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

Certification scheme for *Sambucus*

Specific scope

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

Specific approval and amendment

First approved in September 2007.

Introduction

The genus *Sambucus* belongs to the *Adoxaceae* plant family. In the literature, *Sambucus* was previously assigned to *Caprifoliaceae* or to a narrower plant family, *Sambucaceae*. *Sambucus racemosa* (red elder or European red elder), *S. canadensis* (sweet elder or American elder) and *S. nigra* (European elder) are the species most widely used for agronomical purposes. *S. ebulus*, *S. sieboldiana* and *S. pubens* are used for the production of medicinal components. Widely grown ornamental forms of *Sambucus* are *S. nigra* f. *alba*, *S. nigra* f. *aurea*, *S. nigra* f. *laciniata* and *S. nigra* f. *pendula*. Production of elderberry is mainly located in eastern North America (from the native species *S. canadensis*) and in Central European countries (from the native species *S. nigra*). Traditionally, selections of the best plants collected from the wild were used locally, but high quality cultivars were also developed from *S. canadensis* in the USA and Europe between 1960 and 1980. In Europe the most widely grown cultivar is 'Haschberg', a wild selection of *S. nigra* originating from the region of Vienna, Austria. Fruits of *Sambucus* are used for processing in preserves and for wine-making. Many different *Sambucus* parts are used for pharmaceutical purposes. Anthocyanin pigments of *Sambucus* provide a stable basis for the colour of all kinds of processed food products.

The scheme is presented according to the general sequence proposed by the EPPO Panel on Certification of Pathogen-tested Fruit Crops and approved by EPPO Council (OEPP/EPPO, 1992). This certification scheme covers both fruit producing as well as ornamental species and selections.

Outline of the scheme

For the production of certified pathogen-tested *Sambucus* 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 thermotherapy or meristem tip culture, followed by testing for the pathogens 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, under conditions ensuring freedom from infection, with retesting as appropriate
5 Production of certified plants: semi hard or 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.

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Table 1 *Sambucus* pathogens occurring in the EPPO region

Pest	Geographical distribution	Transmission
A. Pathogens subject to testing during the production of nuclear stock		
Arabis mosaic virus (*Nepovirus*, ArMV)	Europe, America, Japan, New Zealand	*Xiphinema diversicaudatum*
Cherry leafroll virus (*Nepovirus*, CLRV)	Worldwide	*Xiphinema* spp., seed, pollen
Elderberry latent virus (*Carmovirus*, ElLV)	Europe, America	vectorless soil transmission
Elderberry symptomless virus (*Carlavirus*, ESLV)*	Europe, America	aphids
Elderberry vein clearing virus†	Europe, America, Japan	
Strawberry latent ringspot virus (*Sadwavirus*; SLRSV)‡	America	*Xiphinema diversicaudatum*
Verticillium dahliae	Worldwide	
B. Other pathogens of lesser importance or occurring only rarely in *Sambucus*		
Xylella fastidiosa	America	cuttings, graftwood, insects

*syn: Elderberry carlavirus, Elderberry A virus.
†syn: *Sambucus* vein clearing virus.
‡in *Sambucus*, only occurring in North America.

1. Selection of candidates for nuclear stock

Selection should be done in several different plantations of each cultivar or in nature. Such plantations should preferably be free from any symptoms of viruses and of *Verticillium* wilt (caused by *Verticillium dahliae*). Select several vigorous, productive plants which show typical characteristics of the particular cultivar desired and which show no symptoms of serious pests or graft-transmissible diseases[1]. Alternatively, certified starting material may be obtained from other countries. As it is known in practice, that selections of *Sambucus* are not always stable genetically, it is recommended that all candidate nuclear stock plants, which are found suitable for promotion to nuclear stock, should be checked on their pomological and morphological characters, especially when thermotherapy or meristem tip culture has been performed.

2. Production of nuclear stock

General procedure

Semi-hard cuttings are taken in summer during the period of vigorous growth. Cuttings are treated with indole acetic acid and rooted in sand. Rooted cuttings are then potted in containers. Alternatively, hardwood cuttings are taken in autumn, and they can be planted directly in containers. Sterilized growing medium should be used. Containers should not be in direct contact with the soil, in order to prevent any type of contamination. The use of plastic, mist or fog should be avoided, in order to prevent bacterial rot of young plant material. Ornamental types of *Sambucus* may be propagated by grafting instead of cutting. For grafting, seedlings (preferably *Sambucus nigra*) are used, free from the pathogens listed in Table 1. After one year of growth, rooted or grafted plants should be tested for the pathogens specified in Table 1 by the methods given in Appendix 1.

[1]For material originating from the Americas, it should also be free from leaf scorch caused by *Xylella fastidiosa*.

Plants which give a negative result for all tests can be promoted to nuclear stock and transferred to the nuclear stock repository, or nuclear stock material may be propagated from them. The resulting nuclear stock plants are the parent plants for further propagation.

Elimination of pathogens

If all candidate nuclear stock plants of a cultivar give positive test results, thermotherapy can be used as a sanitation procedure (Appendix 3). The treated candidate plants should then be re-tested for pathogen-freedom.

Inspection for other pests

Visual inspections of candidate nuclear stock plants for other pests should be done carefully, particularly for canker (*Botryosphaeria* spp.), dieback (*Nectria cinnaberina* and *Valsa sordida*), powdery mildew (*Phyllactinia guttata*) and Nectriella-galls (*Nectriella pironii*). A particular disease of *Sambucus*, attacking flowering branches more specifically, is a wilt caused by *Fusarium sambucinum* and *Phoma sambuci-nigrae*. An appropriate and effective plant protection programme should be operated in all propagation units.

3. Maintenance of nuclear stock

The nuclear stock plants should be maintained in a repository under conditions excluding pests. Plants should be grown in containers with a soil-free or sterilised growing medium, isolated from the soil and in a suitably designed insect-proof glasshouse or gauzehouse. In the nuclear stock repository each plant should be tested at least every 2 years (annually if possible) for *Cherry leaf roll virus* (*Nepovirus*) and *Elderberry latent virus* (*Carmovirus*), if present in the country. Several visual inspections should be made each year for these viruses and other pests.

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.

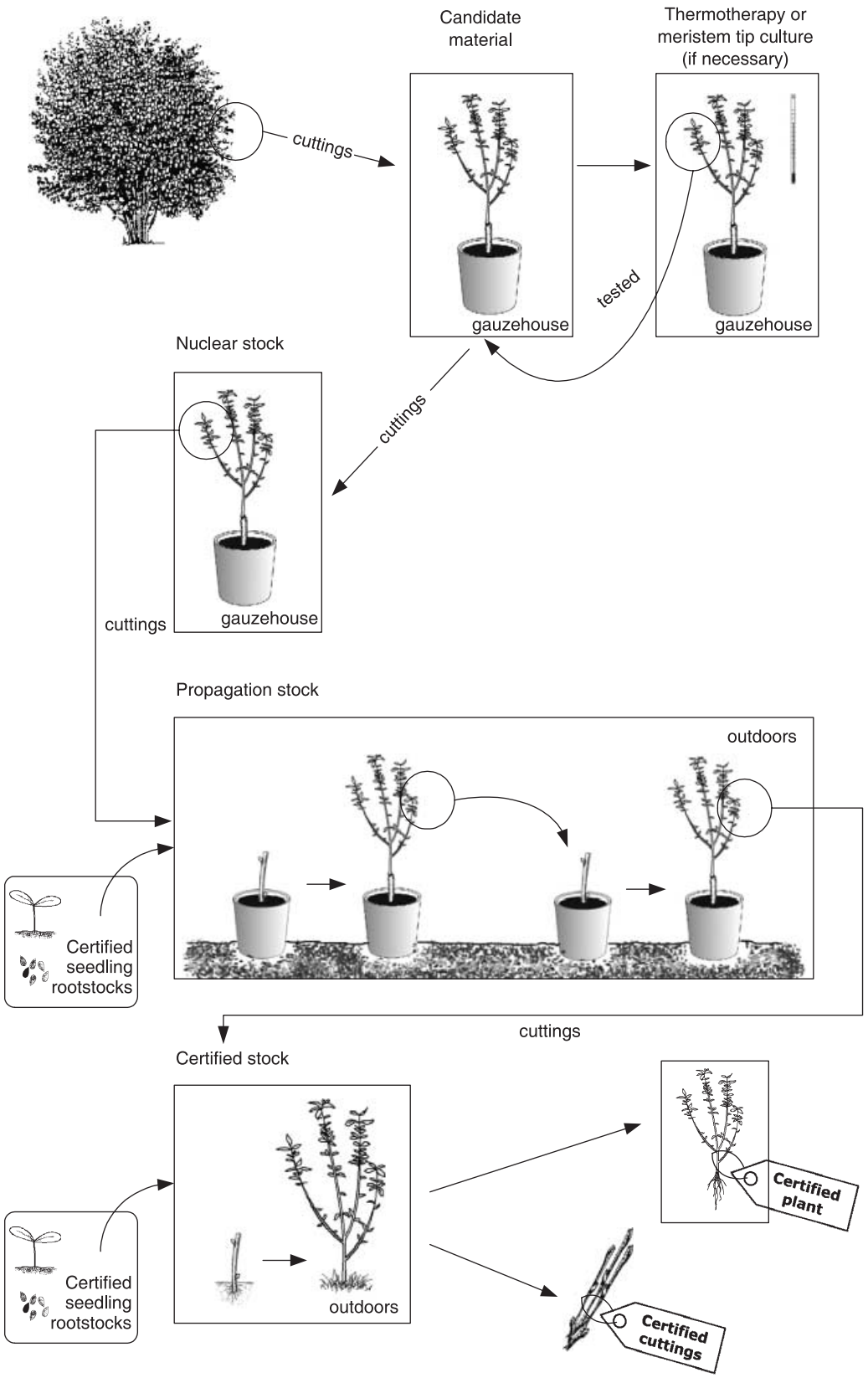

Fig. 1 Diagram of the stages in the *Sambucus* certification scheme.

4. Production of propagation stock

Propagation stock is normally established in the field. Plants produced from rooted cuttings taken from the nuclear stock are established outdoors in isolated plots (e.g. isolation distance of 25 m) and tested. Soil should be tested for the presence of virus-vector nematodes (i.e. *Xiphinema* – see EPPO Standard PM 4/xx, in preparation) and *Verticillium dahliae* (Appendix 2), and should be found substantially free from them. The propagation stock plants should preferably not be kept longer than 5 years because of declining quality, unless acquired infections or other faults require an earlier renewal. The soil should always be retested before new propagation stock plants are established from nuclear stock. Visual inspections of propagation stock should be done regularly for symptoms of the pests listed in Table 1 and for other pests.

5. Production of certified plants

By several alternative propagation methods, certified material can be produced from propagation stock. Either semi hard cuttings are taken in summer or hardwood cuttings are taken in autumn, and grafts are made in winter on *S. nigra* seedlings. Semi hard cuttings are rooted in early summer in sand, and later in the summer once rooted, they are planted in the soil, or, preferably, planted in containers in a container field. Hardwood cuttings are taken in autumn, and are directly planted in the soil or containers. Grafts are made in winter on rootstocks, usually already planted in containers. In most cases, certified planting material will be marketed after one year of growth. Visual inspections and spot checks should be made during the growing season for symptoms of the pests as described in Appendix 4 for the pests listed in Table 3 and for other pests. Recommended certification standards are given in Appendix 4. Provided the level of infections does not exceed these tolerances, the lot can be certified, after elimination of any plant showing symptoms of any pests.

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 into account other important pests that can affect quality, so that the certified plants delivered to the *Sambucus* tree 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.

Appendix 1

Test methods for viruses

Sambucus viruses listed in Table 1 can be detected by inoculation to test plants, ELISA, PCR and grafting onto woody indicators (Table 2).

Inoculation to indicator plants

Candidate nuclear stock material of *Sambucus* should be tested by inoculation to *Chenopodium quinoa*, using a standard phosphate buffer including polyethylene glycol, mol. wt 6000, at 20 g/L in the extraction buffer. Testing during summer season should be avoided, since virus titres can be very low during periods of hot weather. Inoculation to test plants may also be used for the regular retesting of nuclear stock.

Table 2 Test methods for viruses of *Sambucus*

Pathogen	ELISA	Herbaceous test plants	Other
Arabis mosaic virus (*Nepovirus*, ArMV)	+	+	
Cherry leafroll virus (*Nepovirus*, CLRV)	+	+	
Elderberry latent virus (*Carmovirus*, ElLV)		+	
Elderberry symptomless virus (*Carlavirus*, ESLV)		+	
Elderberry vein clearing virus			visual inspection and/or woody indexing on *S. nigra* seedlings
Strawberry latent ringspot virus (*Sadwavirus*, SLRSV)	+	+	

Table 3 Certification standards for *Sambucus*

	% plants		
	Nuclear Stock	Propagation Stock	Certified Stock
Symptoms of virus diseases	0	0	0
Verticillium dahliae	0	0	1
Xylella fastidiosa	0	0	0
Other pests	substantially free	substantially free	substantially free

Grafting onto woody indicators

Elderberry vein clearing virus cannot be detected by laboratory or greenhouse techniques. Only visual inspection has been described. Many species and cultivars of *Sambucus* do not show symptoms of Elderberry vein clearing virus very easily. For this purpose, it is recommended to test all candidate nuclear stock plants by indexing on virus free *S. nigra* seedlings, which are known to show perceptible symptoms of this virus very well. This test can be performed under greenhouse conditions, as long as temperatures do not rise above 25°C.

ELISA

For ArMV, CLRV and SLRSV, ELISA testing can be used instead of inoculation to indicator plants, especially for regular retesting of nuclear stock. The general ELISA procedure for perennial crops can be followed (Barbara *et al*., 1990).

RT-PCR

In certain cases (e.g. importation of selected material from America) it might be necessary to check the presence of *Xylella fastidiosa*. For this purpose, RT-PCR is the preferred method (Rodrigues *et al*., 1993).

Appendix 2

Guidelines on soil sampling for *Verticillium dahliae* analysis

Soil samples are air-dried for 1–2 weeks at 25°C, passed through a 2-mm sieve and thoroughly mixed, 10 g sub-samples are then taken for further processing. The sub-samples are washed through sieves with 125 mm and 37 mm pore size. The residue on the 37 mm sieve is surface-disinfected for 10-s in 0.525% NaOCl, rinsed, and washed into a 50 mL beaker (total volume of residue and water 15–20 mL). Using a spoon, this slurry is distributed onto the surface of 10 Petri dishes (10 cm diameter) containing ethanol-streptomycin-agar medium prepared by adding 1.6 g agar to 200 mL of distilled water and autoclaving the mixture for 20 min (Nadakavukaren & Horner, 1959). After the agar mixture has cooled to 50°C, 27 mg of streptomycin sulphate (75%) is added. This mixture, combined with the water agar, is poured onto 10 dishes. After 7–10 days of incubation at 22°C in darkness, soil is washed from the agar surface with water. Plates are incubated for 2–3 additional days before verifying the identity of the fungal colonies. For the enumeration of *Verticillium* populations in soil, the sum of colonies counted on each set of agar dishes should equal the number of microsclerotia in 10 g of soil (Butterfield & De Vay, 1977; Goud & Termorshuizen, 2003).

Appendix 3

Elimination of viruses from infected *Sambucus* plants

The most effective procedure for the elimination of viruses is thermotherapy, although preliminary experience with meristem tip culture showed that this might be another possibility. Freedom from all of the viruses listed in Table 1 can be achieved by growing infected plants at 37–39°C for 6 to 8 weeks, followed by excising shoot tips about 2–3 mm long. These shoot tips can be side grafted into the stems of young *S. nigra* seedlings, planted in sterilised growing medium (Jongedijk, 2000).

Appendix 4

Recommended tolerances at growing-season inspection

All the plants in a lot, derived from a single plant of the previous certification stage, can remain in the scheme, provided that the level of infection does not exceed the tolerance levels given in Table 3 and provided that all plants showing symptoms of any disorder are removed. Higher grades of stock should normally be free from symptoms of the organism concerned, and substantially free from other pests.

References

Barbara DJ, Clark MF, Thresh JM & Casper R (1990) Rapid detection and serotyping of prunus necrotic ring spot virus in perennial crops by enzyme-linked immunosorbent assay. *Annals of Applied Biology* **90**, 395–399.

Butterfield EJ & De Vay JE (1977) Reassessment of soil assays for *Verticillium dahliae*. *Phytopathology* **67**, 1073–1078.

Goud JK & Termorshuizen AJ (2003) Quality of methods to quantify microsclerotia of *Verticillium dahliae* in soil. *European Journal of Plant Pathology* **109**, 523–534.

Jongedijk GP (2000) Warmtebehandeling vlier gelukt, *Naktuinbouwkrant 2000*, **2**, 7.

Nadakavukaren MJ & Horner CE (1959) An alcohol agar medium selective for determining *Verticillium* microsclerotia in soil. *Phytopathology* **49**, 527–528.

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.

OEPP/EPPO (2008) EPPO Standards PM 4/34 (1) Soil tests for virus-vector nematodes in fruit crops. *Bulletin OEPP/EPPO Bulletin* **38**, 30–51.

Rodrigues JLM, Silva-Stenico ME, Gomes JE, Lopes JRS & Tsai SM (1993) Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S rRNA and *gyrB* Sequences. *Applied and Environmental Microbiology* **69**, 4249–4255.