◆ EPPO Standards ◆

CERTIFICATION SCHEMES

PATHOGEN-TESTED MATERIAL OF *VACCINIUM* SPP.

PM 4/18(1) 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.

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

PM 4/18(1) English

Certification scheme

PATHOGEN-TESTED MATERIAL OF *VACCINIUM* SPP.

Specific scope

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

Specific approval and amendment

First approved in September 1996.
Edited as an EPPO Standard in 1998.

For the production of certified pathogen-tested material of *Vaccinium* spp., the successive steps described below should be taken. They are based on the general sequence adopted by the EPPO Panel on Certification of Pathogen-tested Fruit Crops and approved by the EPPO Council (OEPP/EPPO, 1992). The stages of the certification scheme are illustrated in Fig. 1. Throughout the whole procedure, care should be taken to maintain the pomological characters of the originally selected plants. Checks should be built in on possible mutations or back mutations.

This scheme covers the certification of several species of vaccinium that are being grown on a commercial scale. The species covered included highbush blueberry (*Vaccinium corymbosum* and *V. australe*), lowbush blueberry (*V. angustifolium*), rabbiteye blueberry (*V. ashei*), cranberry (*V. macrocarpon*) and lingonberry (*V. vitis-idaea*). Vegetative propagation may be by:

(a) softwood cuttings, taken in early summer and rooted under plastic. Rooted plantlets are usually replanted in winter in containers and are grown for one more season;

(b) winter cuttings, taken in early winter and planted in early spring, indoors or outdoors, under mist. Rooted plantlets are usually replanted in winter in containers and are grown for one more season;

(c) *in vitro* techniques (Eck, 1988; Mudge, 1994).

1. Selection of material

New or existing cultivars may be selected to become candidate material. The starting material should be selected on the basis of trueness to type, vigour, production, pomological quality and absence of pest symptoms, especially virus symptoms and symptoms of *Exobasidium vaccinii* var. *vaccinii*, *Godronia cassandrae* (anamorph *Topospora myrtilli*) and *Agrobacterium tumefaciens*. Alternatively, starting material may be obtained from existing certification schemes in other EPPO countries.

2. Production of nuclear stock

General procedure

The candidate material for nuclear stock status (which may be derived from cuttings from the candidate material or may be the original plants of the candidate material) 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 containers in sterilized growing medium, with strict precautions to avoid any type of contamination, especially with aphids and leafhoppers that are virus vectors, and to avoid direct contact with the soil. The plants are tested for the pathogens specified in Table 1 by the methods given in Appendix I.

Many of the cultivars used in the EPPO region have a North-American origin. New and existing cultivars are being imported from North America into the EPPO region on a regular basis. From the North-American region, there are several reports of nematode-borne viruses from vaccinium (blueberry leaf mottle nepovirus, tobacco ringspot nepovirus (= blueberry necrotic ringspot virus), peach rosette mosaic nepovirus, tomato ringspot nepovirus). Of these viruses, both tobacco ringspot nepovirus and tomato ringspot nepovirus have been found occasionally in the EPPO region, although never in vaccinium. These nepoviruses form a potential threat to blueberry cultivation in the EPPO region and are all classed as quarantine pests by EPPO. However, until now, no particular measure have been taken to exclude these viruses on vaccinium, so a test for this group of viruses (Table 2) should be included in the general test scheme (Appendix I).

Plants giving negative results in all tests can be promoted to nuclear stock and should be transferred to a separate gauzehouse of similar standard (see section 3) ; alternatively, cuttings taken from such healthy candidate material may be used to produce nuclear stock plants. Plants giving positive results for any virus should be removed immediately.

Elimination of pathogens

If all candidate nuclear stock plants of a cultivar or clone give positive test results, thermotherapy or meristem tip culture can be used as sanitation procedures (Appendix II). The progeny resulting from any such process may be considered to be candidate material and must be re-tested for the viruses mentioned in Tables 1 and 2 and re-assessed for agronomic and varietal characters.

Inspection for other pests

The general status of the plants with respect to *Exobasidium vaccinii* var. *vaccinii*, *Godronia cassandrae* and *Agrobacterium tumefaciens*, and to other diseases or unknown symptoms, should be regularly checked by visual inspection.

3. Maintenance of the nuclear stock

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 pathogen freedom as candidate nuclear stock.

Each plant should be tested every 5 years for the viruses, virus-like diseases and phytoplasmas listed in Table 1, using the methods described in Appendix I. Several visual inspections should be made each year for these and other pests. Tolerances for visual inspection are given in Appendix III. Any plant giving a positive result in one of the tests mentioned, or showing symptoms of any pest on visual inspection should be eliminated.

4. Production of propagation stock

Multiply the nuclear stock in as few steps as possible to obtain the required quantity of propagation stock. Preferably, no more than two steps of multiplication are used to obtain propagation stock II from nuclear stock.

Several visual inspections should be made each year for pests. Tolerances for visual inspection are given in Appendix III. Provided the level of infection does not exceed these tolerances, the lot (i.e. all plants derived from a single nuclear stock plant) can remain in the certification scheme, after elimination of any plant showing symptoms of any pest.

Propagation stock I

Softwood cuttings taken in summer are rooted under plastic in containers in sterilized medium. Alternatively, winter cuttings are rooted, indoors or outdoors, under mist. Direct contact with the soil should be avoided. The plants should be isolated from other vaccinium material (including wild species) by at least 200 m. The stock should be protected by spraying against air-borne virus vectors. Normally after the second growing season, cuttings are taken from these young plants in order to produce propagation stock II.

Propagation stock II

Propagation stock II is produced from propagation stock I either by softwood cuttings taken in summer or by winter cuttings. The same conditions apply as for propagation stock I. Alternatively, propagation stock II may be directly produced from nuclear stock plant by *in vitro* culture. Special checks for agronomic and varietal characters should be carried out on propagation stock II produced in this way and on the certified stock derived from it.

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

5. Distribution of propagation stock and production of certified stock

The material from propagation stock is distributed to nurseries, under strict official control. Ideally, an official or officially authorized organization should perform the distribution. 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). The grower should record the number of mother plants for each category, substantiating the origin of virus-free propagation material by official documents.

Certified stock is directly produced from propagation stock either by softwood cuttings or by winter cuttings. No *in vitro* culture may be used to produce the certified stock. Risks of changes in agronomic or varietal characters are considered to be too high, since checks on these characters cannot be done reliably enough before the certified product has left the scheme.

Sterilized growing medium should be used. Direct contact with the soil should be avoided. The plants should be isolated from other vaccinium material (including wild species) by at least 50 m. The stock should be protected by spraying against air-borne virus vectors. Normally, after the second growing season, the young plants leave the certification scheme and are sold as certified material.

Visual inspection should be made each year for pests. Tolerances on visual inspection are given in Appendix III. Provided the level of infection does not exceed these tolerances, the lot (i.e. all plants derived from a single plant of Propagation stock II) can remain in the certification scheme, after elimination of any plant showing symptoms of any pest.

6. 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 number of pathogen-free plants on the basis of field inspections and of records and documents, presented by the nurserymen.

Visual inspections and spot checks should be made during the growing season for symptoms of the pests listed in Appendix III and for other pests.

7. Certification

Inspection for the granting of certificates should be performed in spring and early summer. Bushes of vaccinium should be in their second growing season at final inspection, in order to assess trueness to type. Recommended certification standards are given under "certified stock" in Appendix III. Certified vaccinium planting 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.

APPENDIX I

Recommended test methods for certified vaccinium in the EPPO region

ELISA

ELISA testing is suitable for blueberry shoestring virus, and also for the North-American nepoviruses (see below).

Herbaceous indicators

Sap inoculation to *Chenopodium quinoa* and *Cucumis sativus* can be used for blueberry leaf mottle nepovirus, tobacco ringspot nepovirus (= blueberry necrotic ringspot virus), peach rosette mosaic nepovirus and tomato ringspot nepovirus. Note: viruses in this group may be further identified by other methods, e.g. ELISA. These viruses do not occur in the EPPO region in vaccinium. See also Table 2 and the main text.

Grafting on woody indicators

Blueberry mosaic agent - Stanley, Cabot (2 y);

Blueberry red ringspot caulimovirus - Cabot (2 y);

Blueberry stunt phytoplasma - Cabot, Jersey (2 y);

Blueberry witches' broom phytoplasma - *V. myrtillus* (2 y).

Visual

Cranberry false blossom phytoplasma and the ringspot agent of cranberry can only be detected by visual examination for symptoms.

APPENDIX II

Elimination of viruses from infected vaccinium plants

Since many of the diseases in vaccinium have phytoplasma etiology, heat treatment is suggested by some authors to be used to produce pathogen-free material. Little information is published on the rate of success of this method nor of any alternative method, e.g. meristem tip culture. Nevertheless, these two techniques are the only ones available at present.

APPENDIX III

Recommended tolerances for visual inspection during the growing season at different stages of certification

All the plants in a lot, derived from a single nuclear stock plant (or from a single plant of propagation stock II, in the case of certified stock), can remain in the scheme, provided that the level of infection, or non-trueness to type, does not exceed the tolerance levels given in Table 3 and provided that all plants showing symptoms of any disorder are removed.

References

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Eck, P. (ed.) (1988) *Blueberry Science*, pp. 130-132. Rutgers University Press, New Jersey (US).

Mudge, K.W. (1994) Rapid propagation of blueberry plants using ex vitro rooting and controlled acclimatization of micropropagules. *HortScience* **29**, 1124-1126.

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) 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.

Table 1. Pathogens occurring in the EPPO region to be tested for during the production of nuclear stock of vaccinium

Pest	Geographical distribution	Transmission
Blueberry shoestring virus	North America, Europe	Aphids
Blueberry stunt phytoplasma	America, Europe	Leafhoppers
Blueberry witches' broom phytoplasma	Europe	Leafhoppers
Cranberry false blossom phytoplasma	North America, Europe	Leafhoppers
Blueberry mosaic agent	North America, Europe	No known vector
Blueberry red ringspot caulimovirus	USA, Europe	No known vector
Cranberry ringspot agent	USA, Europe	No known vector

Table 2. Pathogens not occurring in vaccinium in the EPPO region, but forming a potential risk

Pest	Geographical distribution	Transmission
Blueberry leaf mottle nepovirus[1]	Europe, North America	Pollen
Tobacco ringspot nepovirus[2] (= blueberry necrotic ringspot virus)	America, Australia, Europe	Nematodes
Peach rosette mosaic nepovirus	North America	Nematodes
Tomato ringspot nepovirus[2]	Chile, Japan, New Zealand, North America	Nematodes
Blueberry scorch carlavirus	Canada	Aphids
Blueberry shock ilarvirus	Canada	Pollen

[1] Grapevine Bulgarian latent nepovirus is a distantly related strain of blueberry leaf mottle nepovirus and occurs in Bulgaria, Hungary and Portugal. It has never been reported to infect vaccinium.

[2] Tobacco ringspot nepovirus and tomato ringspot nepovirus have both been reported from the EPPO region, although never found in vaccinium.

Table 3. Certification standards for vaccinium

	% plants			
	Nuclear stock	**Propagation stock I**	**Propagation stock II**	**Certified stock**
Arthropods				
Contarinia vaccinii	0	0.5	0.5	0.5
Fungi				
Armillariella mellea	0	0	0	0
Exobasidium vaccinii var. vaccinii	0	0.5	0.5	1
Godronia cassandrae	0	0.1	0.1	0.5
Bacteria				
Agrobacterium tumefaciens	0	0	0	0
Others				
All virus symptoms	0	0	0	0.5
Not true to variety	0	0	0.1	1
Other pests and diseases	substantially free	substantially free	substantially free	substantially free

Fig. 1 Diagram of the stages in the *Vaccinium* certification scheme