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

Certification scheme for poplar and willow

Specific scope

This standard describes the production of certified pathogen-tested material of *Populus* and *Salix*.

Specific approval and amendment

First approved in September 2007.

Introduction

The genus *Populus* belongs to the *Salicaceae* plant family. A broad range of species and hybrids can be found as nursery crops. Well known species are *P. alba* (white poplar), *P. canescens* (grey poplar), *P. tremula* (trembling aspen), *P. nigra* (black poplar), *P. deltoides* (Eastern cottonwood), *P.* × *canadensis* (Canadian poplar), *P. candicans* (balsam poplar), *P. trichocarpa* (black cottonwood) and *P.* × *euramericana*.

The genus *Salix* also belongs to the *Salicaceae* plant family. Similarly many species of *Salix* are propagated in nurseries, e.g. *S. triandra* (black maul), *S. alba* (white willow), *S. viminalis* (osier), *S. pentandra* (bay willow), *S. smithiana* and *S. caprea* (Kilmarnock willow).

Poplar and willow are grown in the EPPO region on a large scale. Both species are recognized for their quality of the wood, and both species are also used as ornamentals and forest trees. Poplars especially are grown for reforestation and conservation, and as windbreaks and landscape plants. Willows are also used in plantations for producing industrial fuel (biomass). In nurseries, plant material is normally propagated with hardwood cuttings. Some species of willow and poplar may be grafted on rootstocks, especially ornamental forms. *Populus tremula* may be propagated from seed for forestry purposes, but this method of propagation is beyond the scope of this certification scheme. Nevertheless, it is advisable for nurseries propagating *P. tremula* from seeds, to follow the general procedures in this scheme as much as possible.

For the production of pathogen-tested material of *Populus* (poplar) and *Salix* (willow), 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 EPPO Council (OEPP/EPPO, 1992). This certification scheme covers forestry species as well as ornamental species and selections.

Outline of the scheme

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

1 Selection for 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, 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: hardwood cuttings or graftwood taken from propagation stock are grown under conditions minimizing infections to produce certified plants (entire rooted plants or cuttings finally distributed).

Throughout the whole procedure, care should be taken to maintain the 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,

Table 1 *Populus* and *Salix* pathogens occurring in the EPPO region

(a) *Populus* pathogens occurring in the EPPO region

Pest	Geographical distribution	Transmission
A. Pathogens of *Populus* subject to testing during the production of nuclear stock		
Arabis mosaic virus (*Nepovirus*, ArMV)*	Europe, America, Japan, New Zealand	*Xiphinema diversicaudatum*
Poplar mosaic virus (*Carlavirus*, PopMV)	worldwide	natural spread, no vector identified
Tomato black ring virus (*Nepovirus*, TBRV)*	worldwide	*Longidorus elongatus*, *L. attenuatus*
Poplar decline virus (= Populus virus) (Potyvirus, PopDV)	America, (?) Europe	propagation material
Poplar witches' broom phytoplasma	worldwide	natural spread (*Idiocerus populi*)
B. Other pathogens of lesser importance or occurring only rarely in *Populus*		
Poplar vein yellowing virus-like agent	worldwide	propagation material
Tobacco necrosis virus (*Necrovirus*, TNV)*	worldwide	Olpidium brassicae
Tobacco rattle virus (*Tobravirus*, TRV)*	worldwide	*Paratrichodorus* spp., *Trichodorus* spp.

(b) *Salix* pathogens occurring in the EPPO region

Pest	Geographical distribution	Transmission
A. Pathogens of *Salix* subject to testing during the production of nuclear stock		
Poplar mosaic virus (*Carlavirus*, PopMV)	worldwide	natural spread, no vector identified
Tobacco ringspot virus (*Nepovirus*, TRSV)†	worldwide	Xiphinema americanum sensu lato
Salix witches' broom phytoplasma	worldwide	natural spread, no vector identified
Brenneria (= *Erwinia*) *salicis*	worldwide	propagation material
B. Other pathogens of lesser importance or occurring only rarely in *Salix*		
none		

*Only detected in roots.

†Asymptomatic infection.

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

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 or phytoplasmas. 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. Alternatively, certified starting material may be obtained from other countries. *Salix* should be taken from plantations which do not show visible symptoms of *Brenneria salicis* in their vicinity.

2. Production of nuclear stock

General procedure

Normally, hardwood cuttings are taken in late winter, and they can be planted directly in the soil in spring. During winter time, cuttings can be kept in cold storage for several months. In particular cases, poplar will be grafted; *P. canescens* can be grafted onto *P. alba* rootstocks or *P. alba* cuttings ('stenting' technique). Some types of *Salix* may also be propagated by grafting instead of cuttings; *S. caprea* and its cultivars usually will be grafted onto *S. smithiana* rootstocks. For grafting, preferably *Salix* and *Populus* rootstocks are used, free from the pathogens listed in Table 1. Cuttings and graftwood are harvested preferably in late winter time. They can be stored for up to three months at 1.5°C, or even up to one year at −10°C. Before storage, cuttings should be treated with a broad spectrum fungicide, in order to prevent saprophytic fungi from causing problems in the first period after planting. 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.

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 2). 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, especially for pests which might

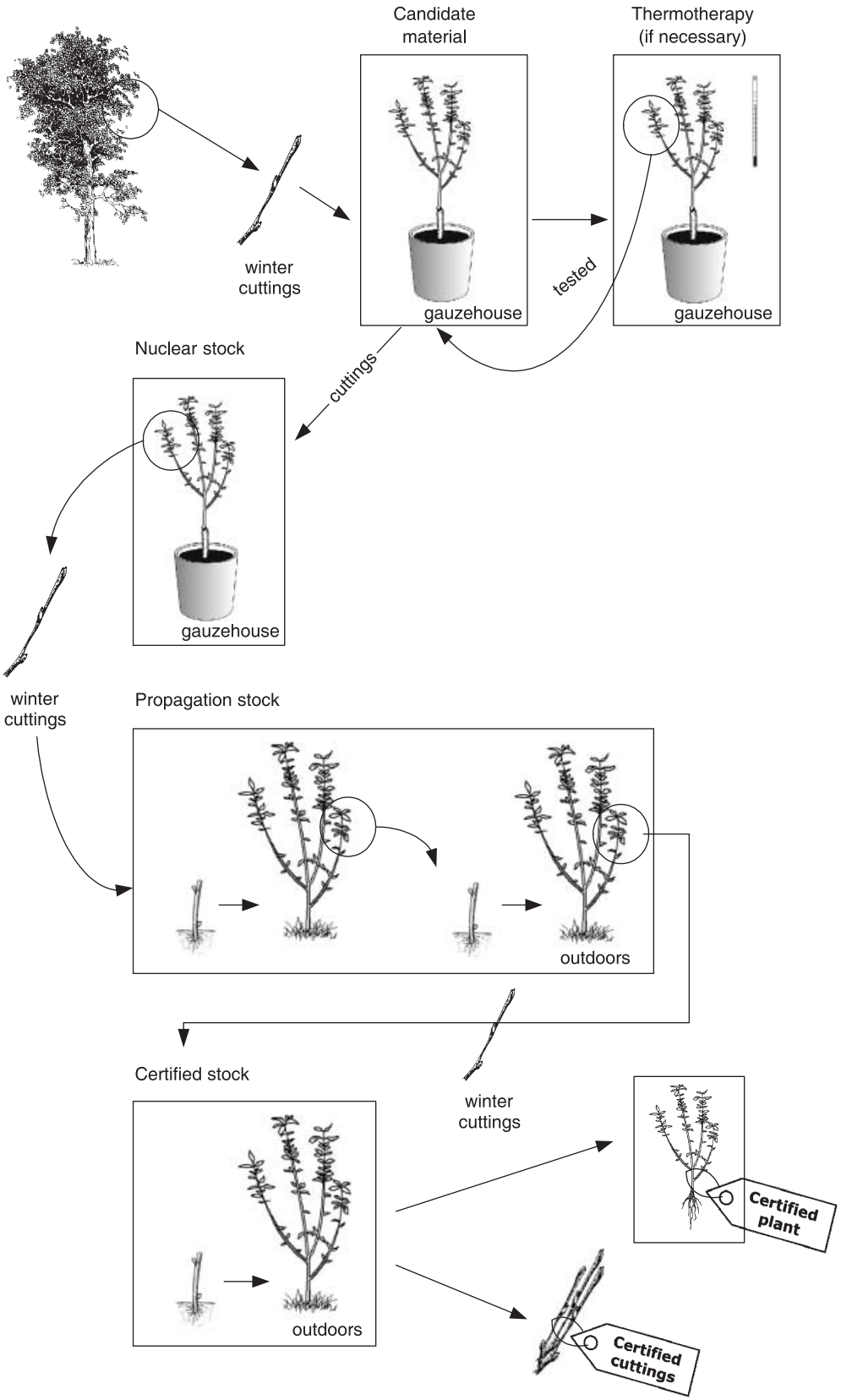

Fig. 1 Diagram of the stages in the *Populus* and *Salix* certification scheme.

be able to spread with planting material. For *Populus*, special attention should be paid to *Xanthomonas populi* (bacterial canker), *Venturia macularis* (leaf blight), *Drepanopeziza populi* and *D. populorum* (leaf spot), *Melampsora larici-populina* and *M. populnea* (leaf rust), *Cryptodiaporthe populea* (trunk scab) and Poplar witches' broom phytoplasma. For *Salix*, pests of most interest are *Melampsora caprearum* (leaf rust), *Venturia saliciperda* (willow scab) and *Glomerella cingulata* (black canker). For both *Populus* and *Salix*, special attention should be paid to infestation with *Paranthrene tabaniformis* (dusky clearwing), as its larvae might spread with propagation material. 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 under conditions ensuring freedom from (re)infection by root contact, pollen or aerial vectors, preferably in pots of sterilized growing medium in a suitably designed aphid-proof house.

In the nuclear stock repository, each plant should be tested at least every 5 years for the pathogens listed in Table 1, if present in the country. Several visual inspections should be made each year for these and other pests.

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. Soil should be tested for the presence of virus-vector nematodes (*Xiphinema*, *Longidorus*, *Trichodorus* and *Paratrichodorus*) and should be found substantially free from them (see EPPO Standard PM4/xx, in preparation).

Propagation stock plants should not be kept longer than 10 years, unless acquired infections or other faults require an earlier renewal. Soil should always be retested before new propagation stock plants are established from nuclear stock. Preferably, propagation stock plantations are surrounded by a zone which is free from any symptoms of *Brenneria salicis* (e.g. 1 km).

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

Certified stock can be produced from propagation stock, usually by using hardwood cuttings but in some cases by grafting. Cuttings should be treated and stored as described under 2. (general procedure). Certified planting material will generally be marketed after one year of growth. Preferably, certified stock plantations should be surrounded by a zone which is free from any symptoms of *Brenneria salicis* (e.g. 100 m). Visual inspections and spot checks should be made several times during the growing season for symptoms of the pests as described in Appendix 3 for the pests listed in Table 3, and for other pests. Recommended certification standards are given in Appendix 3. 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 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 poplar or willow grower are substantially free from these pests. Certified material for export should in any case satisfy the phytosanitary regulations of importing countries. Certified trees 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

The *Populus* and *Salix* viruses listed in Table 1 can be detected by inoculation to test plants, ELISA and PCR (Table 2).

Inoculation to indicator plants

Candidate nuclear stock material of *Populus* and *Salix* should be tested by inoculation to *Chenopodium quinoa*, using a standard phosphate buffer including polyethylene glycol, mol. wt 6000, at 20 g/litre in the extraction buffer. Testing in summer season should be avoided, since virus titres can be very low during periods of hot weather. Inoculation

Table 2 Test methods for viruses of *Populus* and *Salix*

Pathogen	ELISA	Test plants	Other
viruses:			
Arabis mosaic virus (*Nepovirus*, ArMV)	+	+	
Poplar mosaic virus (*Carlavirus*, PopMV)	+	+	
Poplar decline virus (= Populus virus) (Potyvirus, PopDV)		+	
Poplar vein yellowing virus-like agent			visual inspection
Tobacco necrosis virus (*Necrovirus*, TNV)		+	
Tobacco rattle virus (*Tobravirus*, TRV)		+	RT-PCR
Tobacco ringspot virus (*Nepovirus*, TRSV)	+	+	
Tomato black ring virus (*Nepovirus*, TBRV)	+	+	
phytoplasmas:			
Poplar witches' broom phytoplasma			PCR
Willow witches' broom phytoplasma			PCR
bacteria:			
Brenneria (= *Erwinia*) *salicis*			PCR

Note: 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 *Populus* and *Salix*, 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 2. It can be expected that additional PCR tests will become available before the existing scheme may be updated.

Table 3 Certification standards for *Populus* and *Salix*

	% plants		
	Nuclear Stock	Propagation Stock	Certified Stock
virus diseases	0	0	0
Poplar witches' broom [on poplar]	0	0	0
Melampsora spp.*	5	5	5
Brenneria (= *Erwinia) salicis* [on willow]	0	0	0
Xanthomonas populi [on poplar]	0	1	1
Paranthrene tabaniformis	0	0	1
other pests	substantially free	substantially free	substantially free

*Note that *M. medusae* is a quarantine pest for many EPPO countries and subject to specific requirements.

to test plants may also be used for the regular retesting of nuclear stock.

ELISA

For *Arabis mosaic virus (Nepovirus)*, *Poplar mosaic virus (Carlavirus)*, *Tobacco ringspot virus (Nepovirus)* and *Tomato black ring virus (Nepovirus)*, 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*., 1978).

PCR

For the detection of phytoplasmas in woody crops, PCR is the preferred method. Details for sample preparation and testing can be found in the literature (Ahrens & Seemüller, 1992; Hauben *et al*., 1998; Seruga *et al*., 2003).

Appendix 2

Elimination of viruses from infected *Populus* and *Salix*

The most effective procedure for the elimination of viruses is thermotherapy. 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 4–5 mm long. These shoot tips can be rooted in sterilised growing medium.

Appendix 3

Recommended tolerances at growing-season inspection

All the plants in a lot, derived directly from 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.

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