

# ◆ **EPPO Standards** ◆

## **CERTIFICATION SCHEMES**

PATHOGEN-TESTED MATERIAL OF *RUBUS*

**PM 4/10(1) English**



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## **APPROVAL**

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard.

## **REVIEW**

EPPO Standards are subject to periodic review and amendment. The next review date for this set of EPPO Standards is decided by the EPPO Working Party on Phytosanitary Regulations.

## **AMENDMENT RECORD**

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

## **DISTRIBUTION**

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## **SCOPE**

EPPO 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 *RUBUS*

#### Specific scope

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

#### Specific approval and amendment

First approved in September 1994.  
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, 1992a). Certified *Rubus* 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.

#### 1. Selection of material

The scheme concerns mainly raspberry (*Rubus idaeus*) and blackberry (*R. fruticosus*), but may be applied to other *Rubus* spp. and hybrids. 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. Alternatively, starting material may be obtained from existing certification schemes in other EPPO countries.

#### 2. Production of nuclear stock

The candidate material for nuclear stock status should be kept under quarantine in an isolated suitably designed insect-proof gauzehouse, separately from the nuclear stock. All plants should be grown in individual pots in a sterilized growing medium, with strict precautions against infestation by aphids, crown gall (*Agrobacterium* spp.), leafy gall (*Rhodococcus fascians*), downy mildew (*Peronospora rubi*) and raspberry cane midge (*Resseliella theobaldi*), as appropriate to the *Rubus* species or hybrid concerned. 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 I) for the following pathogens (as appropriate to the *Rubus* species or hybrid concerned):

black raspberry necrosis virus, cucumber mosaic cucumovirus, raspberry leaf mottle disease, raspberry leafspot disease, raspberry vein chlorosis rhabdovirus, raspberry yellow spot disease, rubus yellow net virus, arabis mosaic nepovirus, cherry leaf roll nepovirus, raspberry ringspot nepovirus, strawberry latent ringspot nepovirus, tomato black ring nepovirus, apple mosaic ilarvirus, raspberry bushy dwarf virus, rubus stunt MLO, *Phytophthora fragariae* var. *rubi*<sup>1</sup>.

Plants giving negative results in all tests 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, heat treatment and/or meristem-tip culture may be applied to eliminate infection. The progeny resulting from any such 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.

It may be necessary to eliminate *Phytophthora fragariae* var. *rubi* or other *Phytophthora* spp., especially in cool and moist climates. For this purpose, micropropagation culture or stem cuttings are taken from plants shown to be free from the above viruses, at a level of at least 0.5 m above the growing medium. When rooted in soil-less or sterilized growing medium, these plants become the nuclear stock.

The recommended test methods are given in Appendix I.

#### 3. Maintenance of the nuclear stock

The nuclear stock can readily be maintained and multiplied *in vitro* and, in this form, will retain the same status in the scheme. Otherwise, nuclear stock plants should be kept in a suitably designed 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

<sup>1</sup> Detection is performed according to the tests described in Appendix I but specific identification of viruses detected may be done by ELISA

nuclear stock plants. They should be individually re-tested at intervals of 2 years for all the viruses and virus-like agents listed in Section 2 (Production of nuclear stock). Cuttings taken from nuclear stock plants can also be considered as nuclear stock, provided they are grown under the same conditions and are individually tested<sup>1</sup> for all the pests just mentioned. The same applies to plants transferred from *in vitro* culture to pots. In general, any plant giving a positive result in a test or showing symptoms of any disease (fungal, bacterial, viral) should be eliminated. However, in order to check trueness to type and the absence of symptoms of crumbly fruit, material taken from nuclear stock plants may be allowed to fruit (e.g. by using hand pollination) in a separate location.

#### 4. Multiplication of the material (propagation stock)

Root cuttings taken from nuclear stock plants produce the propagation stock. These may then be used to establish spawn beds<sup>2</sup> (for raspberries) or mother plants for leaf-bud or tip propagation (for blackberries or hybrid berries). However, other methods have also been used successfully, such as nodal cuttings (especially for hybrid berries) and micropropagation. Later generations of species required in large numbers are normally field-grown. Outdoor propagation sites should be isolated from non-certified *Rubus* by at least 100 m, especially from fruiting plantations and wild *Rubus* spp. and the soil should be checked for the presence of virus-vector nematodes (*Xiphinema* and *Longidorus*) (Appendix I). Spacing between and within the rows should be sufficient to permit thorough inspection. Spacing between spawn-bed rows should not be less than 3 m, including a 1-m alleyway kept clear of plants.

The propagation stock should be inspected annually for virus symptoms. General precautions against pests should be maintained and any plant showing symptoms of any of the pests (including pathogens) in Table 2 should be eliminated. Recommended certification standards are given in Appendix II.

Spawn beds of raspberry propagation stock I should not be used for more than 4 successive years. A second spawn bed (propagation stock II), derived from the first by transferring a cane with roots to another site, may be established and maintained under the same conditions as above for 4 years of production. Alternatively, a propagation stock I spawn bed can simply be maintained for up to 4 more years as propagation stock II. The filiation of the plants should be recorded, so that each certified plant is known to be derived from nuclear stock by not more than a fixed

number of generations of propagation under the required conditions.

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

#### 5. Production of certified stock

Canes with roots from propagation stock I or II, planted as a spawn bed at another site, are the certified stock from which are taken the certified plants (canes sold for fruit production). At the site at which the stock is planted, there should have been an interval of at least 3 years since the previous *Rubus* crop. If the site has contained other hosts of nepoviruses during the previous 2 years, the soil should be tested for nematodes (see Appendix I) and the site should only be used for the production of certified stock if either no nematodes are found or if those found are shown to be virus-free by a slash test (Appendix I). The site should be isolated from other *Rubus* material by at least 25 m. General precautions against pests should be maintained.

The production of certified plants should only be performed by registered specialized establishments, for which the admission criteria are less stringent than for stages 1-4 (OEPP/EPPO, 1993).

Throughout the production of propagation and certified stock, checks should be made on varietal purity and on possible mutations or back mutations.

#### 6. Certification

Recommended certification standards are given in Appendix II. Inspection for the granting of certificates should be performed in early summer.

Certified *Rubus* material for export should in any case satisfy the phytosanitary regulations of importing countries, especially with respect to any of the pests covered by the scheme which are also quarantine pests.

The stages of the certification scheme are illustrated in Fig. 1.

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<sup>1</sup> The possibility of infection by the other pathogens for which the candidate nuclear stock was tested should be considered; occasional re-testing is advised.

<sup>2</sup> A spawn bed (or primo cane bed) is a bed from which the previous year's canes have been removed, so that it only contains the current year's growth of canes.

## APPENDIX I

### Guidelines on testing procedures

#### *Virus testing methodology*

All the agents concerned can be detected by a combination of three methods: mechanical inoculation to herbaceous indicators (a in Table 1); graft inoculation to *Rubus occidentalis* (b in Table 1); visual observation of symptoms.

Graft tests are normally done in the glasshouse as approach grafts with rooted indicator plants in a container and scions from plants under test maintained in bottles or other containers until a union has formed. They are done at 20-25°C, but after successful union the scion under test can be trimmed and the indicator plant moved to a gauzehouse. Normally only one indicator plant is used for each test done. Duration of the tests is one growing season; if done late the tests should be observed during the following spring.

For herbaceous indicators, tests should be done in the glasshouse using five replicate plants at 20-25°C and observed for up to 4 weeks. Identification of the specific viruses requires serological tests, normally applied to extracts from the herbaceous indicators.

#### *Phytophthora test*

*Phytophthora fragariae* var. *rubi* is a dangerous pathogen which has only recently become important on *R. idaeus* in Europe. It is particularly important to exclude it from certified stock, by testing nuclear stock and by suitable precautions for propagation stock. However, testing is made difficult by the occurrence of several *Phytophthora* spp. in raspberry roots (Duncan *et al.*, 1987). Duncan *et al.* (1993) have recently described bait tests which have been used in UK to monitor populations of *P. fragariae* var. *rubi* in the field, similar to that use for *P. fragariae* var. *fragariae* (see EPPPO Certification Scheme for pathogen-tested strawberry; OEPP/EPPO, 1994). Stammler *et al.* (1993) are also developing a PCR test. By combining these tests, it may be possible to obtain the discrimination, sensitivity and rapidity required for testing nuclear stock plants in a certification scheme.

#### *Soil testing for virus-vector nematodes*

Soil in which propagation stock and certified stock is to be planted should be sampled and the samples found substantially free from the following nematode vector species: *Xiphinema diversicaudatum* (the vector of arabis mosaic and strawberry latent ringspot nepoviruses), *Longidorus macrosoma* and *L. elongatus* (vectors of raspberry ringspot nepovirus). The test procedure to be followed is given in the relevant part of the EPPPO certification scheme for virus-free or virus-tested fruit trees and rootstocks (OEPP/EPPO, 1992b).

The nematodes can be tested directly for the presence of virus by a 'slash test', i.e. breaking up small

numbers of nematodes in phosphate buffer (pH 6.9) and inoculating virus-indicator plants with the suspension (Taylor, 1964).

## APPENDIX II

### Recommended certification standards for *Rubus*

#### *Nuclear stock*

Records should show that all nuclear stock plants were negative when tested for all listed viruses and virus-like agents and for *Phytophthora fragariae* var. *rubi*. No plant may show any symptom of any pest listed in Table 2. All plants should also be substantially free from 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 spawn bed. All plants must also be substantially free from symptoms of infestation by other pests (including pathogens).

#### *Certified stock*

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 spawn bed. All plants should also be substantially free from symptoms of infestation by other pests (including pathogens).

## References

- DUNCAN, J.M., KENNEDY, D.M. & SEEMULLER, B. (1987) Identities and pathogenicities of *Phytophthora* spp. causing root rot of raspberry. *Plant Pathology* **36**, 301-305.
- DUNCAN, J.M., KENNEDY, D.M., CHARD, J., ALI, A. & RANKIN, P.A. (1993) Control of *Phytophthora fragariae* on strawberry and raspberry in Scotland by bait tests. In *Plant Health and the European Single Market* (ed. Ebbels, D.), pp. 301-305. BCPC, Thornton Heath (GB).

OEPP/EPPO (1992a) 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 (1992b) Certification schemes. No. 1. Virus-free or virus-tested fruit trees and rootstocks. Part IV. Technical appendices and table of contents. *Bulletin OEPP/EPPO Bulletin* **22**, 277-283.

OEPP/EPPO (1993) Certification schemes. No. 7. Nursery requirements - recommended requirements for establishments participating in certification of fruit or ornamental crops. *Bulletin OEPP/EPPO Bulletin* **23**, 249-252.

OEPP/EPPO (1994) Certification schemes. No. 11. Pathogen-tested strawberry. *Bulletin OEPP/EPPO Bulletin* **24**, 875-889.

STAMMLER, G., SEEMÜLLER, B. & DUNCAN, J.M. (1993) Analysis of RFLPs in nuclear and mitochondrial DNA and the taxonomy of *Phytophthora fragariae*. *Mycological Research* **97**, 150-156.

TAYLOR, C.E. (1964) Transmission. In Report of the Scottish Horticultural Research Institute for 1963/1964, p. 65. SCRI, Dundee (GB).

**Table 1.** Recommended methods for detection and identification of viruses and virus-like pathogens of *Rubus* occurring in the EPPO region

Pathogen	Host	Test methods
Black raspberry necrosis virus**	<i>Rubus</i> spp. (latent in most cvs of <i>R. idaeus</i> )	(b)
Cucumber mosaic cucumovirus	<i>Rubus</i> spp. & many other genera	(a)
Raspberry leaf mottle**	<i>R. idaeus</i> (latent in most cvs)	(b), (c)
Raspberry leafspot**	<i>R. idaeus</i> (latent in most cvs)	(b), (d)
Raspberry vein chlorosis rhabdovirus	<i>R. idaeus</i> (most cvs)	Visual
Raspberry yellow spot	<i>R. idaeus</i> (latent in most cvs)	(b), (f)
Rubus yellow net virus**	<i>Rubus</i> spp.	(b), (e)
Arabis mosaic nepovirus	<i>R. idaeus</i> , <i>R. fruticosus</i> & other genera	(a)
Cherry leaf roll nepovirus	<i>R. idaeus</i> , <i>R. fruticosus</i> & other genera	(a)
Raspberry ringspot nepovirus	<i>R. idaeus</i> , <i>R. fruticosus</i> & other genera	(a)
Strawberry latent ringspot nepovirus	<i>R. idaeus</i> , <i>R. fruticosus</i> & other genera	(a)
Tomato black ring nepovirus	<i>Rubus</i> spp. (usually latent) & other genera	(a)
Apple mosaic ilarvirus	<i>R. idaeus</i> , <i>R. fruticosus</i> & other genera	(a)
Raspberry bushy dwarf virus	<i>Rubus</i> spp.	(a)
Rubus stunt MLO	<i>Rubus</i> spp.	Visual (c), (d)

\* Test methods denoted by letters above are based on the system described by A.T. Jones (*Crop Research* **26**, 127-171, 1986):

- (a) mechanical inoculation to *Chenopodium quinoa*, *Cucumis sativus* and *Nicotiana clevelandii*;
- (b) graft inoculation to *R. occidentalis*;
- (c) graft inoculation to *R. idaeus* cv. Malling Landmark;
- (d) graft inoculation to *R. idaeus* cv. Norfolk Giant;
- (e) graft inoculation to *R. idaeus* cv. Malling Promise;
- (f) graft inoculation to *R. macraei*.

\*\* The four agents marked, in several different combinations, can lead to symptoms known as rubus (or raspberry) mosaic disease.

**Table 2.** Recommended tolerances for pests of *Rubus*

	% plants		
	Nuclear stock	Propagation stock I & II	Certified stock
Virus and virus-like agents	Nil	0.05	2.0
Phytophthora root rot symptoms	Nil	Nil	Nil
Crown and leafy galls	Nil	0.1	1.0

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

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

