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Efforts to harmonise and promote a stone fruit certification scheme in the Mediterranean countries*

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SUMMARY - The awareness of potentially disastrous consequences to stone fruit crops from virus diseases (i.e. Sharka) had alarmed the scientific community and prompted the initiation of national and international projects for the production of virus-free stocks through certification programmes. To promote a clean stock programme in the Mediterranean region, the Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM) has promoted the "Mediterranean Network for Virus Disease Assessment and Sanitation of Stone Fruit Trees"

(MNFT). Various proposals have been discussed to improve and harmonise current certification schemes starting from EPPO stone fruit protocols now in operation in different countries. Other proposals for more reliable techniques for disease detection used in these programmes were included. A plan for the harmonisation of a stone fruit clonal selection and certification scheme in the Mediterranean Region was submitted to the scientific community and the agricultural authorities of member Governments.

Key words: stone fruits, plant viruses, plant certification, detection, sanitary selection, Mediterranean countries

RESUME - *La connaissance des effets néfastes que les maladies à virus (la Sharka, par exemple) peuvent produire sur les essences à noyaux a mis en état d'alerte la communauté scientifique et a donc encouragé la réalisation de projets nationaux et internationaux pour la production de matériel indemne de virus à travers des programmes de certification. Afin d'appuyer un programme de production de matériel végétal indemne dans la région méditerranéenne, le Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM) a mis en place un réseau dénommé «Mediterranean Network for Virus Disease Assessment and Sanitation of Stone Fruit Trees» (MNFT). De nombreuses propositions ont été discutées dans le but de promouvoir et d'harmoniser les programmes actuels de certification à partir des protocoles de l'OEPP sur les essences à noyaux, adoptés aujourd'hui dans différents pays. D'autres propositions ont été formulées pour inclure des techniques plus fiables de détection des maladies dans lesdits programmes. L'harmonisation d'un programme de sélection clonale et de certification des essences à noyaux dans le pourtour méditerranéen est soumise à l'attention de la communauté scientifique et/ou des autorités compétentes du secteur agricole des différents pays membres.*

Mots-clés: *essences à noyaux, virus des végétaux, certification des plantes, détection, sélection sanitaire, Pays Méditerranéens*

I - Introduction

The Mediterranean region produces about 37% of the world overall production, with 60% for almonds and 47% for peaches (FAO, 1995). A 53% increase in the production of stone fruits in the Mediterranean was recorded from 1980 to 1994, which led to an increased demand for planting materials. However, the available propagative materials were often of poor sanitary quality, being infected by graft-transmissible pathogens.

II - Phytosanitary status of the Mediterranean stone fruit industry

Assessing the current sanitary status of propagative budwood in Mediterranean countries was not easy because of the information available (except for Spain, France and Italy), some limited surveys have been done in some countries (i.e. Turkey, Cyprus, Albania, Lebanon, Tunisia) or virtually absent in other countries (i.e. Libya, Egypt, Algeria, etc).

The stone fruit industry of many countries in the region was threatened by "sharka" disease caused by plum pox potyvirus (PPV), which was recorded in Albania, Croatia, Cyprus, Egypt, France, Greece, Italy, Slovenia, Spain, Syria, Portugal, Turkey and Yugoslavia F.R.

(Roy and Smith, 1994). Other widely distributed viruses were Prunus necrotic ring spot (PNRSV), prune dwarf (PDV) and apple mosaic (ApMV) ilarviruses and apple chlorotic leaf spot trichovirus (ACLSV). Strawberry latent ringspot (SLRV), raspberry ringspot (RRV), cherry leaf roll (CLRV) and arabis mosaic (ArMV) nepoviruses were occasionally detected. Different phytoplasmas (apricot chlorotic leaf roll and plum leptonecrosis); viroids (peach latent mosaic viroid and hop stunt viroid), and other virus-like diseases have been reported from the Northern Mediterranean countries. More detailed information on viruses and other graft-transmissible agents of stone fruits, present in the Mediterranean region, was given by Németh (1986); Dunez (1988); Smith *et al.*, (1988); Diekmann and Putter (1996).

III - Stone fruit certification: state of the art

The main cause for the deterioration of crop health was the uncontrolled distribution of already infected planting materials (both scions and rootstocks) followed by subsequent vector-mediated spread, specifically sharka. This compelled several European and Mediterranean countries to establish both "clean stock programmes" and compulsory eradication measures.

Since 1991, the European and Mediterranean Plant Protection Organization (EPPO) had been involved in establishing certification schemes for a number of different crops. Programmes for fruit tree crops were finalised during 1991-92 and submitted to member countries for their input and approval (Anonymous, 1992a).

1. The Mediterranean situation

The oldest examples of successful fruit certification programmes were demonstrated in the United Kingdom, Netherlands, and France. France organised an official certification programme in 1982 (Dosba, 1993). In Spain, a program was established in 1977 for the selection, multiplication, and control of fruit tree material (Casallo *et al.*, 1988). Technical regulations were approved in 1982, and modified and updated in 1995. In Italy, stone fruit certification activities began in the Emilia-Romagna, Apulia, and Friuli-Venezia Giulia regions and officially extended to the rest of the country in 1992.

In other countries, certification often guarantees only trueness-to-type. In Turkey, the sanitary aspects were only recently undertaken in that modern laboratory facilities were being established and technical staff trained to assay nursery plants for virus and virus-like diseases. In Southern Mediterranean countries, Morocco was the first (1977) to produce a certification scheme initially directed to a single stone fruit species (almond), and later extended to all stone fruit species. In the framework of the UNDP-FAO Regional Project RAB 88/025 "Control of Virus and Virus-like Diseases of Fruit Trees", Tunisia and Algeria are moving towards the adoption of national schemes for stone fruit certification. Albania and

Lebanon are doing the same in collaboration with Italy. In 1996, Albania promulgated by-laws to support future certification activities with a special decree for the eradication of PPV. In Lebanon the technical and legislative protocols for stone fruit certification are now under scrutiny by governmental authorities.

IV - The Mediterranean Network for Virus Disease Assessment and Sanitation of Stone Fruit Trees (MNFT): its role and objectives

Although several initiatives (some at the international level) are underway in the Mediterranean region with the primary objective of producing sanitarily improved materials, it is believed that their effectiveness can be improved by better integration and co-ordination of efforts. In the framework of the Mediterranean research networks promoted by CIHEAM, the Mediterranean Agronomic Institute of Bari in collaboration with the Dipartimento di Protezione delle Piante, University of Bari will be co-ordinating the MNFT with the following objectives:

- exchange of information and training of staff;
- evaluation of the sanitary status of fruit trees in participating countries;
- establishment and/or harmonisation of national sanitary certification schemes;
- organisation of activities for the production and conservation of sanitarily improved stocks.

Scientific institutions from Albania, Cyprus, France, Greece, Italy, Lebanon, Morocco, Spain, Tunisia and Turkey participate in the MNFT.

A comparative review of the state of the art of stone fruit certification in the various countries brought forth the existence of large differences pertaining to: (i) definition of propagating material categories; (ii) the minimum sanitary requirements for materials of "certified" category; (iii) technical requirements for the definition of the different categories of plant material taken into the scheme; and (iv) colour code of the labels identifying different categories. These differences were discussed in detail at MNFT meetings in Bari (1995) and Tunis (1996) during a joint meeting with the UNDP-FAO Regional Project RAB/88/025, using as a preliminary common basis the 1991 EPPO proposal for a stone fruit certification scheme. The scheme that followed, represent the final proposal approved by the MNFT members in the hope that it may constitute the basis for Mediterranean certification protocols and contribute to the advancement of certification programs discussed by EPPO Panels or *ad hoc* committees appointed by national governments.

V - Proposed scheme for stone fruit certification in the Mediterranean countries

1. Definition of propagating material categories

Pre-basic. Propagating materials or plants of "pre-basic" category must be directly derived from the "primary source". Pre-basic materials to be identified by a white label with a blue band.

Basic. Propagating materials or plants of "basic" category must be directly derived from "pre-basic" materials grown under protection (i.e. insect-proof screenhouses). Basic materials or plants carry a solid white label.

Certified. Propagating materials or plants of "certified" category must be derived from mother plants established from "basic" material. Certified materials or plants carry a solid blue label. See organisation chart of the certification program in Tab. 1.

It was recommended that in grafted plants, both the scion and rootstock must be of the same category. The use of either a scion or a rootstock of a lower category shall automatically mark category of the resulting grafted plant.

2. Selection and certification

2.1. Sanitary and clonal selection

Sanitary and clonal selection of native cultivars is an interdisciplinary activity requiring the joint efforts of pomologists and virologists. For each variety, selection shall be made in 10 to 12 year-old orchards in no less than 2 to 3 orchards per variety. Orchards should be located in different ecological areas. Five to ten individual plants may be selected based on general appearance and bearing characteristics typical of the scion cultivar or rootstock. The first selection of a candidate clone must be based on trueness-to-type and absence of apparent disease symptoms. Candidate clone must be observed for at least 2 years and undergo pomological and serological (ELISA) assays. Based on the results of the best performing, individual trees may be selected for further testing (2^{nd} selection).

This approach was being used to identify, obtain, and promote genetic improvements and plant quality control. This will be of primary importance for Mediterranean countries where a large survey of prunus genetic variability must be done: to identify new interesting cultivars, and to enlarge the genetic variability within each collection in order to implement new breeding programmes.

Newly obtained varieties and/or selections must undergo indexing. In these cases, there will not be a need for clonal selection.

2.2. *Repository of selected candidate clones*

Candidate clones derived from the 2nd selection must be conserved in a collection plot under the supervision and responsibility of scientific institutions. Prior to registration, all the plants shall be individually indexed to asses their sanitary status. Only healthy candidate clones will be forwarded into the scheme, whereas infected plants will undergo sanitation by thermotherapy and/or micrografting. Serological and biological tests must be repeated to verify sanitary status.

2.3. *Registration*

After the completion of indexing, selected plant material (candidate clones) must proceed through comparative trueness-to-type and productivity trials in, at least, two different ecological areas. After this, they can be registered in a national catalogue as indicated by law. These plants, virus-tested and true-to-type, represent the "primary source".

2.4. *Conservation of primary sources*

Primary sources must be conserved in an insect-proof screenhouse (Fig. 1) under the responsibility of the breeder to insure freedom from possible re-infection.

2.5. *Conservation for premultiplication*

It consists in the maintenance, under screen, of at least two plants (Fig. 2)derived from the primary source. This is "pre-basic" material and kept in a repository under the responsibility of the certifying authority.

2.6. *Premultiplication*

The pre-multiplication block is made up of plants of "basic" category originated from "pre-basic" material and is to be grown under conditions securing freedom from re-infection (e.g. insect-proof screenhouse) under the responsibility of public institutions. Also nursery activity must be done under screen and budwood produced is intended only for multiplication centres. These activities operate under the auspices and responsibilities of public institutions.

2.7. *Multiplication*

The multiplication phase take place in private nurseries with the establishment of mother plants directly derived from "basic" material, and intended for the production of "certified" budwood. In countries where PPV is present, multiplication plots should not be established

where PPV is endemic. If this is not possible, plots must be located no less than 1,000 metres from PPV-affected stands, according to the decision of certification authority.

2.8. Nursery

Nursery operations produce the certified rootstocks and grafted plants with materials originating from certified mother blocks. The final product must be rechecked to fulfil the conditions of certification required for that species. Nurseries should preferably not be established in areas where PPV is endemic and must be at a distance of no less than 1,000 m from infected stands, according to the decision of certification authority.

Multiplication plots and nurseries producing certified material must be established in soils of good quality, free from soil-borne virus vectors and managed by qualified technicians. Other requirements pertaining to safe distances and cultural practices were codified by specific regulations.

3. Certified material produced and sanitary requirements

Materials produced through certification programmes are defined as follows:

Virus-free: materials free from all known viruses and virus-like agents known for the species considered at the time when by-laws were issued.

Virus-tested: materials free from known viruses or virus-like agents as indicated in the certification scheme.

Minimal sanitary requirements characterising "virus-free" and "virus-tested" categories in the Mediterranean area were listed in Anonymous (1992a). The following tests were proposed for inclusion: PPV for sweet and sour cherry virus-tested and virus-free material; HSVd for apricot, peach and plum virus-free materials; stem pitting disease for almond, apricot, and cherry virus-free materials.

4. Testing for pathogen freedom

The techniques to be used for detection and identification of infectious diseases and their agents include:

4.1. Indexing on woody indicators

The use of woody indicators is a compulsory step in all stone fruit certification programme. It must be included because several graft-transmissible diseases can only be identified on woody indicators. For the main diseases, the use of woody indicators is complementary to ELISA and mechanical transmission procedures. Complete list of woody indicators is

available (Anonymous, 1992b). It is proposed that indexing on GF305, due to its lower cost and shorter time requirements for symptom expression should be used to detect PPV and used eventually in the multiplication phase. Very careful sanitary controls must be carried out where PPV is endemic, e.g. Greece and Albania. In addition to visual inspections, all mother plants must be subjected to ELISA, and 10% of the collection require an indexing on GF 305. In countries where PPV is localised in a few areas (France, Italy, Spain), indexing should be carried out by ELISA on 25% of the plants and on GF305 for those plants with doubtful visual symptoms. In countries where PPV have not been reported (Tunisia, Morocco, Lebanon), ELISA testing may be limited to 10% of the plants. However, two visual inspections per year must be made (Tab. 2).

Recent investigations (Di Terlizzi and Savino, 1995; Di Terlizzi and Savino, 1997; J.K. Uyemoto, personal communication) strongly recommend inspections for wood-marking, such as stem pitting in "virus-free" materials of apricot, almond and cherry grown for two entire vegetative cycles in the field also. Graft-transmission on GF305 may be used to detect the presence of stem pitting.

4.2. Inoculation to herbaceous hosts

A concerted effort must be made to obtain virus-free material by all possible means. Inoculation to herbaceous hosts is regarded as a complement for the detection of mechanically transmissible viruses, but not as a substitute for other diagnostic procedures. *Chenopodium quinoa, Cucumis sativus* and *Nicotiana occidentalis* represent a minimal host range for detection of mechanically transmitted viruses.

4.3. ELISA testing

In principle, ELISA testing is considered a complement to, but not a substitute for other diagnostic procedures. However, it is recommended for PPV, ACLSV, PNRSV, PDV, ApMV and nepoviruses for which antisera are available.

4.4. Electrophoretic and molecular techniques

During recent meetings, the problem was addressed of "dapple fruit" of plum and peach (Sano *et al.*, 1989), which are associated with hop stunt viroid (HSVd). This viroid was detected by molecular hybridisation in apricot in Spain (Astruc *et al.*, 1996) and in Italy also in peach and plum (Loreti *et al.*, 1998; Pallás and Di Terlizzi, unpublished information). Thus, an additional test procedures must be included to detect HSVd in these species.

These molecular techniques (nucleic acid hybridisation, PCR, dsRNA), due to their high efficiency, are proposed for use when available (Tab. 3) especially for primary source material and as positive controls in the first steps of the certification programme

(conservation for premultiplication). These techniques were recommended also for the detection and identification of PPV, viroids, and phytoplasmas. A schematic description of the proposed certification schedule with its steps, category material, outfits, requirements, agencies involved and controls needed, were outlined in Tab. 1.

VI - Conclusions

The MNFT has reviewed the state-of-the art of certification in the Mediterranean and debated the problems. Following a thorough comparative examination of the programmes underway in some of the Mediterranean countries, a preliminary agreement was reached for harmonising certification schemes as a whole and improving the aspects connected with detection and elimination of pathogens. Suggestions and proposals agreed upon during the meetings will be submitted to the attention of EPPO. A great deal of attention was paid to Sharka and to the strategies for restraining its further spread in countries where it occurs or preventing its ingress in countries currently free of the disease. These latter countries were encouraged to immediately establish and enforce programmes for certification and quarantine.

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Tab. 1. Proposal for the organization of a stone fruit certification programme

Steps	Category of plant propagation material	Outfits (localisation)	Requirements	Agencies	Controls	
Obtention of Primary Sources	Primary sources	Scientific Institutions	Screen House	Scientific Institution	Trueness to-type	Sanitary
Conservation for Premultiplication	Pre-Basic	Conservation Center for Premultiplication	Screen House	Certifying Authority	Trueness to-type	Sanitary
Premultiplication	Basic	Premultiplication Center	Screen House	Public Institutions	Trueness to-type	Sanitary
Multiplication	Certified	Mother block	Open Field	Nurserymen's Association and /or individual urseries	Trueness to-type	Sanitary
Propagation	Certifiable ↓controls Certified	Nursery	Open Field	Private nursery		Only Visual

Colour code: Pre-basic → label white with blue band, Basic → label white, Certified→ label blue

Tab. 2. Different Sanitary controls in Mother blocks relative to the PPV situation in the Mediterranean Countries

PPV status		
Present	Present but not in the area	Not present
Visual inspection	Visual inspection	Visual inspection
ELISA (all plants)	ELISA (25% of plants)	ELISA (10% of plants)
Indexing on GF 305 for ELISA negative samples (10% of plants)	Indexing on GF 305 (for doubtful samples)	one visual inspection

ELISA for 10% of plants/annually for other common viruses (PDV, PNRSV, ACLSV and ApMV)

Tab. 3. Proposed methods for the improvement in virus detection and stone fruit certification

Agents and diseases	Diagnostic techniques				
	Current techniques		Proposed techniques		
	Woody indexing	ELISA	Molecular hybridisation*	PCR*	dsRNA*
Viruses:					
PPV	+	+	+	+	
ACLSV	+	+			
PDV	+	+			
PNRSV	+	+			
ApMV	+	+			
CGRMV	+			+	+
LCV	+			+	+
CLRV	+	+			
CRLV	+	+			
ArMV	+	+			
SLRV	+	+			
RRSV	+	+			
TomRSV	+	+			
TBRV	+	+			
MLRSV	+	+			
CVA			+	+	
StPV	+		+		
Viroids:					
PLMVd	+		+	+	
HSVd			+	+	
Phytoplasma:					
ACLR	+		+	+	
Virus-like diseases:					
Necrotic rusty mottle	+				
Rusty mottle (European)	+				
Cherry mottle leaf	+				+
Cherry twisted leaf	+				+
Apricot ring pox	+				+
Peach asteroid spot	+				
Stem pitting	+				

* assays on primary sources and pre-basic materials

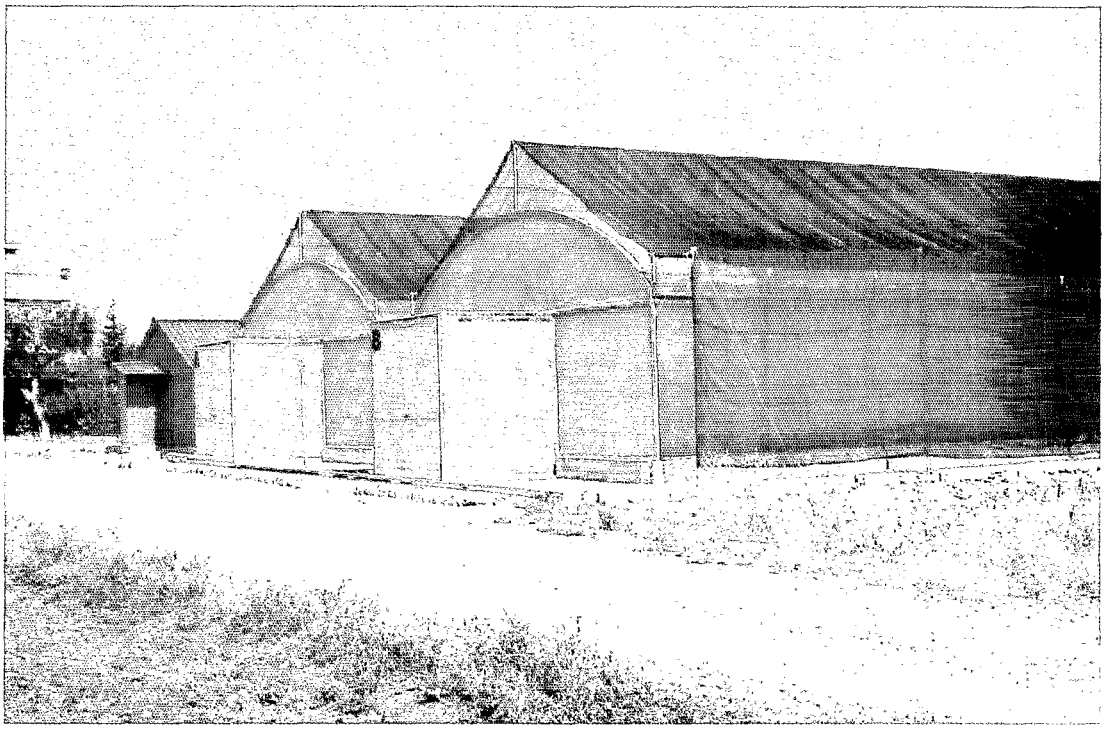

Fig.1. Insect-proof screen house for the maintenance of primary sources, pre-basic or basic material

Fig. 2. Stone fruit mother plants maintained in screenhouse