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

Certification scheme for hop

Specific scope

This standard describes the production of certified pathogen-tested material of hop.

Specific approval and amendment

First approved in 1996–09.
Revised in 2009–09.

The certification scheme for pathogen-tested material of hop (*Humulus lupulus*) provides detailed guidance on the production of vegetatively propagated hop plants. The certification scheme has the aim of providing plants which are true-to-type, free from virus diseases and substantially free from other pests. Plant material produced according to this certification scheme is derived from nuclear stock plants that have been tested and found free from specific pathogens, and produced under conditions minimizing infestation by other 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).

Hop is dioecious, plants being either male or female. Hops for brewing may be produced either seeded or seedless (in which case male plants are not grown). Vegetative propagation may be by: (a) softwood cuttings (from current season's shoots, rooted under mist in propagation houses); (b) strap cuts (shoots with roots and buds cut from the base of the dormant parent plant); (c) cuttings from layered shoots (sections of shoots buried over winter which are lined out for rooting in the nursery); (d) micropropagation from meristems or shoot tips. Most commercial propagation is by softwood cuttings rooted under mist and then grown on, either under protection or in nursery beds outdoors, to produce sets (young plants with thick vigorous roots) for sale as planting material the following autumn.

Outline of the scheme

For the production of certified pathogen-tested hop plants, the following successive steps should be taken:

1. Selection for brewing quality: individual plants of each cultivar to be taken into the scheme are selected.
2. Production of nuclear stock: candidate nuclear stock plants are tested, or submitted to heat or cold treatment or meristem tip culture, followed by testing for the viruses listed in Table 1 (Part A). Only candidate nuclear stock plants that have met all requirements are promoted to nuclear stock plants.
3. Maintenance of the nuclear stock: nuclear stock plants are maintained under conditions ensuring freedom from infection via pollen, aerial or soil vectors, with re-testing as appropriate.
4. Production of propagation stock: propagation stock is produced from nuclear stock material in one or more phases (propagation stock), under conditions ensuring freedom from infection, with retesting as appropriate.
5. Production of certified material: cuttings taken from propagation stock are grown under conditions minimizing infections to produce certified plants.

Throughout the whole procedure, care should be taken to maintain the desired 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 (OEPP/EPPO, 1993) 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.

Selection of material

Selection should be done in several different plantations of each cultivar. Such plantations should be free from any symptoms of verticillium wilt (*Verticillium albo-atrum, V. dahliae*) or fusarium canker (*Gibberella pulicaris*, anamorph *Fusarium sambucinum*) and there should be no history of verticillium wilt in the vicinity. Select several vigorous, productive plants which show typical

Table 1 Hop pathogens occurring in the EPPO region

Pest	Geographical distribution	Transmission
(A) Pathogens subject to testing during production of nuclear stock*		
Apple mosaic virus (*Ilarvirus*, ApMV) [serotype I (intermediate) and serotype H (hop)]	Worldwide	Mechanical
Arabis mosaic virus (*Nepovirus*, ArMV)	Europe, Japan, New Zealand (isolated records elsewhere)	*Xiphinema diversicaudatum*
Hop mosaic virus (*Carlavirus*, HMV)†	Worldwide	Aphids
Hop latent viroid (*Cocadviroid*, HLVd)	Worldwide	Mechanical
Verticillium albo-atrum	Worldwide	
Verticillium dahliae	Worldwide	
(B) Other pathogens of lesser importance or occurring only rarely in hops and which may optionally be tested		
Cherry leaf roll virus (*Nepovirus*, CLRV)	Europe, North America (isolated records elsewhere)	Seed, pollen
Hop latent virus (*Carlavirus*, HLV)	Europe, North America, Australia (isolated records elsewhere)	Aphids
Cucumber mosaic virus (*Cucumovirus*, CMV)	Worldwide	Aphids
Petunia asteroid mosaic virus (*Tombusvirus*, PAMV)	Europe (isolated records elsewhere)	Vector unknown
Tobacco necrosis virus (*Necrovirus*, TNV)	Worldwide	*Olpidium brassicae* (zoospores)
Gibberella pulicaris	Worldwide	

*It is assumed that hop material imported into the EPPO region from elsewhere will have been tested for pests which do not occur in hop in the EPPO region, especially *American hop latent virus* (*Carlavirus*) and *Hop stunt viroid* (*Hostuviroid*).
†HMV is only of importance for Golding cvs and has no significant effect on HMV-tolerant cultivars, therefore testing in tolerant cultivars is optional.

characteristics of the particular cultivar desired and which show no symptoms of serious pests or graft-transmissible disorders. Alternatively, certified starting material may be obtained from other countries.

Production of nuclear stock

General procedure

Softwood nodal cuttings are rooted under mist or fog, and the plants grown on (candidate nuclear stock) in large pots separately from the nuclear stock. For the production of Golding cvs, candidate nuclear stock plants should be kept in an insect-proof house, to avoid infection by the aphid-transmitted virus, *Hop mosaic virus*. The plants should be grown in sterilized growing medium in containers isolated from the soil to avoid any type of contamination. When large enough, the plants should be tested for the pathogens specified in Table 1 (Part A) by the methods given in Appendix 1.

Plants which give a negative result for all tests can be promoted to nuclear stock and transferred to the nuclear stock holding, or nuclear stock material may be propagated from them. The resulting nuclear stock plants are the parent plants used for further propagation.

Elimination of pathogens

If all candidate nuclear stock plants of a cultivar give positive test results, thermotherapy and/or meristem tip propagation can be used as sanitation procedures (Appendix 2). The candidate plants should then be re-tested for freedom from all pathogens specified in Table 1 (Part A).

Inspection for other pests

Visual inspections of candidate nuclear stock plants for other pests should be done carefully, particularly for downy mildew (*Pseudoperonospora humuli*), which can cause systemic infection, and powdery mildew (*Podosphaera macularis*). An appropriate and effective biological and/or chemical plant protection programme should be operated in all propagation units.

Maintenance of nuclear stock

The nuclear stock plants should be maintained in a repository under conditions excluding pests. Plants should be grown in containers with sterilized growing medium, isolated from the soil and in a suitably designed house which is as insect-proof as possible.

In the nuclear stock repository each plant should be tested at least every 2 years (annually, if possible) for the pathogens listed in Table 1 (Part A), if present in the country. Several visual inspections should be made each year for these and other pests.

Production of propagation stock

The nuclear stock is multiplied in as few steps as possible to obtain the required quantity of propagation stock.

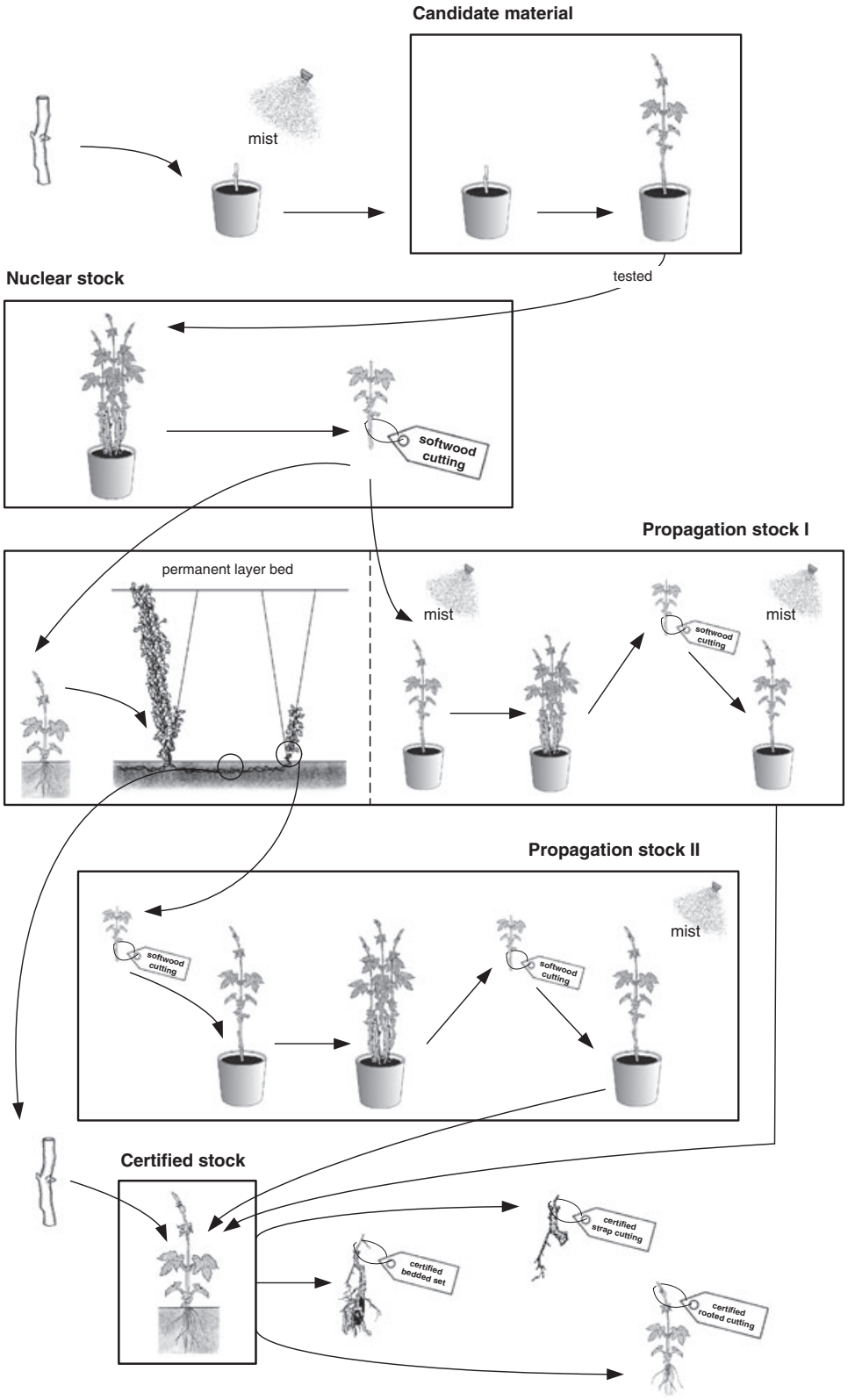

Fig. 1 Diagram of the stages in the hop certification scheme.

Table 2 Test methods for viruses and viroids of hop

Pathogen	ELISA	Test plants	Nucleic acid hybridization and PCR
ApMV (serotype I and H)	+	+	+
Arabis mosaic virus (ArMV)	+	+	
Hop mosaic virus (HMV)	+		+
Hop latent viroid (HLVd)			+
Cherry leaf roll virus (CLRV)	+	+	
Hop latent virus (HLV)	+		+
Hop stunt viroid (HSVd)			+
Cucumber mosaic virus (CMV)	+	+	
Petunia asteroid mosaic virus (PAMV)	+	+	
Tobacco necrosis virus (TNV)	+	+	

Note: Pathogen diagnosis based on PCR has undergone a rapid development over the past decade. This includes nucleic acid extraction technology from almost any plant tissue enabling subsequent enzymatic reactions. As a result, PCR detection is generally available for viruses whenever their genomes have been characterized. However, it should be kept in mind that PCR tests cannot be regarded reliable unless knowledge is available on the variability of individual pathogens and some experience has been gained on the specific crop. For the characterized viruses and viroids in hop, the situation for PCR detection is at different levels of development. Therefore, PCR detection is only mentioned when the Panel had knowledge, that the tests were of equal or superior quality to other recommended methods in Table 1. It can be expected that additional PCR tests will become available before the existing scheme may be updated.

Propagation stock I

A first generation of propagation stock is normally established as a 'permanent layer bed'. Plants produced from rooted cuttings taken from the nuclear stock are established outdoors in isolated plots tested and found free from *Xiphinema diversicaudatum*. In regions where *Verticillium albo-atrum* is known to occur, the plots should be situated at a distance from the infected area sufficient to avoid aerial contamination. Golding cvs should be kept separate from other cultivars (if possible, in an insect-proof house), to avoid contamination by hop mosaic carlavirus. These mother plants should be renewed after 10 years unless acquired infections or other faults require an earlier renewal. The soil should be re-tested before new plants are established. Alternatively, nuclear stock plants may be used directly to produce softwood cuttings which, when rooted under mist or fog, become propagation stock I.

Visual inspections of propagation stock I should be done regularly for symptoms of the pests listed in Table 1 and for the other pests in Table 3.

Propagation stock II

Plants produced from softwood cuttings taken from permanent layer-bed plants in the autumn are grown in containers under protection during the winter in suitably designed structures isolated from hop plants of lower certification status. The plants must be allowed to experience a cold period of not <6 weeks and to become completely dormant, otherwise they will not sprout satisfactorily the following season. In the spring, these plants yield softwood cuttings which are rooted under mist or fog.

Visual inspections of propagation stock II should be done regularly for symptoms of the pests listed in Table 1 and for the other pests in Table 3.

Stages 1–4 should only be carried out by registered specialized establishments, satisfying defined criteria (OEPP/EPPO, 1993).

Table 3 Certification standards for hop

	Plants at visual inspection (%)		
	Nuclear stock	Propagation stock I & II	Certified stock
Symptoms of virus diseases	0	0	0
Verticillium wilt, caused by:			
Verticillium albo-atrum	0	0	0
Verticillium dahliae	0	0	0
Pseudoperonospora humuli	0	0	1
Fusarium sambucinum	0	0	2
Other pests	Substantially free	Substantially free	Substantially free

Production of certified material

The rooted softwood cuttings from propagation stock II (or I, if these mother plants come directly from nuclear stock) are planted out in nursery beds to produce bedded sets for sale the next autumn. Rooted cuttings may also be sold direct from the propagation unit in early summer, if sufficiently vigorous. Alternatively, shoots from permanent layer-bed plants (propagation stock I) may be layered (buried in shallow furrows) in autumn and unearthed in spring to give cuttings. These cuttings are lined out in nursery beds where they root and grow to produce bedded sets for sale. This system gives slower multiplication of stock, but usually results in larger plants. Another possibility is to take strap cuts from mother plants grown from certified stock. These strap cuts are sold for immediate planting. The first system gives the most rapid propagation.

Visual inspections and spot checks should be made during the growing season for symptoms (see Appendix 3) of the pests listed in Table 3 and for other pests.

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 nurser-

ies 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 hop 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.

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Appendix 1

Test methods for viruses and viroids

The hop viruses and viroids listed in Table 1 can be detected by inoculation to test plants, ELISA, and molecular tests (Table 2).

Inoculation to indicator plants

Candidate nuclear stock material of hop should be tested by inoculation to *Chenopodium quinoa, C. amaranticolor* and cucumber (including polyethylene glycol, mol. wt 6000, at 20 g litre^{-1} in the extraction buffer). The tests are best done early in the growing season, as titres of *Apple mosaic virus* (ApMV) especially are greatly reduced in hot weather. Inoculation to test plants may also be used for maintenance of nuclear stock.

Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay testing may be used as an alternative to tests on indicator plants for maintenance of nuclear stock (Barbara *et al.*, 1978; Adams & Barbara, 1980, 1982). It is the only method to test for *Hop mosaic virus* (HMV) and *Hop latent virus* (HLV).

Molecular tests

Hop latent viroid (HLVd) can be detected by nucleic acid hybridization (Barbara *et al.*, 1990) or by RT-PCR tests (Hataya *et al.*, 1992; Nakahara *et al.*, 1999; Grudzin′ska & Solarska, 2004). RT-PCR enables more sensitive testing than hybridization (Knabel *et al.*, 1999). Testing is best done late in the growing season.

Appendix 2

Elimination of viruses from infected hop plants

Virus-free hop plants can be produced from material infected with HMV, HLV and ApMV by meristem culture (Adams, 1975; Kremheller *et al.*, 1989). Thermotherapy is necessary in addition to meristem culture for the elimination of some strains of ApMV. Freedom from ApMV can be achieved by growing the infected plants at 35–38°C for 4–10 days followed by excising shoot tips about 5 mm long. These can be rooted in potting compost without resorting to sterile culture procedures. It may be possible to obtain viroid-free plants from HLVd-infected material by cold treatment followed by meristem culture (Adams *et al.*, 1996).

Appendix 3

Recommended tolerances at growing-season inspection

All the plants in a lot, derived from a single plant of the previous certification stage, can remain in the scheme, provided that the level of infection does not exceed the tolerance levels given in Table 3 at visual inspection and provided that all plants showing symptoms of any disorder are removed. Higher grades of stock should normally be free from symptoms of the organisms named, and substantially free from other pests. Normally it is not possible to check young hop plants in the nursery for trueness to cultivar.