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

Certification scheme for *Ribes*

Specific scope

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

Specific approval and amendment

First approved in September 1994 and revised in 2007.

The certification scheme for pathogen-tested material of *Ribes* spp. provides detailed guidance on the production of vegetatively propagated *Ribes* 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).

Outline of the scheme

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

1 Selection for pomological 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 treatment or meristem tip culture, followed by testing for the viruses listed in Table 1. 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 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 plants: hardwood cuttings taken from propagation stock are grown under conditions minimizing infections to produce certified plants (entire rooted plants or cuttings finally distributed for fruit production).

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 inspections of the plants to verify the apparent health of the stock.

1. Selection of candidates for nuclear stock

The scheme concerns mainly *Ribes nigrum* (black currant), *R. rubrum* (red currant), *R. uva-crispa* (gooseberry) and *R. aureum* (as rootstock) but may be applied to other *Ribes* spp. New or existing cultivars may be selected as candidate material. The starting material should be selected visually on the basis of trueness-to-type, vigour, pomological quality and absence of pest symptoms, especially symptoms of reversion in black currants, *R. nigrum*. Alternatively, starting material may be obtained from existing certification schemes in other EPPO countries. If virus-free starting material is imported from outside the EPPO region it should be tested for the viruses listed in Table 1 and all other viruses occurring naturally in *Ribes* in the region of origin.

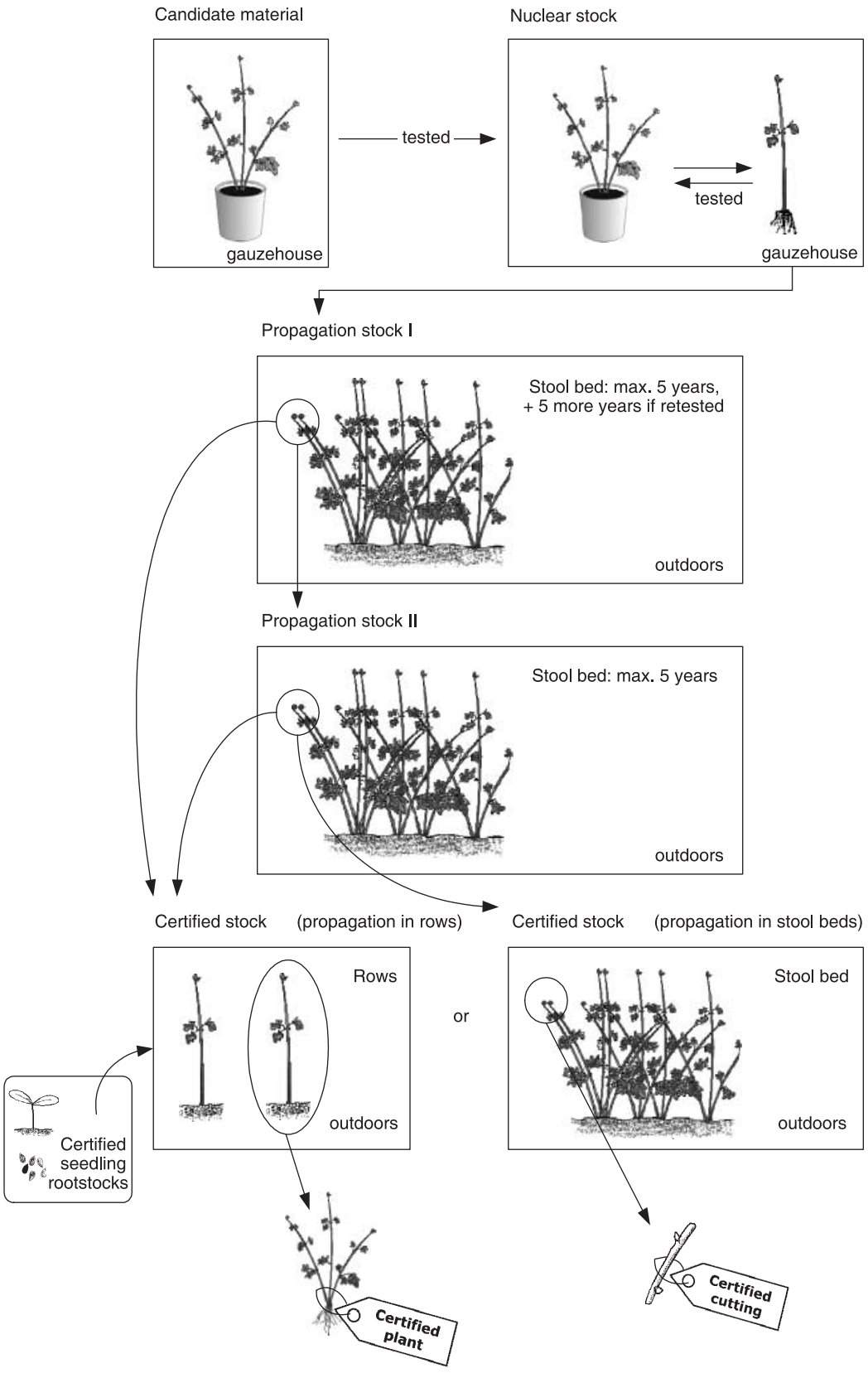

Fig. 1 Diagram of the *Ribes* certification scheme.

Table 1 Recommended methods for detection and identification of *Ribes* viruses and virus-like agents

Pathogen	Host	Test methods	Test methods for confirmation
A. Pathogens occurring in the EPPO region subject to testing during the production of nuclear stock			
Gooseberry vein banding associated virus (Badnavirus, GVBaV*)*	*R. nigrum* *R. rubrum* *R. uva-crispa*	Graft transmission to *R. rubrum* cvs. Jonkheer van Tets, B1385/81, Amos Black, 1385/90 or *R. uva-crispa* cv. Leveller	
Strawberry latent ringspot virus (Sadwavirus, SVBV*)*	*R. rubrum* *R. nigrum*	Mechanical inoculation to test plants*	ELISA
Raspberry ringspot virus (Nepovirus, RpRSV*)*	*R. rubrum*	Mechanical inoculation to test plants or graft transmission on *R. rubrum* cv. Jonkheer van Tets	ELISA
Blackcurrant reversion virus (Nepovirus, BRV*)*	*R. nigrum* *R. rubrum*	Graft transmission to *R. nigrum* cvs. Baldwin, Ojebyn, Amos Black or *R. nigrum* Wellington XXX RT-PCR†	
Arabis mosaic virus (Nepovirus, ArMV*)*	*R. nigrum* *R. rubrum* *R. uva-crispa*	Mechanical inoculation to test plants	ELISA
Cucumber mosaic virus (Cucumovirus, CMV*)*	*R. nigrum* *R. rubrum*	Mechanical inoculation to test plants	ELISA
B. Other pathogens not occurring in the EPPO region or of minor importance			
Vein clearing and vein net of black currant	*R. nigrum*	Graft transmission to *R. nigrum* cv. Amos Black	
Black currant yellows	*R. nigrum*	Graft transmission to *R. nigrum* cv. Amos Black	
Aucuba mosaic (syn. European currant mosaic)	*R. rubrum*	Graft transmission to *R. rubrum* cv. Jonkheer van Tets	
Tomato ringspot virus (Nepovirus, ToRSV*)*	*R. nigrum* *R. rubrum*	Mechanical inoculation to test plants	ELISA

**Chenopodium quinoa*, *Cucumis sativus* and *Nicotiana occidentalis* or *N. clevelandii* (see also Martin, 2004).

†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 pathogens 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 in *Ribes*, the situation for PCR detection is at different level 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.

2. Production of nuclear stock

The candidate material for nuclear stock status should be kept under confinement in an isolated, insect-proof gauzehouse, separately from the nuclear stock. All plants should be grown in individual pots in a sterilized growing medium, with strict precautions, including preventive treatments as appropriate, against infestation by aphids, black currant gall mite (*Cecidophyopsis ribis*), powdery mildews (*Sphaerotheca mors-uvae* and *Microsphaera grossulariae*), black currant leaf midge (*Dasyneura tetensi*) and red spider mite (*Tetranychus urticae*). The general status of the plants with respect to these pests, and to other diseases or unknown symptoms, should be regularly checked by visual inspection.

All plants are individually tested (according to Appendix 1) for the following viruses and virus-like agents: *Gooseberry vein banding associated virus (Badnavirus)*, *Blackcurrant reversion virus (Nepovirus)*, *Strawberry latent ringspot virus (Sadwavirus)*, *Raspberry ringspot virus (Nepovirus)*, *Arabis mosaic virus (Nepovirus)*, *Cucumber mosaic virus (Cucumovirus)*[1]. Plants giving negative results in all tests are promoted to nuclear stock plant status and should be transferred to a separate gauzehouse of similar standard. Plants giving positive results for any virus should be removed immediately. If no plants of a cultivar or clone prove to be free from these pathogens, etc., heat treatment or meristem tip culture may be applied to eliminate infection. The progeny resulting from this process may be considered to be candidate material and should be re-tested for the viruses above and re-assessed for agronomic and varietal characters. The recommended test methods are given in Appendix 1.

[1]Detection is performed according to the tests described in Appendix 1 but specific identification of viruses detected may be done by ELISA.

3. Maintenance of the nuclear stock

Nuclear stock plants should be kept in an insect-proof gauzehouse, containing only nuclear stock plants. They should be maintained under the same conditions and with the same checks on pest freedom as candidate nuclear stock plants. They should be individually re-tested at least at intervals of 4 years for *Blackcurrant reversion virus* and *Gooseberry vein banding associated virus*. For other viruses and virus-like agents listed in Table 2 re-testing can be done according to the risk of re-infection.

Table 2 Recommended tolerances for pests of *Ribes*

	% plants		
	Nuclear stock	Propagation stock I & II	Certified material
Blackcurrant reversion virus	Nil	Nil	Nil
Other viruses and virus-like agents	Nil	0.05	0.5
Gall mite/big bud (*Cecidophyopsis ribis*)	Nil	Nil	Nil
Aphelenchoides ritzemabozi (on buds)	Nil	0.05	0.1

Infection of neighbouring stocks within 50 m by aerially transmissible pathogens over tolerance should disqualify the entered material from certification.

Cuttings taken from nuclear stock plants can also be considered as nuclear stock provided they are grown under the same conditions.

Checks should be made on trueness-to-type. In order to check trueness-to-type, material taken from nuclear stock plants should be allowed to fruit in a separate location.

4. Production of propagation stock

Hardwood cuttings, taken from the nuclear stock and either rooted then planted in stool beds or separated with roots attached ('cuttings with roots') and directly planted in stool beds, become the propagation stock. Alternatively, softwood cuttings may be rooted under the same growing conditions as nuclear stock plants and then planted in stool beds. The rooting and stool beds should be isolated from other non-certified *Ribes* material by at least 200 m. At the site at which the stock is planted, there should have been an interval of at least 3 years since the previous *Ribes* crop. The soil should be tested for nematodes (see Appendix 1) and the site should only be used for the production of propagation stock plants if found substantially free or if those found are shown to be free from virus by a slash or bait test (see EPPO Standard PM 4/34, 2008). The stock should be protected by spraying against air-borne virus vectors. The propagation stock should be inspected twice a year for virus diseases (in spring and in summer). General precautions against pests (including pathogens) should be maintained. Any plant showing symptoms of any of the pests listed in Table 2 should be eliminated. Recommended certification standards are given in Appendix 2.

The stool bed of propagation stock may be maintained for a maximum of 5 years as propagation stock I. It may continue to be used as propagation stock I for a maximum of 5 more years but it should be tested for *Blackcurrant reversion virus* and *Gooseberry vein banding associated virus*. A second stool bed (propagation stock II), derived from the first by hardwood or softwood cuttings (as above), may be established and again maintained under the same conditions for a maximum of 5 years. Alternatively, a propagation stock I stool bed can simply be maintained for up to 5 more years as propagation stock II.

Pomological assessment and trueness-to-type checks should be carried out. During pomological assessment, attention should be paid to virus symptoms occurring in the flowers.

5. Production of certified material

Hardwood cuttings, taken from the propagation stock as above, may be used in two different ways to finally produce certified plants:

(1) hardwood cuttings are planted out in rows for 1 or 2 years to grow into marketable bushes, which are lifted and leave the scheme as certified plants
(2) hardwood cuttings are used to establish stool beds, from which further cuttings leave the scheme as certified plants
(3) grafting on rootstocks (e.g. *Ribes aureum*).

At the site at which hardwood cuttings are planted, there should have been an interval of at least 3 years since the previous *Ribes* crop. If the site has contained other hosts of nepoviruses during the previous 2 years, the soil should be tested for nematodes (see Appendix 1) and the site should only be used for the production of certified plants if found substantially free or if those nematodes found are shown to be free from virus by a slash or bait test (see EPPO Standard PM 4/34, in preparation). The site should be isolated from other *Ribes* material by at least 100 m, or precautions should be taken against the spread by aerial virus vectors. General precautions against pests should be maintained. Any plant showing symptoms of virus disease should be eliminated.

Inspection for the granting of certificates should be performed twice (spring and early summer). Check on varietal purity should be made. Recommended certification standards are given in Appendix 2.

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 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) which indicates the certifying authority, the plant producer and the certification status of the plants.

Appendix 1

Guidelines on testing procedures

Virus testing

Testing *Ribes* for viruses or virus-like agents is done on woody or herbaceous indicators (Table 1), see also Converse (1987). Identification of the specific viruses concerned requires serological tests, normally applied to extracts from the herbaceous indicator, or molecular methods.

Grafting on woody indicators is normally done in the glasshouse at 20–25°C and the grafted plants are then held in a cool glasshouse. When testing for *Blackcurrant reversion virus*, tests on several shoots from large bushes are necessary because of the uneven distribution of the virus in the plant. Tests for reversion are continued for up to 2 years. For other pathogens they continue until the following spring.

For herbaceous test plants, tests should be done in the glasshouse using between two and five replicates of each species (*Chenopodium quinoa*, *Cucumis sativus* and *Nicotiana occidentalis* or *N. clevelandii*) at 20–25°C and the plants observed for up to 4 weeks.

Soil test for virus-vector nematodes

Soil in which propagation stock and plants producing certified material is to be planted should be sampled and the samples found substantially free from the following species of virus-vector nematodes (*Xiphinema diversicaudatum*: vector of *Arabis mosaic virus* and *Strawberry latent ringspot virus*, *Longidorus macrosoma* and *L. elongatus*: vectors of *Raspberry ringspot virus* – see EPPO Standard PM 4/xx, in preparation.

Appendix 2

Recommended certification standards for Ribes

Nuclear stock

Records should show that all nuclear stock plants were negative when tested for all listed viruses and virus-like agents. No plant may show any symptom of infestation by any pest listed in Table 2. All plants should also be substantially free from symptoms of infestation by other pests (including pathogens). If these conditions are not met at the time of the certification inspection, certification will be refused.

Propagation stock

Infestation by various pests should not exceed the limits indicated in Table 2 at the time of the certification inspection. If the limits are exceeded, certification will be refused to the whole stool bed. All plants should also be substantially free from symptoms of infestation by other pests (including pathogens).

Certified material

At the certification inspection, infestation by various pests should not exceed the limits indicated in Table 2. If the limits are exceeded, certification will be refused to the whole stool bed. All plants should also be substantially free from symptoms of infestation by other pests (including pathogens). The same certification standards apply to certified plants leaving the scheme as to the certified stock mother plants.

References

Converse RH (ed.) (1987) Virus diseases of small fruits. United States Department of Agriculture, Agriculture Handbook no. 631.

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OEPP/EPPO (2008) EPPO Standards PM 4/xx (1) Soil test for virus-vector nematodes in fruit crops. In preparation.