◆ EPPO Standards ◆

SCHEMES FOR THE PRODUCTION OF HEALTHY PLANTS FOR PLANTING

CERTIFICATION SCHEME FOR ALMOND, APRICOT, PEACH AND PLUM

PM 4/30(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

EPPO Standards are distributed by the EPPO Secretariat to all EPPO Member Governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

SCOPE

EPPO Schemes for the Production of Healthy Plants for Planting are intended to be used by NPPOs or equivalent authorities, in their capacity as bodies responsible for the design of systems for the 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 (1992) Recommendations made by EPPO Council in 1981: certification of virus-tested fruit trees, scions and rootstocks. *EPPO Technical Documents* **1013**, 42-43.
OEPP/EPPO (1993) 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 prebasic 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 which 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 Schemes for the Production of Healthy Plants for Planting 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 Schemes for the Production of Healthy Plants for Planting 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 EUROPÉENNE ET MÉDITERRANÉENNE POUR LA PROTECTION DES PLANTES

PM 4/30(1) English

Schemes for the production of healthy plants for planting

CERTIFICATION SCHEME FOR ALMOND, APRICOT, PEACH AND PLUM

Specific scope

This standard describes the production of pathogen-tested material of *Prunus armeniaca*, *Prunus domestica*, *Prunus dulcis*, *Prunus persica*, *Prunus salicina* and their rootstocks.

Specific approval and amendment

First approved in September 1990 as part of EPPO Standard PM 4/1.
Approved as a separate standard in September 2000.

The certification scheme for pathogen-tested material of varieties and rootstocks of almond, apricot, peach and plum provides detailed guidance on the production of grafted fruit trees (varieties), vegetatively propagated rootstocks and seedling rootstocks. The species and subspecies covered by the scheme include the following most commonly grown in the EPPO region: *Prunus armeniaca* (apricot), *Prunus domestica* subsp. *domestica* (plum), *Prunus domestica* subsp. *insititia* (damson), *Prunus domestica* subsp. *italica* (greengage), *Prunus domestica* subsp. *syriaca* (mirabelle plum), *Prunus dulcis* (almond), *Prunus persica* (peach/nectarine) and *Prunus salicina* (Japanese plum). In addition, *Prunus besseyi, Prunus cerasifera*, *Prunus davidiana* and interspecific hybrids are used as vegetatively propagated rootstocks. Seedling rootstocks include forms of *P. armeniaca*, *P. persica* and *P. insititia* × *domestica* hybrids. The scheme is also suitable for the certification of related ornamental plants within the genus *Prunus*.

Plant material produced according to this certification scheme is derived from nuclear-stock plants that have been tested and found free from the pathogens listed in Table 1, and produced under conditions minimizing infection from other major pathogens of the species 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 which 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, 1992).

Outline of the scheme

For the production of certified varieties and rootstocks of almond, apricot, peach and plum, the following successive steps should be taken.

1 Selection for pomological quality: individual plants of each species, rootstock type or variety to be taken into the scheme are selected. (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.) 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, 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 nursery or laboratory satisfying defined criteria (see EPPO Standard PM 4/7). The registration requirements for establishments performing the last phase of production (productions of certified plants) are less stringent than for the first four.

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 field trials. Alternatively, virus-free starting material may be imported from other countries. Material imported from outside the EPPO region should be tested by methods recommended by the International Society for Horticultural Science (ISHS) (see Appendix II) for all viruses occurring naturally in the genus *Prunus* 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 rootstock beds and/or from pomological field trials. Alternatively, virus-free starting material may be imported from other countries. Material from outside the EPPO region should be tested as for varieties (above).

2. Production of nuclear stock

Varieties

General procedure

Propagation material from 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, aerial or soil vectors. They should be grown in sterilized growing medium in an isolated, suitably designed, aphid-proof house, separated from the nuclear stock during the testing period. The individual candidate nuclear-stock plants should be tested for the viruses and virus-like diseases specified for each species in Table 1 by the methods 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 for the species can it be promoted to nuclear stock and transferred to the nuclear-stock collection.

Sanitation procedure

For varieties of 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. 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

Selected individual plants and cuttings (candidate nuclear-stock plants) should be kept throughout the period of testing under conditions ensuring freedom from infection by root contact, pollen, aerial or soil vectors. They should be grown in sterilized growing medium in an isolated, suitably designed, insect-proof house, separated from the nuclear stock during the testing period. Individual candidate nuclear-stock plants should be tested for the viruses and virus-like diseases specified for each species in Table 2, by the methods 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 of 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. Only plants giving a negative test result can be promoted to nuclear-stock plants and transferred to the nuclear-stock collection. For a rootstock type which has been long in use, it may be preferable to omit the first testing and proceed directly to heat treatment. However, direct selective testing may save time with newly bred rootstock types.

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 which can be transmitted on propagating material. In particular, this should be done to ensure freedom from *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *morsprunorum* (and, on apricot, *Pseudomonas syringae* pv. *syringae* and *Pseudomonas viridiflava*), *Xanthomonas arboricola* pv. *pruni*, *Phytophthora* spp. and *Quadraspidiotus perniciosus*.

3. Maintenance of the nuclear stock

The nuclear-stock plants should be maintained under conditions ensuring freedom from (re)infection. Because of the risk of aerial infection, plants should preferably be maintained in an aphid-proof house and grown in containers of sterilized growing medium, isolated from the soil. In areas free from PPV and European stone fruit yellows phytoplasma, nuclear-stock plants may be maintained in the open, where they should be separated by approximately 1 km from any cultivated or wild *Prunus* spp. of subgenera *Prunophora* and *Amygdalus* and should be prevented from flowering. In addition, the soil should be tested and found free from virus-transmitting nematodes of the genera *Longidorus* and *Xiphinema* (Appendix IV). The absence of nematodes should be confirmed every 5 years by testing the soil.

Each plant should be checked for trueness-to-type during its vegetative stage. The plants should also be inspected visually every year for possible mutations.

Each nuclear-stock plant should be retested every year for PNRSV, PDV and ApMV. In addition, all plants should be retested for all virus and virus-like diseases, according to the species (Table 1), when the plants are grafted onto new rootstocks. The plants should be inspected visually several times each year for symptoms of virus and virus-like diseases, and for the other pests mentioned above. Any plant giving a positive test result or showing symptoms of viruses, virus-like diseases or other pests mentioned above 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 certified seedling rootstocks. The propagation stock should be kept in fields that have been tested and found free from virus-transmitting nematodes of the genera *Longidorus* and *Xiphinema* (Appendix IV) and isolated from material of the same genus not certified or of lower certification status. Multiplication *in vitro* may be used for rootstocks, and guidelines are given in Appendix V.

Seeds produced on propagation stock of rootstocks[1] may be harvested, tested for seed-transmissible viruses (Appendix I) and germinated to produce seedling rootstocks. These are used as rootstocks for certified trees at the nursery stage. Seedling rootstocks may also be used as rootstocks for propagation stock, provided that the plants on which the seeds are produced are isolated by at least 300 m from any plants of *Prunus* and remain under propagation-stock conditions.

The propagation stock should be inspected visually each year for virus symptoms and for the other pests mentioned above. Particular attention should be given to naturally spreading viruses. For additional security, plants of the first generation of propagation stock may be retested each year by ELISA for PNRSV and PDV. 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.

The plants should be inspected visually for possible mutations. This is the first time that an assessment of fruits can be made, but it should be noted that the type of rootstock can affect fruit characteristics.

5. Production of certified plants and seeds

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. 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 above. Any plants showing symptoms should be removed and certification may be granted to the remainder.

For the production of certified seeds, seeds from propagation stock of rootstocks (see above) should be cleaned, tested for seed-transmissible viruses (Appendix I) and packed in sealed bags.

6. 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 stock should be checked by an official or officially authorized organization which 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

[1] Exceptionally, seeds may be collected from a wild tree of *Prunus cerasifera,* tested for seed-transmissible viruses as for seeds from propagation stock, and used for production of seedling rootstocks for certified plants (but not for propagation stock).

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 or seeds 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

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 of each indicator should be used in the glasshouse. Indicators maintained in the field (3-5 plants for each) should be observed for at least 2 years.

Inoculation to herbaceous hosts (glasshouse)

The use of herbaceous indicators allows the 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 heating and cooling facilities (temperature range 18-25°C). At least five plants should be used for each indicator.

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 could exist in very low concentrations in the tree, may be irregularly distributed or be seasonally undetectable.

Fluorescence and electron microscopy

The DAPI test (based on the DNA-specific fluorescence induced by 4'-6-diamidino-2-phenylindole) can be used for the non-specific detection of phytoplasmas in infected sieve tubes, using fluorescence microscopy. Electron microscopy can also be used for the detection of phytoplasmas.

Molecular hybridization

Molecular hybridization with non-radioactive probes can be applied to the detection of PLMVd. The viroid is uniformly distributed in the plant and can be detected in different tissues (leaves, fruits, bark) during the whole growing season.

PCR

Polymerase chain reaction (PCR) can be used for the detection of phytoplasmas that cause European stone fruit yellows, for viroids (PLMVd) and viruses. Serological and molecular tests can be combined to increase the sensitivity of the single method, e.g. immunocapture reverse transcriptase-PCR (IC-RT-PCR).

Testing seed lots

Various sampling and testing methods are available. A typical method is as follows: from a seed lot (containing 50 kg of seeds), 200 seeds (stones) are sampled and placed in water for at least one night. The stones are cracked, and the kernels (seeds) are extracted. Groups of 3-5 seeds (including integument and embryo) are combined and extracted by standard techniques by ELISA. If any ELISA test is positive, the lot should be rejected.

Appendix II Guidelines on disease detection

The methods for disease 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, RT-PCR, IC-RT-PCR, molecular hybridization.

For the woody tests, the indicators are listed, followed by figures in 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 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 where 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 which, 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 procedure

Methods for elimination of pathogens from stone fruits include or combine thermotherapy, *in vitro* methods and/or chemical treatment. Testing of the treated material for assessment of its health status should follow.

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).

Meristem culture

This technique is routinely used for strawberry, but is not often applied to fruit trees. It is, however, very effective in eliminating viruses, especially when meristems are taken from micropropagated explants.

Shoot-tip grafting

The technique of shoot-tip grafting *in vitro* should be performed with special tools under aseptic conditions and involves the following steps:

- rootstock seedlings are grown in nutrient agar in the dark;
- shoots approximately 1 cm long are collected and disinfected in the laboratory;
- the immature leaves are removed and, then, under the microscope, the very small tip (about 0.1-0.2 mm) consisting of the meristem and two or three leaf primordia are excised;
- the tip is carefully put on the top of a decapitated rootstock seedling.

The young grafted plant is grown in nutrient media for 5-15 weeks under lights and then transplanted into soil.

Heat treatment in vitro

Prunus spp. which are difficult to heat treat normally can receive a longer heat treatment at moderately high temperature (34-38°C) as micropropagated shoots. This technique is currently being examined for apricot and peach.

Chemical treatment in vitro

This method depends on the use of a virucide which may have specific action. Thus, ribavirin is effective in eliminating ACLSV in *Prunus*, while DHT only acts on PNRSV.

Appendix IV Guidelines on nematode analysis

Soil in which certified material is to be planted should be sampled and the samples found free from the nematode vector species listed in Table 6.

Soil samples are taken in the 10-30 cm depth layer, using a semi-cylindrical auger with a diameter of at least 2.5 cm. Screw augers or tools with a diameter of less than this should not be used because of the risk of damaging the nematodes during sampling. If possible, sampling should be performed when the soil is moist; otherwise, it may be necessary to take samples from deeper soil layers, e.g. 30-60 cm. Samples are taken on a grid pattern over the site with, for example, 20 subsamples for sites up to 0.2 ha and 40 for sites between 0.2 and 4 ha. Another possible sampling pattern (more intensive but used in some countries) is to divide the site into units of 0.2 ha and to take 60 subsamples in each of these sample units. Additional samples should be taken from any hedges which surround the site.

Extraction of nematodes from the soil should be performed by a method such as, for example, that of Flegg (1967) which requires little special equipment: mix the soil sample carefully but thoroughly and measure two subsamples of 200 mL by displacement of water. Leave each subsample to soak in water for at least 1 h, then wash it through a 4-mm-pore sieve into a 10-L bucket, which is filled nearly to the brim. Stir the contents of the bucket with the hand in order to put the soil into suspension. Leave for 25 s, then decant the supernatant onto a set of three sieves of 150-µm pore size. Refill the bucket and repeat the stirring and decanting (after leaving for only 15 s).Wash the debris collected off the sieves and transfer to a 110-µm-pore nylon sieve. Place the sieve on a glass funnel with just enough water to submerge the debris on the sieve surface. Leave for 24 h, then collect about 25 mL from the stem of the funnel (this can be achieved by having the funnel stem terminating in a rubber tube closed with a clamp) for examination at 25× magnification. Counting of nematodes can be done at 25× magnification, but identification of species can only be done by a trained taxonomist at considerably higher magnification.

The nematodes can be tested directly for the presence of virus by a 'slash test', i.e. breaking up small numbers of adult nematodes (>5 nematodes) in phosphate buffer (pH 6.9) and inoculating the leaves of *Chenopodium quinoa* with the suspension. An indirect method to test the nematodes for virus is to grow bait plants of *Petunia hybrida* in pots of field soil containing nematodes for 3 weeks and then to test the roots for the presence of virus by inoculation to indicator plants.

Appendix V Guidelines on *in vitro* multiplication

Micropropagation of *Prunus* rootstocks is practised routinely in several European laboratories, either for rapid and intensive multiplication of planting material or for elimination of viruses (see Appendix III). For *Prunus*, *in vitro* multiplication can be used for most rootstocks, and experience is now sufficient for this to be generally recommended. In general, it is easiest for material which is readily multiplied by cuttings, but it is most useful for material which is difficult to multiply otherwise. At present, it can be practised readily for peach, plum, almond and myrobalan but has to be specially worked out for each genotype of apricot. There is much current research on solving these problems for apricot. Numerous interspecific hybrids (e.g. GF677) are also multiplied *in vitro*.

In general, *in vitro* multiplication offers no particular advantage for a scion cultivar which will not be grown on its own roots.

In vitro culture can also be used to maintain material which will be protected from any risk of infection by:

- regular 3-weekly subculturing. Only axillary shoots should be taken as explants, and callus formation should be limited to avoid genetic drift by somaclonal variation;
- storage at 4°C, in the light or dark, for several months without subculturing;
- cryopreservation of apices (meristems plus several leaf primordia) in liquid N_2 at –176°C after cryo-protector treatment or inclusion in alginate pellets.

New rootstock material which has been multiplied *in vitro* should be propagated further to check on pomological and juvenile characteristics before grafting. All material offered for sale which has undergone *in vitro* multiplication should preferably be identified as such.

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Table 1 Viruses and other pathogens of varieties and rootstocks of almond, apricot, peach and plum occurring in the EPPO region and requiring testing in the certification scheme

	Prunus amygdalus (almond)	*Prunus armeniaca* (apricot)	*Prunus persica* (peach)	*Prunus domestica, Prunus insititia* and *Prunus salicina* (plum)	*Prunus besseyi, Prunus cerasifera, Prunus davidiana* and interspecific hybrids
Viruses					
Apple chlorotic leaf spot trichovirus (ACLSV)	×	×	×	×	×
Apple mosaic ilarvirus (ApMV)	×	×	×	×	×
Cherry green ring mottle foveavirus (CGRMV)			×		
Myrobalan latent ringspot nepovirus (MLRSV)				×	×
Plum pox potyvirus (PPV)	×	×	×	×	×
Prune dwarf ilarvirus (PDV)	×	×	×	×	×
Prunus necrotic ringspot ilarvirus (PNRSV)	×	×	×	×	×
Strawberry latent ringspot nepovirus (SLRSV)			×		
Tomato black ring nepovirus (TBRV)	×				
Phytoplasmas					
European stone fruit yellows phytoplasma		×*	×	×†	×
Virus-like diseases					
Peach asteroid spot agent‡		×	×		
Viroids					
Peach latent mosaic pelamoviroid			×		

* Previously known, on apricot, as chlorotic leafroll.

† Previously known, on plum, as leptonecrosis.

‡ Rare and causes little damage.

Table 2 **Methods for detection of viruses of almond, apricot, peach and plum**

ACLSV	
Woody tests (field)	GF305 or Elberta seedling (3/-/2y) (dark-green sunken mottle on leaves)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (dark-green sunken mottle on leaves)
Herbaceous tests	*Chenopodium quinoa, Chenopodium amaranticolor.*
Serological or molecular tests	ELISA, PCR and IC-PCR.
Natural transmission	Unknown
ApMV	
Woody tests (field)	GF305 or Elberta seedling, or plum Ersinger (3/-/2y) (infected leaves show light green, yellowish green or bright yellow rings, spots, bands or oak- leaf patterns)
Woody tests (glasshouse)	GF305 or Elberta seedling (5/20/12w) (infected leaves show light green, yellowish green or bright yellow rings, spots, bands or oak-leaf patterns)
Herbaceous tests	Over 65 herbaceous plant species in 19 families are susceptible to mechanical inoculation. Among these are: *C. quinoa*, *C. amaranticolor*, *Cucumis sativus*, *Cucurbita maxima*, *Nicotiana clevelandii*, *Petunia hybrida*.
Serological or molecular tests	ELISA
Natural transmission	
CGRMV	
Woody tests (field)	Bing, Shirofugen, Kwanzan (3/-/2y) (epinasty of the foliage, necrotic midribs or lateral veins, twisting and curling of infected leaves. The bark is often roughened by the development of longitudinal fissures)
Woody tests (glasshouse)	-
Herbaceous tests	Impossible, at present
Serological or molecular tests	PCR
Natural transmission	Unknown/inconnue
MLRSV	
Woody tests (field)	-
Woody tests (glasshouse)	GF305 or Elberta seedling (5/20/12w) (stunting of the plant, short internodes and rosetting)
Herbaceous tests	*C. quinoa*
Serological or molecular tests	ELISA
Natural transmission	Unknown
PPV	
Woody tests (field)	GF305 or Elberta seedling, or *P. tomentosa* (3/-/2y) (infected leaves are twisted, deformed and show vein yellowing. Severe isolates can induce necrosis and stunting of the whole plant). GF 31 (1/-/1y) (rusty brown corking and cracking of the bark) Ersinger (3/-/2y) (typical ringspots on leaves)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (infected leaves are twisted, deformed and show vein yellowing. Severe isolates can induce necrosis and stunting of the whole plant/ les feuilles infectées sont tordues, déformées et présentent une jaunisse des nervures. Les isolats graves peuvent induire une nécrose et un rabougrissement de la plante entière)
Herbaceous tests	*Chenopodium foetidum*, *N. clevelandii*
Serological or molecular tests	ELISA and/et PCR. Due to the erratic distribution of the virus in infected trees, several samples from one tree should be tested
Natural transmission	Aphids

PDV

Woody tests (field)	Bing (3/-/2y) (chlorotic spots and rings)
	Shirofugen (5/-/6-52w) (necrotic tissue and gummosis around source bud, inserted in 1 year-old shoots)
	GF305 seedling/issu de semence (3/-/2y) (infected leaves are smaller. The plant is stunted and internodes are reduced).
Woody tests (glasshouse)	GF305 seedling/issu de semence (5/20/12w) (infected leaves are smaller. The plant is stunted and internodes are reduced).
Herbaceous tests	*C. sativus, C. maxima*
Serological or molecular tests	ELISA, PCR
Natural transmission	Pollen, seeds

PNRSV

Woody tests (field)	Bing (3/-/2y) (chlorotic spots and rings on leaves; leaf-like enations between the veins near the leaf margins)
	Shirofugen (5/-/6-52w) (necrotic tissue and gummosis around source bud, inserted in 1 year-old shoots)
	GF305 seedling (3/-/2y) (necrotic irregular areas on infected leaves; shoot necrosis)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (infected leaves are smaller. The plant is stunted and internodes are reduced).
Herbaceous tests	*C. quinoa, C. sativus, C. maxima*
Serological or molecular tests	ELISA, PCR
Natural transmission	Pollen, seeds

SLRSV

Woody tests (field)	GF305 or Elberta seedling (3/-/2y) (stunting of the plant, short internodes and rosetting)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (stunting of the plant, short internodes and rosetting)
Herbaceous tests	*C. quinoa, C. amaranticolor, C. sativus*
Serological or molecular tests	ELISA
Natural transmission	*Xiphinema diversicaudatum*

TBRV

Woody tests (field)	GF305 or Elberta seedling (3/-/2y) (stunting of the plant, short internodes and rosetting)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (stunting of the plant, short internodes and rosetting)
Herbaceous tests	*C. quinoa, C. amaranticolor, C. sativus*
Serological or molecular tests	ELISA
Natural transmission	*Longidorus attenuatus, L. elongatus*

Table 3 **Methods for detection of viroids in almond, apricot, peach and plum**

PLMVd	
Woody tests (field)	-
Woody tests (glasshouse)	GF305 seedling. Latent strains can be detected by cross-protection. GF305 are inoculated by chip-budding and 2 months later, reinoculated by budding with a severe strain able to produce the foliar mosaic. Absence of the characteristic symptoms of the severe strain on the indicator plant demonstrates the presence of a latent strain (Desvignes, 1976).
Serological or molecular tests	PCR, hybridization
Natural transmission	Unknown

Table 4 **Methods for detection of phytoplasmas in almond, apricot, peach and plum**

European stone fruit yellows phytoplasma	
Woody tests (field)	GF305 seedling, Luizet. Grafting should be performed during the summer (general decline of the plant; yellowing and rolling of leaves)
Woody tests (glasshouse)	GF305 seedling, Luizet (5/20/12w) Grafting should be performed during the summer (general decline of the plant; yellowing and rolling of leaves)
Serological or molecular tests	PCR
Microscopy	DAPI
Natural transmission	*Cacopsylla pruni*

Table 5 **Methods for detection of virus-like diseases in almond, apricot, peach and plum**

Peach asteroid spot agent	
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (sooty ringspots after 4 months)
Serological or molecular tests	None
Natural transmission	Unknown

Table 6 **Nematode vectors of viruses of almond, apricot, peach and plum**

Nematode vector	**Viruses**
Xiphinema diversicaudatum	*Strawberry latent ringspot nepovirus*
Longidorus attenuatus	*Tomato black ring nepovirus*
Longidorus elongatus	*Tomato black ring nepovirus*

Fig. 1 Diagram of the stages in the certification scheme for almond, apricot, peach and plum: scion material.

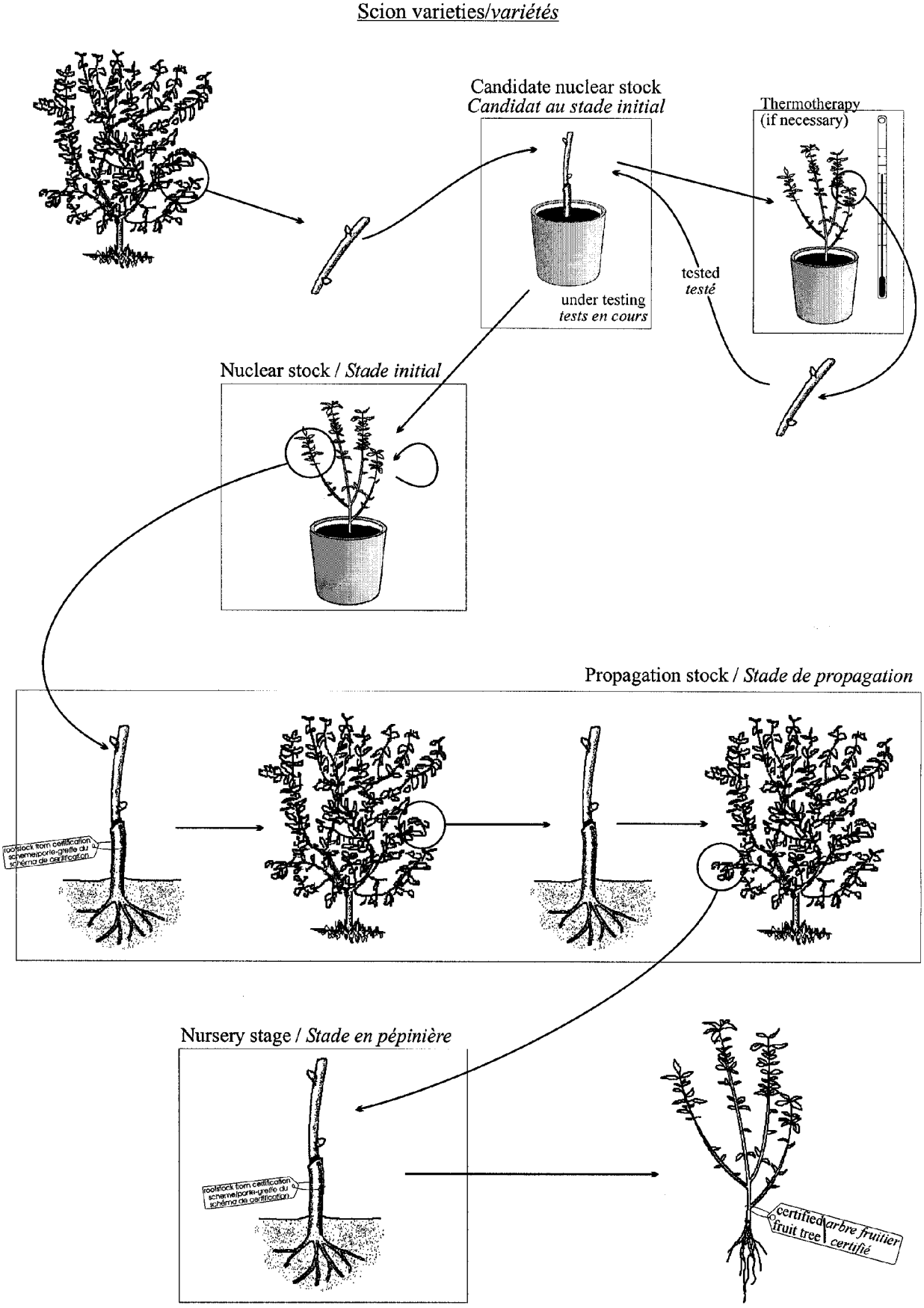

Fig. 2 Diagram of the stages in the certification scheme for almond, apricot, peach and plum: rootstocks.