



The Minister of Agriculture, Food and Forestry Policies

Having regard to the Ministerial Decree of 14 April 1997 published in Supplement no. 112 to the *Official Journal* of the Italian Republic no. 126 of 2 June 1997, transposing the Commission Directives no. 93/48/EEC of 23 June 1993, no. 93/64/EEC of 5 July 1993 and no. 93/79/EEC of 21 September 1993, on the marketing of fruit plant propagating material and fruit plants intended for fruit production;

Having regard to the Ministerial Decree of 24 July 2003 published in the *Official Journal* of the Italian Republic, general series, no. 240 (*Gazzetta Ufficiale della Repubblica italiana*, serie generale, n. 240) of 15 October 2003 organising the national (Italian) service for voluntary certification of fruit plant propagating material;

Having regard to the Legislative Decree no. 214 of 19 August 2005 published in Supplement no. 169/L to the *Official Journal* of the Italian Republic no. 248 of 24 October 2005 on the implementation of Directive 2002/29/EC on protective measures against the introduction and spread into the Community of organisms harmful to plants or plant products;

Having regard to the Ministerial Decree of 4 May 2006, published in the *Official Journal* of the Italian Republic, general series, no. 168 of 21 July 2006 releasing general provisions for the production of propagating material of fruit plants and shrubs as well as for agamically-propagated herbaceous species;

Having identified the opportunity of laying down special provisions for the production of certified plant propagating material of Citrus;

Having regard to the proposal about the technical protocols for the production of certified propagating material of Citrus approved by the National (Italian) Certification Committee (Comitato nazionale per la certificazione) in the session held on 30 January 2006, in accordance with Article 3 of the Ministerial Decree of 24 July 2003;

Having received the favourable opinion of the Phytosanitary Committee referred to in Article 52 of the Legislative Decree no. 214 of 19 August 2005, in accordance with Article 11 of the Ministerial Decree of 4 May 2006, at the meeting held on 18 July 2006;

Orders:

Article 1
Subject



The Minister of Agriculture, Food and Forestry Policies

1. The rules set forth in this Decree apply to certification of plant propagating material belonging to the genera *Citrus*, *Poncirus*, *Fortunella*, as well as other genera of *Aurantioideae* and their hybrids.
2. For the purposes of this decree, the Ministerial Decree of 4 May 2006, mentioned in the premises, will be hereinafter referred to as the "decree".

Article 2

Registration of Primary Sources

1. For the registration of Primary sources with the National (Italian) Certification Service, the plant breeder must fulfil the obligations set forth in Article 13 of the Ministerial Decree of 24 July 2003 and Article 2 of the "decree". The pomological data sheet and the phytosanitary data sheet must be prepared according to the patterns in Annex 1 of this decree.
2. For the registration of new cultivars, the pomological data sheet must comply with that provided for in UPOV or CPVO protocols.
3. New selections are allowed in the Conservation and Pre-multiplication steps, provided that they comply with the phytosanitary characteristics required and that there exists a genetic description distinguishing them from existing varieties.

Article 3

Means and Facilities

1. Means and facilities necessary to *in vivo* conservation and production of "Pre-basic" and "Basic" propagating material referred to in Article 4 and 5 of the "decree", must meet the requirements listed in Annex 2 of this decree.
2. Means and facilities necessary to *in vivo* growing and production of "Certified" propagating material referred to in Article 6 of the "decree", must meet the requirements listed in Annex 3 of this decree.

Article 4

Certification of Propagating Material

1. Pursuant to Article 11 of the Ministerial Decree of 24 July 2003, for the purposes of the issuance of certification of nursery productions according to Article 12 of the Ministerial Decree of 24 July 2003 and Article 8 of the "decree", "Pre-basic", "Basic" and "Certified" propagating material which is virus-free (VF) or virus-tested (VT), must be free from the diseases and pathogens listed in Annex 6 of this decree.

Article 5



The Minister of Agriculture, Food and Forestry Policies

Tests and Controls

1. “Pre-basic, “Basic” and “Certified” propagating material must be subjected to phytosanitary controls and tests and genetic trueness-to-type checks as referred to in Article 5.2, (b) of the Ministerial Decree of 24 July 2003, and in Articles 4.3, 5.3 and 6.4 of the “decree”, as provided for in Annexes 7 and 8 of this decree.

Article 6

Increase blocks

1. Increase blocks established in accordance with article 3.2 (c) of the “decree” must fulfil, according to the step in which they are set up, the requirements listed in Annex 2 for Pre-multiplication and in Annex 3 for Multiplication.

Article 7

Provisional Regulations

1. Until 31 December 2011, propagating material belonging to the genera *Citrus*, *Poncirus* and *Fortunella* as well as to other genera of *Aurantioideae* and their hybrids even if not compliant with this decree, provided that it derives from primary sources included in the National or Regional Certification programmes and already existing at the time of entry into force of this decree, is admitted to national (Italian) certification.

This decree is sent to the Supervisory body for registration and will enter into force the day after its publication in the *Official Journal* of the Italian Republic.

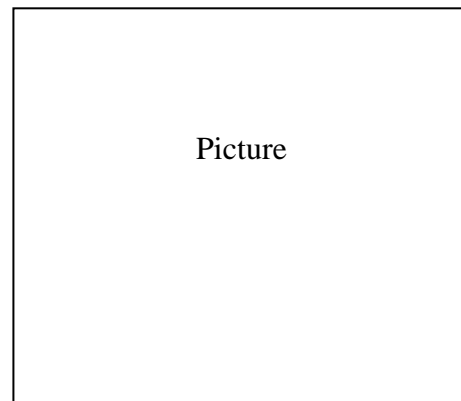
Rome, 20 November 2006

The Minister: De Castro

DATA SHEETS FOR THE REGISTRATION OF THE CITRUS PRIMARY SOURCE

Part I – Pomological Data Sheet**Genus:****Species:****Cultivar:****Clone:****Genetic origin:****Plant characteristics:**

- Development
- Vigour
- Growth
- Habit
- Thorns
- Leaf:
 - Size
 - Shape
 - Apex shape
 - Leaf margin shape
 - Leaf blade pattern
 - Colour of the upper leaf side
 - Colour of the lower leaf side
 - Length of the leaf petiole
 - Petiole wings
 - Wing size
- Flower
 - Size
 - Distribution
 - Pollen presence

**External fruit characteristics**

- Epicarp colour
- Epicarp surface
- Oil glands
- Fruit shape
- Average weight
- Cross diameter
- Longitudinal diameter
- Base
- Calyx
- Peduncle
- Attachment to the peduncle
- Navel

Internal fruit characteristics

- Grind
- Pulp:
 - Colour
 - Texture

- Vescicules
- Juice content
- % soluble solids
- Acidity
- Seeds

Production characteristics

- Fruit-setting
- Productivity
- Ripening time
- Fruit persistence on the plant

Behaviour towards the main physiological disorders and diseases:
(facultative)

Belonging to GMO **YES** **NO**

Pomological characterisation:
According to UPOV or CPVO (www.cpvo.europa.eu) standards

Molecular characterisation

Conservation of the Primary Source:

.....
(Responsible body)

.....
(Location)

Date

The Manager

Part II – Testing protocols for plant health assessment

Causal agent / Disease	Acronym	Biological assays (woody indicators)		Microscopic / Serological tests		Biomolecular Tests	
		+	-	+	-	+	-
VIRUSES							
<i>Citrus tristeza virus</i>	CTV	<input type="checkbox"/> Mexican lime	<input type="checkbox"/>	<input type="checkbox"/> ELISA	<input type="checkbox"/>	<input type="checkbox"/> Hybridisation	<input type="checkbox"/>
				<input type="checkbox"/> DTBIA	<input type="checkbox"/>	<input type="checkbox"/> RT-PCR	<input type="checkbox"/>
<i>Citrus leaf rugose virus</i>	CiLRV	<input type="checkbox"/> Grapefruit	<input type="checkbox"/>				
<i>Citrus variegation virus / Citrus crinkly leaf virus</i>	CVV / CCLV	<input type="checkbox"/> Lemon	<input type="checkbox"/>	<input type="checkbox"/> ELISA	<input type="checkbox"/>	<input type="checkbox"/> Hybridisation	<input type="checkbox"/>
		<input type="checkbox"/> Etrog citron	<input type="checkbox"/>			<input type="checkbox"/> RT-PCR	<input type="checkbox"/>
<i>Citrus psorosis virus</i>	CPsV	<input type="checkbox"/> Sweet organe cv <i>Madam Vinous</i>	<input type="checkbox"/>	<input type="checkbox"/> ELISA	<input type="checkbox"/>	<input type="checkbox"/> Hybridisation	<input type="checkbox"/>
				<input type="checkbox"/> DTBIA	<input type="checkbox"/>	<input type="checkbox"/> RT-PCR	<input type="checkbox"/>
Satsuma dwarf virus	SDV	<input type="checkbox"/> Dweet Tangor	<input type="checkbox"/>				
		<input type="checkbox"/> Troyer citrange	<input type="checkbox"/>				
Citrus tatter leaf virus	CTLV	<input type="checkbox"/> Dweet Tangor	<input type="checkbox"/>				
		<input type="checkbox"/> Troyer citrange	<input type="checkbox"/>				
Indian citrus ring spot virus	ICRSV	<input type="checkbox"/> Grapefruit	<input type="checkbox"/>				
		<input type="checkbox"/> 861-S1 Etrog citron	<input type="checkbox"/>				
		<input type="checkbox"/> Troyer citrange	<input type="checkbox"/>				
		<input type="checkbox"/> Mexican lime	<input type="checkbox"/>				
Citrus vein enation virus	CVEV	<input type="checkbox"/> Grapefruit	<input type="checkbox"/>				
		<input type="checkbox"/> Etrog citron 861-S1	<input type="checkbox"/>				
		<input type="checkbox"/> Troyer citrange	<input type="checkbox"/>				
		<input type="checkbox"/> Mexican lime	<input type="checkbox"/>				
VIROIDS							
<i>Citrus exocortis viroid</i>	CEVd	<input type="checkbox"/> 861-S1 Etrog citron	<input type="checkbox"/>			<input type="checkbox"/> Hybridisation	<input type="checkbox"/>
		<input type="checkbox"/> Parson' special mandarin onto Rough lemon	<input type="checkbox"/>			<input type="checkbox"/> RT-PCR	<input type="checkbox"/>
<i>Citrus cachexia viroid</i>	HSVd	<input type="checkbox"/> 861-S1 Etrog citron	<input type="checkbox"/>			<input type="checkbox"/> Hybridisation	<input type="checkbox"/>
		<input type="checkbox"/> Parson' special mandarin onto Rough lemon	<input type="checkbox"/>			<input type="checkbox"/> RT-PCR	<input type="checkbox"/>
VIRUS-LIKE AGENTS							
Concave gum	CG	<input type="checkbox"/> Sweet organe cv <i>Pineapple</i>	<input type="checkbox"/>				
		<input type="checkbox"/> Grapefruit	<input type="checkbox"/>				
		<input type="checkbox"/> Rough lemon	<input type="checkbox"/>				
Cristacortis	CCr	<input type="checkbox"/> Sweet organe cv <i>Pineapple</i>	<input type="checkbox"/>				
		<input type="checkbox"/> Grapefruit	<input type="checkbox"/>				
		<input type="checkbox"/> Rough lemon	<input type="checkbox"/>				
Impietratura	CI	<input type="checkbox"/> Sweet organe cv <i>Pineapple</i>	<input type="checkbox"/>				
		<input type="checkbox"/> Grapefruit	<input type="checkbox"/>				
		<input type="checkbox"/> Rough lemon	<input type="checkbox"/>				
Kumquat disease	KdV	<input type="checkbox"/> Sweet organe cv <i>Pineapple</i>	<input type="checkbox"/>				
		<input type="checkbox"/> Grapefruit	<input type="checkbox"/>				
		<input type="checkbox"/> Rough lemon	<input type="checkbox"/>				
Rough lemon incompatibiliy	RLeI						

(Part II to be continued)

(Part II continued)

FUNGI	ISOLATION		YEAR/S
	Result		
	+	-	
Foot rot <i>Phytophthora citrophthora</i>			
Citrus root rot <i>Phytophthora nicotianae</i>			
Mal secco <i>Phoma tracheiphila</i>			
SPIROPLASMAS	ISOLATION		YEAR/S
	Result		
	+	-	
<i>Spiroplasma citri</i> Stubborn			

HEALTH STATUS: Virus-free VF Virus-tested VT

Date

The Laboratory Manager

*MEANS FOR IN VIVO GROWING AND PRODUCTION OF
“PRE-BASIC” and “BASIC” MATERIAL*

Facilities

The Conservation and Pre-multiplication steps shall be carried out in an insect-proof screenhouse. The screenhouse size shall allow the proper development of plants proportioned to the container volume; moreover, the following requirements shall be fulfilled:

- a. isolation of growing containers from the ground or the flooring by:
 - i. a suitably designed French drain covered with fine gravel or any inert material providing for effective drainage;
 - ii. a layer of concrete or different material. In such a case containers, flats for seedling beds and acclimatation benches shall be kept on supports at least 20cm high off the ground;
- b. a French drain, all around the screenhouse, at least 80 cm wide and at least 20 cm deeper than the inside flooring;
- c. isolation from surface water flow through a kerb or a similar isolating structure, declared appropriate by the locally competent Regional Phytosanitary Service;
- d. walls with a double 20/10 mesh (20 wires/cm warp and 10 wires/cm weft) net and entrance with a double door;
- e. plants with a different health status (Virus-free VF and Virus-tested VT) may be grown under the same screenhouse provided that they are isolated by a double net;
- f. an anti-hail net.

Growing and production

- a. The “Pre-basic” and “Basic” material shall be maintained and propagated in a screenhouse and grown in containers of appropriate volume;
- b. when the plants are brought in, they shall be numbered on the spot in a progressive order and in an indelible fashion;
- c. the soil or growing medium shall be found free from *Phytophthora citrophthora* and *P. nicotianae*; freedom from the above shall be substantiated by an official document;
- d. irrigation water shall be found free or cleaned up from propagules of *Phytophthora citrophthora* and *P. nicotianae*;
- e. any delivery of material by the Pre-multiplication Centre (PC) shall be at all time recorded and immediately notified (by fax and/or email) to the locally competent Regional Phytosanitary Service and to the relevant Pytosanitary Service of the final user;
- f. records shall be kept of all operations in a special Farm Book;
- g. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

Increase blocks

- a. “Basic” material from increase blocks shall be propagated in a screenhouse and grown in containers of appropriate volume;
- b. the soil or medium used for seedling beds and growing containers shall be found free from *Phytophthora citrophthora* and *P. nicotianae*; freedom from the above shall be substantiated by an official document;
- c. irrigation water shall be found free or cleaned up from propagules of *Phytophthora citrophthora* and *P. nicotianae*;

- d. to make graftings in the nurseries, certifiable propagating material can be collected from increase block plants twice and no later than 24 months after the grafting date;
- e. material of “Tarocco” cultivars can be collected once in eighteen months;
- f. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

MEANS FOR *IN VIVO* GROWING OF MOTHER PLANTS AND PRODUCTION
OF “CERTIFIED” MATERIAL

Part I - Mother Plant Blocks (MPB)

Certified Scion Mother Plant (ScMP) and Seed Mother Plant (SMP) blocks shall meet the following requirements:

- a. they shall be located in areas declared suitable by the locally competent Regional Phytosanitary Service, found free from Tristeza (Citrus Tristeza Virus) foci and from other quarantine pests unless otherwise provided by the Phytosanitary Service;
- b. they shall be established on soils which respond to the normal agronomic and health requirements and found free from *P. nicotianae* and *Phytophthora citrophthora*; freedom from the above shall be substantiated by an official document;
- c. they shall be established on soils on which none of the citrus trees have been grown for at least 5 years;
- d. in areas where the presence of *mal secco* disease has been reported by the locally competent Regional Phytosanitary Service, Mother Plants of susceptible species (lemon, lemon relatives, citron, lime, sour orange and bergamot) shall be covered with a 50% shading protective net;
- e. the plantings shall be at least 100m apart from any type of citrus except for plants grown in isolation, under a screenhouse;
- f. they shall be separated by a surrounding zone at least 2m wide, kept free from any vegetation;
- g. they shall be isolated from surface water flow;
- h. irrigation water shall be found free or cleaned up from propagules of *Phytophthora nicotianae* and *P. citrophthora*;
- i. plants shall be numbered on the spot, in a progressive order and in an indelible fashion;
- j. in the plot, the rows shall be complete and distinct per plant accession (species, cultivar and clone); if different accessions are grown in the same row, they shall be separated by a double inter-space; in any case, the planting distance shall not be lower than 4x3m; the planting layout shall be reported in a specific document and notified to the locally competent Regional Phytosanitary Service;
- k. scion mother plants (ScMP) shall not be kept for more than 20 years since their establishment;
- l. seed mother plants (SMP) shall not be kept for more than 30 years since their establishment;
- m. from each scion mother plant (ScMP) not more than 1500 scions, for a maximum of 6000 buds, can be annually collected excepting “Tarocco” cultivars for which this annual limit is 1000 scions and 4000 buds;
- n. the blocks shall be kept under continuous surveillance to control pathogens, pests and weeds;
- o. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

Part II- Increase blocks

The increase blocks shall be located in areas declared, by the locally competent regional Phytosanitary Service, free from Tristeza (Citrus Tristeza Virus) foci and from other quarantine pests, unless further instructions are provided by the Phytosanitary Service.

II.A – Increase blocks in open field

- a. They shall be established on soils which respond to the normal agronomic and health requirements and found free from *Phytophthora nicotianae* and *P. citrophthora*; freedom from the above shall be substantiated by an official document;
- b. they shall be established on soils on which none of the citrus trees have been grown for at least 5 years;
- c. the plantings shall be located in isolated areas or at a distance of at least 100m from any commercial citrus groves and “CAC” plant nurseries, except for plantings under insect-proof nets;
- d. in areas where the presence of *mal secco* disease has been reported by the locally competent Regional Phytosanitary Service, plants of susceptible species (lemon, lemon relatives, lime, citron, sour orange and bergamot) shall be covered with a 50% shading protective net;
- e. irrigation water shall be found free or cleaned up from propagules of *Phytophthora citrophthora* and *Phytophthora nicotianae*;
- f. accessions under multiplication shall be distinct in easily identifiable plots; the planting layout shall be reported in a specific document and notified to the locally competent Regional Phytosanitary Service;
- g. in the plot, the rows shall be complete and distinct per plant accession (species, cultivar and clone); if different accessions are grown in the same row, they shall be separated by a double inter-space; in any case, the planting distance shall not be lower than 2x1m; the planting layout shall be reported in a specific document and notified to the locally competent Regional Phytosanitary Service;
- h. seedlings shall be grafted at not less than 40cm from the crown;
- i. in case of grafting failure and when appropriate, top-grafting shall be made by using material of the same accession; in this instance a grafting height of at least 35cm is admitted;
- j. well-lignified propagating material can be collected from increase block plants three times since the grafting or the establishment date except for “Tarocco” cultivars for which collection is allowed only twice, with one-year interval, and after the trueness-to-type checks;
- k. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

II.B – Increase blocks in containers

- a. The plants shall be at least 100m apart from any commercial citrus groves and “CAC” plant nurseries, except for plantings under insect-proof nets;
- b. in areas where the presence of *mal secco* disease has been reported by the locally competent Regional Phytosanitary Service, plants of susceptible species (lemon, lemon relatives, lime, citron, sour orange and bergamot) shall be covered with a 50% shading protective net;
- c. the soil or growing medium shall be found free from *Phytophthora nicotianae* and *P. citrophthora*; freedom from the above shall be substantiated by an official document;
- d. the growing containers, of appropriate volume (at least 8 l), can be either placed on the ground, - and in this instance freedom from *Phytophthora nicotianae* and *Phytophthora citrophthora* shall be ascertained - or isolated from the ground by ;
 - i. a layer of fine gravel or any inert material providing for effective drainage, at least 10 cm high; when mulching films are used, the minimum height of the French drain is reduced to 5 cm;
 - ii. a layer of concrete or different material; in such a case the containers shall be placed on supports at least 20 cm high;
- e. irrigation water shall be found free or cleaned up from propagules of *Phytophthora citrophthora* and *P. nicotianae*;
- f. the planting density shall not exceed 8 plants per square meter;

- g. the area intended for growing plants in pots shall be separated by a surrounding zone at least 2 m wide, constantly tilled or kept free from any vegetation;
- h. the plants shall be subdivided in homogeneous lots (per species, cultivar, clone and rootstock), easily identifiable; the planting layout shall be reported in a specific document and notified to the locally competent Regional Phytosanitary Service
- i. seedlings shall be grafted at not less than 40cm from the crown onto rootstocks 0.8cm in diameter;
- j. in case of grafting failure and when appropriate, top-grafting shall be made by using material of the same accession; in this instance a grafting height of at least 35cm is admitted;
- k. well-lignified propagating material can be collected from increase block plants two times except for “Tarocco” cultivars for which collection is allowed only once ;
- l. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

Part III- Nurseries (Seedling, Rootling and Sapling beds)

The nurseries of certifiable plants shall be located in areas declared appropriate by the locally competent Regional Phytosanitary Service, found free from Tristeza (Citrus Tristeza Virus) foci and from other quarantine pests, unless further instructions are provided by the Phytosanitary Service.

To produce certifiable plants only soil-less culture is allowed. The nurseries shall fulfil the following requirement:

- a. the growing medium shall be free from *P. nicotianae*, *Phytophthora citrophthora* and *Pratylenchus vulnus*, *Tylenchulus semipenetrans*; freedom from the above shall be substantiated by an official document;
- b. irrigation water shall be found free or cleaned up from propagules of *Phytophthora citrophthora* and *P. nicotianae*;
- c. the flats for seedling beds shall be isolated from surface and sub-surface water flow and shall not be placed on the ground but kept at 10cm at least off the ground;
- d. the flats shall preventively be disinfected with a 2% sodium hypochlorite solution;
- e. the growing containers, of appropriate volume, can be either placed on the ground, - and in this case freedom from *Phytophthora nicotianae* and *Phytophthora citrophthora* shall be substantiated by an official document- or isolated from the ground by ;
 - i. a layer of fine gravel or any inert material providing for effective drainage, at least 10cm high; when mulching films are used, the minimum height of the French drain is reduced to 5 cm;
 - ii. a layer of concrete or different material; in such a case the containers shall be placed on supports at least 20 cm high;
- f. seedlings of species susceptible to *mal secco* shall be covered by a 50% shading net if they are less than 50m apart from other lemon groves;
- g. seedlings which are to be moved to the graftling bed shall exhibit at least 4 to 6 fully developed leaves so as to distinguish natural hybrids from nucellar seedlings;
- h. the plants shall be subdivided in homogeneous lots (per species, cultivar, clone and rootstock), made up of a maximum of 4 rows, easily identifiable and reported on a map;
- i. the growing containers shall be placed at a distance of at least 20cm in the row and lots shall be spaced out by at least 50 cm;
- j. the grafting shall be made at a height not lower than 30cm from the crown onto rootstocks 0.6 cm at least in diameter. The Bodies responsible for inspection can authorise a lower height only when dwarfing rootstocks are used. In case of grafting failure and when

- appropriate, top-grafting shall be made by using material of the same accession; in this instance a grafting height of at least 25cm is admitted;
- k. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

“VIRUS-FREE” AND “VIRUS-TESTED” HEALTH STATUS
TABLES FOR “PRE-BASIC”,
“BASIC” AND “CERTIFIED” MATERIAL:
DISEASES AND HARMFUL ORGANISMS COVERED BY THE SCHEME.

Official/ scientific name	Health Status		
	Acronym	Virus -free (VF)	Virus-tested (VT)
VIRUSES			
Citrus tristeza virus	CTV	X	X
Citrus leaf rugose virus	CiLRV	X	X
<i>Citrus variegation virus / Citrus crinkly leaf virus</i>	CVV / CCLV	X	X
<i>Citrus psorosis virus</i>	CPsV	X	X
Satsuma dwarf virus	SDV	X	
Citrus tatter leaf virus	CTLV	X	
Indian citrus ring spot virus	ICRSV	X	
Citrus vein enation virus	CVEV	X	
SPIROPLASMAS			
Stubborn		X	
VIROIDS			
Citrus exocortis viroid	CEVd	X	X
<i>Citrus cachexia viroid</i>	HSVd	X	X
VIRUS-LIKE AGENTS			
<i>Concave gum</i>	CG	X	X
Cristacortis	CCr	X	X
Impietratura	CI	X	X
Kumquat disease	KdV	X	
Rough lemon incompatibility	RLeI	X	

SANITARY CHECKS

Part I – On “Prebasic” and “Basic” and “Certified” material

Viruses, Spiroplasmas, Viroids, Virus-like agents and Fungi

Two types of checks shall be carried out:

- a. Visual inspections: every year on all plants, at the appropriate time, when symptoms are likely to be most visible for each single disease, *mal secco* included;
- b. Laboratory testing: according to the procedures indicated in tables 1 and 2 of the present annex.

In the increase blocks and in the nurseries, visual inspections shall be carried out every year on all plants at the appropriate time, when symptoms are likely to be most visible for each single disease, *mal secco* included.

All material descended from the first multiplication of the primary source when brought into the Conservation Centre for Pre-multiplication or in the other steps shall be individually submitted to sanitary and trueness-to-type checks according to the procedure reported in tables 1 and 2 of the present annex.

Part II- On soil and growing media in all steps

Mycological analysis through isolation on selective media for *P. nicotianae* and *Phytophthora citrophthora* on samples collected according to the following methods:

- growing media: a sample shall be collected every 5m³, made up of 10 sub-samples, for a total volume of at least 1 litre.
- soil: before planting and, at any time, before any deep tillage, 1 sample per hectare shall be collected, made up of 10 sub-samples, for a total volume of at least 1 litre;

Nematological analysis through isolation techniques for *Pratylenchus vulnus* and *Tylenchulus semipenetrans* on samples collected according to the following methods:

- growing media: a sample shall be collected every 5m³, made up of 5 sub-samples, for a total volume of at least 1 litre.
- soil: before planting and, at any time, before any deep tillage, 1 sample per hectare shall be collected, made up of 5 sub-samples, for a total volume of at least 1 litre.

Table 1: Procedure for the assessment of “Virus-free” and “Virus-tested” health status of Primary Sources and of “Pre-basic” and “Basic” Mother Seed Plants (MSP) and Mother Scion Plants (MScP)

Disease or harmful organism	CHECKS					
	Visual inspections		Biological assay		Laboratory*testing : serological or molecular	
	Time	Frequency	Indicator recommended	Frequency	Frequency	Sampling time and type
VIRUSES						
CTV	From growth recovery until a temperature of 25°C	Annual	Mexican lime	Every 5 years	On all plants every year	<u>Leaves</u> : collected in spring and autumn (until a temperature of 25°C)
CiLRV	From growth recovery until a temperature of 25°C	Annual	Grapefruit	Every 3 years from the 3 rd year		
CVV/CCLV			Lemon Etrog citron		On all plants within 3 years	Flowers and leaves: from growth recovery until a temperature of 25°
CPsV	From growth recovery until a temperature of 25°C	Annual	Sweet orange cv <i>Madam vinous</i>	Every 3 years from the 3 rd year	On all plants within 3 years	<u>Flowers</u> : collected in spring <u>Leaves</u> : from vegetative growth until a temperature of 25°C (spring and autumn)
SDV CTLV	From growth recovery until a temperature of 25°C	Annual	Dweet tangor Troyer citrange	On all plants within 6 years		
ICRSV CVEV	From growth recovery until a temperature of 25°C		Grapefruit Etrog citron 861-S1 Troyer citrange Mexican lime	On all plants within 6 years		
SPIROPLASMAS						
<i>Spiroplasma citri</i>	From growth recovery	Annual			On all plants within 6 years	
VIROIDS						
CEVd HSVd	From growth recovery	Annual	Etrog citron 861-S1 Parson'Special Mandarin onto rough lemon	On all plants within 5 years from the 5 th year	On all plants within 5 years from the 5 th year	<u>Mature leaves</u> : collected in summer-early autumn
VIRUS-LIKE AGENTS						
CG, CCr, CI, KdV and RLeI	From growth recovery until a temperature of 25°C	Annual	Sweet orange cv. <i>Pineapple</i> Grapefruit Rough lemon	On all plants within 6 years From the 6 th year since their establishment		

* only for *Spiroplasma citri*, laboratory testing consists in isolation and culturing

Table 2: Procedure for the assessment of “Virus-free” and “Virus-tested” health status of “Certified” Mother Seed Plants (MSP) and Mother Scion Plants (MScP)

Disease or harmful organism	CHECKS					
	Visual checks		Biological assay		Laboratory testing*: serological or molecular	
	Time	Frequency	Indicator recommended	Frequency	Frequency	Sampling time and type
VIRUSES						
CTV	From growth recovery until a temperature of 25°C	Annual			On all plants every year	<u>Leaves:</u> collected in spring and autumn (until a temperature of 25°C)
CiLRV CVV CCLV	From growth recovery until a temperature of 25°C	Annual				
CPsV	From growth recovery until a temperature of 25°C	Annual			On all plants within 5 years	<u>Leaves:</u> from vegetative growth until a temperature of 25°C (spring and autumn)
SDV CTLV	From growth recovery until a temperature of 25°C	Annual				
SPIROPLASMAS						
<i>Spiroplasma citri</i>	From growth recovery	Annual				
VIROIDS						
CEVd HSVd	From growth recovery	Annual			On all plants within 5 years from the 5 th year	<u>Mature leaves:</u> collected in summer-early autumn
VIRUS-LIKE AGENTS						
CG, CCr, CI, KdV e RLeI	From growth recovery until a temperature of 25°C	Annual				

* only for *Spiroplasma citri*, laboratory testing consists in isolation and culturing

TRUENESS-TO-TYPE CHECKS

Part I – On “Pre-basic” and “Basic” material

The trueness-to-type certification for cultivars and rootstocks is issued by the competent Regional Phytosanitary Service after observing one growing and production cycle so as to assess the conformity to the phenotype at appropriate time, when the phenological characteristics are likely to be best expressed.

Later, at ripening time, a visual inspection shall be carried out every year to assess the production characteristics.

Part II – on “Certified” Mother Plants

The trueness-to-type certification for cultivars and rootstocks is issued by the competent Regional Phytosanitary Service after observing one growing and production cycle, before the collection of certified material.

Later, at ripening time, a visual inspection shall be carried out every year to assess the production characteristics.

Part III – In the Increase Blocks

Visual inspections shall be carried out to assess the plant growing characteristics.

