

◆ **EPPO Standards** ◆

CERTIFICATION SCHEMES

PATHOGEN-TESTED MATERIAL OF GRAPEVINE VARIETIES AND ROOTSTOCKS

PM 4/1-26 English



European and Mediterranean Plant Protection Organization
1, rue Le Nôtre, 75016 Paris, France

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 Certification and Classification Schemes are intended to be used by National Plant Protection Organizations or equivalent authorities, in their capacity as bodies responsible for the design of systems for production of healthy plants for planting, for the inspection of such plants proposed for phytosanitary 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* no. 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

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

Certified stock: Material which is produced from propagation stock under appropriate conditions.

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 from a defined parent plant.

Nuclear stock: Material individually tested by the most rigorous procedure in the scheme. Material propagated from nuclear stock may remain nuclear stock under appropriate conditions. All such material must be maintained at all times under strict conditions ensuring freedom from infection.

Propagation stock: Material derived from the multiplication of nuclear stock, under conditions ensuring freedom from infection. Pathogen freedom is checked by an appropriate procedure. Material derived from propagation stock under the same conditions remains propagation stock, but, according to the plant species concerned, a maximum number of generations of propagation may be fixed at this stage.

OUTLINE OF REQUIREMENTS

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

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

Certification scheme

PATHOGEN-TESTED MATERIAL OF GRAPEVINE VARIETIES AND ROOTSTOCKS

Specific scope

This standard describes the production of certified pathogen-tested material of grapevine varieties and rootstocks.

Specific approval and amendment

First approved in September 1993.
Edited as an EPPO Standard in 1998.

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). Certified grapevine 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.

Table of contents

Outline of the scheme

Definition of propagation categories

1. Selection for pomological and health quality
2. Maintenance of the candidate material
3. Production of nuclear stock plants
4. Maintenance of nuclear stock
5. Propagation stock
6. Distribution of propagation stock and production of certified stock
7. Control on the use and status of certified material
8. Certification and labelling

Appendix I - Guidelines on testing procedures

1. Testing on *Vitis* indicators
 - (a) Whip or cleft grafting in the field
 - (b) Chip-bud grafting
 - (c) Machine grafting
 - (d) Green grafting
2. Inoculation to herbaceous hosts
3. ELISA testing
4. Detection of individual diseases
 - Fanleaf
 - Grapevine degeneration (European nepoviruses)
 - Leafroll
 - Rugose wood
 - Enation

Fleck
Vein necrosis
Vein mosaic
Flavescence dorée
Bois noir and other European yellows diseases

Appendix II - Guidelines on sanitation procedures

1. Heat treatment
 - Hot-air treatment
 - Hot-water treatment
2. Shoot (meristem) tip culture *in vitro*

Tables

Table 1. Virus and virus-like diseases of grapevine occurring in the EPPO region and covered by the scheme

Table 2. Pathogens, transmissibility, geographical distribution and vectors for the diseases cited in Table 1

Table 3. Soil-borne (nematode) and aerial vectors of grapevine diseases recorded in EPPO countries

Table 4. Main indicators for virus and virus-like diseases of grapevine

References

Outline of the scheme

For the production of certified grapevine varieties and rootstocks, the following successive steps should be taken:

1. Selection for pomological and health quality of individual plants of each scion variety or rootstock type to be taken into the scheme.
2. Assessment of health status of visually selected plants by testing, or production of healthy plants (nuclear stock) by heat treatment and/or meristem-tip (shoot-tip) culture followed by testing.

3. Maintenance of the nuclear stock (including the plants resulting from selective testing) under conditions ensuring freedom from re-infection by aerial or soil vectors, with retesting as appropriate.

4. Multiplication of the nuclear stock in one phase (propagation stock), under conditions ensuring freedom from re-infection, with retesting as appropriate.

5. Distribution of propagation stock to nurseries.

6. Production of certified (virus-tested) stock by nurseries under strict official control, with random tests on virus status by the official organization as appropriate¹.

Points 1-3 are considered to be carried out by a government agency, an official organization or under strict official control; points 4 and 5 by or under the strict control of an official organization; point 6 under strict control only.

Definition of propagation categories

1. *Propagation stock*. Propagating materials or plants (for grapevine, commonly known as plants of basic category) directly derived from nuclear stock plants and grown in propagation blocks. They have the same health status as the original source and can only be delivered to nurseries that have the necessary qualifications.

2. *Certified stock*. Propagating materials or plants derived from mother vines established in commercial nurseries from propagation stock and delivered to the growers. The production of new mother vines from certified mother vines is forbidden. To do so, the nurseryman must obtain new propagation stock.

Trueness to type should be monitored and maintained for all categories. Propagation stock and certified stock categories assure, in addition, health status, as declared for individual scion variety, clone or rootstock type.

1. Selection for pomological and health quality

Select vines of each variety or rootstock according to procedures which ensure trueness to type and high pomological quality. An effort should be made to select vines with no apparent symptoms of, or the least affected by, infectious graft-transmissible diseases. However, according to world experience, freedom from viruses, virus-like agents and viroids is a very rare condition. Thus, selection for health status should generally be complemented by procedures for eliminating disease.

¹ Certified grapevine 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.

2. Maintenance of the candidate material

Collect cuttings from the selected vines and cold-store (e.g. at 2-4°C) until use. If conditions are favourable (e.g. sandy soils, low levels of infestation by phylloxera, *Viteus vitifolii*), force cuttings of scion varieties or rootstocks to root in a glasshouse and transplant rooted cuttings as such in the field. Alternatively, for scion varieties, graft buds or bud sticks from selected vines onto vegetatively propagated certified rootstocks or seedling rootstocks. Prior to use, rootstocks should be tested for freedom from the harmful diseases and pathogens specified in Tables 1-2. However, seedlings are considered to be free from all viruses occurring in EPPO countries, virus-like agents, viroids and prokaryotes.

The soil should be free from nematode vectors (Table 3) and vines should be carefully protected from aerial vectors (the mealybugs and leafhoppers listed in Table 3) in areas where these may occur. For the repository, a safety distance from commercial vineyards or mother-vine plots is not strictly necessary. Contiguity between repository and other grapevine stands should, however, be avoided.

3. Production of nuclear stock plants

To become nuclear stock plants, selected vines should undergo testing for the harmful diseases and pathogens occurring in the EPPO region specified in Tables 1-2 and/or sanitation according to the procedures of Appendices I and II. Material imported from outside the EPPO region should also be tested by EPPO-recommended methods for all other viruses or virus-like pathogens occurring naturally in *Vitis* in the region of origin. Testing on the woody indicators specified in Table 4 is essential for material to be classified as nuclear stock, but the other procedures given in the disease-detection summaries of Appendix I may be useful for preliminary screening or for retesting. Regardless of the type of treatment used, sanitized vines should be retested. Plant rooted explants from heat chamber or tissue culture into separate pots, and grow them in a glasshouse or screenhouse to ensure freedom from aerial vectors. If vigorous growth is obtained, serological tests and testing on woody indicators can be performed within a year after transplanting. If candidate nuclear stock plants of a given variety or rootstock give negative results in all tests, it can be promoted to a nuclear stock plant and moved to the nuclear stock repository.

If, for a given variety or rootstock, 100% virus infection can be expected, it is advisable not to waste time with the first testing, but to proceed directly with sanitation.

Inspection for other pests

Besides the diseases and pathogens specifically considered above, all material should also be checked for the presence of other pests which can be carried on propagation material and affect its quality. In particular, this should be done for freedom from crown

gall (*Agrobacterium tumefaciens*), *Xylophilus ampelinus*, and canker-type diseases (*Phomopsis viticola*, *Eutypa* spp., *Stereum* spp.), and mites (*Calepitrimerus vitis*, *Panonychus ulmi*, *Eotetranychus carpini*). It should be noted that latent infections of *A. tumefaciens* may occur.

4. Maintenance of nuclear stock

Pot individually a limited number (2-5) of stock plants of each source (clone) of each variety or rootstock type taken into the scheme, and grow them under conditions ensuring freedom from re-infection by aerial or soil vectors. For this purpose, double-door entry, insect-proof screenhouses with gravel floor, heavy plastic or tarpaulin or any other material preventing contact of the roots with soil are suitable. Nuclear stock plants should be kept under continuous surveillance and be sprayed regularly with appropriate pesticides, to control the normal quality pests of grapevine.

In vitro storage of a duplicate set of each nuclear stock plant can be envisaged when reliable procedures for *in vitro* maintenance of *Vitis* germplasm become available.

Vines in the repository should be checked each year for virus symptoms and other pathogenic disorders. Retesting is advisable if new and better detection techniques, antisera or indicators become available, or whenever visual inspections suggest tests should be carried out.

5. Propagation stock

Propagation blocks are outdoor plantings that constitute the source of propagation stock. Propagation blocks are established with material propagated directly from nuclear stock. These blocks should be established, preferably, in soils with no grapevine history, or soils that have not hosted grapevines for at least 6 years and, in any case, are found free from virus-transmitting nematodes (Table 3). The blocks should have a safety distance of 15-20 m from any vineyard made up of material of lower category (certified), but this may be reduced if the soil in the adjacent fields (vineyards or orchards) has been found to be free from virus-transmitting nematodes.

(a) *Rootstocks*. Place cuttings from each rootstock type in the nuclear block in a glasshouse for rooting, and plant rooted cuttings directly in the field, each source in a separate plot, or row, and labelled so as to be readily distinguished from one another.

(b) *Varieties*. Graft each variety taken into the scheme onto rootstocks of the same certification level or onto seedling rootstocks, and transplant the grafted vines into the field.

Check stocks visually each year for symptoms of graft-transmissible diseases and retest vines if suggested by visual inspections.

6. Distribution of propagation stock and

production of certified stock

(a) *Varieties*. Distribute scion material from propagation blocks to nurseries under strict official control. If possible, an official or officially authorized organization should perform the distribution. For the production of mother-vine plants from which certified propagative material is to be derived, propagation stock scion material should be grafted by the nurseries onto rootstocks of the propagation stock category only. Nurseries should declare their production of certified stock every year to the official organization concerned, recording the number of plants for each variety/rootstock combination and substantiating the origin of certified scions and rootstocks by certificates, bills or delivery notes with appropriate remarks.

(b) *Rootstocks*. Distribute rooted cuttings destined for the establishment of mother-vine plants for the production of certified cuttings or rooted cuttings to nurseries under strict official control. If possible, an official or officially authorized organization should perform the distribution. The production of certified rootstocks should be announced by the nurseries to the official organization concerned. Nurseries should record the number of mother plants and substantiate the origin of certified material by official certificates or delivery notes with appropriate remarks.

Mother vines for the production of certified stock bud sticks of scion varieties, rooted cuttings of rootstocks, grafted vines, should be established in plots at a minimum distance of 8-10 m from other vineyards or orchards and in soils free from virus-transmitting nematodes. To this effect, the nurseries should produce a certificate of nematological analysis issued by an official, or officially authorized, organization.

7. Control on the use and status of certified material

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 certified plants on the basis of field inspections and of the records and documents presented by the nursery. During production of certified stock in nurseries, some random tests on virus status should also be performed using available short-time testing methods (e.g. ELISA test, green grafting).

8. Certification and labelling

The certifying authority issues nurseries with certificates on the basis of points 4, 5 and 6 and supplies the appropriate labels in the required numbers. Grafted rooted cuttings and rootstock rooted cuttings are graded by the nursery and labelled in bundles. The authority inspects and checks on correct labelling before delivery.

APPENDIX I

Guidelines on testing procedures

1. Testing on *Vitis* indicators

The use of *Vitis* indicators is still a compulsory step in any grapevine certification programme. It cannot be excluded because there are several diseases, some of major importance, which cannot be identified except on woody differential hosts. Testing is performed by grafting on the indicators listed in Table 4. Since at least three replicates of any variety or rootstock type taken into the scheme are grafted on each indicator, a total of 12-18 grafts is required for each candidate vine. Various grafting techniques can be used:

(a) *Whip or cleft grafting in the field*

(b) *Chip-bud grafting*. This technique is recommended for detection of rupestris stem pitting because the pits induced by the disease develop on the indicator stem below the grafted chip and extend basipetally in a band or stripe.

(c) *Machine grafting*

(d) *Green grafting* (Walter *et al.*, 1990)

It is recognized that green grafting has distinct advantages over other techniques. Therefore, an effort should be made to encourage its use.

2. Inoculation to herbaceous hosts

The use of herbaceous indicators allows detection of mechanically transmissible viruses (Table 2), including some which are of minor or negligible importance. Whereas an effort should be made to obtain nuclear and propagation stock free from all these viruses, inoculation to herbaceous hosts is 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.

3. ELISA testing

The use of ELISA is recommended for grapevine fanleaf nepovirus and other European nepoviruses where they occur, and for closteroviruses for which antisera are available. It can also be applied to detection of grapevine phloem-limited isometric virus (Boscia *et al.*, 1990). Sources of antigens for ELISA tests can be grapevine buds, roots, leaves and wood shavings. Wood shavings, however, are advantageous because: (i) they can be used throughout the year without apparent loss of efficiency due to the seasonable variation of antigen titre in vegetative organs; (ii) give low and consistent background readings; (iii) are much more reliable for identification of closteroviruses in American rootstocks, especially *Vitis rupestris* and its hybrids. Use of ELISA testing is 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.

4. Detection of individual diseases

Fanleaf

Graft transmission

| | |
|----------------------|---|
| Indicator: | <i>Vitis rupestris</i> St George |
| No. plants per test: | 3-5 rooted cuttings |
| Inoculum: | Wood chips, single buds, bud sticks, shoot tips |
| Temperature: | 22-24°C |
| Symptoms: | (a) Acute phase (shock) symptoms. Chlorotic spots, rings and lines, localized necrosis 3-4 weeks after grafting (chip-bud or green grafting) (b) Chronic symptoms. Reduced growth, severely deformed leaves with prominent teeth (distorting strains), bright yellow discolorations and mild deformation of the leaves (chromogenic strains) |

Transmission to herbaceous hosts

| | |
|-------------------|---|
| Diagnostic hosts: | <i>Gomphrena globosa</i> , <i>Chenopodium amaranticolor</i> , <i>C. quinoa</i> |
| Inoculum: | Tissues from young symptomatic leaves or succulent roots |
| Extraction: | Grind in 2.5% aqueous nicotine |
| Temperature: | Below 25°C |
| Symptoms: | Chlorotic local lesions soon turning reddish in 7-8 days, twisting of the upper leaves in 10-12 days (<i>G. globosa</i>). Chlorotic local lesions on inoculated leaves in about a week followed by systemic mottling and mild leaf deformation (<i>Chenopodium</i> spp.) |

Other tests

Serology (ELISA, ISEM). Molecular probes.

Grapevine degeneration (European nepoviruses)

Graft transmission

Indicators: Several *Vitis vinifera* cultivars: Pinot noir, Jubileum 75 (GCMV); Siegfriedrebe (ArMV, RRV, TBRV)

No. plants per test: 3-5 rooted cuttings

Inoculum: Wood chips, single buds, bud sticks, shoot tips

Temperature: 22-24°C

Symptoms: Severe stunting and necrosis of the apex of Pinot noir in second year of vegetation (GCMV); foliar discolorations and cane deformations of Siegfriedrebe within the first year after inoculation.

Transmission to herbaceous hosts

Diagnostic hosts: *Datura stramonium* (GCMV)
Chenopodium quinoa (TBRV and GBLV)
Nicotiana clevelandii (RRV)
Cucumis sativus (SLRV)
Nicotiana glutinosa (ArMV)

Inoculum: Tissue from young symptomatic leaves

Extraction: Grind in 2.5% aqueous nicotine

Temperature: Below 25°C

Symptoms: *D. stramonium* (CGMV), transient, systemic, yellowish zonate spots.
C. quinoa (TBRV), necrotic local lesions in 6-8 days followed by mosaic and necrosis of the plant tip in about 2 weeks.
C. quinoa (GBLV), necrotic local lesions in 3-4 days, systemic chlorotic mottle and necrosis.
Nicotiana clevelandii (RRV), necrotic local spots and rings in 5-7 days, systemic veinal necrosis.
Cucumis sativus (SLRV), chlorotic local lesions in 5-7 days, systemic interveinal chlorosis or necrosis in 10-12 days.
Nicotiana glutinosa (ArMV), chlorotic ringspots.

Other tests

Serology (ELISA, ISEM). Molecular probes are being developed.

Leafroll

Graft transmission

Indicators: Several cultivars of red-fruited *V. vinifera* (Pinot noir, Cabernet franc, Merlot, Barbera, Mission)

No. plants per test: 3-5 rooted cuttings

Inoculum: Wood chips, single buds, bud sticks, shoot tips

Temperature: 22°C (green grafting)

Symptoms: Rolling and reddening of the leaves in 4-6 weeks (green grafting) or 6-8 months to 2 years (field testing)

Transmission to herbaceous hosts (grapevine closterovirus A)

Diagnostic host: *Nicotiana clevelandii* or *N. benthamiana*

Inoculum: Virus preparations micropurified from grapevine leaves or cortical tissues; viruliferous mealybugs; tissues from young leaves (with some virus isolates only)

Extraction: Grind leaf tissues in 2.5% aqueous nicotine

Temperature: Below 25°C

Symptoms: Systemic vein clearing and yellowing in 10-12 days

Other tests

Serology (ELISA, ISEM) and Western blot for closteroviruses for which antisera are available. PAGE electrophoresis for dsRNA pattern. Molecular probes are being developed.

Rugose wood complex

Graft transmission

Indicators: *V. rupestris* St George: rupestris stem pitting
LN 33: corky bark
Kober 5BB: Kober stem grooving
LN 33: LN 33 stem grooving

No. plants per test: 3-5 rooted cuttings

Inoculum: Wood chips or single buds (recommended for rupestris stem pitting), bud sticks

Temperature: 22°C (green grafting)

Symptoms: Basipetal pitting below grafted bud (rupestris stem pitting), internodal swellings and stem grooving on LN 33 (corky bark); stem grooving in Kober 5BB only (Kober stem grooving); stem grooving in LN 33 only (LN 33 stem grooving).

Enation

Graft transmission

Indicator: LN 33 or *V. vinifera* cv. Italia
No. plants per test: 3-5 rooted cuttings
Inoculum: Single buds, bud sticks
Temperature: Field conditions
Symptoms: Enations and leaf deformation 1-3 years after grafting.

Fleck

Graft transmission

Indicator: *Vitis rupestris* St George
No. plants per test: 3-5 rooted cuttings
Inoculum: Wood chips, single buds, bud sticks, shoot tips
Temperature: 22°C (green grafting or growth chamber)
Symptoms: Clearing of veinlets in 4-6 weeks according to growing conditions

Other tests

Serology (ELISA).

Vein necrosis

Graft transmission

Indicator: American *Vitis* hybrid 110R
No. plants per test: 3-5 rooted cuttings
Inoculum: Wood chips, single buds, bud sticks, shoot tips
Temperature: 26°C (green grafting)
Symptoms: Necrosis of the veinlets, stunting and necrosis of the shoot tips.

Vein mosaic

Graft transmission

Indicator: *Vitis riparia*
No. plants per test: 3-5 rooted cuttings
Inoculum: Wood chips, single buds, bud sticks, shoot tips
Temperature: 22°C (green grafting)
Symptoms: Chlorotic blotches and green mosaic along the veins, leaf deformation in 4-6 weeks.

Flavescence dorée

Note: the methods given below are available, but visual inspection only is all that is currently recommended.

Graft transmission

Indicators: Hybrid Baco 22A and *V. vinifera* cv. Chardonnay, Aramon
No. plants per test: 3-5 rooted cuttings
Inoculum: Wood chips, single buds, bud sticks
Temperature: Field conditions

Symptoms: Stunting, leaf yellowing and necrosis (white-berried varieties), leaf reddening and necrosis (red-berried varieties) 2-3 months or more after inoculation

Other tests

Serology (ELISA).

Bois noir and other European yellows diseases

Note: the methods given below are available, but visual inspection only is all that is currently recommended.

Graft transmission

Indicator: *Vitis vinifera* cv. Chardonnay, Riesling
No. plants per test: 3-5 rooted cuttings
Inoculum: Wood chips, single buds, bud sticks
Temperature: Field conditions
Symptoms: Yellowing or reddening of the leaves and rolling of the blades followed by necrosis of the veins usually within the first year after inoculation.

APPENDIX II

Guidelines on sanitation procedures

1. Heat treatment

All known graft-transmissible infectious agents of grapevine, except viroids, can be eliminated from parts of infected plants with varying levels of efficiency by heat therapy. Heat treatment can be performed in several ways but, regardless of the procedure used, testing of the treated material for assessment of its health status should follow. A sufficient interval between sanitation of the material and the conclusion of virus testing is necessary in order to avoid false negatives.

Hot-air treatment

(a) Place pot-grown vegetative vines (e.g. rooted cuttings 2-year-old or older) of each variety or rootstock type to be taken into the scheme into a heat cabinet and hold at constant temperature of $38\pm 1^\circ\text{C}$ and 16-18 h artificial illumination.

Collect tips 0.5-1 cm long from vegetative shoots after 4 weeks or more (up to 300 days if the vines survive) from the beginning of the treatment, and root in a heated (25°C) sand bench under mist or, after surface sterilization, in agarized nutrient medium under sterile conditions.

Pot rooted explants and let them grow in a glasshouse until ready for testing.

For further details, see Goheen & Luhn (1973), Martelli (1979), Ottenwaelter *et al.* (1973), Stellmach (1980).

(b) Graft a bud from the candidate vine to be heat-treated into the main shoot of a pot-grown, 2-year-old healthy LN 33 indicator. Transfer budded LN 33 to the heat cabinet 12-15 days after grafting and expose for 60 days to $37\pm 1^{\circ}\text{C}$. Move treated vines out of the cabinet, cut LN 33 shoot above grafted bud, allow the bud to develop into a shoot and check for health status.

For more details, see Goheen (1977).

Hot-water treatment

Hot water is used for eliminating intracellular prokaryotes like the MLO agent of flavescente dorée, from infected grapevine cuttings. Collect dormant cuttings and immerse in water according to the method of Caudwell *et al.* (1991).

2. Shoot (meristem) tip culture in vitro

Collect shoot tips or axillary buds from vines grown at $36-38^{\circ}\text{C}$, surface-sterilize by dipping explants for 20 min in a 5% solution of commercial sodium hypochlorite and 0.1% Tween 20. Rinse thoroughly with 2-3 changes (10 min each) of sterile distilled water.

Dissect 0.4-0.6 mm-long explants comprising the meristematic dome and the first pair of leaf primordia and transfer to sterile test tubes in agarized Murashige and Skoog medium supplemented with 0.5 ppm benzylaminopurine. Allow explants to grow for 45 days at 25°C in a cabinet with 16 h artificial illumination (about 4000 lux).

Separate actively growing shoots and transfer individually to a medium containing 1 ppm benzylaminopurine for 45-50 days for elongation. Transfer elongated shoots (3 nodes long or more) individually to a medium containing 0.5-1 ppm indolbutyric acid for root production. Transfer rooted explants to small pots containing vermiculite under saturated humidity conditions, then to pots with soil compost and protect plantlets with a polyethylene bag for as long as necessary (usually 2-3 weeks). Grow plantlets in a glasshouse until ready for testing.

For further details, see Barlass *et al.* (1982).

References

BARLASS, M., SKEENE, K.G.M., WOODHAM, R.C. & KRAKE, L.R. (1982) Regeneration of virus-free grapevines using *in vitro* apical culture. *Annals of Applied Biology* **101**, 291-295.

BOSCIA, D., SAVINO, V., ELICIO, V., JEBABI, S.D. & MARTELLI, G.P. (1990) Detection of closteroviruses in grapevine tissues. In *Proceedings of the 10th Meeting of ICVG*. Volos (GR).

CAUDWELL, A., LARRUE, J., VOLOT, C. & GREANAN, S. (1991) Hot-water treatment against flavescente dorée on dormant wood. In *Proceedings of the 10th Meeting of ICVG*. Volos (GR).

GOHEEN, A.C. (1977) Virus and virus-like diseases of grapes. *HortScience* **12**, 465-469.

GOHEEN, A.C. & LUHN, C.F. (1973) Heat inactivation of viruses in grapes. *Rivista di Patologia Vegetale* **9**, 287-289.

MARTELLI, G.P. (1979) Identification of virus diseases of grapevine and production of disease-free plants. *Vitis* **18**, 127-136.

OEPP/EPPO (1992) Recommendations made by EPPO Council in 1981: certification of virus-tested fruit trees, scions and rootstocks. *EPPO Technical Documents* no. 1013, 42-43.

OTTENWAELETER, M.M., HEVIN, H. & DOAZAN, J.P. (1973) Amélioration du rendement du bouturage des extrémités après thérapie sur plantes en pots par l'utilisation de la culture sur milieu gélosé stérile. *Vitis* **12**, 46-48.

STELLMACH, G. (1980) Moderate heat propagation of grapevines for elimination of graft transmissible disorders. In *Proceedings of the 7th Meeting of ICVG*, pp. 325-328. Agriculture Canada, Ottawa (CA).

WALTER, B. & ETIENNE, L. (1987) Detection of the grapevine fanleaf virus away from the period of vegetation. *Journal of Phytopathology* **120**, 355-364.

WALTER, B., BASS, P., LEGIN, R., VERNON, R., COLLAS, A. & VESSELLE, G. (1990) The use of a green grafting technique for the detection of virus-like disease of the grapevine. *Journal of Phytopathology* **128**, 137-145.

Table 1. Virus and virus-like diseases of grapevine occurring in the EPPO region and covered by the scheme (see Table 2 for full details)

-
1. Grapevine degeneration complex, caused by grapevine fanleaf nepovirus and other European nepoviruses
 2. Grapevine leafroll complex
 3. Grapevine rugose wood complex (corky bark, rupestris stem pitting, Kober stem grooving, LN 33 stem grooving)
 4. Grapevine fleck disease
 5. Grapevine enation disease
 6. Grapevine diseases associated with closteroviruses (material found to contain closteroviruses is not admitted for certification)
 7. Grapevine diseases caused by MLOs (visual inspection only - material visibly affected by MLOs is not admitted for certification)
-

Other graft-transmissible diseases known to occur in the EPPO region are tolerated for the moment, but every effort should be made to eliminate them, especially grapevine vein mosaic disease and grapevine vein necrosis disease.

Table 2. Pathogens, transmissibility, geographical distribution and vectors for the diseases cited in Table 1

| Virus | Geographical distribution | Vector |
|---|--|---|
| <i>A. Grapevine degeneration complex</i> (all mechanically transmissible) | | |
| 1. Artichoke Italian latent nepovirus (AILV) | Bulgaria | <i>Longidorus apulus</i> <i>Longidorus fasciatus</i> |
| 2. Arabis mosaic nepovirus (ArMV) | Europe (Switzerland, Germany, Hungary, Yugoslavia, Bulgaria, France, Italy), Japan | <i>Xiphinema diversicaudatum</i> |
| 3. Grapevine Bulgarian latent nepovirus (GBLV) | Bulgaria, Hungary, Portugal Yugoslavia | Unknown |
| 4. Grapevine chrome mosaic nepovirus (GCMV) | Hungary, Yugoslavia | Unknown |
| 5. Grapevine fanleaf nepovirus (GFLV) | Worldwide | <i>Xiphinema index</i> <i>Xiphinema italiae</i> |
| 6. Grapevine Tunisian ringspot nepovirus (GTRV) | Tunisia | Unknown |
| 7. Raspberry ringspot nepovirus (RRV) | Germany | <i>Longidorus macrosoma</i> <i>Longidorus elongatus</i> |
| 8. Strawberry latent ringspot nepovirus (SLRV) | Germany, Italy, Turkey | <i>Xiphinema diversicaudatum</i> |
| 9. Tomato black ring nepovirus (TBRV) | Germany, Israel, Canada (Ontario), Hungary | <i>Longidorus attenuatus</i> <i>Longidorus elongatus</i> |

B. *Grapevine leafroll complex* (only grapevine closterovirus A mechanically transmissible)

| | | | |
|-----|--|----------------------------|---|
| 10. | Grapevine closterovirus A | Europe, Mediterranean | <i>Planococcus ficus</i> <i>Planococcus citri</i> <i>Pseudococcus longispinus</i> |
| 11. | Grapevine leafroll- associated closterovirus I | Europe, Mediterranean, USA | Unknown |
| 12. | Grapevine leafroll- associated closterovirus II | Europe, Mediterranean | Unknown |
| 13. | Grapevine leafroll- associated closterovirus III | Europe, Mediterranean, USA | <i>Planococcus ficus</i> <i>Pseudococcus longispinus</i> |
| 14. | Grapevine leafroll- associated closterovirus IV | Mediterranean, USA | Unknown |
| 15. | Grapevine leafroll- associated closterovirus V | France | Unknown |

Two additional closteroviruses, associated with leafroll, have been found which, apparently, are serologically unrelated to all the above.

C. *Grapevine rugose wood complex* (not mechanically transmissible)

Occurs worldwide, with no known vector. The diseases known as corky bark, rupestris stem pitting, Kober stem grooving and LN 33 stem grooving belong to this complex. No pathogen associated with this virus-like disease has yet been characterized, but a closterovirus serologically unrelated to the leafroll-associated closterovirus has been reported to be associated with corky bark.

D. *Grapevine fleck disease* (not mechanically transmissible)

| | | | |
|-----|--|-----------------------|---------|
| 16. | Grapevine phloem-limited isometric virus (GPLIV) | Europe, Mediterranean | Unknown |
|-----|--|-----------------------|---------|

E. *Grapevine enation disease* (not mechanically transmissible)

Occurs in Europe, North America (USA), South America (Venezuela), South Africa, New Zealand, Australia, with no known vector. No pathogen associated with this virus-like disease has not yet been identified.

F. *Grapevine diseases caused by MLOs*

| | | | |
|-----|--|---|--------------------------------|
| 17. | Grapevine flavescence dorée MLO | France, Italy | <i>Scaphoideus titanus</i> |
| 18. | Grapevine bois noir and other yellows MLOs | Europe (France, Germany, Italy, Switzerland, Greece, Romania, Bulgaria), Chile, Israel, New Zealand | Unknown (probably leafhoppers) |

Table 3. Soil-borne (nematode) and aerial vectors of grapevine diseases recorded in EPP0 countries

| Vector | Pathogen | Geographical distribution |
|----------------------------------|---|--|
| <i>Xiphinema index</i> | Grapevine fanleaf nepovirus | Worldwide associated with grapevine |
| <i>Xiphinema italiae</i> | ? ¹ | Mediterranean region |
| <i>Xiphinema diversicaudatum</i> | Arabis mosaic nepovirus Strawberry latent ringspot nepovirus | Throughout Europe and the Middle East |
| <i>Longidorus attenuatus</i> | Tomato black ring nepovirus | Patchy distribution throughout Europe, but more concentrated in north-central, i.e. PL, DE, NL, BE, GB (England) |
| <i>Longidorus elongatus</i> | | Mainly northern Europe but also rarely in ES, IT, BG and south FR |
| <i>Longidorus macrosoma</i> | Raspberry ringspot nepovirus | Western Europe |
| <i>Xiphinema vuittenezi</i> | ? ² | Mainly central and southern Europe |
| <i>Planococcus ficus</i> | Grapevine closterovirus A Grapevine leafroll-associated closterovirus III | Mediterranean region |
| <i>Planococcus citri</i> | Grapevine closterovirus A | Mediterranean region |
| <i>Pseudococcus longispinus</i> | Grapevine closterovirus A Grapevine leafroll-associated closterovirus III | Mediterranean region |
| <i>Scaphoides titanus</i> | Grapevine flavescence dorée MLO | Introduced to SW Europe from N. America. Spreading eastwards |

¹ *Xiphinema italiae*, although reported in the literature as a vector of grapevine fanleaf nepovirus, does not seem to have any vectoring efficiency in the field.

² *Xiphinema vuittenezi* has not been proved experimentally to transmit any virus. However, it has been found associated with the spread of certain nepoviruses (e.g. grapevine chrome mosaic nepovirus) in the field. For this reason, it should be regarded as a potentially dangerous nematode.

Table 4. Main indicators for virus and virus-like diseases of grapevine¹

| Indicator | Disease identified |
|---|---|
| 1. <i>Vitis rupestris</i> St George | Degeneration ² , fleck, rupestris stem pitting |
| 2. <i>Vitis vinifera</i> Cabernet franc, Pinot noir and other red-berried cultivars | Leafroll ³ |
| 3. Kober 5BB (<i>Vitis berlandieri</i> × <i>Vitis riparia</i>) | Kober stem grooving |
| 4. LN 33 (Couderc 1613 × <i>Vitis berlandieri</i>) | Corky bark, enation, LN33 stem grooving |
| 5. <i>Vitis riparia</i> Gloire de Montpellier | Vein mosaic ⁴ |
| 6.110 R (<i>Vitis rupestris</i> × <i>V. berlandieri</i>) | Vein necrosis ⁴ |

¹ Appendix I provides full details of the conditions for the tests and suggests some alternative indicators.

² In countries where degeneration is also caused by nepoviruses other than grapevine fanleaf nepovirus, Siegfriedrebe (FS4 201/39) may be used as an indicator.

³ The choice of the most suitable indicator for leafroll depends on climatic conditions of the region where the testing is done.

⁴ As noted in Table 1, these are optional for the moment but strongly recommended.