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 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 Pome fruits ;

Having regard to the proposal about the technical protocols for the production of certified propagating material for Pome fruits approved by the National (Italian) Certification Committee (Comitato nazionale per la certificazione) in the session held on 15 and 16 June 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 propagating material for the fruit plants listed below as well as their rootstocks even if of a different species or hybrids:
   - Apple tree *(Malus domestica* L.*)*,
   - Pear tree *(Pyrus communis* L.),
   - Quince tree *(Cydonia* sp.*)*,

other Pome fruits and their hybrids of interest to agriculture.
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 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” propagating material referred to in Article 4 of the “decree”, must meet the requirements listed in Annex 2 of this decree.
2. Means and facilities necessary to *in vivo* conservation and production of “Basic” propagating material referred to in Article 5 of the “decree”, must meet the requirements listed in Annex 3 of this decree.
3. Means and facilities necessary to *in vivo* production of “Certified” propagating material referred to in Article 6 of the “decree”, must meet the requirements listed in Annex 4 of this decree.
4. Means, facilities and modes necessary to *in vitro* production of “Pre-basic”, ”Basic” and “Certified” propagating material referred to in Article 7 of the “decree”, must meet the requirements listed in Annex 5 of this decree.

Article 4
Certification of Propagating Material

The Minister of Agriculture, Food and Forestry Policies

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
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, 5 and 6 of the "decree", as provided for in Annexes 7 and 8 of this decree.

Article 6
Provisional Regulations

1. Until 31 December 2011, propagating material belonging to the genera *Malus, Pyrus* and *Cydonia* as well as 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, date

THE MINISTER

ANNEX 1

DATA SHEET FOR THE REGISTRATION OF POME FRUIT PRIMARY SOURCE

Part I – Pomological Data Sheet

Production of Primary Source:

□ **Cross: Year: _____ made by: _____**

_____

Parent ♀ _____ x ♂ _____

□ **Sanitary selection: Years from _____to_____ carried out by: _____**

_____

Representative picture

□ **Mutant or Clonal selection: Year: _____ identified by: _____**

in _____ in the Cultivar: _____

Conservation of Primary source:

_____

(Responsible body)

_____

(Location)

Belonging to GMO □ **YES** □ **NO**

Origin:_____

Pursuant to Article 2 (2) of Directive 2001/18/EC of 12/03/2001

Pomological characterisation:

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

Molecular characterisation

Year:_____Laboratory:_____

Molecular Markers	Number of combinations per Primer Or enzyme systems	Bibliographic reference
□ SSR		
□ AFLP		
□ Isozymes:		
□ Other		

□ Tick if compliant

Date ..... The Laboratory Manager

II. A Apple *

Causal agent / Disease	Acronym	Biological assays (woody indicators) **		Microscopic / Serological tests	Biomolecular** Tests
		Greenhouse	Field		
Test result		+ -	+ -	+ -	+ -
VIRUSES					
Apple mosaic virus	**ApMV**	□ *M .pumila* Charden □	□ *M. pumila* Golden D. □ □ *M. pumila* L Lambourne □	□ ELISA □	□ RT-PCR □ □ RT-PCR- ELISA □
Apple stem pitting virus	**ASPV**	□ *Pyronia veitchii* □ □ *M .pumila* Spy 227 □ □ *M. pumila* Virginia Crab □ □ *M. pumila* Kola □ □ *M. pumila* Radiant □	□ *Pyronia veitchii* □ □ *M. pumila* Spy 227 □ □ *M. pumila* Virginia Crab □		□ RT-PCR □
Apple chlorotic leaf spot virus	**ACLSV**	□ *Malus platycarpa* □ □ *M. sylvestris* R12740 7A □ □ *Cydonia oblonga* C7/1 □ □ *Cydonia oblonga* Pigwa □	□ *Malus platycarpa* □ □ *Malus sylvestris* R12740 7A □	□ ELISA □	□ IC-RT-PCR □ □ RT-PCR □
Apple stem grooving virus	**ASGV**	□ *M. pumila* Virginia Crab □ □ *M. micromalus* GMAL273 □	□ *M pumila* Virginia Crab □	□ ELISA □	□ RT-PCR □ □ IC-RT-PCR □ □ RT-PCR- ELISA □
VIROIDS					
Apple dimple fruit viroid	**ADFVd**		□ *M. pumila* Red delicious □		□ RT-PCR □ □ Hybridisation □
Apple scar skin viroid = *Dapple apple*	**ASSVd** = **DAVd**	□ *M. pumila* Stark's Earliest □ □ *M. pumila* Sugar Crab □	□ *M. pumila* Red delicious □		□ RT-PCR □ □ Hybridisation □
BACTERIA					
Fire blight/Erwinia amylovora	**Ea**			According to EPPO standards	
PHYTOPLASMAS					
Apple Proliferation, Candidatus phytoplasma mali	**AP**	□ *M. pumila* Charden □	□ *M. pumila* Golden D. □	□ IF □ □ ELISA □ □ DAPI □	□ PCR □ □ PCR-ELISA □
VIRUS-LIKE AGENTS					
Apple rubbery wood = *Apple flat limb* = *Apple chat fruit*	**ARW** **AFL** **ACF**	□ *Prunus avium* Mazzard □ □ *Prunus avium* F12/1 □	□ *M. pumila* L .Lambourne □ □ *M. pumila* Gravensteiner □		
DISEASES CAUSING FRUIT ALTERATIONS					
Russet ring *Green crinkle* *Rough skin* *Star crack* *Russet wart* *Ring spot*	**ARRV** **GCV** **ARSk** **ASC** **ApRWa** **ApRS**		□ *M. pumila* Golden D. □		

* Testing methods

** For the primary source registration both biomolecular testing and biological assays shall be conducted.

HEALTH STATUS: ☐ **Virus-free VF** ☐ **Virus-tested VT**

Date .....

The Laboratory Manager

II.B Pear and Quince*

Causal agent/ Disease	Acronym	Biological assays (woody indicators) **		Microscopic/ Serological tests	Biomolecular** Tests
		Greenhouse	Field		
Test result		+ -	+ -	+ -	+ -
VIRUSES					
Apple stem pitting virus	**ASPV**	☐ *Pyronia veitchii* ☐ ☐ *Malus pumila* Spy 227 ☐ ☐ *M. pupila* Virginia crab ☐ ☐ *P. communis* Noveau Poiteau ☐ ☐ *P. communis* Julesd'Airolles ☐ ☐ *Pyrus communis* A 20 ☐	☐ *Pyronia veitchii* ☐ ☐ *M. pumila* Spy 227 ☐ ☐ *Malus pumila* ☐ Virginia crab		☐ RT-PCR ☐
Apple chlorotic leaf spot virus	**ACLSV**	☐ *Malus sylvestris* R12740 7A ☐ ☐ *Cydonia oblonga* C7/1 ☐ ☐ *Cydonia oblonga* Pigwa ☐ ☐ *Malus platycarpa* ☐ ☐ *Pyronia veitchii* ☐ ☐ *P. communis* Noveau Poiteau ☐ ☐ *Pyrus communis* A 20 ☐ ☐ *P. communis.* Beurre Hardy ☐	☐ *Malus platycarpa* ☐ ☐ *Malus sylvestris* ☐ R12740 7A	☐ ELISA ☐	☐ IC-RT-PCR ☐ ☐ RT-PCR ☐
Apple stem grooving virus	**ASGV**	☐ *M. pumila* Virginia Crab ☐ ☐ *M. micromalus* GMAL273 ☐ ☐ *Pyronia veitchii* ☐	☐ *Malus pumila* ☐ Virginia crab	☐ ELISA ☐	☐ RT-PCR ☐ ☐ IC-RT-PCR ☐ ☐ RT-PCR-ELISA ☐
VIROIDS					
Pear blister canker viroid	**PBCVd**		☐ *Pyrus communis* ☐ Fieud 37 ☐ *P. communis* A 20 ☐		☐ RT-PCR ☐ ☐ Hybridisation ☐
Apple scar skin viroid	**ASSVd**		☐ Stark's Earliest ☐ ☐ Sugar Crab ☐ ☐ Red Delicious ☐ ☐ Starkrimson ☐		☐ RT-PCR ☐ ☐ Hybridisation ☐
BACTERIA					
Fire blight/Erwinia amylovora	**Ea**			According to EPPO standards	
Crown gall/Agrobacterium tumefaciens					
Bacterial blast/Pseudomonas syringae pv s.					
PHYTOPLASMAS					
Candidatus Phytoplasma pyri associated to Pear decline	**PD**			☐ DAPI ☐	☐ PCR ☐ ☐ PCR-ELISA ☐
VIRUS-LIKE AGENTS					
Apple rubbery wood = *Apple flat limb* = *Apple chat frui)*	**ARW** **AFL** **ACF**	☐ *Prunus avium* Mazzard ☐ ☐ *Prunus avium* F12/1 ☐	☐ *Malus pumila* ☐ L .Lambourne ☐ *Malus pumila* ☐ Gravensteiner		
Quince yellow blotch *Pear rough bark* *Pear bark split* *Pear bark necrosis* *Pear bud drop*	**QYB** **PRB** **PBS** **PBN** **PBD**		☐ *P. communis* A 20 ☐ ☐ *Pyrus communis* ☐ B. Hardy ☐ *Pyrus communis* ☐ Doyenne du Comice		

* Testing methods

** For the primary source registration both biomolecular testing and biological assays shall be conducted

HEALTH STATUS: ☐ **Virus-free VF** ☐ **Virus-tested VT**

Date .....

The Laboratory Manager

ANNEX 2

MEANS FOR *IN VIVO* GROWING AND PRODUCTION OF
"PRE-BASIC MATERIAL"

Facilities

The Conservation for Pre-multiplication step 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 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 seed flats and acclimatation benches shall be placed off the ground on supports at least 20 cm high;
b. a French drain, all around the greenhouse, at least 80 cm wide and at least 20 cm deeper than the inside flooring;
c. isolation from the surface water flow through a kerb or a similar isolating structure, declared appropriate by the locally competent Regional Phytosanitary Service;
d. hard roof, walls and ceiling with a double 20/10 mesh (20 wires/cm warp and 10 wires/cm weft) net and entrance with double net and 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.

Growing and production

a. The "Pre-basic" material shall be maintained and propagated in a screenhouse and grown in containers of appropriate volume;
b. the soil or growing medium shall be sterilised and found free from *Chondrostereum purpureum, Verticillium dahliae, V. albo-atrum, Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae* pv *Syringae*; freedom from the above shall be substantiated by an official document;
c. when the plants are brought in, they shall be numbered on the spot in a progressive order and in an indelible fashion;
d. the containers and flats for rooting, acclimatisation and seedling beds shall be kept at 20 cm at least off the ground;
e. before use, the containers and flats for rooting, acclimatisation and seedling beds shall be disinfected with a 2% sodium hypochlorite solution for at least 20/30 minutes;
f. mother plants shall not be kept more than 15 years since they have been established and shall be renewed after checking all requirements for the primary source registration;
g. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

ANNEX 3

MEANS FOR *IN VIVO* GROWING AND PRODUCTION
OF "BASIC" MATERIAL

Part 1 – Facilities

I. A Pear, rootstocks and other pome fruit trees or their hybrids

The Pre-multiplication step shall be conducted in an insect-proof screenhouse which responds to the requirements and characteristics set out in Annex 2 of the present decree.

I. B. Apple and quince

The Pre-multiplication step shall be carried out under an insect-proof screenhouse. Upon authorisation this step can be implemented in mother plant blocks provided that:

a. they are located in areas declared appropriate by the locally competent Regional Phytosanitary Service, in accordance with the phytosanitary regulations in force and, in any case, at a distance of 1000m from any *Erwinia amylovora* host plants, on soils on which none of the tree crops have been grown for at least 4 years and in regions where pome fruit orchards are not intensively cultivated;
b. they are established on soils which respond to the normal agronomic and health requirements and found free from *Chondrostereum purpureum*, *Verticillium dahliae*, *V. albo-atrum*, *Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae* pv *syringae*; freedom from the above shall be substantiated by an official document;
c. they are protected by anti-hail nets.

Part II – Growing and Production

II.A. Pear, rootstocks and other pome fruit trees or their hybrids

a. The "Basic" material shall be maintained and propagated under an insect-proof screenhouse and grown in containers of appropriate volume;
b. "Basic" mother plants shall not be kept for more than 20 years since they have been brought into the screenhouse, unless otherwise provided by the locally competent Regional Phytosanitary Service;
c. the soil or growing medium used for maintenance and propagation must be sterilised and found free from *Chondrostereum purpureum*, *Verticillium dahliae, V. albo-atrum, Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae* pv *syringae*; freedom from the above shall be substantiated by an official document;
d. when the plants are brought into the house, they shall be numbered on the spot in a progressive order and in an indelible fashion;
e. the containers and flats used for rooting, acclimatation and seedling beds shall be kept at 20 cm at least off the ground;
f. the flats used for rooting, acclimatation and seedling beds shall be preventively disinfected with a 2% sodium hypochlorite solution for at least 20-30 minutes;
g. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

II.B. Apple and Quince

The "Basic" material shall be maintained and propagated under an insect-proof screenhouse according to the procedure specified in annex 2 of the present decree.

Upon authorisation this step can be implemented in mother plant blocks provided that:

a. they are located in areas declared appropriate by the locally competent Regional Phytosanitary Service, in accordance with the phytosanitary regulations in force and, in any case, at a distance of 1000m from any *Erwinia amylovora* host plants, on soils where none of the tree crops have been grown for at least 4 years and in regions where pome fruit orchards are not intensively cultivated;
b. they are established on soils which respond to the normal agronomic and health requirements and found free from *Chondrostereum purpureum, Verticillium dahliae, V. albo-atrum, Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae* pv *syringae*; freedom from the above shall be substantiated by an official document;
c. plants are grafted onto dwarfing rootstocks;
d. the number of basic mother plants is not lower than 5 plants per variety or clone;
e. individual scion mother plants (ScMP) or seed mother plants (SMP) are numbered on the spot, in a progressive order and in an indelible fashion when they are established;
f. blocks are protected by an anti-hail net;
g. plants are not kept for more than 10 years since they have been established.

II.C. Stool bed

"Basic" rootstocks are descended, by cutting (agamic propagation), from "Pre-basic" material which is collected from conservation or primary source, upon authorisation of the National Certification Committee (NCC) and according to the following procedure:

a. a maximum of two pre-multiplication steps can be implemented;
b. to carry out the first pre-multiplication step (PC1), bench-grafted cuttings on seedling rootstocks or alternatively self-rooted cuttings, planted in "bins" or similar containers as the stool bed, according to the procedure indicated in annex 2; the plants derived from this step shall be grown as follows:
   i. apple and quince outdoors, to obtain the first "increase" stool bed (PC1) in the same conditions as the variety;
   ii. whereas for pear, the increase stool bed (PC1) shall be grown in the screenhouse, in "bins" or similar containers as the stool bed, according to the procedure indicated in annex 2;
c. rooted cuttings derived from the first Pre-multiplication (PC1) shall be collected to establish the "basic" stool bed in the field (PC2) in accordance with the requirements set out for the basic variety;
d. in the field, the plots shall be complete and distinct per species, variety and clone; different species, varieties or clones are not admitted in the same row.

The stool bed rootstock production is carried out outdoors on soils which respond to the following requirements:

a. they shall be located in areas declared appropriate by the locally competent Regional Phytosanitary Service, in accordance with the phytosanitary regulations in force and, in any case, at a distance of 1000m from any *Erwinia amylovora* host plants and in regions where fruit orchards are not intensively cultivated;
b. they shall respond to the normal agronomic and health requirements and be found free from *Chondrostereum purpureum, Verticillium dahliae, V. albo-atrum, Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae* pv *syringae*; freedom from the above shall be substantiated by an official document;
c. they shall be protected by an anti-hail net;
d. the plants shall not be kept for more than 10 years since their establishment.

ANNEX 4

MEANS FOR *IN VIVO* GROWING AND PRODUCTION
OF "CERTIFIED" MATERIAL

Part I - Scion Mother Plant (ScMP) Blocks

They shall meet the following requirements:

a. they shall be located in areas declared appropriate by the locally competent regional Phytosanitary Service, according to the phytosanitary regulations in force and be, in any case, at a distance of 500m from any *Erwinia amylovora* host plants and in regions where pome fruit orchards are not intensively cultivated; the regional Phytosanitary Service can otherwise provide more stringent instructions, having heard the opinion of the National Certification Committee (NCC);
b. they shall be established on soils which respond to the normal agronomic and health requirements;
c. they shall be established on soils on which none of the tree crops have been grown for at least 4 years;
d. they shall be protected by an anti-hail net;
e. genetically unstable cultivars or mutants shall be grafted only onto dwarfing rootstocks belonging to basic or higher category;
f. mother plants of genetically "unstable" varieties shall not be kept for more than 10 years since their establishment;
g. mother plants of genetically "stable" varieties shall not be kept for more than 15 years since their establishment;
h. individual plants shall be numbered in a progressive order and in an indelible fashion when they are established;
i. the rows shall be complete and distinct per plant accession; if different accessions are grown in the same row, they shall be separated by a double inter-space; the planting layout shall be reported on a map;
j. the blocks shall be kept under continuous surveillance to control pathogens, pests and weeds;
k. irrigation water shall be found free or cleaned up from harmful organisms as set out by the Community Directive on the marketing of fruit plants (MD of 14 April 1997) and specified in the technical annexes of the present decree;
l. the planting distance shall be proportioned so that the normal cultural operations and the related checks can be carried out;

Part II – Seed Mother Plant Blocks (SMP) and stool bed

They shall meet the following requirements:

a. they shall be located in areas declared appropriate by the locally competent Regional Phytosanitary Service, according to the phytosanitary regulations in force, and be, in any case, at a distance of 500m from any *Erwinia amylovora* host plants and in regions where pome fruit orchards are not intensively cultivated; the Regional Phytosanitary Service can otherwise provide more stringent instructions, heaving heard the opinion of the National Certification Committee (NCC);
b. they shall be established on soils on which none of the tree crops have been grown for at least 4 years, conforming to the normal agronomic and health requirements and found free from *Chondrostereum purpureum, Verticillium dahliae, V. albo-atrum, Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae* pv *syringae* and from

the nematodes *Pratylenchus vulnus P. penetrans*, *Meloidogyne hapla* and *M. incognita;* freedom from the above shall be substantiated by an official document;

c. irrigation water shall be found free or cleaned up from harmful organisms as set out by the Community Directive on the marketing of fruit plants (MD of 14 April 1997) and specified in the technical annexes of the present decree;
d. the seed mother plant (SMP) blocks shall be complete and distinct per species, variety and clone and, in any case, different species or varieties are not admitted in the same row; every year the planting layout shall be made available to the locally competent Regional Phytosanitary Service and kept updated;
e. the seed mother plants (SMP) shall not be kept for more than 18 years since their establishment;
f. the stool beds shall not be kept for more than 15 years since their establishment;
g. the plantings shall be kept under continuous surveillance to control pathogens, pests and weeds.

The National Certification Committee (NCC) can otherwise provide, heaving heard the opinion of the locally competent Regional Phytosanitary Service, delivered at the request of the Multiplication Centre (MC) manager.

Part III- Nursery

The certified material shall be grown and produced in the nursery according to the following procedure:

a. the nursery shall be located in areas declared appropriate by the locally competent Regional Phytosanitary Service, in accordance with the phytosanitary regulation in force, and , in any case, at a distance of 500m from any pome fruit orchards; the regional Phytosanitary Service can otherwise provide more stringent instructions, heaving heard the opinion of the National Certification Committee (NCC);
b. the nursery shall be established on soils on which none of the tree crops have been grown for at least 2 years; they shall respond to the normal agronomic and health requirements and be found free from *Chondrostereum purpureum, Verticillium dahliae, V. albo-atrum, Armillariella mellea, Nectria galligena, Phytophthora cactorum* and *Pseudomonas syringae* pv *syringae* and from the nematodes *Pratylenchus vulnus P. penetrans*, *Meloidogyne hapla* and *M. incognita*; freedom from the above shall be substantiated by an official document;
c. the area intended for growing plants in containers shall be isolated from surface water flow and separated by a surrounding zone at least 2 m wide, kept free from any vegetation;
d. the plots shall be kept under continuous surveillance to control pathogens, pests and weeds;
e. the plants shall be subdivided in homogeneous lots, easily identifiable and reported on a map;
f. the plots shall be homogeneous, easily identifiable and isolated from any "CAC" nursery material by a zone at least 2 wide and include complete and distinct rows per species, variety and clone; different varieties and clones are admitted in the same row, provided that they are clearly spaced not less than 1m apart;
g. the production cycle of the plants to be certified shall not last more than 3 years since their establishment;
h. the soil shall be isolated from surface and subsurface water flow;
i. irrigation water shall be found free or cleaned up from harmful organisms as set out by the Community Directive on the marketing of fruit plants (MD of 14 April 1997) and specified in the technical annexes of the present decree;
j. the rooting and acclimatation containers shall be isolated from surface and subsurface water flow and kept at 10 cm at least off the ground;
k. containers shall preventively be disinfected with a 2% sodium hypochlorite solution.

ANNEX 5

MEANS FOR *IN VITRO* PRODUCTION OF "PREBASIC", "BASIC" AND "CERTIFIED" PROPAGATING MATERIAL OF PEAR AND RELATED ROOTSTOCKS

Part I
A – *In vitro* production of "Prebasic" and "Basic" material

a. *In vitro* pre-multiplication can be carried out at the Pre-multiplication Centre (PC) or in one or several micro-propagation laboratories registered with the regional Phytosanitary Service; to this end the Pre-multiplication Centre and the laboratory concerned shall subscribe to a specific convention and a specific request shall be submitted to the Phytosanitary Service for each plant accession.
b. The material derived from in *vitro* production can be acclimatised at the Pre-multiplication Centre (PC) or in one or several acclimatation facilities registered with the Regional Phytosanitary Service; to this end the Pre-multiplication Centre and the acclimatation facility concerned shall subscribe to a specific convention.
c. The "Basic" material shall be separated from any propagating material by means/facilities providing phytosanitary isolation (greenhouses, insect-proof houses, etc.).
d. The soil mix for acclimatation shall be free from any pathogen and to this end tested peat-moss of known origin shall be used or, instead, chemically or physically sterilised media.
e. Initial explants for micro-propagation (axillary buds for *in vitro* propagation) shall be collected only from plants grown at the Conservation Centres for Pre-multiplication (CCP);
f. The next stage can also include a period of *in vitro* establishment of not more than 3 months, followed by a maximum of 8 sub-cultures.
g. Within 2 years since the initial explant, the material under pre-multiplication shall be renewed, regardless of the number of sub-cultures attained.

Part II – Production of "Certified" material

The multiplication cycle shall start with "Pre-basic" or "Basic" material derived from pre-multiplication and can develop over a maximum of 12 sub-cultures (pre-multiplication + multiplication).

If necessary, to obtain a large amount of starting material to propagate, at the request of the National Certification Committee (NCC), a further multiplication of 8 sub-cultures will be admitted for a maximum of 20 transplantings (from establishment to rooting).

However, the material under multiplication shall be renewed within 2 years after the beginning of the multiplication step, regardless of the number of sub-cultures attained.

Part III - Growing rules

Micro-propagation of chimeric clones is not allowed due to the high risk of obtaining micro-propagated plants which do not conform to the initial phenotype.

During all stages of *in vitro* culture (multiplication, elongation and rooting), laboratories shall fulfil the following requirements:

a. the initial explant shall not be too small, that is to say not lower than 0.5 mm;
b. the culture medium used in all micro-propagation stages (collection, establishment, multiplication) shall not exceed, in any case, 1 mg/l cytokinin content;
c. it is not allowed to supply TDZ (Thidiazuron) to the culture medium nor any substances with possible mutagenic effect;
d. any culture displaying proliferation of undifferentiated tissue (callus) shall be removed;

e. at transplanting the basal part of the shoot tuft, where undifferentiated tissues can more likely proliferate, shall be removed;
f. only shoots derived from axillary buds shall be used;
g. vitrescent cultures and/or those with other morpho-physiological abnormalities (in particular, fasciations) shall be eliminated.

Growing containers of material under pre-multiplication and multiplication shall be maintained in a specific and clearly identified laboratory compartment and individually labelled so as to be easily recognised. The label shall report a number and all information needed to identify the content (variety, clone, date of clone entry, sub-culture number, transfer date). Growing operations shall be recorded daily in a book of original entry and, weekly, in a stock book, with non removable, progressively numbered pages, endorsed by the locally competent Regional Phytosanitary Service. The above book shall be stored at any time in the laboratory and made available for inspection, when appropriate. The containers eliminated following contaminations and/or morpho-physiological abnormalities of the culture and the containers moved into the refrigerator, will also be recorded in the book. If necessary, corrections shall be made by crossing out and allowing to read what is written below.

The "Basic" and the "Certified" material shall be acclimatised in a special greenhouse or tunnel and therefore, the acclimatation of non-certified material in the same environment is not allowed.

At the end of acclimatation, upon authorisation of the Regional Phytosanitary Service, a label, which guarantees trueness-to-type and the health status, shall be placed on the 60-plantlets honeycombed package; if, for technical reasons, the plantlets can not be transported in honeycombed containers, the same label can be used for the packaging of 60-plantlets bundles; moreover, labels can be used for rootstocks in bundles of 25 and multiples of 25 up to 100 plantlets.

ANNEX 6

"VIRUS-FREE" AND "VIRUS-TESTED"
HELATH STATUS OF POME FRUIT "PREBASIC",
"BASIC" AND "CERTIFIED" MATERIAL:
DISEASES AND HARMFUL ORGANISMS COVERED BY THE SCHEME.

Part I – Apple

Official /scientific name	Acronym	Health status	
		VF	VT
VIRUSES			
Apple mosaic virus	**ApMV**	**X**	**X**
Apple stem pitting virus	**ASPV**	**X**	**X**
Apple chlorotic leaf spot virus	**ACLSV**	**X**	**X**
Apple stem grooving virus	**ASGV**	**X**	**X**
VIROIDS			
Apple dimple fruit viroid	**ADFVd**	**X**	**X**
Apple scar skin viroid	**ASSVd**	**X**	**X**
PHYTOPLASMAS			
Candidatus Phytoplasma mali (associated to Apple proliferation)	**AP**	**X**	**X**
BACTERIA			
Erwinia amylovora	**Ea**	**X**	**X**
VIRUS-LIKE AGENTS			
Apple rubbery wood (= *Apple flat limb*) (= *Apple chat fruit*)	**ARW** **AFL** **ACF**	**X**	**X**
DISEASES CAUSING FRUIT ALTERATIONS			
Russet ring	**ARRV**	**X**	
Green crinale	**GCV**	**X**	
Rough skin	**ARSk**	**X**	
Star crack	**ASC**	**X**	
Russet wart	**ApRWa**	**X**	
Ring spot	**ApRS**	**X**	
FUNGI			
Chondrostereum purpureum,		**X**	**X**
Verticillium dahliae and V. albo-atrum		**X**	**X**
Armillariella mellea		**X**	**X**
Nectria galligena		**X**	**X**
Phytophthora cactorum		**X**	**X**
NEMATODES			
Pratylenchus vulnus and P. penetrans		**X**	**X**
Meloidogyne hapla and M. incognita		**X**	**X**

Part II – Pear and Quince

Official /scientific name	Acronym	Health status	
		VF	VT
VIRUSES			
Apple stem pitting virus	**ASPV**	**X**	**X**
Apple chlorotic leaf spot virus	**ACLSV**	**X**	**X**
Apple stem grooving virus	**ASGV**	**X**	**X**
VIROIDS			
Pear blister canker viroid	**PBCVd**	**X**	**X**
Apple scar skin viroid	**ASSVd**	**X**	**X**
PHYTOPLASMAS			
Candidatus phytoplasma pyri (associated to Pear decline)	**PD**	**X**	**X**
BACTERIA			
Erwinia amylovora	**Ea**	**X**	**X**
Agrobacterium tumefaciens		**X**	**X**
Pseudomonas syringae pv Syringae		**X**	**X**
VIRUS-LIKE AGENTS			
Apple rubbery wood	**ARW**	**X**	
Apple flat limb	**AFL**	**X**	
Apple chat fruit	**AFN**	**X**	
Quince yellow blotch	**QYB**	**X**	
Pear rough bark	**PRB**	**X**	
Pear bark split	**PBS**	**X**	
Pear bark necrosis	**PBN**	**X**	
Pear bud drop	**PBS**	**X**	
FUNGI			
Chondrostereum purpureum,		**X**	**X**
Verticillium dahliae and *V. albo-atrum*		**X**	**X**
Armillariella mellea		**X**	**X**
Nectria galligena		**X**	**X**
Phytophthora cactorum		**X**	**X**
NEMATODES			
Pratylenchus vulnus and *P. penetrans*		**X**	**X**
Meloidogyne hapla and *M. incognita*		**X**	**X**

ANNEX 7

PHYTOSANITARY CHECKS

Part I – On "Prebasic" and "Basic" material

Virus, viroids, phytoplasmas, virus-like agents and bacteria

Visual inspection: every year on all plants, at the appropriate time, when symptoms are likely to be most visible;

Laboratory testing:

a. All plants under conservation for pre-multiplication shall be tested within three years since they have been established according to the procedure specified in Table 1 for the apple and 2 for the pear and the quince; they shall be retested within eight years since they have been established.
b. All plants under pre-multiplication shall be tested within 3 years since they have been established, according to the procedure specified in Table 1 of the present annex for the apple and in Table 2 of the present annex for the pear and the quince.
c. In the event that the material tested (assessed and checked) is found unsuitable, the Centre manager shall inform the Regional Phytosanitary Service and remove it, according to the procedure established by the Phytosanitary Service.

Part II – On "Certified" material

II.A. On material in scion and seed mother plant blocks

Virus, viroids, phytoplasmas, virus-like agents and bacteria

Visual inspection: every year on all scion mother plants, at the appropriate time, when symptoms are likely to be most visible.

If the material tested is found (assessed and checked) unsuitable, the Centre manager shall inform the Regional Phytosanitary Service and remove it, according to the procedure established by the Phytosanitary Service.

II.B. On nursery material

Virus, viroids, phytoplasmas, virus-like agents and bacteria

Visual inspection: every year on all scion mother plants, at the appropriate time, when symptoms are likely to be most visible.

The check inspections and the plant management and protection programme are the responsibility of the nurseryman.

If the material tested is found (assessed and checked) unsuitable, the Centre manager shall inform the Regional Phytosanitary Service and remove it, according to the procedure established by the Phytosanitary Service.

Table 1 **APPLE** Procedure to assess the health status of Apple "virus-free" and "virus-tested" "Prebasic", "Basic" and certified seed mother plants and scion mother plants

Harmful organism /Disease	Visual inspection		Biological assays*		Laboratory serological/molecular testing	
	Time	Frequency	Indicator	Sampling time and sample type	Time, sample type, test	Frequency
VIRUSES						
ApMV	Spring up to a temperature of 25° C	Annual	*Malus pumila* Charden *Malus pumila* Golden Delicious *Malus pumila* Lord Lambourne	Grafting: August or at growth recovery with hardwood cuttings	End winter / spring cuttings/ young leaves ELISA or RT-PCR or RT-PCR-ELISA	Within three years after establishment
ASPV	May – September	Annual	*Pyronia veitchii* *Malus pumila* Spy 227 *Malus pumila* Virginia Crab *Malus pumila* Kola *Malus pumila* Radiant	Grafting: August or at growth recovery with hardwood cuttings	End winter / spring cuttings/ young leaves RT-PCR	Within three years after establishment
ACLSV	May – September Summer	Annual	*Malus sylvestris* R12740 7A *Cydonia oblonga* C7/1 *Cydonia oblonga* Pigwa *Malus platycarpa*	Grafting: August or at growth recovery with hardwood cuttings	End winter / spring cuttings/ young leaves ELISA or RT-PCR or IC-RT-PCR	Within three years after establishment
ASGV	May – September	Annual	*Malus pumila* Virgiania Crab *Malus micromalus* GMAL273 *Pyronia veitchii*	Grafting: August or at growth recovery with hardwood cuttings	End winter / spring cuttings/ young leaves ELISA or RT-PCR or IC-RT-PCR or RT-PCR-ELISA	Within three years after establishment
VIROIDS						
ADFVd **ASSVd**	End summer and spring	Annual	*Malus communis* Stark's Earliest *Malus communis* Sugar Crab *Malus communis* Red Delicious *Malus communis* Starkrimson	Grafting: August or at growth recovery with hardwood cuttings For ASSVd check 3 fruit-settings	During the growing season cuttings/ leaves / fruit RT-PCR or Hybridisation	Within three years after establishment
PHYTOPLASMAS						
AP	At growth recovery, bud burst, Summer, Autumn Leaf colouring	Annual	*Malus communis* Charden *Malus com.* Golden Delicious *Malus com.* Lord Lambourne	Grafting: Summer – Autumn with hardwood cuttings, Spring with roots	Summer /autumn cuttings/ leaves IF or ELISA or DAPI or PCR- ELISA or PCR	Within three years after establishment
BACTERIA						
Ervinia amylovora		Annual				
Agrobacterium tumefaciens	At uprooting					
P. syringae pv *syringae*		Annual				
VIRUS-LIKE AGENTS						
ARW **AFL** **ACF**	Spring – Summer	Annual	*Prunus avium* Mazard F12/1 *Malus com* Lord Lambourne *Malus com* Gravensteiner *Cydonia oblonga* C 7/1	Grafting: from August to April with hardwood cuttings		
ARRV, GCV, ARSk, ASC, ApRWa e ApRS	Summer, until fruit ripening	Annual	Not included	Not included		

* to be carried out on all plants in conservation (Pre-basic category) within three years since their establishment and, only for pre-multiplication (Basic category), at least once in 3 years, for all plants from which the material has been collected

Table 2 **PEAR and QUNICE:** Procedure to assess the health status of Pear and Quince "virus-free" and "virus-tested" Prebasic, Basic and Certified seed and scion mother plants

Harmful organism/Disease	Visual inspection		Biological Assays *		Laboratory serological/molecular testing	
	Time	Frequency	Indicator	Sampling time and sample type	Time, sample type, test	Frequency
VIRUSES						
ASPV	May – July	Annual	*Pyronia veitchii* *Malus pumila* Spy 227 *Malus pumila* Virginia crab *Pyrus communis* Noveau Poiteau, *Pyrus communis* Julesd'Airolles, *Pyrus communis* A 20	Grafting: August or at growth recovery with hardwood cuttings	End winter / spring cuttings/ young leaves RT-PCR	Within three years after establishment
ACLSV	May – July	Annual	*Malus sylvestris* R12740 7A *Cydonia oblonga* C7/1 *Cydonia oblonga* Pigwa *Malus platycarpa* *Pyronia veitchii* *Pyrus communis* Noveau Poiteau *Pyrus communis* A 20, *Pyrus communis* Beurre Hardy	Grafting: August or at growth recovery with hardwood cuttings	End winter / spring cuttings/ young leaves Elisa or RT-PCR or IC- RT-PCR	Within three years after establishment
ASGV	At growth recovery up to a temperature of 25° C	Annual	*Malus pumila* Virgiania Crab *Malus micromalus GMAL273* *Pyronia veitchii*	Grafting: August or at growth recovery with hardwood cuttings	End winter / spring cuttings/ young leaves Elisa or RT-PCR or IC- RT-PCR	Within three years after establishment
VIROIDS						
PBCVd	End summer and spring	Annual	*Pyrus communis* Fieud 37 *Pyrus communis* A 20	Grafting: August or at growth recovery with hardwood cuttings	During the growing season cuttings/ young leaves RT-PCR or Hybridisation	Within three years after establishment
ASSVd	End summer and spring	Annual	*Malus pumila* Stark's Earliest *Malus pumila* Sugar Crab *Malus pumila* Red Delicious *Malus pumila* Starkrimson	Grafting: August or at growth recovery with hardwood cuttings	During the growing season cuttings/ young leaves RT-PCR or Hybridisation	Within three years after establishment
PHYTOPLASMAS						
PD	End summer- autumn (on varieties and indicator plants)	Annual			During the growing season Lignified branches, leaf petioles and veins DAPI or PCR or PCR-Elisa	Within three years after establishment
BACTERIA						
Erwinia amylovora		Annual				
Agrobacterium tumefaciens	At uprooting					
P. syringae pv syringae		Annual				

(Table2 to be continued)

(Table 2 continued)

Harmful organism/Disease	Visual inspection		Biological Assays *		Laboratory serological/molecular testing	
	Time	Frequency	Indicator	Sampling time and sample type	Time, sample type, test	Frequency
VIRUS-LIKE AGENTS						
ARW **AFL** **QYB**	Spring – summer	Annual	*Prunus avium* Mazard F12/1 *Malus com* L. Lambourne *Malus com.* Gravensteiner *Cydonia oblonga* C 7/1	Grafting: August or at growth recovery with hardwood cuttings		
PRB, PBS, **PBN e PBD**	Spring – summer	Annual	*Pyrus communis* A 20 *Pyrus communis* Beurre Hardy, *P. communis* Doyenne du Comice	Grafting: August or at growth recovery with hardwood cuttings		

* to be carried out on all plants in conservation (Pre-basic category) within three years after they have been established and, only for pre-multiplication (Basic category), at least once in 3 years, for all plants from which the material has been collected

ANNEX 8

TRUENESS-TO-TYPE CHECKS

Part I – On material under conservation for pre-multiplication (CCP)

The phenological and pomological checks in the conservation step shall be conducted during the main stages of the growing cycle.

If the material inspected is found not true-to-type i.e. unsuitable, the Centre manager shall inform the Regional Phytosanitary Service and remove it, according to the procedure established by the Phytosanitary Service.

For pome fruit cultivars, trueness-to-type certification can be issued only after observing at least one fruit-setting so as to check the conformity to the phenotype.

In order to asses trueness-to-type it is necessary to grow outdoors at least 4 control plants for each mother plant, descended from the agamic propagation of the plant in conservation and grafted onto "certified" rootstocks which shall:

- be dwarfing for the apple
- belong to *Cydonia* species, with the related "certified" interstock", for the pear.

When pre-multiplication is directly carried out in the field, with fruit-bearing mother plants, plants in conservation must not be monitored.

The seed source plant trueness-to-type shall be certified at fruit harvest and after observing, for a whole growing cycle in the nursery, at least 200 seedlings obtained from the seed collected on the seed source plant.

The trueness-to-type certification for clonal rootstocks can be issued after observing at least 1 complete growing cycle, both in the stool bed and on the mother plant for cutting (softwood or hardwood) production so as to check the conformity to the phenotype. For the purposes of this certification, the finger-printing technique can be used as well, where appropriate.

Part B – On material in pre-multiplication (PC)

In the pre-multiplication step the phenological and pomological checks shall be conducted during the main stages of the growing cycle.

The variety or clone trueness-to-type certification shall be issued only after observing at least one fruit-setting so as to check the conformity to the phenotype:

- Pre-multiplication outdoors: for genetically stable varieties the Basic material shall be collected from the whole mother plant, whereas for genetically unstable varieties only cuttings on the fruit-bearing wood with true-to-type fruits shall be collected. In this step the pomological checks shall be carried out every year for each plant maintained at the Pre-multiplication Centre (PC), prior to collecting the propagating material for summer/autumn cultivars and during the year before cutting for winter cultivars.
- Pre-multiplication in the screen-house: inspection of control plants as for conservation. In this step the pomological checks shall be carried out, in any case, for at least 2 fruit-settings.

The trueness-to-type of seed source plants shall be certified at fruit harvest and after observing, for a whole growing cycle in the nursery, at least 200 seedlings obtained from the seed collected on seed source plants.

The trueness-to-type certification for clonal rootstocks shall be issued after observing at least 1 complete growing cycle, both in the in stool bed and in the nursery, so as to check the conformity to the phenotype

If the material inspected is found not true-to-type, i.e. unsuitable, the nurseryman shall inform the Regional Phytosanitary Service and remove it, according to the procedure established by the Phytosanitary Service.

Part C – On material in Scion mother plants (ScMP) and Seed mother plants (SMP) blocks

The trueness-to-type certification shall be issued only after observing the mother plant phenotype every year.

- For genetically unstable cultivars, the phenotype checks shall be integrated with fruit inspections repeated every year for each plant maintained in the Mother plant block (MB), prior to collecting the propagating material for summer/autumn cultivars and in the year before cutting for winter cultivars.
- The trueness-to-type certification for clonal rootstocks or rootstocks propagated by cutting shall be issued after inspections, for at least 1 complete growing cycle, in the stool bed or on the mother plants so as to check the conformity to the phenotype. For the purposes of this certification, the finger-printing technique can be used as well, where appropriate

The trueness-to-type certification of seed rootstocks and seed source varieties shall be issued according to the procedure specified for fruit tree cultivars and, moreover, after observing, for a whole growing cycle in the nursery, at least 200 seedling rootstocks obtained from the seed collected on seed source plants.

If the material checked is found not true-to-type, i.e. unsuitable, the nurseryman shall inform the Regional Phytosanitary Service and remove it, according to the procedure established by the Phytosanitary Service.

Part D – On material in the nurseries.

The phenological and pomological checks in the nursery shall be carried out during the main stages of the growing cycle along with sanitary checks.