◆ EPPO Standards ◆

SCHEMES FOR THE PRODUCTION OF HEALTHY PLANTS FOR PLANTING

CERTIFICATION SCHEME FOR CHERRY

PM 4/29(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 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 4/29(1) English

Schemes for the production of healthy plants for planting

CERTIFICATION SCHEME FOR CHERRY

Specific scope

This standard describes the production of pathogen-tested material of *Prunus avium*, *Prunus cerasus* 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.

This certification scheme for pathogen-tested material of sweet cherry (*Prunus avium*), sour cherry (*Prunus cerasus*) and their rootstocks (*P. avium, P. cerasus, Prunus mahaleb* and interspecific hybrids) 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 flowering cherry species.

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 by 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 cherry varieties and rootstocks, 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 (production 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 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, 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 in Table 1 by the methods outlined 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 virus possibly 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, aphid-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 in Table 1 by the methods outlined 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 their descendants) 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*, *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. Nuclear-stock plants may also be maintained in the open, where they should be separated by approximately 1 km from any cultivated or wild *Prunus* spp. of subgenus *Cerasus* 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 (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. 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.

[1] Exceptionally, seeds may be collected from a wild tree of *Prunus avium,* 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).

The plants should be inspected visually for possible mutations. 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 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 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 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 2 years.

Testing on 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. Tests on herbaceous indicators should be conducted in a glasshouse with heating and cooling facilities (temperature range 18-25°C). At least five plants from each indicator should be used.

ELISA testing

The ELISA method allows large-scale testing for fruit-tree viruses for which polyclonal and/or monoclonal antisera are available. However, as for any antibody technique, the method is subject to certain limitations, e.g. viruses at very low concentrations in the tree, irregularly distributed or seasonally undetectable.

PCR

The polymerase chain reaction (PCR) can be used for the detection of some viruses or virus-like diseases. Serological and molecular tests can be combined to increase the sensitivity of each method on its own, 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 three to five seeds (including integument and embryo) are combined and extracted by standard ELISA techniques. If any ELISA test is positive, the lot is rejected.

Appendix II Guidelines on disease detection

The methods for disease detection are specified in Tables 2 and 3 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: use of ELISA, RT-PCR, IC-RT-PCR.

For the woody tests, the indicators are listed, followed by figures in brackets representing number of replicates, temperature in °C (for glasshouse testing), duration of test (d, days; w, weeks; y, years; c, fruit cropping years) and, finally, a short description of the symptoms. In general, testing on woody indicators is always needed to establish virus freedom for nuclear stock, so a test on woody indicators is always specified. Tests on herbaceous indicators, serological tests or RT-PCR 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. 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 is the widely used method for elimination of pathogens from cherry varieties and rootstocks. There is little experience with use of meristem culture or shoot-tip grafting (see EPPO Standard PM 4/30). 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).

Heat treatment in vitro

Cherry varieties which are difficult to heat treat can normally receive a longer heat treatment at moderately high temperature (34-38°C) as micropropagated shoots.

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 4. 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 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 that of Flegg (1967), which requires little special equipment: mix the soil sample carefully but thoroughly and measure two sub-samples of 200 mL by displacement of water. Each subsample is left to soak in water for at least 1 h, then washed through a 4-mm-pore sieve into a 10-L bucket, which is filled nearly to the brim. The contents of the bucket are stirred with the hand in order to put the soil into suspension, left for 25 s, then the supernatant is decanted onto a set of three sieves of 150-µm pore size. The bucket is refilled and the stirring and decanting repeated (after leaving for only 15 s). The debris collected on the sieves is washed and transferred to a 110-µm-pore nylon sieve. The sieve is placed on a glass funnel with just enough water to submerge the debris on the sieve surface, left for 24 h, then about 25 mL is collected 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 cherry rootstocks is practised routinely in several European laboratories for rapid and intensive multiplication of planting material. For cherry, *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 with *P. avium, P. cerasus* and *P. mahaleb*. Numerous interspecific hybrids (e.g. Colt, Maxma 14) 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 sub-culturing. Only axillary shoots should be taken as explant, and callus formation should be limited, to avoid genetic drift by somoclonal 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 cryoprotecting 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.

References

Anon (1970) *La Thermothérapie des Espèces Ligneuses*. Station de cultures fruitières et maraîchères, Gembloux (BE).

Anon (1998) International Working Group on fruit-tree viruses – ISHS. Detection of virus and virus-like diseases of fruit trees: laboratory assays, bioassays and indicators. *Acta Horticulturae* No. 472, 761-783.

Flegg JJM (1967) Extraction of *Xiphinema* and *Longidorus* spp. from soil by a modification of Cobb's decanting and sieving technique. *Annals of Applied Biology* **60**, 429-437.

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).

Table 1 Viruses and other pathogens of cherry varieties and rootstocks (*Prunus avium, P. cerasus, P. mahaleb* **and interspecific hybrids) occurring in the EPPO region and requiring testing in the certification scheme**

Virus	**Acronym**
Apple chlorotic leaf spot trichovirus	ACLSV
Apple mosaic ilarvirus	ApMV
Arabis mosaic nepovirus	ArMV
Petunia asteroid mosaic tombusvirus and *Carnation Italian ringspot tombusvirus*, causing cherry detrimental canker	PAMV, CIRV
Cherry green ring mottle foveavirus	CGRMV
Cherry leaf roll nepovirus	CLRV
Little cherry closteroviruses 1 and *2*	LChV-1, LChV-2
Cherry mottle leaf trichovirus	ChMLV
Prune dwarf ilarvirus	PDV
Prunus necrotic ringspot ilarvirus	PNRSV
Raspberry ringspot nepovirus	RpRSV
Strawberry latent ringspot nepovirus	SLRSV
Tomato black ring nepovirus	TBRV
Virus-like diseases	
Necrotic rusty mottle	
Rusty mottle (European)	

Table 2 **Methods for detection of viruses of cherry**

ACLSV	
Woody tests (field)	GF305 seedling (or Elberta) (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 seedling (3/-/2y) (infected leaves show light green, yellowish green or bright yellow rings, spots, bands or oak-leaf patterns)
Woody tests (glasshouse)	GF305 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, PCR
Natural transmission	Unknown
ArMV	
Woody tests (field)	Bing (3/-/2y) (enations, leafy outgrowth on the lower surface of the leaves)
	GF305 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*

Detrimental canker (caused by tombusviruses such as PAMV and CIRV)

Woody tests (field)	Bing, Sam (3/-/2y) (necrosis of midribs and main vein of leaves, sharp twist of leaf blade, shoot necrosis leading to rectangular bending)
Woody tests (glasshouse)	-
Herbaceous tests	*C. quinoa, C. amaranticolor, C. Sativus*
Serological or molecular tests	ELISA
Natural transmission	Unknown

CGRMV

Woody tests (field)	Kwanzan, Shirofugen (3/-/2y) (epinasty of the foliage, necrotic midribs or lateral veins, twisting and curling of infected leaves. Bark often roughened by development of longitudinal fissures)
Woody tests (glasshouse)	-
Herbaceous tests	Impossible, at present
Serological or molecular tests	PCR
Natural transmission	Unknown

CLRV

Woody tests (field)	Bing (3/-/2y) (chlorotic rings on leaf, rosetting) GF305 seedling (3/-/2y) (stunting of the plant, short internodes and rosetting, slight leaf rolling)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (stunting of the plant, short internodes and rosetting, slight leaf rolling)
Herbaceous tests	*C. quinoa, Nicotiana* spp., *C. sativus*
Serological or molecular tests	ELISA
Natural transmission	Transmission by nematodes doubtful

LChV-1 and LChV-2

Woody tests (field)	Sam, Canindex (3/-/2y) (red colouring or bronzing of the leaves from the end of August, tissues along midrib and main veins remain green)
Woody tests (glasshouse)	-
Herbaceous tests	-
Serological or molecular tests	PCR
Natural transmission	Unknown

CMLV

Woody tests (field)	Sam, Bing (3/-/2y) (irregular chlorotic mottle and distortion of the leaves)
Woody tests (glasshouse)	GF305 seedling/issu de semence (5/20/12w) (severe strains cause yellowish mottle along the leaf edges. Latent strains can be detected by cross protection. Absence of symptoms on indicators inoculated with the severe strain demonstrates the presence of a latent CMLV strain)
Herbaceous tests	*C. quinoa, C. amaranticolor, Nicotiana occidentalis*
Serological or molecular tests	ELISA (polyclonal and monoclonal antibodies), PCR[2]
Natural transmission	*Eriophyes inaequalis*

[2] According to present European experience, these methods only give a positive result for North American isolates.

PDV

Woody tests (field)	Bing (3/-/2y) (chlorotic spots and rings on leaves; in the first year, enations along the midrib)
	Shirofugen (5/-/16-52w) (necrotic tissues and gummosis around source bud, inserted in 1-year-old shoots)
Woody tests (glasshouse)	GF305 seedling/issu de semence (5/20/12w) (infected leaves smaller; plant stunted and internodes reduced)
	Shirofugen (5/22-26/8w)(necrotic tissues and gummosis around source bud, inserted in 1-year-old shoots)
Herbaceous tests	*C. sativus, C. maxima*
Serological or molecular tests	ELISA, PCR
Natural transmission	Pollen, seed

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/-/16-52w) (necrotic tissues and gummosis around source bud, inserted in 1-year-old shoots)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (necrotic irregular areas on infected leaves; shoot necrosis)
	Shirofugen (5/22-26/8w)(necrotic tissues and gummosis around source bud, inserted in 1-year-old shoots)
Herbaceous tests	*C. quinoa*, *C. sativus, C. maxima*
Serological or molecular tests	ELISA, PCR
Natural transmission	Pollen, seed

RpRSV (causing Pfeffinger disease)

Woody tests (field)	Bing (3/-/2y) (yellowish green oil flecks, leaves distorted with deep sinuses. Secondary symptoms include rasp leaves, narrow, stiff and brittle)
Woody tests (glasshouse)	GF305 seedling (5/20/12w) (yellow chlorotic mottle along the midrib, wavy and narrow leaves)
Herbaceous tests	*C. quinoa*, *C. amaranticolor*
Serological or molecular tests	ELISA, PCR
Natural transmission	Seed, nematodes (*Longidorus macrosoma* and *L. elongatus*)

SLRSV

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

TBRV

Woody tests (field)	GF 305 seedling, Elberta, Bing (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. sativus, C. amaranticolor*
Serological or molecular tests	ELISA
Natural transmission	*Longidorus elongatus, L. attenuatus*

Table 3. **Methods for detection of virus-like diseases of cherry**

Cherry necrotic rusty mottle	
Woody tests (field)	Sam (3/-/2y) (yellowing of the young leaves, small line pattern and ringspots followed by necrosis)
Woody tests (glasshouse)	-
Herbaceous tests	-
Serological or molecular tests	PCR with CGRMV related primers
Natural transmission	Unknown
Cherry rusty mottle (European)	
Woody tests (field)	Sam, Bing (3/-/2y) (affected leaves gradually develop a pale green colour. By the end of August rusty red or bronzing mottling appears)
Woody tests (glasshouse)	-
Herbaceous tests	Impossible, at present
Serological or molecular tests	PCR with CGRMV-related primers
Natural transmission	Unknown

Table 4. **Nematode vectors of cherry viruses**

Nematode vector	Viruses
Xiphinema diversicaudatum	ArMV, SLRSV
Longidorus macrosoma	RpRSV
Longidorus attenuatus	TBRV
Longidorus elongatus	TBRV, RpRSV

Fig. 1 Diagram of the stages in the certification scheme for cherry: scion material

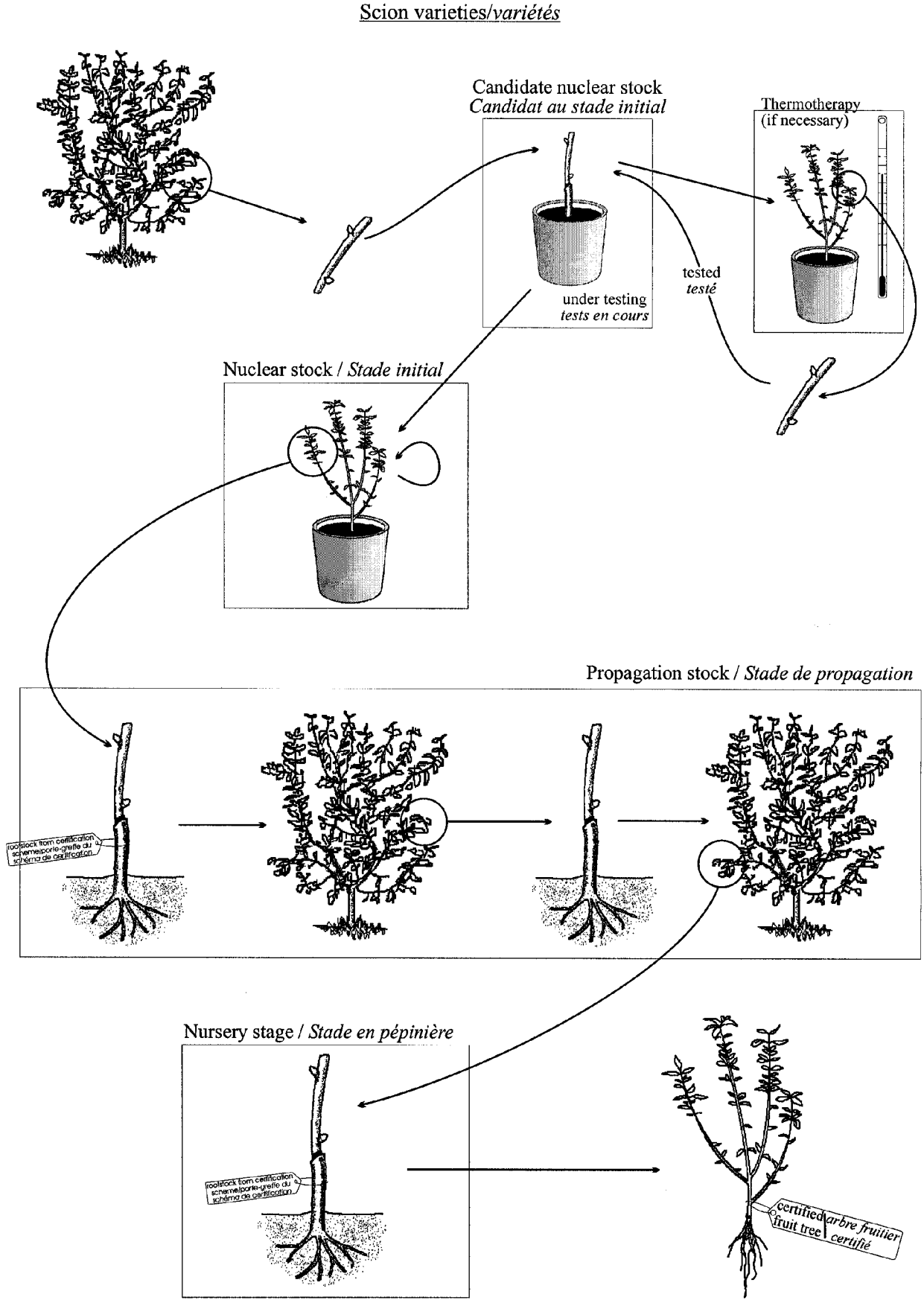

Fig. 2 Diagram of the stages in the certification scheme for cherry: rootstocks