**Scheme for the production of healthy plants for planting**
**Schemás pour la production de végétaux sains destinés á la plantation**

# Certification scheme for *Rubus*

## Specific scope

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

## Specific approval and amendment

First approved in 2004–09.
Revised in 2009–09.

The certification scheme for pathogen-tested material of *Rubus* spp. provides detailed guidance on the production of vegetatively propagated *Rubus* 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 *Rubus* 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 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 (rooted canes 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, in particular crumbly fruit.

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, 2009 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 candidates for nuclear stock

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. If 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 *Rubus* in the region of origin.

## 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

 

**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 | Test methods for confirmation |
|---|---|---|---|
| Black raspberry necrosis virus* | *Rubus* spp. (latent in most cvs of *R. idaeus*) | *R. occidentalis* | |
| *Cucumber mosaic virus* (*Cucumovirus*) | *Rubus* spp. & many other genera | Mechanical inoculation to test plants† | ELISA‡ |
| Raspberry leaf mottle* | *R. idaeus* (latent in most cvs) | *R. occidentalis* | |
| Raspberry leafspot* | *R. idaeus* (latent in most cvs) | *R. occidentalis* | |
| Raspberry vein chlorosis virus | *R. idaeus* (most cvs) | Visual symptoms<br>*R. idaeus* cv. 'Norfolk Giant',<br>*R. idaeus* cv. 'Malling Delight',<br>*R. idaeus* cv. 'Baumforth's seedling'<br>*R. idaeus* cv. 'Lloyd George' | |
| Raspberry yellow spot | *R. idaeus* (latent in most cvs) | *R. occidentalis*<br>*R. idaeus* cv. 'Malling Promise' | |
| *Rubus yellow net virus** (*Badnavirus*) | *Rubus* spp. | *R. occidentalis*<br>*R. macraei* | |
| *Arabis mosaic virus (Nepovirus)* | *R. idaeus*, *R. fruticosus* & other genera | Mechanical inoculation to test plants† | ELISA |
| *Cherry leaf roll virus (Nepovirus)* | *R. idaeus*, *R. fruticosus* & other genera | Mechanical inoculation to test plants† | ELISA‡ |
| *Raspberry ringspot virus (Nepovirus)* | *R. idaeus*, *R. fruticosus* & other genera | Mechanical inoculation to test plants† | ELISA‡ |
| *Strawberry latent ringspot virus (Sadwavirus)* | *R. idaeus*, *R. fruticosus* & other genera | Mechanical inoculation to test plants† | ELISA |
| *Tomato black ring virus (Nepovirus)* | *Rubus* spp. (usually latent) & other genera | Mechanical inoculation to test plants† | ELISA‡ |
| *Apple mosaic virus (Ilarvirus)* | *R. idaeus*, *R. fruticosus* & other genera | Mechanical inoculation to test plants† | ELISA |
| *Raspberry bushy dwarf virus (Idaeovirus)* | *Rubus* spp. | Mechanical inoculation to test plants† | ELISA‡ |
| Rubus stunt phytoplasma | *Rubus* spp. | Visual symptoms<br>*R. idaeus* cv. 'Malling Landmark',<br>*R. idaeus* cv. 'Norfolk Giant'<br>PCR§ | |

*The four agents marked, in several different combinations, can lead to symptoms known as rubus (or raspberry) mosaic disease.
†*Chenopodium quinoa*, *Cucumis sativus* and *Nicotiana clevelandii*.
‡Virus is quite variable and a single antiserum may not detect all isolates. This is especially true when using monoclonal antibodies.
§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 as 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 *Rubus*, the situation for PCR detection is at a 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.

(*Agrobacterium tumefaciens*), leafy gall (*Rhodococcus fascians*), downy mildew (*Peronospora rubi*) and raspberry cane midge (*Resseliella theobaldi*), as appropriate to the *Rubus* sp. 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 1) for the following pathogens (as appropriate to the *Rubus* sp. or hybrid concerned): Black raspberry necrosis virus, *Cucumber mosaic virus* (*Cucumovirus*), Raspberry leaf mottle disease, Raspberry leafspot disease, Raspberry vein chlorosis virus, Raspberry yellow spot disease, *Rubus yellow net virus* (*Badnavirus*), *Arabis mosaic virus* (*Nepovirus*), *Cherry leaf roll virus* (*Nepovirus*), *Raspberry ringspot virus* (*Nepovirus*), *Strawberry latent ringspot virus* (*Sadwavirus*), *Tomato black ring virus* (*Nepovirus*), *Apple mosaic virus* (*Ilarvirus*), *Raspberry bushy dwarf virus* (*Idaeovirus*), Rubus stunt phytoplasma, *Phytophthora rubi* and all other *Phytophthora* spp. infecting *Rubus*.

Plants giving negative results in all tests are promoted to nuclear stock plant status and should be transferred to a separate

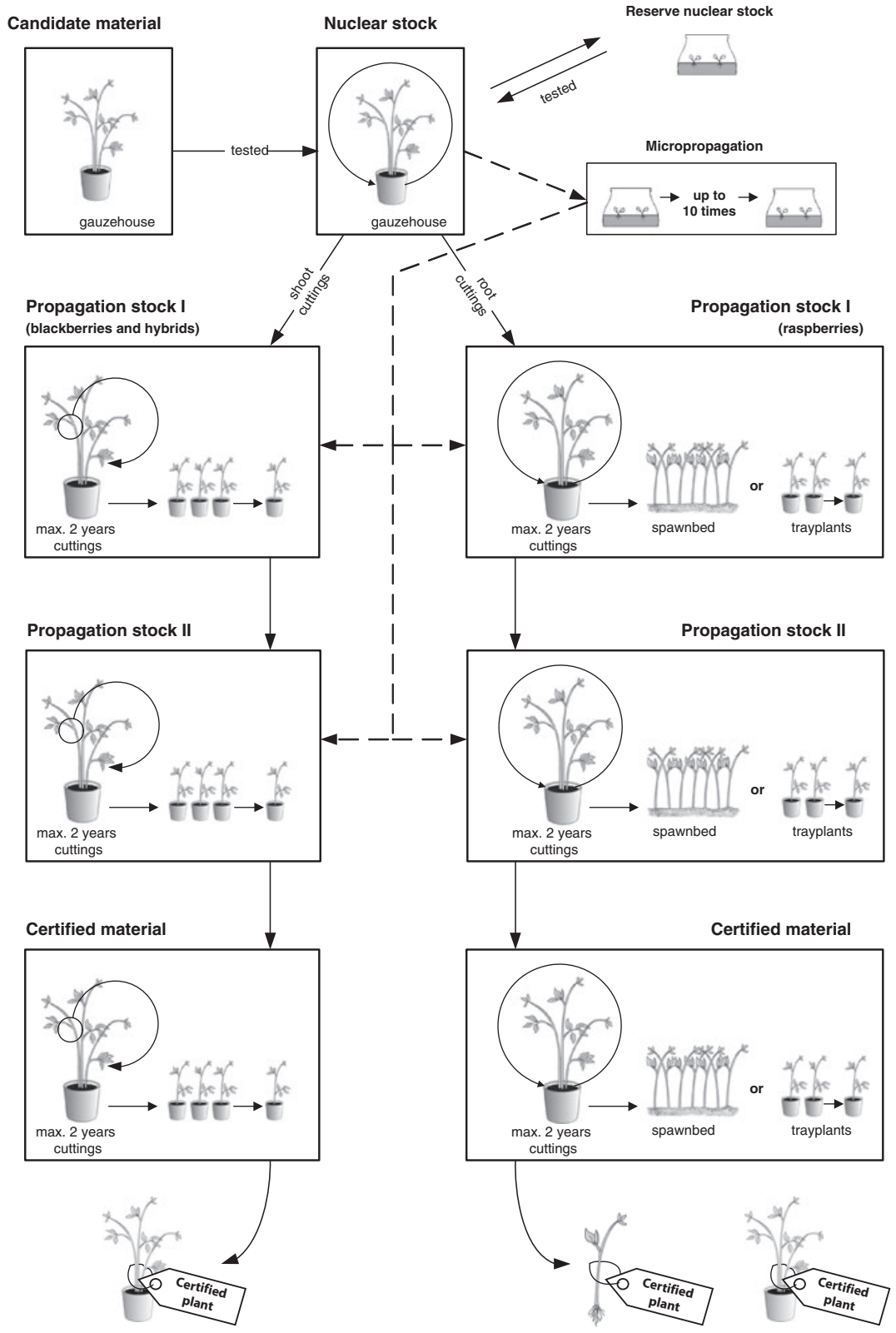


**Fig. 1** Diagram of the *Rubus* certification scheme.

gauzehouse of similar standard. Plants giving positive results in any test 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 rubi* or other *Phytophthora* spp., especially in cool and moist climates. For this purpose, micropropagation culture or stem cuttings are taken from plants, at a level of at least 0.5 m above the growing medium. When rooted in soil-less or sterilized growing medium and tested free from *P. rubi* and all other *Phytophthora* sp., these plants become the nuclear stock.

The recommended test methods are given in Appendix 1.

## 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 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[1] for all the pests mentioned above. 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 should be allowed to fruit (e.g. by using hand pollination) in a separate location.

## Production of propagation stock

Propagation stock of *Rubus* can be produced in two generations: propagation stock I and propagation stock II. At the site at which the propagation 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 virus vector nematodes (see Appendix 1). The site should only be used for the production of certified material if found to be substantially free from nematodes or if those nematodes found are shown to be free from viruses [see (OEPP/EPPO, 2009) Standard PM 4/35, **39**, 284–288].

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

### Raspberry

*Raspberry propagation stock I*

Usually propagation stock of raspberry starts with root material directly derived from nuclear stock. The roots are used to establish propagation stock I mother plants. These mother plants are used to produce cuttings for establishing spawn beds or a generation of propagation stock II mother plants. The mother plant generation should have a maximum age of two years. The entire propagation stock I (mother plants plus spawn bed) should not exceed a maximum age of four years. Rooted canes from the spawn beds can be used for establishing propagation stock II spawn beds. New plantings are allowed a year of establishment which will not count towards the age of the bed if the planting is not able to be certified in the first year.

*Raspberry propagation stock II*

A second generation (propagation stock II), derived from the first by transferring canes 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.

Flowering should preferably be prevented. In the case of primocane varieties, in which flowering cannot be prevented, the official organization may require additional sampling and testing for possible infestations with raspberry bushy dwarf virus.

### Blackberry and hybrids

*Blackberry and hybrids propagation stock I*

Usually propagation stock of blackberry starts with shoot material directly derived from nuclear stock. The roots are used to establish propagation stock mother plants. These mother plants are used to produce cuttings for young plants or a generation of propagation stock II mother plants. Mother plants should not be older than two years. Plants produced in this step can be used to establish propagation stock II mother plants.

*Blackberry and hybrids propagation stock II*

Propagation stock II of blackberry and hybrids is produced in the same way as propagation stock I.

### Alternative production methods

*Tray-plants*

In the EPPO region, tray-plants have become an alternative way of growing propagation stock instead of production from spawn beds and stem cuttings, either in aphid-proof gauzehouses or in specifically designed tray-plant areas.

In vitro *production*

Multiplication can entirely take place *in vitro*, beginning with meristems, apical tips or axillary buds (Appendix 3) from nuclear stock plants. The maximum number of reproduction cycles should be 10 (see Appendix 3). The rooted plants transplanted

out of the *in vitro* conditions become propagation stock I or II, depending on the demand for the number of plants. All plants produced by *in vitro* multiplication must be clearly designated as such. However, this designation is not needed for the progeny obtained from these plants, as trueness-to-type can be adequately determined within one multiplication step, provided an appropriate number of plants produce fruit.

### Isolation requirements

Propagation stock is usually produced in well isolated spawn beds or in aphid-proof greenhouses.

Outdoor propagation sites should be isolated from non-certified *Rubus* by at least 100 m, especially from fruiting plantations and wild *Rubus* spp. The soil should be tested for virus-vector nematodes (*Xiphinema* and *Longidorus;* see Appendix 1) and the site should only be used for the production of propagation stock plants if found substantially free from these nematodes or if those found are shown to be free from virus by a slash or bait test [see (OEPP/EPPO, 2009) Standard PM 4/35 **39**, 284–288].

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 2.

## Production of certified material

Cuttings taken from propagation stock plants produce the certified material. For raspberry, root cuttings or rooted canes may then be used to establish spawn beds or kept as mother plants for root cutting production. For blackberries and hybrid berries, cuttings taken from propagation stock plants can be used to produce mother plants for leaf-bud or tip propagation. The mother plants (or the spawn beds) should not exceed a maximum age of four years.

Canes with roots from propagation stock I or II are planted as a spawn bed at another site to produce the certified material (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 virus vector nematodes (see Appendix 1). The site should only be used for the production of certified material if found to be substantially free from nematodes or if those nematodes found are shown to be free from viruses [see (OEPP/EPPO, 2009) Standard PM 4/35]. The site should be isolated from other *Rubus* material by at least 25 m. General precautions against pests should be maintained.

As for propagation stock, tray-plants are an alternative way of growing certified material for any *Rubus* spp.

Recommended certification standards are given in Appendix 2. Inspection for the granting of certificates should be performed in early summer.

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

| | Plants (%) | | |
|---|---|---|---|
| | Nuclear stock | Propagation stock I & II | Certified material |
| 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.

## 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 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 check inspections should also take account of other important pests that can affect quality, so that the certified plants delivered to the *Rubus* 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.

## References

Bonants P, Hagenaar-de Weerdt M, van Gent-Pelzer M, Lacourt I, Cooke D & Duncan J (1997) Detection and identification of *Phytophthora fragariae* by polymerase chain reaction. *European Journal of Plant Pathology* **103**, 345–355.

Duncan JM, Kennedy DM & Seemuller B (1987) Identities and pathogenicities of *Phytophthora* spp. causing root rot of raspberry. *Plant Pathology* **36**, 301–305.

Duncan JM, Kennedy DM, Chard J, Ali A & Rankin PA (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 (1992) Recommendations made by EPPO Council in 1981: certification of virus-tested fruit trees, scions and rootstocks. *EPPO Technical Documents* **1013**, 42–43.

OEPP/EPPO (1993) Certification schemes. PM 4/7 Nursery requirements – recommended requirements for establishments participating in certification of fruit or ornamental crops. *Bulletin OEPP/EPPO Bulletin* **23**, 249–252.

OEPP/EPPO (2009) EPPO Standards PM 4/35 (1) Soil test for virus-vector nematode in fruit crops. *OEPP/EPPO Bulletin* **39**, 284–288.

Schlenzig A, Cooke DEL & Chard JM (2005) Comparison of a baiting method and PCR for the detection of *Phytophthora fragariae* var. *rubi* in certified raspberry stocks. *Bulletin OEPP/EPPO Bulletin* **35**, 87–91.

Stammler G, Seemüller B & Duncan JM (1993) Analysis of RFLPs in nuclear and mitochondrial DNA and the taxonomy of *Phytophthora fragariae*. *Mycological Research* **97**, 150–156.

Taylor CE & Brown DJF (1976) The geographical distribution of *Xiphinema* and *Longidorus* nematodes in the British Isles and Ireland. *Annals of applied Biology* **84**, 383–402.

## Appendix 1

### 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; graft inoculation to woody indicators; visual observation of symptoms (Table 1).

Graft tests are normally done in the glasshouse as bottle or leaf 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 carried out. Duration of the tests is at least one growing season; if done late in the season the tests should be observed during the following spring.

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

*Phytophthora* spp. *test*

*Phytophthora rubi* is a serious pathogen which has become important on *R. idaeus* in Europe. It is particularly important to exclude this and other species from certified material, by testing nuclear stock and by suitable precautions for propagation stock. Testing is made difficult by the occurrence of several *Phytophthora* spp. in raspberry roots (Duncan *et al.*, 1987) and can be carried out either by a bait test alone or PCR or a combination of both bait test and PCR methods. Bait tests are described by Duncan *et al.* (1993) and Schlenzig *et al.* (2005) can be used to monitor populations of *P. rubi* in the field. Development and use of PCR tests for *P. rubi* and other species are described in Stammler *et al.* (1993) and Schlenzig *et al.* (2005) and the required primers by Bonants *et al.* (1997).

*Soil testing for virus-vector nematodes*

Soil in which propagation stock II and plants producing certified material is to be planted should be sampled and the samples found substantially free from the following species of nematode vector: *Xiphinema diversicaudatum* (the vector of *Arabis mosaic virus* and *Strawberry latent ringspot virus*), *Longidorus macrosoma* (*Raspberry ringspot virus*), *L. attenuatus* (*Tomato black ring virus*) and *L. elongatus* (*Raspberry ringspot virus* and *Tomato black ring virus*). The nematodes can be tested directly for the presence of virus [see (OEPP/EPPO, 2009) Standard PM 4/35]. Alternatively a bait test can be carried out on a duplicate sample of soil taken from the field (Taylor & Brown, 1976).

## Appendix 2

### 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 rubi* and all other *Phytophthora* sp. infecting *Rubus*. 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 lot. All plants must 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 lot. All plants should also be substantially free from symptoms of infestation by other pests (including pathogens).

## Appendix 3

### Guidelines on sanitation procedures

Viruses and virus like organisms, and also fungi and bacteria, are readily removed by a combination of hot air treatment and meristem culture. To this end, plants in containers are exposed to high temperatures (37 to 38°C) for a period of 4 to 8 weeks. Shoots then are collected and surface sterilized and meristems of 0.1–0.3 mm are collected and cultured.

The microplants obtained from meristem culture are then transferred to a proliferation medium containing growth regulators (indole butyric acid, benzylaminopurine, gibberellic acid).

Subsequently, explants are pricked out on a medium without benzylaminopurine, containing indole butyric acid to favour rooting. When the plants reach 3–4 cm and are well rooted, they are transplanted into peat blocks in the glasshouse at high relative humidity.

Plants produced in this way are considered to be candidate plants, and should always be retested individually according to the methods described in section 2 (Production of nuclear stock). The success rate of this method is variable, but can be as high as 90–95%. When all test mentioned above have shown to give negative results, the material can be promoted to nuclear stock.