Schemes for the production of healthy plants for planting
Schémas pour la production de végétaux sains destinés à la plantation

Certification scheme for hazelnut

Specific scope
This standard describes the production of pathogen-tested material of hazelnut (*Corylus avellana*).

Specific approval and amendment
Approved in 2003-09.

Introduction

The certification scheme for pathogen-tested material of hazelnut (*Corylus avellana*) provides detailed guidance on the production of propagated varieties to be grown on their own roots. Plant material produced according to this certification scheme is derived from nuclear-stock plants that have been tested and found free from the following pathogens: *Apple mosaic ilarvirus* (ApMV), *Prunus necrotic ringspot ilarvirus* (PNRSV) and *Hazelnut maculatura lineare phytoplasma* (HML phytoplasma), and produced under conditions minimizing infestation by other pests.

Certified hazelnut 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 the following successive steps should be taken.

1 Selection for pomological quality: individual plants of each variety to be taken into the scheme are selected.
2 Production of nuclear stock: candidate nuclear-stock plants are propagated by layering or by suckers. The candidate plants are kept isolated from the nuclear stock. The candidate nuclear stock is tested. 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, with retesting as appropriate. The plants should be grown in containers of sterilized growing medium, isolated from the soil.
4 Production of propagation stock: propagation stock is produced from nuclear-stock material under conditions ensuring freedom from infection.
5 Production of certified plants: certified plants are produced from the propagation stock as one-year-old rooted stems after one or two stages of propagation.

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. The scheme is represented diagrammatically in Fig. 1.

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). 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 candidate nuclear stock

A number of productive plants, with the typical characters (trueness to type) of each variety to be taken into the scheme, should be selected in orchards and/or from pomological field trials. Plants with no apparent symptoms should be selected. Varieties are propagated on their own roots and there is therefore no production of rootstocks.

Alternatively, starting material may be imported from other countries. Material imported from outside the EPPO region should be free from all viruses or phytoplasmas occurring

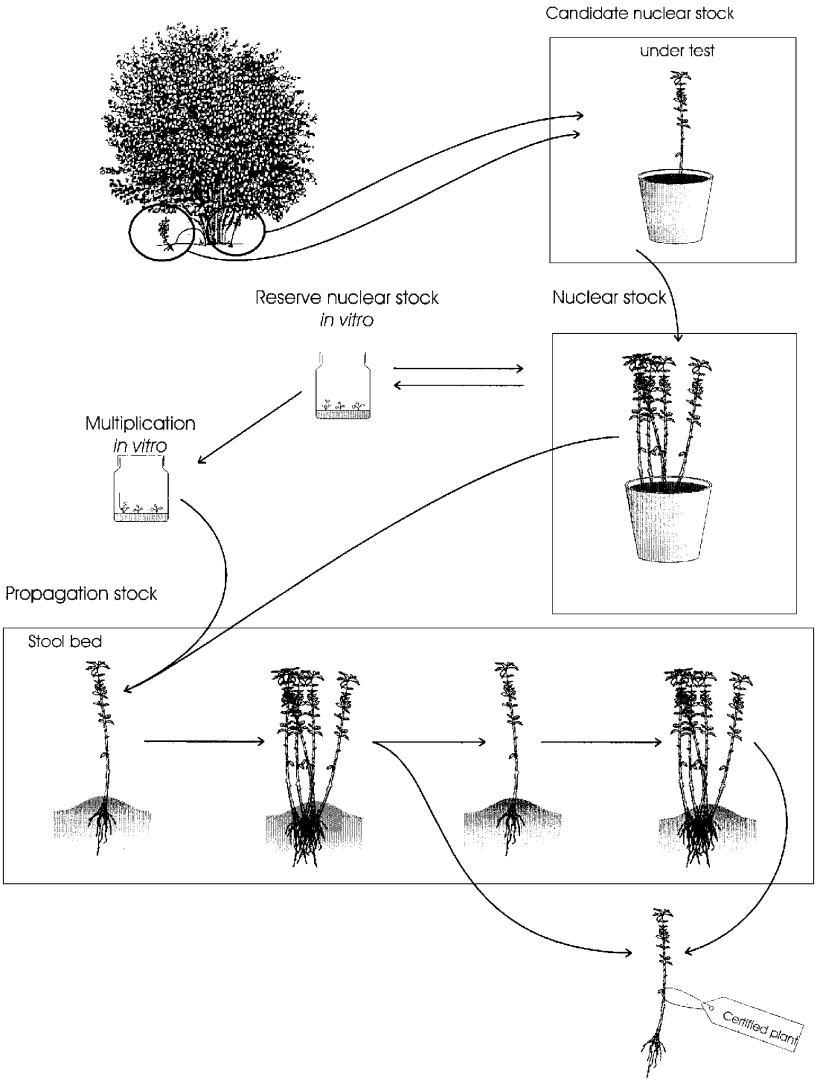

Fig. 1 Diagram of the stages in the certification scheme for hazelnut.

naturally in the genus *Corylus* in the region of origin, and from any other regulated pests.

2. Maintenance and testing of candidate nuclear stock

2.1 Growing conditions

The pomologically selected plants are propagated by layering or by suckers. Greater success in propagation can be achieved under glasshouse conditions. The plants obtained (candidate nuclear-stock plants) should, during the period of testing, be kept in an isolated, suitably designed, insect-proof house separately from the nuclear stock. They should be grown in sterilized growing medium in containers isolated from the soil. Adequate control of pests should be ensured, in particular *Mikomyia coryli*, *Cryptosporiopsis coryli*, *Chondrostereum purpureum*, *Nectria galligena*, *Phyllactinia guttata*.

2.2 Testing requirements

The individual candidate nuclear-stock plants should be tested for *Apple mosaic ilarvirus* (ApMV), *Prunus necrotic ringspot ilarvirus* (PNRSV) and *Hazelnut maculatura lineare phytoplasma* (HML phytoplasma) (Appendix I) by the methods of Appendix II. The plants should be visually inspected for *Phytoptus avellanae*, *Xanthomonas arboricola* pv. *corylina*, *Pseudomonas avellanae*, *Armillariella mellea* and *Verticillum* spp. Any plant found to be infected, by testing or by inspection, should be recorded and immediately removed.

2.3 Promotion to nuclear stock

Plants that give negative results in all tests and inspections can be promoted to nuclear stock and transferred to the nuclear-stock collection. There is no experience with thermotherapy or other sanitation techniques for hazel so production of nuclear

stock relies on the selection of virus-free material. Treatment of plants which gave a positive result at testing may be a possibility for the future.

3. Maintenance of the nuclear stock

3.1 Growing conditions

The nuclear-stock plants should be maintained in a suitably designed insect-proof house under conditions ensuring freedom from (re)infection. Each plant should be grown in containers of sterilized growing medium, isolated from the soil. Some material may be stored *in vitro* (Appendix IV) as a reserve stock, but will need to be checked for agronomic characters, in particular trueness to type, after leaving the *in vitro* conditions. Flowering of the nuclear-stock plants should be prevented to minimize virus infection. Trueness to type can be verified by observing fruiting on plants propagated from the nuclear stock which should be kept in a different place from the nuclear stock. General precautions against infection should be taken, and adequate control of other pests should be ensured.

3.2 Testing requirements

Because of the possibility of re-infection, each nuclear-stock plant should be regularly re-tested for ApMV, PNRSV and HML phytoplasma. In the absence of detailed information on re-infection of hazel, the suggestion for frequency of re-testing is every 5 years. Nuclear-stock plants should also be regularly inspected for viruses, virus-like symptoms, *Phytoptus avellanae*, *Xanthomonas arboricola* pv. *corylina*, *Pseudomonas avellanae*, *Armillariella mellea* and *Verticillum* spp. Any plant found to be infected, by testing or inspection, should be immediately recorded and removed.

3.3 Certification

Before a nuclear-stock plant may be propagated further in the certification scheme, the passage to the next stage should be authorized by the official organization on the basis of records of the tests and observations performed during production, and of one or more certification (visual) inspections. Recommended certification standards are given in Appendix III.

4. Propagation stock

4.1 Growing conditions

The propagation stock is produced from nuclear stock by stooling. It should be planted in stool beds in which no symptoms of *Agrobacterium tumefaciens* have been observed in the previous 5 years. If necessary for obtaining a sufficient quantity of certified plants, a second generation of stool beds may be planted. It is advisable not to keep the stool beds for more than 15 years, due to the increasing risk of re-infection. Multiplication *in vitro* may also be used (Appendix IV), but care should be taken to limit the number of propagation steps and to prevent the formation of callus. General precautions against infection should be maintained, and appropriate control measures should be taken if any pests are observed.

4.2 Testing requirements

The propagation stock should be visually inspected each year for viruses, virus-like symptoms, *Phytoptus avellanae*, *Xanthomonas arboricola* pv. *corylina*, *Pseudomonas avellanae*, *Armillariella mellea* and *Verticillum* spp. Any plant found to be infected should be immediately recorded and removed.

4.3 Certification

Certification of propagation stock should be granted on the basis of records of the tests and observations performed during production, and of a certification (visual) inspection. Recommended certification standards for propagation stock are given in Appendix III.

5. Production of certified plants

Certified plants to be sold to growers are taken directly from the first or second generation of propagation stock as one-year old rooted stems.

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 plants 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 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

Viruses and phytoplasmas of hazelnut tested for in the certification scheme

Apple mosaic ilarvirus
ApMV is the most important of the pathogens on hazelnut. It causes mosaic on the leaves and may have an effect on yield and vigour. It should be tested for by ELISA or RT-PCR.

Prunus necrotic ringspot ilarvirus
PNRSV produce few symptoms on its own, but it has a synergetic effect when present with ApMV. It is transmitted by pollen. It should be detected by ELISA or RT-PCR.

Hazelnut maculatura lineare phytoplasma
HML phytoplasma causes elongated spots on the leaves. It has mainly been observed in Italy, with a low importance. However, its vector is not known and it should be kept out of propagation.

Appendix II

Guidelines on testing procedures

Testing for viruses
ELISA can be used to detect ApMV and PNRSV. Polyclonal antibodies should be used. The test should be performed on young growing leaves. Samples can be prepared following a standard method. All stages of the ELISA test should be performed according to the published procedures or by following the instructions accompanying the proprietary reagents.

The polymerase chain reaction (PCR) can be used for the detection of ApMV and PNRSV. Serological and molecular tests can be combined to increase the sensitivity of each method on its own, e.g. immunocapture PCR (IC-RT-PCR).

Testing for phytoplasmas

PCR can be used to test for HML phytoplasma and other phytoplasmas using universal primers. 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 III

Recommended certification standards

Certification will be granted on the basis of records of the tests and observations performed during production and of one or more certification (visual) inspections. In general, certification inspection is done on the plants from which the corresponding category of material will be taken. The assessor should verify that the standards mentioned below are fulfilled.

Candidate nuclear stock

Records should show that the candidate nuclear-stock plant was negative for ApMV, PRNSV and HML phytoplasma in the tests performed, and that any plant showing symptoms of viruses, virus-like diseases, *Phytoptus avellanae*, *Xanthomonas arboricola* pv. *corylina*, *Pseudomonas avellanae*, *Armillariella mellea* and *Verticillum* spp. was removed. The plant should show no symptom of pest attack. If these conditions are not met at the time of the certification inspection, certification will be refused to the plant concerned.

Nuclear stock

Records should show that all tests on the nuclear-stock plant were negative for ApMV, PNRSV and HML phytoplasma, and that no symptoms of viruses, virus-like diseases, *Phytoptus avellanae*, *Xanthomonas arboricola* pv. *corylina*, *Pseudomonas avellanae*, *Armillariella mellea* or *Vertillicium* spp. were observed. If these conditions are not met at the time of certification inspection, certification will be refused to the plant concerned.

Propagation stock

Results should show that any plant showing symptoms of viruses, virus-like diseases, *Phytoptus avellanae*, *Xanthomonas arboricola* pv. *corylina*, *Pseudomonas avellanae*, *Armillariella mellea* and *Verticillum* spp. was removed. Visual inspection at certification should show absence of symptoms of viruses and virus-like diseases in each stoolbed. If these conditions are not met at the time of certification inspection, certification will be refused to the whole stoolbed concerned.

Appendix IV

In vitro maintenance and multiplication of hazelnut

Hazelnut can be maintained and micropropagated *in vitro* using the medium of Quoirin *et al*. (1977). Al Kaï *et al*. (1984) give a description for micropropagation of this species: The multiplication should be done with explants taken from plants grown in the glasshouse. Explants are axillary buds with 1–2 cm stem taken on non-lignified parts of growing stems. For disinfection, the material is place in 70° alcohol for 30–60 s, then dipped in $HgCl_2$ at 1 gL^{-1} (with a few drops of Tween-20) for 10 min. It is then washed, first in a solution of $CaCl_2$ (2.5 gL^{-1}) and then three times in sterile distilled water.

After growth has started on M & S medium (Murashige & Skoog, 1962) diluted by half, the following multiplication and elongation medium is used: modified M & S medium containing Fe EDDHA (200 mgL^{-1}), Zuccherelli vitamin mix, gibberellic acid (0.1 mgL^{-1}), naphthalene acetic acid (0.01 mgL^{-1}) and benzylaminopurine (5 mgL^{-1}), with pH adjusted to 5.5. The medium

should then be sterilized at 115 °C for 20 min. Note that using Fe in its EDDHA form (instead of EDTA as for some other plants) is specific to *in vitro* multiplication of hazelnut in order to avoid specific problems arising with this species. For rooting, M & S medium containing Zuccherelli vitamin mix, indole butyric acid (0.1 mgL^{-1}) and Fe EDDTA (200 mgL^{-1}) is used and has given 90% success. The plants are then transferred to a glasshouse at 25 ± 2 °C on sterilized compost, and under plastic cover to maintain high relative humidity for a few days.

References

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