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 of agamically-propagated herbaceous species;

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

Having regard to the proposal about the technical protocols for the production of certified propagating material of Olive tree 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

1. The rules set forth in this Decree apply to certification of plant propagating material belonging to the species *Olea europea* L.

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* growing 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* growing 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* growing and 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

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 4.3, 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, respectively

Article 7

Provisional Regulations

1. Until 31 December 2011, propagating material belonging to the Olive species, 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

ANNEX 1

DATA SHEETS FOR THE REGISTRATION OF THE OLIVE PRIMARY SOURCE

Part I –Pomological Data Sheet

Genus: **Species:** **Cultivar:** **Clone:**

POMOLOGICAL CHARACTERISTICS

Observations made for n°_____ years

Picture

INFLORESCENCE

Shape:
Average length (mm):
N° flowers:

TREE

Vigour:
Habit:
Canopy:

ENDOCARP

Shape:
Symmetry:
Size:
Max diameter position:
Surface:
Fibro-vascular bundles:
Fibro-vascular bundles pattern:
Fibro-vascular bundles depth:
Base shape:
Apex shape:
Apex end:

Belonging to GMO □ **YES** □ **NO**

MOLECULAR CHARACTERISATION

YEAR/S:_____

MOLECULAR MARKERS

SSR – N° primer combinations:_____ Bibliographic reference:_____

RAPDs- N° primer combinations:_____ Bibliographic reference:_____

AFLP- N° primer combinations:_____ Bibliographic reference:_____

Isozymes - N° enzyme systems: _____ Bibliographic reference:_____

Other (specify)

POMOLOGICAL CHARACTERISATION:

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

CONSERVATION OF THE PRIMARY SOURCE:

.....

(Responsible body)

.....

(Location)

Date **.....** The Manager

Part II – Testing protocols for plant health assessment

Causal agent / Disease	Acronym	Biomolecular tests + *result* -
VIRUSES		
Arabis mosaic	***ArMV***	☐ RT-PCR ☐ ☐ Hybridisation ☐
Cherry leafroll	**CLRV**	☐ RT-PCR ☐ ☐ Hybridisation ☐
Strawberry latent ringspost	**SLRV**	☐ RT-PCR ☐ ☐ Hybridisation ☐
Cucumber mosaic	**CMV**	☐ RT-PCR ☐ ☐ Hybridisation ☐
Olive latent 1	**OLV-1**	☐ RT-PCR ☐ ☐ Hybridisation ☐
Olive latent 2	**OLV-2**	☐ RT-PCR ☐ ☐ Hybridisation ☐
Olive yellow leaf associated	**OLYaV**	☐ RT-PCR ☐ ☐ Hybridisation ☐
Tobacco necrosis	**TNV**	☐ RT-PCR ☐ ☐ Hybridisation ☐
PHYTOPLASMAS		
Phytoplasmas		☐ PCR ☐

FUNGI	**ISOLATION**		**YEAR/S**
	Result		
	+	**-**	
Verticillium wilt: *Verticillium dahliae*			
BACTERIA			
Olive knot *Pseudomonas savastanoi pv savastanoi*			

☐ tick the box of the test performed

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

Date .....

The Laboratory Manager

ANNEX 2

MEANS FOR IN VIVO GROWING AND PRODUCTION OF
"PRE-BASIC" PROPAGATING 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 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 placed on supports at least 20 cm high to provide isolation from 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. hard roof and walls with a double 20/10 mesh (20 wires/cm warp and 10 wires/cm weft) net and entrance with double-net walls and double door.

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 for containers, seedling beds, rooting and acclimatation shall be found free from the nematodes *Meloidogyne incognita, M. javanica, Pratylenchus vulnus, Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae*; freedom from the above shall be substantiated by an official document;
c. the containers and flats for rooting, acclimatation and seedling beds shall be kept at 20 cm at least off the ground ground;
d. before use, the containers and flats for rooting, acclimatisation and seedling beds shall be disinfected with a 2% sodium hypochlorite solution;
e. 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" PROPAGATING MATERIAL

Part I - Facilities

I.A Mother Plant Blocks

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

a. they shall be established on soils which respond to the normal agronomic and health requirements and found free from the nematode *Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae*; freedom from the above shall be substantiated by an official document;
b. they shall be established on soils on which none of the tree crops have been grown for at least 5 years;
c. they shall be separated from neighbouring plots by a surrounding zone, at least 20 m wide, kept free from any vegetation; such a limit
    i. is increased to 30m when any tree crops are present;
    ii. is reduced to 10m when freedom from the nematode *Xiphinema diversicaudatum* is ascertained by the Regional Phytosanitary Service or in case protective barriers are created (e.g. ditches, furrows, etc.);
d. they shall be isolated from surface water flow;
e. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulation on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document;
f. the planting distance shall allow the normal cultural operations and the related checks;
g. the plants shall be numbered on the spot in a progressive order and in an indelible fashion;
h. in the plot, 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;
i. scion mother plants (ScMP) shall not be kept for more than 30 years since their establishment;
j. seed mother plants (SMP) shall not be kept for more than 40 years since their establishment;
k. the blocks shall be kept under continuous surveillance to control pathogens, pests and weeds;
l. 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;
m. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

I.B Increase blocks

In the increase blocks plants shall be grown in containers:

a. the growing containers, of appropriate volume can be either placed on the ground - provided that the soil is found free from the nematode *Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae* - 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

b. the soil or medium used for growing plants in containers shall be found free from nematode *Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae;* 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 plants shall be individually numbered on the spot in an indelible fashion;
e. the plants shall be subdivided in homogeneous lots per accession, easily identifiable; the planting layout shall be reported on a map;
f. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulation on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document
g. propagating material can be collected from increase block plants to produce certified mother plants for 7 years at most, starting from the 3rd year when trueness-to type-checks are made on fruit or from the 1st year, when molecular testing methods are applied;
h. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

Part II- Production

"Basic" material in the increase blocks (grafted and self-rooted plants) shall be produced soil-less.

II.A. Seedling beds in flats

a. Soil-less flats shall not be placed on the ground, but kept at 10 cm at least off the ground**;**
b. the growing medium shall be found free from the nematodes *Meloidogyne incognita, M. javanica, Pratylenchus vulnus, Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae;* freedom from the above shall be substantiated by an official document;
c. before use, the flats shall be disinfected with a 2% sodium hypochlorite solution.

II.B Graftling and Sapling beds

a. The area intended for the establishment of graftling and sapling beds shall be isolated from surface water flow and separated by a surrounding zone at least 2 m wide, kept free from any vegetation;
b. the soil or growing medium for plants in containers shall be found free from the nematodes *Meloidogyne incognita, M. javanica, Pratylenchus vulnus, Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae;* freedom from the above shall be substantiated by an official document;
c. the growing containers, of appropriate volume shall be 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;
d. the plants shall be subdivided and numbered in homogeneous lots per accession, easily identifiable; the planting layout shall be reported on a map;
e. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulations on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document;
f. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

II.C. Rooting and acclimatation structures

a. Rooting and acclimatation structures shall be kept at 20 cm at least off the ground or properly isolated;
b. the growing medium for rooting shall be sterilised; the growing medium for acclimatation shall be found free from the nematodes *Meloidogyne incognita, M. javanica, Pratylenchus vulnus, Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae;* freedom from the above shall be substantiated by an official document;
c. before use, the flats shall be disinfected with a 2% sodium hypochlorite solution.

ANNEX4

MEANS FOR *IN VIVO* GROWING AND PRODUCTION
OF "CERTIFIED" PROPAGATING 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 established on soils which respond to the normal agronomic and health requirements and found free from the nematode *Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae*; freedom from the above shall be substantiated by an official document;
b. they shall be established on soils on which none of the tree crops have been grown for at least 3 years;
c. they shall be isolated from surface water flow;
d. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulations on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document;
e. plants shall be numbered on the spot in a progressive order and in an indelible fashion;
f. 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;
g. they shall be separated from neighbouring plots by a surrounding zone, at least 10 m wide, kept free from any vegetation; such a limit
    i. is increased to 20m when any tree crops are present;
    ii. is reduced to 5m when freedom from the nematode vector *Xiphinema diversicaudatum* is ascertained by the Regional Phytosanitary Service or in case special protective barriers are created (e.g. ditches, furrows, etc.);
h. scion mother plants (ScMP) shall not be kept for more than 30 years since their establishment;
i. seed mother plants (SMP) shall not be kept for more than 40 years since their establishment;
j. the blocks shall be kept under continuous surveillance to control pathogens, pests and weeds;
k. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

Part II- Increase blocks

In the increase blocks plants can be grown in open field and soil-less.

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 the nematode *Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae*; freedom from the above shall be substantiated by an official document;
b. they shall be established on soils on which none of the tree crops have been grown for at least 3 years;
c. they shall be separated from neighbouring plots by a surrounding zone, at least 10 m wide, kept free from any vegetation; such a limit
    i. is increased to 20m when any tree crops are present;
    ii. is reduced to 5m when freedom from the nematode vector *Xiphinema diversicaudatum* is ascertained by the Regional Phytosanitary Service or in case special protective barriers are created (e.g. ditches, furrows, etc.);

d. the soil shall be isolated from surface water flow;
e. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulations on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document;
f. plants shall be numbered on the spot in a progressive order and in an indelible fashion;
g. plant accessions under multiplication shall be distinct, in easily identifiable plots; the planting layout shall be reported on a map;
h. in the plot 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;
i. propagating material can be collected from increase block plants to produce certified mother plants for 7 years at most, starting from the 3rd year when trueness-to type-checks are made on fruit or from the 1st year, when molecular testing methods are applied;
j. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

II.B – Increase blocks in containers

a. The area intended for growing soil-less plants shall be separated by a surrounding zone, at least 2 m wide, kept free from any vegetation;
b. the growing medium for plants in containers shall be found free from the nematode *Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae*; freedom from the above shall be substantiated by an official document;
c. the growing containers, of appropriate volume can be either placed on the ground - provided that the soil is found free from the nematode *Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae* - 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;
d. 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;
e. plants shall be numbered and subdivided in homogeneous lots per accession, easily identifiable; the planting layout shall be reported on a map;
f. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulations on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document;
g. propagating material can be collected from increase block plants to produce certified mother plants for 7 years at most, starting from the 3rd year when trueness-to type-checks are made on fruit or from the 1st year, when molecular testing methods are applied;
h. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

Part III- Nurseries

III.A. Seedling, Graftling and Sapling beds in open field

a. The soil or growing medium for the establishment of seedling, graftling and sapling beds shall be found free from the nematodes *Meloidogyne incognita, M. javanica, Pratylenchus vulnus, Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae;* freedom from the above shall be substantiated by an official document;

b. the area intended for growing certified olive plants in open field (grtaftling and sapling beds) and for establishing seedling beds shall be separated from neighbouring plots by a surrounding zone, at least 2m wide, kept free from any vegetation; such a limit is increased to 10m when any tree crops are present;
c. the plants shall be subdivided in homogeneous lots, easily identifiable, entirely and exclusively intended for growing olive plants; the planting layout shall be notified to the locally competent Regional Phytosanitary Service;
d. the area intended for growing plants shall be isolated from surface and subsurface water flow;
e. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulations on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document.

III.B. Soil-less Seedling, Graftling and Sapling beds

a. The containers and flats for seed planting, rooting and acclimatisation and the area intended for growing certified soil-less plants shall be isolated from surface and subsurface water flow;
b. the containers and for seed planting, rooting and acclimatisation shall not be placed on the ground but kept at 10 cm at least off the ground;
c. before use, the containers and flats shall be disinfected with a 2% sodium hypochlorite solution;
d. plants shall be grown in containers of appropriate volume;
e. the area intended for growing certified soil-less olive plants shall be separated by a surrounding zone at least 2 m wide, kept free from any vegetation;
f. the growing containers, shall be isolated from the ground by:
    i. a layer of fine gravel at least 10 cm high or 5cm high when mulching films are used;
    ii. a layer of concrete or different material; in such a case the containers shall be placed on supports at least 20 cm high;
g. containers can be placed on the ground provided that the soil is found free from the nematodes *Meloidogyne incognita, M. javanica, Pratylenchus vulnus, Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae;* freedom from the above shall be substantiated by an official document;
h. the soil or growing medium for the establishment of seedling beds, acclimatation, rooting and growing shall be found free from the nematodes *Meloidogyne incognita, M. javanica, Pratylenchus vulnus, Xiphinema diversicaudatum* and from the fungus *Verticillium dahliae;* freedom from the above shall be substantiated by an official document;
i. the plants shall be subdivided in homogeneous lots, easily identifiable, entirely and exclusively intended for growing olive plants; the planting layout shall be notified to the locally competent Regional Phytosanitary Service;
j. irrigation water shall be found free or cleaned up from harmful organisms in accordance with the Community regulations on the marketing of fruit plants (Ministerial Decree of 14 April 1997) and with the technical annexes of the present decree; freedom from the above shall be substantiated by an official document;
k. implements shall be at all time disinfected with a 10% sodium hypochlorite solution between cuttings.

ANNEX 5

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

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

a. Initial explants for micro-propagation (*in vitro* multiplication through axillary buds) shall be collected only from plants grown at the Conservation Centres for Pre-multiplication (CCP);
b. transplanting 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;
a. the total duration of proliferation sub-cultures in conservation and pre-multiplication shall not exceed 4 years, whereas, when appropriate, cold storage shall not exceed 12 months in total. After this period, new explants shall be collected from the Conservation Centre for Pre-multiplication (CCP);
b. to prepare *in vitro* cultures in active multiplication intended for laboratories, a maximum of 10 (ten) subcultures cans be made in pre-multiplication (even spaced out by one period –at most- of cold storage) after the initial one which is necessary to start the sterile culture;
c. micropropagation of chimeric clones is not allowed due to the high risk of obtaining micropropagtaed plants which are not conform to the initial phenotype;
d. It is not allowed to use substances with possible mutagenic effect nor culture systems with bacterial organisms, to facilitate any specific stage;
e. in the multiplication and rooting stages, laboratories will take the following precautions:
    i. remove any shoots originated from undifferentiated tissues (callus);
    ii. at transplanting, remove the basal part of the shoot tuft, where the proliferation of undifferentiated tissues can more likely proliferate;
    iii. use only shoots originated from axillary buds;
    iv. remove vitrescent cultures and/or those with other morpho-physiological abnormalities (in particular, fasciations).
f. growing containers shall be maintained in a specific and clearly identified laboratory compartment and individually labelled so as to be easily recognised. The label shall report the date, the sub-culture progressive number and the growing stage: proliferation, elongation or rooting.
g. the acclimatation benches shall fulfil the requirements listed in Annex3 and 4 of the present protocol.

Part II – *In vitro* production of "Certified" material

a. By certified mail, commercial laboratories shall request to the Pre-multiplication Centre (PC) the initial number of sterile shoots for each selection. The Pre-multiplication Centre (PC) shall deliver the cultures under active multiplication within 6 months after the request. It is allowed to attain a maximum of 36 (thirty-six) subcultures for *in vitro* commercial multiplication (even when they are spaced out by one period- at most - of cold storage). At the end of the 36th subculture, the shoots shall be moved to the elongation or rooting stage (during which or after which one period of cold storage is allowed although cold has previously been applied);

b. in the multiplication step, the proliferation sub-cultures shall not last more than 4 years in total, whereas, when appropriate, cold storage shall not exceed 12 months. After this period new sterile shoots shall be used;
c. Growing containers shall be maintained in a specific and clearly identified laboratory compartment and individually labelled so a to be easily recognised. The label shall report the date, the sub-culture progressive number and the growing stage: proliferation, elongation or rooting.
d. transplanting operations shall be recorded daily in a special 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.

ANNEX 6

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

Disease / Harmful organism	**Health status**		
	Acronym	**Virus-free (VF)**	**Virus-tested (VT)**
VIRUSES			
Arabis mosaic	***ArMV***	**X**	**X**
Cherry leafroll	**CLRV**	**X**	**X**
Strawberry latent ringspot	**SLRV**	**X**	**X**
Cucumber mosaic	**CMV**	**X**	
Olive latent 1	**OLV-1**	**X**	**X**
Olive latente 2	**OLV-2**	**X**	
Olive leaf yellow associated	**OLYaV**	**X**	**X**
Tobacco necrosis	**TNV**	**X**	
PHYTOPLASMAS			
FUNGI			
Verticillium wilt: *Verticillim dahliae*		**X**	**X**
BACTERIA			
Olive knot		**X**	**X**
NEMATODES			
Meloidogyne incognita		**X**	**X**
Meloidogyne javanica		**X**	**X**
Pratylenchus vulnus		**X**	**X**
Xiphinema diversicaudatum		**X**	**X**

ANNEX 7

SANITARY CHECKS

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

Viruses, phytoplasmas and fungi

Two types of checks shall be carried out:

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

In the increase blocks and in the nurseries, visual inspection 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.

Part II- On soil and growing media in all steps

Mycological analysis through isolation on selective media for *Verticillium dahliae* on samples collected according to the following methods:

- 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;
- growing media: a sample shall be collected every $5m^3$, made up of 10 sub-samples, for a total volume of at least 1 litre.

Nematological analysis through isolation techniques for *Xiphinema diversicaudatum, Meloidogyne incognita, M. javanica, Pratylenchus vulnus,* and on samples collected according to the following methods:

- 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;
- growing media: a sample shall be collected every $5m^3$, made up of 5 sub-samples, for a total volume of at least 1 litre.

Table 1: Procedure for the assessment of "Virsu-free" and "Virus-tested" health status of Primary Sources and "Pre-basic"and "Basic" Seed Mother Plants (SMP) and Scion Mother Plants (ScMP)

Disease and/or Harmful organism	CHECKS				
	Visual inspection		Laboratory testing		
	Time	Frequency	Sampling type and time	Technique	Frequency
VIRUSES					
ArMV **CLRV** **SLRV** **OLV-1** **OLYaV** **OLV-2** **OLRV** **CMV** **TNV**	Spring and Autumn	Annual	Cortical scrapings from well-lignified branches: in Spring or early Autumn	RT- PCR or molecular hybridisation	On 10% of plants every year from the 5th year
PHYTOPLASMAS					
Phytoplasmas	Spring	Annual		Polymerase chain reaction (PCR).	In doubtful cases
FUNGI					
Verticillium wilt: *Verticillium dahliae*	From April to September	Annual	Vascular tissues of 1-2 year branch portions.	Isolation	In doubtful cases
BACTERIA					
Olive knot: *Pseudomonas savastanoi* pv *savastanoi*	Spring and Autumn	Annual			

Table 2: Procedure for the assessment of "Virus-free" and "Virus-tested" health status of "Certified" Seed MotherPlants (SMP) and Mother Scion Plants (MScP)

Disease and/or harmful organism	CHECKS				
	Visual inspection		Laboratory testing		
	Time	Frequency	Sampling type and time	Technique	Frequency
VIRUSES					
ArMV **CLRV** **SLRV** **OLV-1** **OLYaV** **OLV-2** **OLRV** **CMV** **TNV**	Spring and Autumn	Annual	Cortical scrapings from well-lignified branches in Spring or early Autumn	RT- PCR or molecular hybridisation	From the 5th year on all plants, within 30 years for ScMPs ad 40 years for SMPs
PHYTOPLASMAS					
Phytoplasmas	Spring	Annual		Polymerase chain reaction (PCR).	In doubtful cases
FUNGI					
Verticillium wilt: *Verticillium dahliae*	From April to September	Annual	Vascular tissues of 1-2 year branch portions.	Isolation	In doubtful cases
BACTERIA					
Olive knot: *Pseudomonas savastanoi* pv *savastanoi (Rogna)*	Spring and Autumn	Annual			

ANNEX 8

TRUENESS-TO-TYPE CHECKS OR CLONAL SELECTION

The trueness-to-type certification is based on pomological and agronomic observations. Alternatively, molecular techniques can also be applied when the primary source brought into the national certification scheme is accompanied by appropriate molecular documentation.

Part I – On "Pre-basic" and "Basic" material

The trueness-to-type certification of olive cultivars and clones intended for fruit production can be issued provided that:

i. at least one fruit-setting is observed or else,
ii. DNA analysis is performed through one or more techniques (RAPD, RFLP, AFLP etc) recognised as suitable, according to the instructions provided by the plant breeder at the registration of the primary source, capable of distinguishing the cultivar or the clone, depending on whether a variety or a new clone is to be registered.

The trueness-to-type certification for clonal rootstocks can be issued provided that:

i. at least two annual growing cycles of nursery propagation are carried out and the conformity to the phenotype is checked or else,
ii. DNA analysis is performed through one or more techniques recognised as suitable, according to the instructions provided by the plant breeder (RAPD, RFLP, AFLP etc.) at the registration of the primary source.

If trueness-to-type is assessed by using a morphological key, in the first one-two blooming and fruit-setting years, at least two checks shall be performed and repeated annually on all the above material, during the growing cycle, in the following phenological stages: blooming and fruit harvesting.

Part II – On "Certified" Mother Plants

The trueness-to-type certification for all plants shall be issued by the competent Regional Phytosanitary Service before collecting the certified material, provided that:

iii. at least one fruit-setting is checked or else,
iv. DNA analysis is performed through one or more techniques recognised as suitable, according to the instructions provided by the plant breeder (RAPD, RFLP, AFLP etc.) at the registration of the primary source.

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