◆ EPPO Standards ◆

CERTIFICATION SCHEMES

PATHOGEN-TESTED MATERIAL OF *MALUS*, *PYRUS* AND C*YDONIA*

PM 4/27(1) English

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APPROVAL

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard.

REVIEW

EPPO Standards are subject to periodic review and amendment. The next review date for this set of EPPO Standards is decided by the EPPO Working Party on Phytosanitary Regulations.

AMENDMENT RECORD

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

DISTRIBUTION

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SCOPE

EPPO Certification Schemes are intended to be used by NPPOs or equivalent authorities, in their capacity as bodies responsible for the design of systems for production of healthy plants for planting, for the inspection of such plants proposed for certification, and for the issue of appropriate certificates.

REFERENCES

OEPP/EPPO (1991) Recommendations made by EPPO Council in 1990: general scheme for the production of certified pathogen-tested vegetatively propagated ornamental plants. *Bulletin OEPP/EPPO Bulletin* **21**, 757.
OEPP/EPPO (1992a) Recommendations made by EPPO Council in 1981: certification of virus-tested fruit trees, scions and rootstocks. *EPPO Technical Documents* 1013, 42-43.
OEPP/EPPO (1992b) EPPO Standards PM 4/1(1) Certification schemes. Virus-free or virus-tested fruit trees and rootstocks. Part I. Basic scheme and its elaboration. *Bulletin OEPP/EPPO Bulletin* **22**, 267-277.
OEPP/EPPO (1993a) EPPO Standards PM 4/7(1) Certification schemes. Nursery requirements - recommended requirements for establishments participating in certification of fruit or ornamental crops. *Bulletin OEPP/EPPO Bulletin* **23**, 249-252.
OEPP/EPPO (1993b) Recommendations made by EPPO Council in 1992: scheme for the production of classified vegetatively propagated ornamental plants to satisfy health standards. *Bulletin OEPP/EPPO Bulletin* **23**, 735-736.

DEFINITIONS

Basic material

Propagation stock material from all but the last stage of propagation stock, satisfying the recommended certification standards and certified for sale. According to the number of stages of propagation stock, there may be several grades of basic material.

Candidate nuclear stock

Any plant that may become or may be propagated to produce nuclear stock. Testing for specified pests is required before the plant can be accepted as nuclear stock. Until testing is complete and negative, the plant remains candidate nuclear stock.

Certification scheme

System for the production of vegetatively propagated plants for planting, intended for further propagation or for sale, obtained from nuclear stock after several propagation stages under conditions ensuring that stated health standards are met. The filiation of the material is recorded throughout the scheme.

Certified material

Propagating material from the last stage of propagation stock, satisfying the recommended certification standards and certified for sale. In the case of plants that are sold grafted onto rootstocks, the rootstocks must also be at least of the last stage of propagation stock, and the plants must be held under approved conditions between grafting and sale.

Certified material may, according to the plant concerned, be referred to more specifically as, for example, certified plants, certified cuttings, certified bulbs, etc.

Classification scheme

System for the production of vegetatively propagated plants for planting, intended for further propagation or for sale, obtained from selected candidate material after one or several propagation stages under conditions ensuring that stated health standards are met. Different classes may be defined according to the inspections and tests used, the tolerance levels applied and the precautions taken. The filiation of classified material is not considered.

Filiation

The line of descent by vegetative propagation from a defined parent plant.

Nuclear stock

Plants individually tested by the most rigorous procedure in a certification scheme and found free from specified pests. All such plants must be maintained at all times under strict conditions ensuring freedom from infection. According to the crop concerned, plants propagated from nuclear stock material may remain nuclear stock provided that they do not leave the nuclear stock conditions. In the case of plants that are maintained by grafting onto rootstocks, the rootstocks must also be nuclear stock.

Nuclear stock material

Propagating material derived from nuclear stock, which may be further propagated without change of ownership or certified for sale as pre-basic material.

Prebasic material

Nuclear stock material, satisfying the recommended certification standards and certified for sale.

Propagation stock

Plants derived from nuclear stock, propagated and maintained under conditions ensuring freedom from infection. Pathogen freedom is checked by appropriate procedures. Propagation may be done in a number of successive stages under different approved conditions. The plants are then known as propagation stock I, propagation stock II, etc. There may be several generations within each of these stages, provided that the plants do not leave the approved conditions. The number of stages and/or generations allowed within propagation stock is generally limited and will depend on the crop concerned. In the case of propagating material that is maintained by grafting on a rootstock, the rootstock should be at least of the corresponding stage of propagation stock.

Propagation stock material

Propagating material derived from propagation stock, which may be further propagated without change of ownership, or certified for sale as basic or certified material, according to the stage of propagation stock concerned.

OUTLINE OF REQUIREMENTS

EPPO Certification Schemes describe the steps to be followed for the production of vegetatively propagated planting material of a particular cultivated plant, whose health status is attested by an official certificate. Certification and classification represent distinct alternative approaches to the production of healthy planting material. In a typical certification scheme, the certified material is descended by not more than a fixed number of steps from individual plants each of which is tested and found free from pests, and is then maintained and propagated under rigorous conditions excluding recontamination. In a classification scheme, the classified material is descended by one or more steps from material which, as a population, meets certain health standards and is maintained and propagated under conditions minimizing recontamination. In both cases, however, health status is attested by an official certificate. Which of the approaches is appropriate for a given cultivated plant depends on considerations of cost and resources, health status required, practical possibilities for testing, rate of recontamination, value of the final material.

EPPO Certification Schemes give details on the selection, growth and maintenance of the candidate material, and on the propagation of this material in several stages under conditions ensuring that stated health standards are met. Appropriate checks on specified pests are specified throughout the scheme. Information is provided, as necessary, on relevant pests, cultural practices, inspection and testing methods, recommended certification standards.

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

PM 4/27(1) English

Certification schemes

PATHOGEN-TESTED MATERIAL OF *MALUS*, *PYRUS* AND C*YDONIA*

Specific scope

This standard describes the production of pathogen-tested material of *Malus, Pyrus* and *Cydonia*.

Specific approval and amendment

First approved in September 1990 as part of EPPO Standard PM 4/1.
Approved as a separate standard in September 1999.

The certification scheme for pathogen-tested material of varieties and rootstocks of *Malus*, *Pyrus* and *Cydonia* provides detailed guidance on the production of grafted fruit trees (varieties), vegetatively propagated rootstocks and seedling rootstocks. The scheme is also suitable for the certification of ornamental plants of these genera.

Plant material produced according to this certification scheme is derived from nuclear stock plants that have been tested and found free from specified pathogens, and produced under conditions minimizing infection by other major pathogens of the genera concerned. Certified fruit-tree material for export should in any case satisfy the phytosanitary regulations of importing countries, especially with respect to any of the pathogens covered by the scheme that are also quarantine pests. The scheme is presented according to the general sequence proposed by the EPPO Panel on Certification of Fruit Crops and adopted by EPPO Council (OEPP/EPPO, 1992a).

Outline of the scheme

For the production of certified varieties and rootstocks of *Malus Pyrus and Cydonia*, the following successive steps should be taken.

1 selection for pomological quality: individual plants of each species, rootstock type or variety[1] to be taken into the scheme are selected. Alternatively, virus-free starting material is imported from other countries.

2 production of nuclear stock: candidate nuclear stock plants are established by budding or grafting this material onto rootstocks of nuclear stock status. The plants are kept under conditions ensuring freedom from infection. The candidate nuclear stock is tested by the most rigorous procedures in the scheme. Alternatively, virus-free plants (candidate nuclear stock) are produced by heat treatment followed by testing. Only candidate nuclear stock plants that have met all requirements are promoted to nuclear stock plants.

3 maintenance of nuclear stock: nuclear stock plants are maintained under conditions ensuring freedom from infection by root contact, pollen or aerial vectors, with retesting as appropriate.

4 production of propagation stock: propagation stock is produced from nuclear stock material in as few steps as possible under conditions ensuring freedom from infection, with retesting as appropriate.

5 production of certified plants: certified plants are produced by grafting propagation stock material onto rootstocks of at least propagation stock standard.

Throughout the whole procedure, care should be taken to maintain the pomological characters of the originally selected plants. Checks should be built in for possible mutations or back mutations, especially for varieties. The scheme is represented diagrammatically in Figs 1 and 2.

The certification scheme should be carried out by an official organization, or by an officially registered, specialized establishment satisfying defined criteria (OEPP/EPPO, 1993). The admission criteria for establishments performing the last phase of production (5) are less stringent than for stages 1-4.

All tests and inspections during production should be recorded. If the stages of the certification scheme are conducted by a registered nursery, certification will be granted by the official organization on the basis of the records of the tests and inspections performed during production, and of visual inspections to verify the apparent health of the stock.

1. Selection of candidates for nuclear stock

Varieties

One or more fruiting trees, with typical agronomic characters, of each variety to be taken into the scheme should be selected in orchards and/or from pomological

1 In this scheme, the terms variety and rootstocks are used in the traditional fruit-growing sense: the variety is the scion cultivar, while the rootstock may be a cultivar or a species

field trials. Alternatively, virus-free starting material may be imported from other countries. Material imported from outside the EPPO region should also be tested by methods recommended by the International Society for Horticultural Science (ISHS) (see Appendix II) for all other viruses occurring naturally in the genus concerned in the region of origin.

Vegetatively propagated rootstocks

Healthy-looking, vigorous and well-rooted individual plants of known agronomic characters of each rootstock type to be taken into the scheme should be selected in different stoolbeds or stoolbushes. Alternatively, virus-free starting material may be imported from other countries. Material from outside the EPPO region should be tested as for varieties (above).

Seedling rootstocks

Seeds of *Malus*, *Pyrus* and *Cydonia* are considered to be virus-free. However, selected trees for the production of seeds should be free from virus symptoms and should preferably be chosen in areas known to be free from fruit tree viroids. They should be known to produce uniform progeny, or else this should be investigated. When germinated, the seedlings should be grown to suitable size under conditions similar to those for varieties and vegetatively propagated rootstocks of either nuclear stock (see point 3) or propagation stock (see point 4).

2. Production of nuclear stock

Varieties

General procedure

Propagation material of the pomologically selected trees is collected and budded or grafted onto nuclear stock rootstocks. These plants (potted candidate nuclear stock plants) should, during the period of testing, be kept under conditions ensuring freedom from infection by root contact, pollen or aerial vectors. They should be grown in sterilized growing medium. The individual candidate nuclear stock plants should be tested for the viruses, phytoplasmas and virus-like diseases specified in Table 1 by the methods mentioned in Appendices I and II. Only if the candidate nuclear stock plant gives a negative test result for all the pathogens listed in Table 1 can it be promoted to nuclear stock and transferred to the nuclear stock collection.

Sanitation procedure

For varieties for which none of the selected trees gave a negative test result, material should be prepared for heat treatment by budding or grafting propagation material onto a number of potted rootstocks. These plants should then be heat-treated (Appendix III) and the newly produced plants (in general, shoot tip grafts) tested after one growing season, which allows time for any possible virus present to develop. For eliminating viroids, heat treatment alone is not always effective, and it may therefore be necessary to use alternative methods (Appendix III). Only plants giving a negative test result can be promoted to nuclear stock and transferred to the nuclear stock collection.

If, for a given variety, it is likely that all candidate nuclear stock plants are infected with viruses, time can be saved by omitting the first testing and proceeding directly to heat treatment.

Vegetatively propagated rootstocks

General procedure

Individual plants or cuttings should be selected and grown either on their own roots or budded or grafted onto an easily distinguishable rootstock type. These potted candidate nuclear stock plants should be kept throughout the period of testing under conditions ensuring freedom from infection by root contact or aerial vectors. They should be grown in sterilized growing medium. Individual candidate nuclear stock plants should be tested for the viruses, phytoplasmas and virus-like diseases specified in Table 1 by the methods mentioned in Appendices I and II. Only candidate nuclear stock plants giving a negative test result can be promoted to nuclear stock and transferred to the nuclear stock collection.

Sanitation procedure

For rootstock types for which none of the selected plants gave a negative test result, a number of the plants or descendants from them should be placed in pots for heat-treatment after a certain time (Appendix III). They should then be tested (as above) after one growing season, which allows time for any viruses present to multiply. For elimination of viroids, heat treatment alone is not always effective and it may therefore be necessary to use alternative methods (Appendix III). Only plants giving a negative test result can be promoted to nuclear stock plants and transferred to the nuclear stock collection.

Inspection for other pests

All candidate nuclear stock (varieties and vegetatively produced rootstocks) should, besides the diseases and pathogens mentioned in Table 1, be inspected for the presence of other pests that can be transmitted on propagating material. In particular, this should be done to ensure freedom from *Agrobacterium tumefaciens, Erwinia amylovora* (OEPP/EPPO, 1992), *Pseudomonas* spp., *Armillariella mellea, Chondrostereum purpureum, Glomerella cingulata, Pezicula malicorticis* and *P. alba, Nectria galligena*, *Phytophthora* spp., *Roessleria pallida, Verticillium* spp., *Quadraspidiotus perniciosus* and *Eriosoma lanigerum*.

3. Maintenance of the nuclear stock

The nuclear stock plants should be maintained under conditions ensuring freedom from (re)infection by root contact, pollen or aerial vectors, preferably in pots of sterilized growing medium in a suitably designed aphid-proof house. Some material of each source of each species, variety or rootstock type may be stored *in vitro* as a reserve stock, but any such material will need to be checked for agronomic characters, especially trueness to type, after leaving the *in vitro* conditions.

Flowering of the nuclear stock plants should be prevented to minimize infection, especially with *E. amylovora*. Trueness to type can be verified by observing fruiting on plants propagated from the nuclear stock but kept in a different place from the nuclear stock.

The plants should be inspected visually several times each year for symptoms of virus and virus-like diseases, and for the other pests mentioned in section 2. The plants should also be visually inspected each year for possible mutations. It is considered advisable to retest the individual nuclear stock plants at least once during the useful life of the plants for the pathogens in Table 1. Any plant giving a positive test result or showing symptoms of viruses, virus-like diseases or other pests mentioned in section 2 should be removed immediately from the nuclear stock collection.

4. Production of propagation stock

The nuclear stock should be multiplied in as few steps as possible to obtain the required quantity of propagation stock. Nuclear stock material should be budded or grafted onto rootstocks of equivalent certification status or onto seedling rootstocks produced under nuclear stock conditions. Propagation stock should be kept in fields isolated from potential sources of infection, particularly host plants of phytoplasmas or *E. amylovora*.

The propagation stock should be visually inspected each year for virus symptoms and for the other pests mentioned in section 2. It is advisable to retest randomly the propagation stock of *Malus* regularly for apple proliferation phytoplasma especially in areas where the disease is prevalent. For *Pyrus*, in areas where pear decline phytoplasma is prevalent, it is advisable to retest the propagation stock randomly and then to test any plant suspected of being infected. Any infected plant should be removed and, if there is an indication that infection may have derived from the previous generation, it is advisable to remove all the plants in the lot and to retest the possible source plant.

Flowering of the trees may be needed to check pomological characteristics, but it should be noted that flowering can lead to risk of infection by *E. amylovora*, especially in areas where this pathogen is prevalent. The plants should be inspected visually for possible mutations, especially the varieties for fruit colour, spur type and genetic disorders (chimeras, etc.). This is the first time that an assessment on fruits can be made, but it should be noted that the type of rootstock can affect fruit characteristics.

5. Production of certified plants

For the production of certified fruit trees, the scion material should be grafted or budded onto rootstocks of equivalent or higher certification status only. These plants should be kept in fields isolated from potential sources of infection, particularly host plants of phytoplasmas or *E. amylovora*. To be certified, the plants should be inspected by the official organization for symptoms of virus, virus-like diseases or any of the pests mentioned in section 2. Any plants showing symptoms should be removed and certification may be granted to the remainder.

Administration of the certification scheme

Monitoring of the scheme

An official organization should be responsible for the administration and monitoring of the scheme. If officially registered nurseries carry out the different stages of the scheme, the official organization should confirm that all necessary tests and inspections have been performed during production, and should verify the general health status of the plants in the scheme by visual inspections. Otherwise, certification will not be granted and/or the plants concerned will not be permitted to continue in the certification scheme.

Control on the use and status of certified material

Throughout the certification scheme, the origin of each plant should be known so that any problems of health or trueness to type may be traced. The use of propagation material in nurseries to produce certified plants should be checked by an official or officially authorized organization that controls the health, origin and amount of such material on the basis of field inspections and of the records and documents presented by the nursery. The nursery plant-protection programme and the check inspections should also take account of other important pests that can affect quality, so that the certified plants delivered to the fruit grower are substantially free from these pests. Certified fruit-tree material for export should in any case satisfy the phytosanitary regulations of importing countries.

Certified plants leaving the scheme should carry an official certificate (which may be a label) indicating the certifying authority, the plant producer and the certification status of the plants.

Appendix I

Guidelines on testing procedures

1. Testing on woody indicators (field and glasshouse)

The use of woody indicators is still a compulsory step in any certification programme. This is because there are diseases, some of major importance, which can only be identified on woody differential hosts. The method consists of graft-inoculating indicator plants with budwood from candidate nuclear stock plants or plants suspected to be infected and observing the new growth and/or fruits on the indicator plants for symptoms; such symptoms are normally specific and highly diagnostic for many diseases.

If testing is conducted in a glasshouse, heating and cooling facilities (temperature range 18-25°C) should be available in order to ensure correct temperatures for symptom expression (Appendix II). At least three plants from each indicator should be used in the glasshouse. Indicators maintained in the field (3-5 plants for each) should be observed for at least two years or, for some diseases, for at least two fruiting periods (4-5 years).

2. Testing on herbaceous hosts (glasshouse)

The use of herbaceous indicators allows detection of mechanically transmissible viruses, including those of minor importance. The method should be regarded as a complement to, but not as a substitute for, other diagnostic procedures. It may be useful, for example, for preliminary screening or for random testing. Herbaceous tests should be conducted in a glasshouse, with heat and cooling facilities (temperature range 18-25°C). At least five plants for each indicator should be used.

3. ELISA testing

The ELISA method allows large-scale testing for fruit-tree viruses for which polyclonal and/or monoclonal antisera are available. However, there are certain limitations in any antibody technique, such as the fact that some viruses may exist in very low concentrations in the tree, may be irregularly distributed or be seasonally undetectable.

4. PCR

Polymerase chain reaction (PCR) can be used for the detection of some viruses, viroids and of apple proliferation and pear decline phytoplasmas; it can also detect the virus-like diseases spy epinasty and decline and platycarpa scaly bark. Serological and molecular tests can be combined to increase the sensitivity of each method on its own, e.g. immunocapture PCR (IC-RT-PCR).

5. Molecular hybridization

Molecular hybridization can be used for the detection of viroids and some viruses.

6. DAPI

The DAPI method (using fluorescent microscopy after staining with the nucleic acid dye 4,6-diamino-2-phenylindole) allows rapid small-scale testing for phytoplasma diseases but is not as sensitive as PCR.

Appendix II

Guidelines on virus or disease detection

Introduction

The methods for detection are specified in Tables 2-5 for each virus or disease under the headings:

- woody tests (field) = tests on woody indicators in the field;
- woody tests (glasshouse) = tests on woody indicators in the glasshouse;
- herbaceous tests = glasshouse testing on herbaceous indicators;
- serological or molecular tests = the use of ELISA, reverse transcriptase-polymerase chain reaction (RT-PCR), immunocapture PCR (IC-RT-PCR), molecular hybridization;
- microscopy = DAPI test or electron microscopy.

For the woody tests, the indicators are listed, followed by figures between brackets representing number of replicates, the temperature in °C (for glasshouse testing) and duration of test (d = days, w = weeks, y = years, c = fruit cropping years); then a short description of the symptoms is given. In general, testing on woody indicators in the field is always needed to establish virus freedom for nuclear stock, and a test on woody indicators is thus always specified. Tests on herbaceous indicators, serological tests, RT-PCR or DAPI are mainly used in screening candidate material rapidly and economically to eliminate infected plants or in the retesting of propagation stock.

The information on tests is mainly taken from the publications of the ISHS Working Group on Fruit Tree Viruses, which appear in *Acta Horticulturae* after every three-yearly meeting (Anon, 1998). Readers are advised to consult the most recent ISHS recommendations, where key references to techniques are also given, in particular for the PCR technique in which rapid technological development is taking place at present. The ISHS recommendations also include comments on the advantages and limitations of the methods. The EPPO Panel on Certification of Pathogen-tested Fruit Crops, reviewing the ISHS recommendations, has identified those woody indicators that, on the basis of its experience, it particularly recommends for effectiveness and ease of use. Nevertheless, this does not exclude the use of

others that may be listed by the ISHS or found satisfactory by individuals under their own conditions.

Appendix III

Guidelines on sanitation procedures

Thermotherapy

Because of the wide range of methods available for heat treatment of fruit crops depending on the type of material to be treated, details of methods are not provided here but can be obtained by reference to Anon (1970), Németh (1986) or Fridlund (1989).

In vitro methods for the elimination of virus and viroid infections

In general, in *vitro* methods should be applied with care for apple and pear varieties, as these methods may introduce genetic and/or phenotypic aberrations to a greater extent than traditional horticultural methods. Normally, heat treatment is the preferred method for the removal of viruses. Where viroid infections are involved, *in vitro* methods should be applied, as viroids in general are not susceptible to heat treatment. A general method is described by Zimmermann (1989). The normal procedure typically includes the following steps:

1 dissection of a meristem from lateral buds, preferably 0.3-0.5 mm;

2 shoot development for 6-12 weeks;

3 root development with 3- to 4-weeks interval on fresh medium;

4 acclimatization of the explants in the glasshouse for several weeks;

5 retesting for virus and viroid infections after at least 1 year of growth, allowing the viruses and viroids present to develop;

6 retesting of pomological and dendrological characters.

Cultures can be stored at 4°C for several months without subculturing. Apices can be freeze-dried in liquid N_2 at –176°C after dimethylsulphoxide (DMSO) treatment or included in alginate pellets.

References

Anon (1970) *La Thermothérapie des Espèces Ligneuses*. Station des cultures fruitières et maraîchères à Grand-Manil, Gembloux (BE).

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Fridlund P (1989) Thermotherapy. In *Virus and Virus-like Diseases of Pome Fruits and Simulating Noninfectious Disorders* (ed. Fridlund P), pp. 284-295. Cooperative Extension, Washington State University, Pullman (US).

Németh M (1986) *Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees*, pp. 135-139. Martinus Nijhoff, Dordrecht (NL).

OEPP/EPPO (1992) EPPO Standards PM 3/40 *Erwinia amylovora* - sampling and test methods. *Bulletin OEPP/EPPO Bulletin* **22**, 225-231.

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Zimmerman RH (1989) In *Virus and Virus-like Diseases of Pome Fruits and Simulating Noninfectious Disorders* (ed. Fridlund P), pp. 278-283. Cooperative Extension, Washington State University, Pullman (US).

Table 1. Pathogens in the EPPO region requiring tests in the certification scheme*

Host	Type of pathogen	Name	Acronym
Malus spp.	Viruses	Apple chlorotic leaf spot trichovirus	ACLSV
		Apple mosaic ilarvirus	ApMV
		Apple stem-grooving capillovirus	ASGV
		Apple stem-pitting foveavirus	ASPV
	Phytoplasmas	Apple proliferation phytoplasma	AP
	Virus-like diseases	Rubbery wood, flat limb	
		Horseshoe wound	
		Fruit disorders: chat fruit, green crinkle, bumpy fruit of Ben Davis, rough skin, star crack, russet ring, russet wart	
	Viroids	Apple scar skin viroid†	ASSVd
Pyrus and *Cydonia* spp.	Viruses	Apple chlorotic leafspot trichovirus	ACLSV
		Apple stem-grooving capillovirus	ASGV
		Apple stem-pitting foveavirus	ASPV
	Phytoplasmas	Pear decline phytoplasma	PD
	Virus-like diseases	Bark split, bark necrosis	
		Rough bark	
		Quince sooty ringspot (probably caused by ASPV)	
		Pear stony pit (probably caused by ASPV)	
		Rubbery wood, quince yellow blotch	
	Viroids	Pear blister canker viroid	PBCVd

* The virus-like diseases listed are distinct symptoms whose causal agent is not known or, at most, only suspected. For other distinct symptoms that are now known to be caused by some of the pathogens listed, see Appendix II.

† Apple dimple fruit viroid (ADFVd) belongs to the same genus as ASSVd. It has been found only once near Napoli (IT), and insufficient information is available on testing to include it in this scheme.

Table 2. Methods for detection of viruses of *Malus*, *Pyrus* and *Cydonia*

Apple chlorotic leaf spot trichovirus (ACLSV)	
Woody tests (field) for *Malus*	*Malus platycarpa* (3/-/2 y) (chlorotic rings and line pattern on leaves)
	and
	Malus sylvestris R 12740 7A (3/-/2 y) (terminal dieback, leaf distortion)
	Pyronia veitchii (3/-/2 y) (ring and line pattern mosaic)
Woody tests (field) for *Pyrus/Cydonia*	*Cydonia oblonga* C7/I (3/-/2 y) (ring and line pattern mosaic)
	A20 (3/-/2 y) (ring and line pattern mosaic)
	Beurré Hardy (3/-/2 y) (ring and line pattern mosaic)
	Pyronia veitchii (3/-/2 y) (ring and line pattern mosaic)
Woody tests (glasshouse) for *Malus*	*Malus platycarpa* (3/20/8 w) (chlorotic rings and line pattern on leaves)
	and
	Malus sylvestris R 12740 7A (3/22/4 w) (terminal dieback, leaf distortion)
Woody tests (glasshouse) for *Pyrus/Cydonia*	Nouveau Poiteau (3/22/10 w) (ring and line pattern mosaic)
Herbaceous tests	*Chenopodium quinoa*, *C. amaranticolor, Nicotiana occidentalis* '37B'
Serological or molecular tests	ELISA, RT-PCR, IC-RT-PCR.
Natural transmission	Unknown
Apple mosaic ilarvirus (ApMV)	
Woody tests (field)	Golden Delicious(3/-/2 y) (chlorotic mosaic on leaves)
	Lord Lambourne (3/-/2 y) (chlorotic mosaic on leaves)
Woody tests (glasshouse)	No indicator recommended
Herbaceous tests	Over 65 herbaceous plant species in 19 families are susceptible to mechanical inoculation. Among these are *Chenopodium quinoa*, *C. amaranticolor, Cucumis sativus*, *Cucurbita maxima*, *Nicotiana clevelandii*, *Petunia hybrida*. However, transmission to herbaceous indicators can be difficult.
Serological or molecular tests	ELISA
Natural transmission	Unknown
Apple stem-grooving capillovirus (ASGV)	
Woody tests (field) for *Malus*	Virginia Crab (3/-/3y) (necrotic grooves on woody cylinder)
Woody tests (field) for *Pyrus/Cydonia*	Virginia Crab (3/-/3y) (necrotic grooves on woody cylinder)
	Pyronia veitchii (3/-/3y) (necrotic grooves on woody cylinders)
Woody tests (glasshouse) for *Malus*	Virginia Crab (3/26/4w) (necrotic grooves on woody cylinder)
	Malus micromalus GMAL273 (4/26-32/4w) (chlorotic/necrotic spots, epinasty, stem necrosis)
Woody tests (glasshouse) for *Pyrus/Cydonia*	Virginia Crab (3/26/8w) (necrotic grooves on woody cylinder)

Herbaceous tests	*Chenopodium quinoa*
Serological or molecular tests	ELISA, RT-PCR
Natural transmission	Unknown
Apple stem-pitting foveavirus (ASPV)	
Woody tests (field) for *Malus*	*Pyronia veitchii* (3/-/2 y) (pits in the xylem)
	Spy 227 (3/-/2 y) (epinasty and decline)
	and
	Virginia Crab (3/-/3 y) (pits in the xylem)
Woody tests (field) for *Pyrus*/*Cydonia*	Jules d'Arolles (3/-/2 y) (vein yellows/red mottling along the veins)
	Pyronia veitchii (3/-/2 y) (pits in the xylem)
	Virginia Crab (3/-/3 y) (pits in the xylem)
Woody tests (glasshouse) for *Malus*	*Pyronia veitchii* (3/22/8 w) (pits in the xylem)
	Spy 227 (3/24/12 w) (epinasty and decline)
	Virginia Crab (3/26/4 w) (pits in the xylem)
Woody tests (glasshouse) for *Pyrus/Cydonia*	*Pyronia veitchii* (3/22/8 w) (pits in the xylem)
	Virginia Crab (3/26/8 w) (pits in the xylem)
Herbaceous tests	*Nicotiana occidentalis* ssp. *obliqua*
	Nicotiana occidentalis '37B'
Serological or molecular tests	RT-PCR, IC-RT-PCR
Natural transmission	Unknown

Table 3. Methods for detection of phytoplasmas

Apple proliferation phytoplasma	
Woody tests (field)	Golden Delicious*, use three buds (5/-/2 y) (witches' brooms, enlarged stipules)
Serological or molecular tests	PCR
Microscopy	DAPI
Natural transmission	Possibly by leafhoppers
Pear decline phytoplasma	
Woody tests (field)	Doyenné du Comice or *Pyronia veitchii* (3/-/2 y) (leaf curl, early autumn coloration of leaves) (on pear seedling rootstock; three buds)
Serological or molecular tests	PCR
Microscopy	DAPI
Natural transmission	pear psyllids

* There are doubts about the reliability of Golden Delicious to detect apple proliferation phytoplasma, and, therefore, PCR is recommended as the most sensitive test.

Table 4. Methods for detection of viroids

Apple dapple apple (ADAVd)	
Woody tests (field)	Golden Delicious (3/-/3 c) (pale circular spots, that stand out against the colour of the fruits)
Apple scar skin viroid (ASSVd)	
Woody tests (field)	Golden Delicious (3/-/3 c) (scar patches on fruit or pale green circular spots on fruits)
Pear blister canker viroid (PBCVd)	
Woody tests (field)	*Pyrus* A20 (3/-/3 y) (blister canker)

The diseases apple dapple apple and apple scar skin may be caused by molecular variants of the same agent (ASSVd). Hybridization and, in some cases, RT-PCR are ISHS-recommended laboratory tests for viroids.

Table 5. **Methods for detection of virus-like diseases**

*** Apple bumpy fruit of Ben-Davis disease**	
Woody tests (field)	Lord Lambourne (5/-/3c) (yellow leafspots, deformation of leaf blade)
Apple chat fruit	
Woody tests (field)	Lord Lambourne (5/-/3c) (small, brownish-red fruit with dark green spots)
†Apple flat limb disease	
Woody tests (field)	Gravensteiner (3/-/3y) (flattening on shoots, causing deep furrows)
Apple green crinkle disease	
Woody tests (field)	Golden Delicious (3/-/3 c) (dwarfed, malformed fruits)
***Apple horseshoe wound disease**	
Woody tests (field)	Golden Delicious (3/-/3 y) (horseshoe-shaped wounds on bark below or around buds)
Apple rough skin disease	
Woody tests (field)	Schone van Boskoop (3/-/3 c) (rough, corky spots on fruit skin, which often crack open) Golden Delicious‡ (3/-/3 c) (rough, corky spots on fruit skin)
Apple rubbery wood disease	
Woody tests (field)	Lord Lambourne (5/-/3 y) (abnormal flexibility of stem and branches)
Apple russet ring	
Woody tests (field)	Golden Delicious (3/-/3 c) (brown, circular rings on fruits)
Apple russet wart disease	
Woody tests (field)	Golden Delicious (3/-/3 c) (russet warts with necrotic spots on fruits)

***Apple star crack disease**	
Woody tests (field)	Golden Delicious (3/-/3 c) (cracks on the fruit skin, reduction in size)
Pear ring (pattern) mosaic	See apple chlorotic leafspot trichovirus
Pear bark necrosis disease	See pear bark split disease
Pear bark split disease	
Woody tests (field)	Beurré Hardy (3/-/3 y) (bark scabby with cracks) *Pyrus* A20 (3/-/3 y) (bark scabby with cracks)
Pear blister canker disease	See pear rough bark disease
Pear rough bark disease	
Woody tests (field)	Williams (3/-/3 y) (bark rough with blisters) Doyenné du Comice (3/-/2 y) (bark rough with blisters) *Pyrus* A20 (3/-/3 y)) (bark rough with blisters)
Pear stony pit disease (apple stem pitting virus ?)	
Woody tests (field)	Beurré Hardy (3/-/3 c) (stony pits on fruits) Durondeau (3/-/3 c) (stony pits on fruits)
Pear vein yellows/red mottle	See apple stem-pitting foveavirus
§**Platycarpa scaly bark disease**	
Woody tests (field)	*Malus platycarpa* (3/-/2 y) (bark roughened and scaly, trees often dwarfed) Other tests as for apple stem-pitting foveavirus
Quince sooty ringspot (apple stem-pitting ?)	
Woody tests (field)	C 7/1 (3/-/2 y) (severe leaf epinasty, sooty ringspots on leaf, often die-back of shoots)
Quince yellow blotch (see apple rubbery wood disease)	
Woody tests (field)	C 7/1 (3/-/2 y) (diffuse yellow blotches on leaf, longitudinal flat depression on stem)
§Spy epinasty and decline	Testing as for apple stem-pitting foveavirus

***** May be caused by the same agent as apple green crinkle disease

† May be caused by the same agent as apple rubbery wood disease

‡ There is some evidence to indicate that Golden Delicious may not detect all strains.

§ May be caused by apple stem-pitting foveavirus

Fig. 1. Diagram of the stages in the certification scheme for *Malus*, *Pyrus* and *Cydonia*: scion material

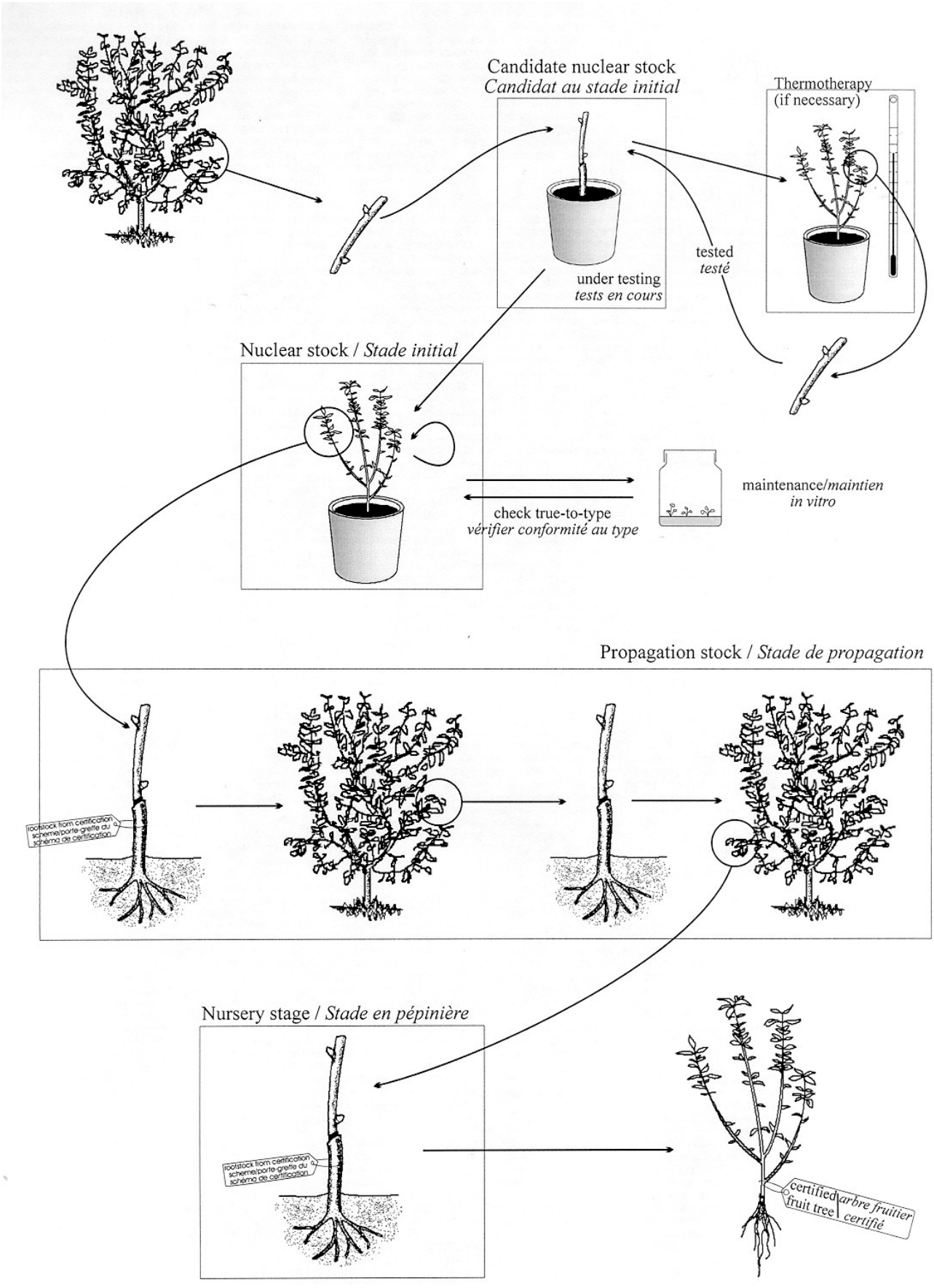

Fig. 2. Diagram of the stages in the certification schemes for *Malus*, *Pyrus* and *Cydonia*: rootstocks