia)*	ELISA, ISEM
Vein enation/woody gall	Mexican lime	
Rugose leaf	Mexican lime	ELISA, mechanical inoculation
Infectious variegation	citron (*C. medica*)	ELISA, mechanical inoculation
Exocortis, Citrus viroids	citron 861S1	PAGE
Satsuma dwarf	Dweet tangor (*C. reticulata x C. sinensis*)	ELISA
Psorosis complex, Cristacortis, Impietratura	Sweet orange (*C. sinensis*) cvs Pineapple, Hamlin, Madame vinous, Dweet tangor	
Cachexia	Parson's special mandarin (*C. reticulata*)	PAGE
Stubborn	Pineapple and Madame vinous oranges, Dweet tangor	Culturing

Table II – List of virus and virus-like diseases affecting citrus quality by EC Dir. 93/48 of 1993 (CAC).

Virus and virus-like diseases of citrus
Virus: tristeza, infectious variegation, leaf rugose
Viroids: exocortis, cachexia
Diseases inducing psorosis-like symptoms on the leaves: psorosis, ringspot, cristacortis, impietratura, concave gum
Spiroplasma: stubborn

Pomological and sanitary selection

To be really effective selection must be an integral part of «certification» which is an interdisciplinary endeavour encompassing phytopathological (primarily virological) and pomological competences. Thus, it aims at quality improvement and guarantees a given sanitary status, the varietal conformity and the clonal origin of the citrus material.

This approach is useful in order to promote a genetic improvement under plant quality control and should be of primary importance for the Mediterranean countries where a large survey of citrus genetic variability is needed.

1. Field selection

As schematically reported in Table V, selection must be made in aged orchards (more than 10 to 12 year-old), identified as suitable plantations in typical growing areas, in no less than 2 to 3 orchards per variety.

Five to ten individual plants per orchard are selected based on their general appearance and bearing characteristics typical of the cultivar or rootstock.

The selection of a suitable tree must be based on observations, for a period of at least 2 years, for trueness-to type (bearing time) and lack of apparent disease symptoms (spring and late summer).

The following agronomical characters may be included in the selection: size, colour and organoleptic characteristics, fruit-bearing; tree size, cold and pathogen resistence etc..

All symptoms which may be correlated to diseases or mineral deficiencies (leaf mottling, mosaic, chlorosis, malformations, enations; gumming, concavities, bark scaling, pitting, galls in the trunk and branches; abnormalities at the budunion) have to be taken into account.

2. Data processing

Based on the results of the best performing (comparative pomological processing and sanitary data), candidate stocks will be submitted to testing procedures (Table III) to complete the assessment of their sanitary status, which can be defined in one of the following two categories :

Virus-free Material free from all graft-transmissible pathogens known for citrus *spp.* at the time when by laws are issued.

Virus-tested Material free from all graft-transmissible pathogens indicated in the certification scheme.

3. Sanitary assays

At the end of field observations, candidate stocks shall undergo at first serological (ELISA, immunoprinting) and molecular assays (Dot-blot) as reported in Table III-a. These tests, which are

rapid and handy, can serve for a preliminary screening of the trees under selection and will prevent contamination of other stocks in the maintenance phase.

Electrophoretical assays will be carried out for the detection of citrus viroids (cachexia, exocortis and citrus viroids) only in those candidate stocks which were viroid-negative in the previous sanitary screening.

Graft-transmissions can be performed only at a cool temperatures (20-24°C), using a restricted number of indicator plants (about 5).

Madame vinous sweet orange at warm temperatures (32-35°C) could be included only for the detection of *Spiroplama citri* when it occurs in the country.

Since for gummy bark and bud-union abnormalities 3 to10 years field indexing is needed, first primary sources may be registered as virus-tested until field indexing is finished.

In consideration of other severe citrus graft-transmissible pathogens which are not yet present in the Mediterranean region (mosaic, huanglongbing, citrus variegated chlorosis, citrus canker, witches'broom) and of the unsatisfactory quarantine measures of most countries in this area, it would be advisable to include in this phase the detection, mainly by ELISA, of these diseases (Table III) .

Of utmost importance is making this protocol applicable in a very short time. So it will initially meet the requirements of virus-tested category which include only the graft-transmissible pathogens of Mediterranean interest, reported in bold character in Table III.

4. Maintenance of candidate stocks

It is advisable to maintain candidate stocks in a block as a germoplasm collection (see characteristics of mother block) otherwise in pots, containing sterilized soil mixture, which may be placed in insect-proof screenhouse (in areas where CTV is present).

5. Sanitation of candidate stocks

Infected candidate stocks can be sanitated by *in vitro* shoot tip grafting that, in combination with thermotherapy at 30-35°C (Table IV), gives the best results for virus elimination. The advantages of combining both techniques are many (plants are identical to the source tree and come into production the 2nd to the 3rd year after micrografting).

Due to the poor sanitary conditions of most Mediterranean citrus groves, especially concerning viroid infections, sanitation assays could start during sanitary controls. This procedure will reduce the time needed for the production of healthy candidate stocks and guarantee sanitation from other unknown pathogens.

Countries where sanitation facilities are not available, can ask for selected stocks to be sanitated in authorized laboratories located in other Mediterranean countries.

Plants obtained by shoot tip grafting must be sanitarily controlled only for those diseases detected in the source material.

Table III - Sanitary controls in data processing phase: (a) preliminary screening; (b) further testings for virus-free sanitary status

PATHOGEN/DISEASE	ASSAY
(a) Preliminary screening	
Tristeza, Psorosis, Vein enation-woody gall	ELISA
Exocortis, Cachexia, Citrus Viroids, *S. citri*	Dot-blot
(b) Further testings	
Infectious variegation, crinkly leaf	citron 861-S or Eureka lemon
Complex of the oak leaf patterns diseases (Concave gum/blind pocket, Cristacortis , Impietratura) Psorosis	Dweet tangor and Madame vinous sweet orange
Tristeza, , Leaf rugose, Vein enation-woody gall *Turkish citrus chlorotic dwarf disease*	Mexican lime
Satsuma dwarf	D.tangor or White sesame
Exocortis, Citrus viroids	sPAGE or citron 861-S (32-35°C)
Cachexia,	sPAGE or Parson's special mandarin/Rough lemon (32-35°C)
Tatterleaf	Cowpea or ELISA or Citrange
Kumquat disease	Citrange
Stubborn*	Madame vinous sweet orange (*C. sinensis*) 30-35°C
Bud union abnormality**	Sweet orange/Rough lemon (in the field 3-10 years)
Gummy bark**	Sweet orange/Sour orange (in the field 3-10 years)
Diseases not present in the Mediterranean region	
Mosaic	Mexican lime
Witches' broom	ELISA
Huanglongbing	ELISA/Mexican lime (25°-32°C)
Variegated Chlorosis	ELISA
Canker	ELISA

* Indexing test will be carried out when the pathogen is in the country

** Field indexing for these diseases can be carried out, in the countries where they are present, during the maintenance of primary source so as to obtain virus-free material.

Table IV - Shoot-tip grafting and thermotherapy phases for virus elimination

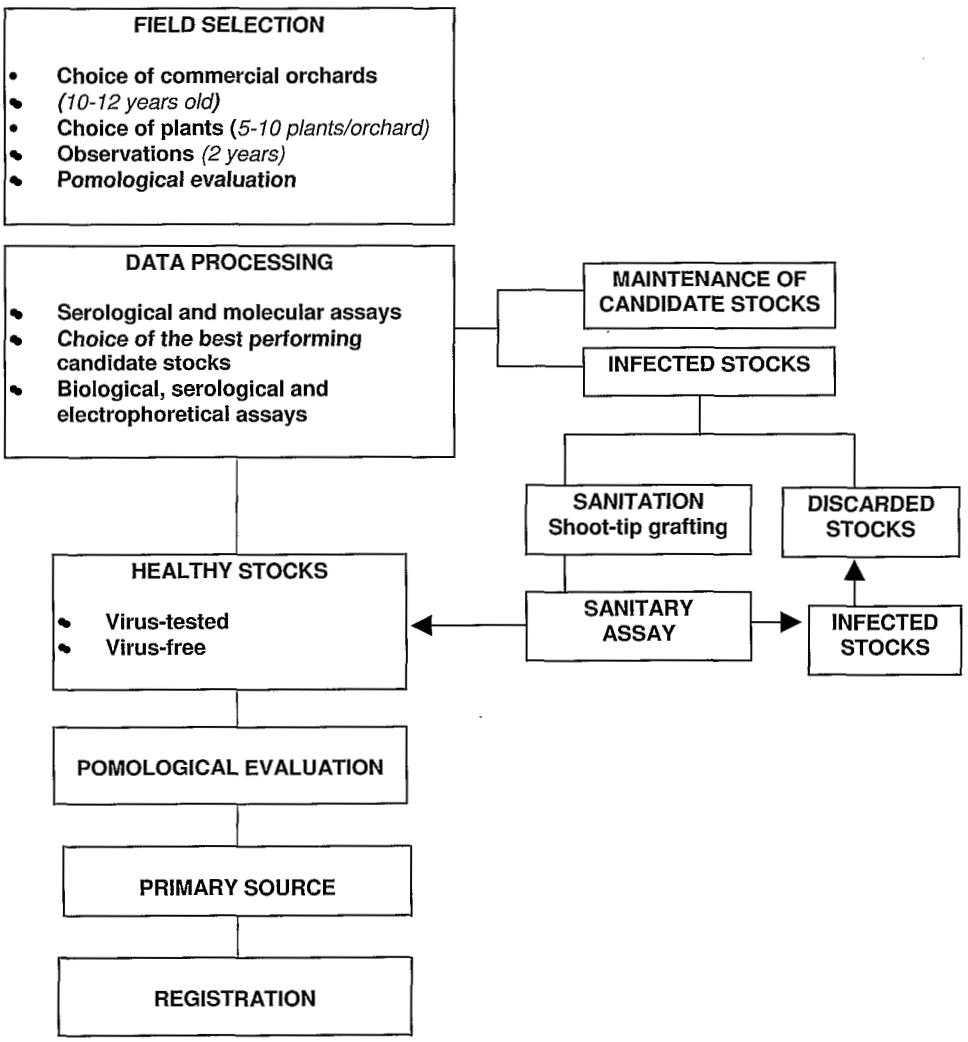
❑ Defoliated plants for shoots production are kept at warm temperatures (32-35°C) for about 20 days;
❑ Rootstock seedlings are prepared in MS media, kept in the dark for about 15 days;
❑ Shoots, of approximately 1 cm long, are collected from the thermotreated plants and disinfected under aseptic conditions;
❑ Shoot tip (about 0.2mm), consisting of the meristem and 2-3 leaf primordia, is excised under the microscope and inserted into a T-cut of a decapitated rootsock seedling;
❑ The grafted plant is grown in a liquid medium under artificial light (1000lux) for at least 5 weeks and then transplanted or grafted on a rootstock for acclimatation.

6. Registration

At the end of sanitary controls, selected stocks must go through comparative trueness-to-type and productivity trials by grafting them on certified rootstocks, grown in the field. In this way selected stocks will be productive enough early for varietal evaluation. The so-called « primary sources » can be registered in a national catalogue by a governmental agency. Two plants/clone or variety must be kept in an insect-proof screenhouse, ensuring isolation from surface water and outdoor condition, under the responsibility of the breeder.

Registered true-to-type stocks can enter certification schemes (Table V) as *virus-tested* or *virus-free* category.

Table V - Scheme for the production of the « Primary Source »



Certification

1. Organization

Table VI reports general indications for the organization of a possible certification scheme, defining the technical characteristics of citrus propagating material in terms of sanitary status and varietal trueness within the framework of certification service tasks.

Table VI – Organization of the citrus certification programme

Phases	Material category	Colour code	Controls		Cropping conditions	Agencies
			Trueness to type	Sanitary		
Conservation for premutiplication	Pre-basic	White with a blue band	Phenologic	Test	Screenhouse	Certifying Authority
Premultiplication	Basic	White	Phenologic	Test	Screenhouse	Public Agencies
Multiplication	Certified	Blue	Phenologic	Test	Open field	Nursery Associations
Nursery Propagation	Certified	Blue	Biometric characteristics	Test*	Open field	Nurseries

*In countries where CTV is present, 1 % of the plants are tested every year for CTV by immunoprinting

2. Definition of propagating material categories

In order to guarantee the quality of grafted plants in a certification phase, both the scion and rootstock must be of the same category. The use of one member of a lower category will lower the category of the grafted plant

"Primary source". Plants originally obtained by the conservative breeder (i.e. the physical or juristic person who has identified the plant selection or clone).

"Pre-basic". Propagating material or plants directly derived from the "primary source" and identified by a white label with a blue band.

"Basic". Propagating material or plants directly derived from the multiplication of pre-basic stocks. Basic material is intended for delivery only to nurseries that have the necessary qualifications and is identified by a white label .

"Certified". Propagating material or plants produced from basic stocks by authorized nurseries under appropriate growing conditions. Certification is granted at this stage and labels (blue tags) are issued by the certifying authority. Then, the material is delivered to the growers and quits the control of the certification system.

The material which has never entered the certification system is called "standard" (usually identified by a yellow-orange label). It is true-to-type but represents a sanitary hazard.

3. Certification phases

The certification scheme is divided in the following phases in which the production of certified material will be sanitarily and pomologically controlled . The final product has to be checked to fulfill the conditions of certification required for these species.

- *Conservation for premultiplication*

At least 2 plants from the primary source, «*pre-basic category*», must be maintained in an insect-proof screenhouse, ensuring isolation from surface water and outdoor condition, under the responsibility of the certifying authority.

- *Premultiplication*

The premultiplication phase is made up of plants of «*basic category*» originated from «pre-basic» material. The plants are grown in an insect-proof screenhouse, ensuring isolation from surface water and outdoor condition, under the responsibility of public institutions.

The preparation of propagating materials, intended only for multiplication centres, must be always carried out under an insect-proof screenhouse or in isolated conditions to prevent recontaminations.

- *Increase Block*

The number of basic plants can be variable depending on the economical importance of each variety and on the time needed to establish a producing multiplication block. In this case an increase block is advisable so as to temporarily meet the nursery demand for specific varieties.

It must be under insect proof screen the same as the basic material.

This block can be also included at the multiplication phase and located under net.

- *Multiplication*

The establishment of mother plant plot for the production of «*certified*» material is made with plants derived from «*basic*» material in private nurseries.

- *Nursery*

The production of certified rootstocks and grafted plants is made in the nursery using material coming from certified mother plots.

4. Controls

This programme is aimed at providing growers with healthy and true-to-type nursery plants. Sanitary and varietal controls must be carried out in each certification stage at given intervals.
Visual inspections will be carried out annually for detection of virus symptoms (at the appropriate time when symptoms are likely to be most visible), pests (e.g. *A. floccosus* and *Parabemisia myricae*) and possible mutations.

• *Sanitary controls*

From the experience made by different MNCC laboratories through comparative trials and taking into account the literature concerning detection techniques of graft-transmissible pathogens of citrus (Roistacher, 1991), the best method for each disease must met the following requirements: to be user friendly, reliable, inexpensive and with a low environmental impact.

– Serological, electrophoretical and molecular assays are rapid and reliable but handling and costs are extremely variable according to the country.

Usually if a laboratory is well equipped for ELISA testing in terms of facilities and human resources, also molecular and electrophoretical techniques are likely to be applied with a little effort. Their application is very important to reduce the time, space, costs and labour required by biological indexing.

The use of these techniques, however, is conditioned by the availability of specific and good quality reagents on the market.

– The utilization of citrus indicators is still compulsory in any citrus certification programme for the detection of most citrus graft-transmissible diseases.

Biological indexing basically requires climatized greenhouses at two different temperatures (cool and warm), excellent indicator plants and long time for symptom appearence. Since viroids and spiroplasmas can reliably be detected by sPAGE and dot-blot hybridization respectively, biological indexing can be performed only at a cool temperatures (22°-24°C), using no more than 5 citrus indicators (Table VII).

For certification biotests can be limited to the detection of OLP diseases and of pathogens for which ELISA kits are not commercially available.

Of utmost importance is emphasize that desinfection of pruning tools with 10% sodium hypochlorite solution is mandatory to prevent possible recontaminations.

– For obtaining comparable results with the detection of infectious agents, attention must be paid to the type of samples and timing of collection.

For instance, the detection of viroids and phloem-restricted prokaryotes requires samples, leaves and phloem respectively, collected at the end of summer when temperatures have been high (more than 30°C) for extended periods of time.

On the contrary, samples for virus and virus-like diseases (usually leaves or cortical scrapings in the case of closteroviruses), are best collected in spring or in autumn, when tempertaures do not go below 12°C.

– Diseases not yet present in the Mediterranean should not be included in the sanitary controls if they have been tested for in the course of the production of the primary source.

From the above it ensues that the main diagnostic procedures for cheking graft-transmissible pathogens included in certification protocols can be limited to five (Tables VII-VIII).

Table VII - Diagnostic techniques used for certification

Technique	Working area	Materials (controls are compulsory)	Pathogen/disease
Graft-transmissions to citrus indicators *	Greenhouse (22-24°C)	M. lime., D. tangor, E. citron., Citrange, M.V. orange	CTV, CVEV, CPV, SDV, CVV, CiLRV, CTLV, OLP complex, Turkish citrus chlorotic dwarf disease
Mechanical transmissions to herabaceous hosts	Greenhouse (22-24°C)	Cowpea,White sesame	CTLV, SDV, CVV, CiLRV
Serological tests (ELISA or immunoprinting)	Laboratory	Commercial kits	CTV, CVV, CTLV, SDV, CPV
Electrophoresis (sPAGE)	Laboratory	Markers	CVds, CEVd, CCaVd
Molecular assays (Dot-blot)	Laboratory	Specific probes	*S. citri*

*Grafting of chip bud, blind buds or other inoculum tissue are performed directly in the indicator seedling or in the rootstock supporting the indicator.

Table VIII - Sanitary controls required by the certification protocol of citrus propagating material

Diseases	Agent	Biological indexing (22-24°C)	Laboratory assay
Virus			
– Tristeza	CTV	Mexican lime	ELISA
– Rugose leaf	CiLRV	Mexican lime	ELISA*
– Vein enation-woody gall	CVEV	Mexican lime	ELISA**
– Infectious variegation-Crinkly leaf	CVV	Etrog citron 861S1	ELISA*
– Psorosis	CPV	Dweet tangor	ELISA
– Satsuma dwarf	SDV	White sesame, Dweet tangor	ELISA*
– Tatter leaf	CTLV	Cowpea, Citrange	ELISA*
Viroids			
– Cachexia, Exocortis, Citrus viroids	CCaVd, CEVd, CVds		sPAGE
Virus-like			
– Concave gum/Blind pocket, Cristacortis, Impietratura		Dweet tangor	
– Turkish citrus chlorotic dwarf disease		Mexican lime	
Phloem-restricted prokaryotes			
– Stubborn	*S. citri*	Madame vinous***	ELISA/Dot blot

*ELISA can be applied if antibodies are commercially available, although no comparative trials to test their reliability have been carried out by the use of several Mediterranean isolates in the framework of MNCC activity;
** ELISA kit for the detection of barley yellow dwarf luteovirus (BYDV-RMV) gives strong positive reaction with CVEV-infected bark extracts ;
***Biological indexing will be carried out at 30°-35°C only if the pathogen is present in the country.

Testing intervals

Plants must be sanitarily controlled in each phase, following the testing intervals as shown in Table IX.

- CPV and CVEV must be detected by ELISA every 3 years in all phases, except for the nursery phase. ELISA for CPV has been recently done using monoclonal and polyclonal antibodies, which should be soon available on the market. Serological controls at short test intervals are necessary because of the unknown epidemiology of this virus, that may have a vector (e.g. the Argentinian strain).
  Indexing can be done every 10 years as with other diseases.

- CTV must be annually tested for by ELISA (using both monoclonal and polyclonal antibodies) in the first three certification phases, and by immunoprinting in the nursery if the pathogen is in the country. Both techniques are equally reliable for CTV detection, but immunoprinting alone can be used if ELISA facilities are not available and where a large number of plants need to be examined.
  In the countries where CTV is present, plants must undergo indexing in the first three phases every 5 years, otherwise every 10 years.

- Viroids (CCaVd, CEVd and CVds) can be checked every 3 years by sPAGE in the prebasic, basic and certified mother trees phases.

- *S. citri* can be detected by ELISA or Dot-blot hybridization in the first three phases every 3 years otherwise annually where the pathogen is present, in which case biological indexing with Madame vinous at 30-35°C is to be done every 5 years.

- All other virus and virus-like agents included in the protocol must be indexed every 10 years.

Table IX - Testing intervals (years) in the certification categories

Category	Test intervals (years)							
	CPV CVEV		CTV		Viroids	*S. citri*		Other virus virus-like
	ELISA	Indexing	ELISA*	Indexing*	sPAGE	ELISA/ Dot-blot*	Indexing*	Indexing
Prebasic	3	10	1/3	5/10	3	1/3	5/0	10
Basic	3	10	1/3	5/10	3	1/3	5/0	10
Certified mother trees	3	10	1/3	5/10	3	1/3	10/0	10
Certified plants			1**					

* The first figure is the time interval for testing when the pathogen occurs in a country. The second figure is the time interval if the pathogen is absent.

** 1% of commercial citrus in the nursery by immunoprinting in countries where CTV is present

• *Inspections for trueness to type*

The certification of varietal trueness for the cultivars of *"Pre-basic and basic category"* may be issued by a public institution, after observations for at least three growing and productive seasons for evaluating trueness of the material to the phenotype.

The certification for varietal trueness of seed-propagated rootstocks may be issued after observation, for an entire growing cycle in the nursery, a quantity of at least 200 seedlings from any seedstock.

5. Technical characteristics

Selected stocks submitted to certification are highly valuable. They must be grown with care and be protected as much as possible from re-infection, especially during the multiplication and nursery phase.

For prebasic and basic stocks insect-proof screenhouse conditions are compulsory given the high number of vector-transmitted pathogens, which can threaten the health of this material.

In countries where vector-transmitted diseases are present, multiplication plots and nurseries producing certified propagative material must be located as far as possible from infected areas to avoid contamination. They must be established in soils of good quality, free from soil-borne virus-vectors and be under superior management by highly qualified technicians.

Pathogens and pests should be accurately controlled and working tools must be disinfected in a 10% sodium hypochlorite solution to avoid viroid contamination.

These and other requirements pertaining to safety distances and cultural practices are usually codified by official regulations.

The technical characteristics should fulfill the following conditions in each certification phase:

Insect-proof screenhouse

"Primary source, prebasic and basic" materials must be grown in containers with sterilized growing medium and maintained in insect-proof screenhouses, under isolated conditions to prevent any recontamination from the outdoor enviroment and from surface waters (Figure I);

The gravel or other material on the floor must ensure an efficient drainage and the isolation from the soil;

Double insect-proof screen and the double door are to be used to prevent the entrance of all types of virus-vectors.

Mother blocks

Scion mother plants and seed mother plants must be grown on good arable land, where no citrus plants species have been grown for at least 3 years, free from citrus stumps, from the nematodes and fungi

included in the protocol (see analysis of soils of mother blocks) and isolated from the flow of surface water

Lemon mother plants are to be established in a field that has not hosted plants of the same species from at least five years and be covered with a white net (normally used to avoid wind and hail damages) to prevent contamination of *D. tracheiphila* (in the areas where the fungus is present);

Plots, thoroughly protected against pathogens and pests, must be homogenous (each row must contain a single specie), located 100 metres from any citrus orchard (and under screen in the countries where CTV is present) with a borderline of at least 3 metres, constantly tilled and kept free from vegetation;

Scion mother plants cannot be kept for more than 30 years; for the seed mother plants, this limit equals 40 years.

Increase blocks

Plants (rootstocks must belong to the same category of the scions) must be grown in containers or in good quality and "healthy" soil.

Each row in a plot must contain a single cultivar or clone. Plots are established under covered sheds, fifty metres away from citrus orchards (Figure II). The life of an increase block is 18 months if the plants are grown in containers or 3 years if the plants are grown in soil.

Nurseries

Seed and planting beds must be made with soil and/or growing media free from nematodes and fungi (meeting the same characteristics of mother blocks), at least 15 metres from citrus orchards or nurseries obtained with unqualified propagating material. Each nursery must be mapped so as to identify each plot (Figure III).

Seed beds

Only genetically and sanitarily certified and homogeneous seed lots must be used.

Planting bed

The seedlings of species susceptible to "mal secco" are to be placed under a white net if grown less than 50 m from lemon plantings (in areas where the fungus is present).

Homogenous plots with single rootstocks must be well established using the same cultivar or clone in each row of the plot. The scions for grafting must cause from authorized mother or increase blocks. Top graftings can only be executed with material of the same cultivar and sanitary category;

The plot, kept free from plant and animal pests, must be bordered by a border of at least 2 m kept free from any vegetation;

6. Analysis of soils of mother blocks and nurseries

Nematodes

Certified trees are grown in soils where citrus *spp.* have not been cultivated for the last five years, which are free from *Pratylenchus vulnus* and *Radopholus similis*.
Ten samples (each made up of no more than 5 different subsamples per hectare) are to be taken in spring and autumn before any tillage and sent to an authorized laboratory.

When the mentioned nematodes are identified, the soil has to be immediately disinfested following the laboratory protocol.

The efficacy of the treatment must be confirmed by a further nematological analysis carried out six months afterwards.

In the nursery a sample of 1 kg of soil mix (composed by 10 subsamples) is to be taken per each cubic meter.

Phytophthora spp.

The inoculum of *Phytophthora spp* in the soil where the certified material is grown must not exceed three propagules per gram of dry soil.

Twenty samples (each of 10 specimens per hectare) are to be taken in the spring before planting.

Periodical inspections must be conducted to check any type of contamination.

Conclusions

Most virus and virus-like diseases of citrus are not transmitted by vectors and can be effectively controlled by the use of clean certified stock. Additional measures should be taken to control vector-transmitted diseases which, in the Mediterranean region, are only represented by tristeza, vein enation and stubborn. Tristeza is one of the most serious citrus diseases and is controlled by the use of tolerant rootstocks. Vein enation does not produce important losses, but hampers the use of susceptible rootstocks like rough lemon or *Citrus volkameriana*. Stubborn is difficult to control in heavily infected areas.

Some severe diseases are not yet recorded in the area while other diseases are present in some countries but not in others.

Because of this situation is important that all countries become aware of the high risk of importing citrus propagative material without adequate controls.

In some countries certifcation programmes are being successfully carried out, whereas in others such programmes do not yet exist. The general recommendations coming out from this proposal of a

Mediterranean certification protocol should be submitted to all governments and to the international organizations of this area, which are responsible for the establishments of certification programmes in the Mediterranean.

Only the harmonization of certifcation procedures will guarantee the exchanging of citrus certifed material with the same sanitary quality and trueness to type, enhancing not only the quality of the material itself and its productivity but defending the Mediterranean citrus area from the introduction and the spreading of exotic diseases.

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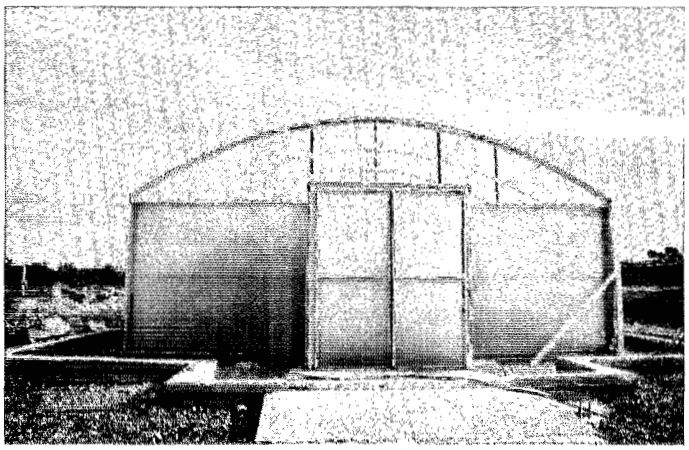

Figure I - Screenhouse for the maintenance of primary source, pre-basic and basic materials

Figure II - Increase block in the premultiplication or multiplication phase

Figure III - Growing of certified plants in the nursery phase