◆ EPPO Standards ◆

CERTIFICATION SCHEMES

SEED POTATOES

PM 4/28(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 Schemes are intended to be used by NPPOs 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 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 (1992a) Recommendations made by EPPO Council in 1981: certification of virus-tested fruit trees, scions and rootstocks. *EPPO Technical Documents* 1013, 42-43.
OEPP/EPPO (1992b) EPPO Standards PM 4/1(1) Certification schemes. Virus-free or virus-tested fruit trees and rootstocks. Part I. Basic scheme and its elaboration. *Bulletin OEPP/EPPO Bulletin* **22**, 267-277.
OEPP/EPPO (1993a) EPPO Standards PM 4/7(1) Certification schemes. Nursery requirements - recommended requirements for establishments participating in certification of fruit or ornamental crops. *Bulletin OEPP/EPPO Bulletin* **23**, 249-252.
OEPP/EPPO (1993b) 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

Basic material

Propagation stock material from all but the last stage of propagation stock, satisfying the recommended certification standards and certified for sale. According to the number of stages of propagation stock, there may be several grades of basic material.

Candidate nuclear stock

Any plant that may become or may be propagated to produce nuclear stock. Testing for specified pests is required before the plant can be accepted as nuclear stock. Until testing is complete and negative, the plant remains candidate nuclear stock.

Certification scheme

System for the production of vegetatively propagated plants for planting, intended for further propagation or for sale, obtained from nuclear stock after several propagation stages under conditions ensuring that stated health standards are met. The filiation of the material is recorded throughout the scheme.

Certified material

Propagating material from the last stage of propagation stock, satisfying the recommended certification standards and certified for sale. In the case of plants that are sold grafted onto rootstocks, the rootstocks must also be at least of the last stage of propagation stock, and the plants must be held under approved conditions between grafting and sale.

Certified material may, according to the plant concerned, be referred to more specifically as, for example, certified plants, certified cuttings, certified bulbs, etc.

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 by vegetative propagation from a defined parent plant.

Nuclear stock

Plants individually tested by the most rigorous procedure in a certification scheme and found free from specified pests. All such plants must be maintained at all times under strict conditions ensuring freedom from infection. According to the crop concerned, plants propagated from nuclear stock material may remain nuclear stock provided that they do not leave the nuclear stock conditions. In the case of plants that are maintained by grafting onto rootstocks, the rootstocks must also be nuclear stock.

Nuclear stock material

Propagating material derived from nuclear stock, which may be further propagated without change of ownership or certified for sale as pre-basic material.

Prebasic material

Nuclear stock material, satisfying the recommended certification standards and certified for sale.

Propagation stock

Plants derived from nuclear stock, propagated and maintained under conditions ensuring freedom from infection. Pathogen freedom is checked by appropriate procedures. Propagation may be done in a number of successive stages under different approved conditions. The plants are then known as propagation stock I, propagation stock II, etc. There may be several generations within each of these stages, provided that the plants do not leave the approved conditions. The number of stages and/or generations allowed within propagation stock is generally limited and will depend on the crop concerned. In the case of propagating material that is maintained by grafting on a rootstock, the rootstock should be at least of the corresponding stage of propagation stock.

Propagation stock material

Propagating material derived from propagation stock, which may be further propagated without change of ownership, or certified for sale as basic or certified material, according to the stage of propagation stock concerned.

OUTLINE OF REQUIREMENTS

EPPO Certification 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 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 4/28(1) English

Certification scheme

SEED POTATOES

Specific scope

The EPPO certification scheme for seed potatoes is intended to be used by National Plant Protection Organizations and official organizations in charge of certification, in their capacity as bodies responsible for the design of systems for the production of healthy seed potatoes, for the inspection of such potatoes proposed for certification and for the issue of certificates.

This scheme complements the existing UN/ECE standard on the production and marketing of seed potatoes (UN/ECE, 1994) and attempts to be compatible with it. It presents requirements for the production of certified seed potatoes to a certain standard with regard to a number of important pests. The scheme takes account of the fact that a number of these important pests are quarantine pests for many countries. Moreover, some of these important pests may be subject to national regulations, which have the objective of containing or eradicating the pest concerned. As a consequence, seed potatoes produced for domestic use or export in a particular country may have to satisfy additional requirements for such pests. This scheme cannot include all such requirements, which will differ according to the countries concerned. However, the scheme draws attention to the probable existence of such requirements when it refers to the pests that are regulated in this manner in many EPPO countries. In particular, the scheme refers to the requirements for seed potatoes moved within the EU (EU, 1977, 1966, 1993a) and to the EU "Control Directives" for the individual pests *Synchytrium endobioticum* (EU, 1969a), *Globodera* spp. (EU, 1969b), *Clavibacter michiganensis* subsp. *sepedonicus* (EU, 1993b) and *Ralstonia solanacearum* (EU, 1998).

The certification standards presented in this scheme (Table 3) are considered to be the minimum requirements for the practical production of healthy seed potatoes, but national authorities may decide to set stricter standards in national certification schemes based on the EPPO scheme, in order to take account of different conditions in their territories in relation to the prevalence of certain pests.

Specific approval and amendment

First approved in September 1999.

Specific definitions

Seed potatoes

Tubers and microplants of cultivated tuber-forming *Solanum* spp. which are produced under an official certification system to meet specified requirements.

Microplants of potato

Plants (including tubers) in tissue culture of tuber-forming *Solanum* spp.

Minitubers of potato

Tubers produced by microplants of potato in a growing medium meeting specified requirements.

Outline of the scheme

The scheme has the aim of providing seed potatoes that are free from certain pests and meet specified tolerances for others, and whose health status is attested by an official certificate. It does not cover farm-saved tubers or potato germplasm (tubers or microplants to be used as breeding material or true potato seeds). For the production of certified seed potatoes, the following successive steps should be followed by an official organization or under its control:

1 Selection for quality of individual candidate nuclear stock plants of each cultivar to be taken into account in the scheme. Optional selection for virus freedom among these plants by testing.

2 Micropropagation of these plants. Selection for freedom from viruses and bacteria among microplants by testing or production of virus-free plants by treatment or *in vitro* methods, followed by testing. The microplants thus shown to be free from the given viruses and bacteria are designated as nuclear stock.

3 Maintenance of nuclear stock as microplants.

4 Multiplication of nuclear stock in two phases, propagation stock I and II, respectively, under protected conditions and in the field, respectively, with retesting as appropriate, under rigorous conditions excluding reinfestation by certain pests and reducing reinfestation by others.

5 Production of propagation stock III and propagation stock IV.

6 Issue of certificates for tubers from propagation stock I, II, III or IV.

Material produced at the stages of propagation stock III and IV is covered by the UN/ECE Standard (UN/ECE, 1994). These stages are illustrated in Fig. 1.

In the scheme, a specific terminology has been used for the successive stages of multiplication and certification: "candidate nuclear stock", "nuclear stock" and "propagation stock". These terms have been defined for all EPPO certification schemes and can be related as follows to alternative terms used for seed potato certification as defined by the UN/ECE standard (UN/ECE, 1994): material produced from propagation stock I corresponds to "prebasic seed - TC", while material produced from propagation stock II corresponds to "prebasic seed", material produced from propagation stock III corresponds to "basic seed" and material produced from propagation stock IV corresponds to "certified seed". Because of the greater number of distinct steps in the propagation of seed potatoes, this UN/ECE terminology does not exactly correspond with the general terminology used in other EPPO Certification Schemes.

At all stages of the scheme, propagation may be performed under more stringent conditions than those described (for example, propagation stock II may be produced in the glasshouse instead of outdoors).

Throughout the whole procedure, care should be taken to maintain the characters of the originally selected plants. Checks should be built in to detect the occurrence of possible mutations.

1. Selection of material

New or existing cultivars of potato (*Solanum tuberosum*)[1] may be selected as candidate nuclear stock (as tubers or microplants). This starting material should have been derived from plants selected visually on the basis of trueness to type and freedom from pests. Starting material may be obtained from existing certification schemes in other EPPO countries.

2. Production of nuclear stock

The candidate nuclear stock should be kept under containment in an officially approved micropropagation and insect-proof glasshouse facility, separately from the nuclear stock. All material should be micropropagated, following methods such as those in Appendix IV. This micropropagation is primarily intended to eliminate certain bacterial and fungal pathogens, especially *Erwinia* spp. A testing programme should be applied to ensure that all material held as individual microplants is free from the pests listed in Table 1.

The recommended test methods are given in Appendix°I. According to the pathogen, the test is applied to the microplant, to a plant grown from the microplant under containment in a glasshouse or to tubers produced by such a plant.

Preliminary testing of leaves from an eye plug or mother tuber plant may be useful to detect tubers infected by viruses (Appendix I, 1). The trueness to type of the cultivar and freedom from pests may be evaluated on a plant grown from the mother tuber.

Material imported from outside the EPPO region should also be inspected and tested under quarantine for all EPPO quarantine pests of potato according to the relevant EPPO phytosanitary procedures (OEPP/EPPO, 1984, 1990a, b, 1991; EPPO/CABI, 1996) and generally inspected for any other pests.

Plants giving negative results in all tests should be transferred to a separate micropropagation facility of similar standard (see section 3) to become nuclear stock. Plants giving positive results in any test should be removed immediately, and consideration should be given to retesting other plants in the facility.

If no plants of a cultivar prove to be free from these pests, procedures may be applied to eliminate infection (Appendix V). The microplants resulting from this treatment are considered again as candidate nuclear stock and should be retested for the pathogens above and reassessed for agronomic and varietal characters. It should be noted that meristem culture is also very effective in eliminating fungal and bacterial diseases of potato.

3. Maintenance of nuclear stock

Nuclear stock microplants should be kept in an officially approved micropropagation facility, containing only nuclear-stock plants. Microplants should be maintained in such a way as to avoid the formation of callus tissue or die-back and under conditions to prevent their contamination by pests. The tissue cultures should be stored on a medium without growth hormones; M & S medium with 3% mannitol is suitable. The cultures should be checked for trueness to type, for example by growing five or six microplants from each culture in the field every 2 years and examining the plants visually for trueness to type.

4. Multiplication of the material

4.1 Propagation stock I (for production of UN/ECE-equivalent 'prebasic seed – TC')

Propagation stock I includes one or two steps: micropropagation of microplants from nuclear stock in tissue culture, optionally followed by the production of minitubers from the microplants.

Micropropagation

Laboratories should be officially approved for the production of microplants in tissue culture for planting, either directly in the field or to produce minitubers. These laboratories should demonstrate a proficiency in aseptic techniques for such production and should not

[1] The scheme may be extended to other tuber-forming species of *Solanum* or their hybrids with *S. tuberosum*.

handle other plant material likely to carry potato pests. Laboratories should ensure sterile conditions and maintain a record system that documents the source of the material and the volume of production. This propagation stock I may be maintained indefinitely in tissue culture provided the above requirements are met.

Minitubers

Minitubers are grown in a suitably designed aphid-proof facility isolated from other plant material not derived from tissue culture. Additional security can be achieved if the production takes place at a time of year when there is little or no risk of introduction of insects. In certain parts of Europe, minitubers can be grown outdoors to satisfy these conditions.

The growing medium should be pest-free. The growing crop should be kept free from aphids and other pests at all times. The occurrence, development or spread of pests should have been prevented by appropriate husbandry practices, and insecticides and fungicides can be used to control the pests. Measures should be applied as follows to avoid recontamination: use of protective clothing; disinfection or change of shoes; use of pest-free soil; use of water free from potato pests (e.g. tap water or rain water). Each crop should be officially inspected at least once during the growing season and found to be free from potato pests.

The filiation of plants should be recorded so that each propagation stock I plant is known to be derived from nuclear stock by no more than a fixed number of generations (indefinite for micropropagation but limited to one generation for minitubers).

Certification of 'prebasic seed – TC' material will be granted on the basis of records and inspections carried out on the growing crop and on harvested minitubers. The material should conform to the recommended certification standards in Appendix II. It should be noted that confirmation of varietal identity or trueness to type will depend on inspection of the crop derived from this material.

4.2 Propagation stock II (for production of UN/ECE-equivalent 'prebasic seed')

Plants of propagation stock II are produced in the field from propagation stock I minitubers, or microplants from propagation stock I, in as few generations as possible.

The plants are produced under conditions that reduce the risk of virus spread by aphids (including suitably timed insecticide or oil sprays against aphids, early haulm-killing, roguing). Precautions should be taken to minimize the spread of mechanically transmitted diseases through good hygiene and clean equipment. The crop should be planted in a plot not known to be infected with *Synchytrium endobioticum*, *Ditylenchus destructor*, *Meloidogyne chitwoodi* or *M. fallax*, and the soil should have been sampled and the samples found free from potato cyst nematodes *Globodera pallida* and *Globodera rostochiensis* (see Appendix I, 7). The crop should be free from *Synchytrium endobioticum*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Ralstonia solanacearum*, PSTVd and potato stolbur phytoplasma. Any other national regulations concerning the pests mentioned here should have been respected, as well as any phytosanitary requirements of other countries to which material from the certification scheme may be exported. General precautions against pests should be maintained (especially against those pathogens that affect the tubers directly and are subject to fixed tolerances at certification). The crop should be officially inspected a number of times during the growing season on occasions appropriate to detection of the target pests. The number of inspections depends on the period during which the crop is exposed to a risk of infection, and may be three inspections in the south of the EPPO region and as little as one inspection in the north.

The filiation of plants should be recorded so that each propagation stock II plant is known to be derived from nuclear stock by no more than a fixed number of generations.

The material should conform with the recommended certification standards in Appendix II. These include virus and off-type tolerances for the progeny produced from the tubers. These standards may be met by the application of suitable measures during the growing season (timing of aphid control and/or haulm killing, roguing, etc.) enforced by the certification authority. Compliance with the standards may be checked, if appropriate, by post-harvest tuber tests for common aphid-transmissible viruses or by later monitoring of the progeny in the field. The certifying authority can decide whether there is a need for post-harvest tuber tests according to the probability that the crop has been infected by viruliferous aphids; this, in turn, depends on such factors as the climate, the cultivar, the proximity to other potato crops, the prevalence of aphids at the appropriate time of year, etc. Certification will be granted on the basis of records of the tests/inspections performed on the growing crop, of post-harvest tuber tests (if any) and of inspections of harvested tubers intended for marketing.

Notwithstanding the above, tubers from individually selected plants of the first three generations of propagation stock II can, under special conditions defined in Appendix III, be used in place of the initial minitubers or microplants to start a new cycle of propagation stock II. This process has been described as 'clonal selection'.

4.3 Propagation stock III (for production of UN/ECE-equivalent 'basic seed')

After the allowed number of generations of propagation stock II, or if the certification standards for propagation stock II are not met, the material passes to the stage of propagation stock III. The conditions for soil testing, inspection and protection against pests during production of propagation stock III are essentially the same as for propagation stock II (see 4.2), except that the certification standards are set at a lower level.

The material should conform to the recommended certification standards in Appendix II, set for basic category seed potatoes in the UN/ECE standard.

4.4 Propagation stock IV (for production of UN/ECE-equivalent 'certified seed')

After the allowed number of generations of propagation stock III, or if the certification standards for propagation stock III are not met, the material passes to the stage of propagation stock IV. The conditions for soil testing, inspection and protection against pests during production of propagation stock IV are essentially the same as for propagation stock II and III, except that the certification standards are set at a lower level. An allowed number of generations is also fixed for propagation stock IV.

The material should conform with the recommended certification standards in Appendix II, set for certified category seed potatoes in the UN/ECE Standard.

4.5 Number of field generations

The maximum number of generations allowed in propagation stock II, III and IV is decided by the certification authority but, in general, the total for all these field generations should not exceed 10.

Throughout the stages of propagation stock, official checks should be made on varietal identity and purity and on possible mutations.

Appendix I

Guidelines on testing procedures

1. Testing for viruses in candidate material

Tests are performed on leaves from potato plants grown from a microplant of candidate material in a quarantine glasshouse. The list of viruses to be tested for and of methods to be used appears in Table 2.

ELISA

ELISA tests are available for all viruses but are recommended in this scheme only for PVA, PVM, PVS, PVX, PVY and PLRV. Each plant of the candidate material should be tested separately.

Use of indicator plants

The potato viruses covered by the scheme (except PLRV) can be detected by sap inoculation to a range of suitable indicator plants. Table 2 gives an example of the use of the following five indicator plants, *Chenopodium quinoa, Nicotiana benthamiana, N. glutinosa, N. tabacum* cv. White Burley and *Phaseolus vulgaris* cv. The Prince; other combinations that allow the detection of all the viruses may also be used. Screening with these indicator plants will detect potato viruses without necessarily identifying them, including PVA, PVM, PVS, PVX and PVY, which are tested for by ELISA in any case. It should be noted that some isolates of PVS can be detected by *Chenopodium quinoa*, but that *C. murale* can detect more isolates. The reliability of bioassays may be affected by a number of factors and some viruses may require special inoculation buffers.

2. Post-harvest tuber tests

Virus tests may be carried out on tubers or on sprouts or leaves grown from tubers or from eye-plugs. A dormancy-breaking compound is usually used to encourage sprout growth (De Bokx, 1987) and/or increase virus concentration.

Gibberellic acid is commonly used to encourage sprout growth, by dipping tubers, or, most often, eye plugs, in a 1-ppm GA3 solution for about 15 min. After draining, eye plugs may be immersed in a suitable protectant fungicide in order to prevent attack by *Rhizoctonia solani*. They are stored overnight at room temperature, then planted in sterilized compost or a soil-less medium to develop sprouts or sprouts with leaves. Sap from sprouts or leaves is then tested by ELISA. Rindite (a mixture of ethylene chlorohydrin, dichloroethane and carbon tetrachloride, 7:3:1 in volume) is also available for this purpose, but its use is not authorized in some countries. It is applied to tubers in a fumigation chamber, as described by Ehlers *et al.* (1983), to encourage sprout growth and increase virus concentration. Tubers are then incubated in a growth chamber at 22-25 °C, with high humidity and low light to encourage sprout development. Sprout sap is used for ELISA.

The number of tubers in a sample will depend on the tolerance to be satisfied, the confidence level used and the heterogeneity of the lot.

3. Testing for potato spindle tuber viroid (PSTVd)

The test for PSTVd is applied to leaves from potato plants grown either from a microplant of candidate material or from an original candidate tuber, under containment in a glasshouse. The test can be done by return-polyacrylamide gel electrophoresis (RT-PAGE) (OEPP/EPPO, 1984; modified by Huttinga *et al*., 1987; Schroeder & Weidemann, 1989), RNA probes (EU, 1997a) or PCR (Shamloul *et al*., 1997; Weidemann & Buchta, 1998).

4. Testing for Clavibacter michiganensis subsp. sepedonicus

The standard immunofluorescence (IF) test (OEPP/EPPO,1990a; EU, 1993b) is applied to a potato tuber, either the candidate tuber itself or a daughter tuber of a plant grown from a microplant of candidate material under containment in a glasshouse. A positive IF result (without further confirmation) is sufficient to reject the material but, in many EPPO countries, ring rot regulations require that any positive test result should be investigated further.

5. Testing for Erwinia spp.

Microplants are tested as individual 6 to 12-week plants growing in agar in plastic tubes. The basal section of the initial microplant is allowed to regrow to produce sufficient plant material for testing. Aseptic techniques should be used throughout the test procedure for testing, and positive controls included with each test.

Potato microplants are removed from agar using forceps, and excess agar is removed by gently scraping the root tissue. The plant is macerated with sterile pestle and mortar. Aliquots (100 µL) of the macerate are plated onto plates of nutrient agar and potato dextrose agar and incubated at 27°C for 3 days. Any bacteria may be further identified, and the test should be abandoned if too many bacteria are present. The remaining macerate is placed in 5 mL of PT broth (Burr & Schroth, 1977) and incubated anaerobically for 48 h at 27°C. Undiluted broth (100 µL) and a 10-fold serial dilution are spread onto crystal violet pectate agar plates (Pérombelon & Burnett, 1991) and incubated at 27°C for 3 days before examining for the presence of pitted colonies indicative of pectolytic *Erwinia* spp. The *Erwinia* colonies can be purified on nutrient agar plates, and the subspecies identified by standard biochemical tests. The soft rot *Erwinia* species can also be detected by the use of specific antibodies or polymerase chain reaction (PCR)-based methods (Pérombelon *et al*., 1999; Toth *et al*., 1999).

6. Testing for Ralstonia solanacearum

The test is applied to a potato tuber, either the candidate tuber itself or a daughter tuber of a plant grown from a microplant of candidate material, under containment in a glasshouse. Immunofluorescence (IF) and/or selective plating should be used, with optional additional tests (ELISA, PCR, enrichment, bioassay on tomato or aubergine). A test procedure is provided by OEPP/EPPO (1990b), but has since been improved (EU, 1997b). A revised EPPO procedure is in preparation. A positive IF or plating result (without further confirmation) is sufficient to reject the material but, in many EPPO countries, brown rot regulations require that any positive test result should be investigated further.

7. Testing soil for potato cyst nematodes Globodera pallida and Globodera rostochiensis

Procedures are described in OEPP/EPPO (1991b). The normal sampling procedure is to take 100 cores of 4-5 mL of soil with a half-cylindrical sampling tool, from not deeper than 5 cm in the soil, distributed on a grid pattern throughout the plot and collected in a polyethylene bag to provide a sample of 400 mL (500 g). The definition of the plot to be sampled in this way differs according to local practices.

The soil samples are then processed in the laboratory. Various methods are available and are considered equivalent: flotation method, flask and paper-strip methods, Fenwick can, elutriation method, Wye washer or centrifugal method.

Appendix II

Recommended certification standards for seed potatoes

Certification will be granted on the basis of records of the tests/inspections performed on the growing crop, of post-harvest tuber tests (if any) and of inspections of harvested tubers. The assessor will verify that the standards mentioned below are fulfilled.

Nuclear stock

The micropropagation facilities must satisfy official requirements. Records must show that all nuclear stock microplants gave a negative test result for AMV, CMV, PVA, PAMV, PLRV, PVM, PMTV, PVS, PVV, PVX, PVY, TMV, TNV, TRV, TBRV, ToMV, TSWV, PSTVd, *Ralstonia solanacearum*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Erwinia* spp. No microplant may show any symptom of fungal, bacterial or viral diseases. If these conditions are not met, certification at this grade will be refused.

Propagation stock I

In the case of micropropagation, the micropropagation facilities must satisfy official requirements. In the case of minituber production, all plants and tubers must be free from pests and from any symptoms of attack by pests. The percentage of minitubers showing physical defects and dirt must not exceed 3% and 1% respectively (see Table 3). If these conditions are not met at the time of the growing season inspection and tuber inspection, certification at this grade will be refused.

Propagation stock II

Records must show that the plot in which the material is planted is free from *Globodera pallida* and *G. rostochiensis,* and is not known to be infected with *Synchytrium endobioticum*, *Ditylenchus destructor*, *Meloidogyne chitwoodi* or *M. fallax*. The crop must be free from *Synchytrium endobioticum*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Ralstonia solanacearum*, PSTVd and potato stolbur phytoplasma Any other national regulations concerning the pests mentioned here must have been respected, as well as any phytosanitary requirements of other countries to which material from the certification scheme may be exported. The incidence of other pests and disorders in the growing crop and on inspection of tubers intended for marketing must not exceed the tolerances given in Table 3. The results of post-harvest tuber tests, if any, must not exceed the direct progeny tolerances given in

Table 3. If these conditions are not met, certification at this grade will be refused.

Propagation stock III and IV

Records must show that the plot in which the material is planted is free from *Globodera pallida* and *G.°rostochiensis*, and is not known to be infected with *Synchytrium endobioticum*, *Ditylenchus destructor*, *Meloidogyne chitwoodi* or *M. fallax*. The crop must be free from *Synchytrium endobioticum*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Ralstonia solanacearum*, PSTVd and potato stolbur phytoplasma. Any other national regulations concerning the pests mentioned here must have been respected, as well as any phytosanitary requirements of other countries to which material from the certification scheme may be exported. The incidence of pests and disorders in the growing crop and on inspection of tubers intended for marketing must not exceed the tolerances given in Table 3. The results of post-harvest tuber tests, if any, must not exceed the direct progeny tolerances given in Table 3. If these conditions are not met, certification at this grade will be refused.

Appendix III

Details on the selection of mother plants in clonal selection

Clonal selection is a system of seed potato propagation that starts from selected plants of propagation stock II and satisfies prebasic standards. Propagation stock II of a particular cultivar descended from a clonally selected mother plant may be called a "clonal stock". Clonally selected mother plants should be taken from:

- the direct progeny of propagation stock I; or
- plants of the first three generations of clonal stock.

The new clonal stock (progeny of selected mother plants) is subjected to an intensive inspection and testing regime:

- visual (throughout propagation);
- testing for at least PVA, PVM, PVS, PVX, PVY and PLRV (leaf samples, zero tolerance) of all plants in the first year and of at least 50 plants in the second year;
- testing for *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *sepedonicus* in the third year;
- verification of trueness to type.

Appendix IV

Micropropagation of potato

Material submitted as tubers should be washed, surface-sterilized in 0.3% available chlorine for 15 min and incubated in the dark to produce sprouts 3-7 cm long. Sprouts should also be surface-sterilized and rinsed in sterile water before excising the axillary buds. These are placed on a growing medium, such as Murashige and Skoog (M & S) medium with 30 g L^{-1} sucrose and 8 g L^{-1} Oxoid no. 3 agar. The subsequent microplants should be subdivided into nodal segments every 4-6 weeks and transferred to fresh medium. All *in vitro* work should be done by aseptic techniques in sterile air cabinets. Cultures should be incubated at 18-20°C under cool-white fluorescent tubes at a 16 h day-length.

Appendix V

Guidelines on procedures for eliminating pathogens

Virus-infected material submitted as tubers should be washed and surface-sterilized as described in Appendix IV before proceeding to eliminate the virus by two possible methods.

In the first method (MacDonald, 1973), the tuber is incubated in a humid chamber at 32-35°C until sprouts are produced. Sprouts are then removed, surface-sterilized in 0.3% available chlorine for 15 min and washed in sterile water. The apical meristem (150-200°µm) is excised, placed on M & S medium and incubated at 18-20°C under cool-white fluorescent tubes at a 16 h day-length. The meristems are subcultured onto new M & S medium at intervals of 4-6 weeks until a plantlet is produced.

In the second method (Jeffries, 1998), microplants are produced as described in Appendix IV. Nodal sections from the microplants are transferred to M & S medium containing 20 mg°L^{-1} ribavarin. These nodes are incubated for 10 days at 18°C under lights at a 16-h day length to allow the microplants to grow and root. The cultures are then subjected to an alternating temperature regime of 40°C for 4 h followed by 25°C for 4 h. After 4 weeks, each microplant is subdivided into nodes. The first node beneath the tip is grown on for virus testing, and the second node is transferred to fresh ribavarin-amended M & S medium and the treatment repeated as described above until plantlets are found free from viruses.

Références

Burr TJ & Schroth MN (1977) Occurrence of soft-rot *Erwinia* spp. in soil and plant material. *Phytopathology* **67**, 1382-1387

De Bokx JA (1987) Biological properties. In: *Viruses of Potatoes and Seed Potato Production* (eds de Bokx JA & van der Want JPH), p. 79. Pudoc, Wageningen (NL).

Ehlers U, Vetten HJ & Paul HI (1983) Detection of potato leafroll virus in primarily infected tubers by enzyme-linked immunosorbent assay, *Phytopathologische Zeitschrift* **107**, 37-46.

EPPO/CABI (1996) *Quarantine Pests for Europe* (2nd edn). CAB International, Wallongford (GB).

EU (1966) Directive 66/403/EEC of 14 June 1966 on the marketing of seed potatoes. *Official Journal of the European Communities* 125, 154-160.

EU (1969a) Directive 69/464/EEC of 8 December 1969 on control of potato wart disease. *Official Journal of the European Communities* L 323, 1-2.

EU (1969b) Directive 69/465 of 8 December 1969 on control of potato cyst eelworm. *Official Journal of the European Communities* L 323, 3-4.

EU (1977) Directive 77/93/EEC of 21 December 1976 on protective measures against the introduction into the Member States of harmful organisms of plants or plant products *Official Journal of the European Communities* L 26, 20-54.

EU (1993a) Directive 93/17/EEC of 30 March 1993 determining Community grades of basic seed potatoes, together with the conditions and designations applicable to such grades. *Official Journal of the European Communities* L 106, 7-10.

EU (1993b) Directive 93/85/EEC of 4 October 1993 on the control of potato ring rot. *Official Journal of the European Communities* L 259, 1-25.

EU (1997a) Directive 97/46 of 25 July 1997 amending Directive 95/44/EC establishing the conditions under which certain harmful organisms, plants, plant products and other objects listed in Annexes I to V to Council Directive 77/93/EEC may be introduced into or moved within the Community or certain protected zones thereof, for trial or scientific purposes and for work on varietal selection. *Official Journal of the European Communities* L 204, 43-46.

EU (1997b) Commission Decision 97/647/EC of 9 September 1997 detailing an interim scheme for the diagnosis, detection and identification of *Pseudomonas solanacearum* (Smith) Smith in potatoes. *Official Journal of the European Communities* L 273, 1-25.

EU (1998) Directive 98/57/EC of 20 July 1998 on the control of *Ralstonia solanacearum* (Smith) Yabuuchi *et al*. *Official Journal of the European Communities* L 235, 1-39.

Huttinga H, Mosch WHM & Treur A (1987) *Comparison of bi-directional electrophoresis and molecular hybridization methods to detect potato spindle tuber viroid and chrysanthemum stunt viroid. Bulletin OEPP/EPPO Bulletin* **17**, 37-43.

Jeffries CJ (1998) *Technical Guidelines for the Safe Movement of Germplasm, No. 19 Potato*. FAO/IPGRI, Rome (IT).

MacDonald DM (1973) Heat treatment and meristem culture as a means of freeing potato varieties from virus X and S. P*otato Research* **16**, 263-269.

OEPP/EPPO (1984) EPPO Standard PM 3/21(1). Phytosanitary inspection procedure for potato viruses (non-European) and potato spindle tuber viroid. *Bulletin OEPP/EPPO Bulletin* **14**, 73-76.

OEPP/EPPO (1990a) EPPO Standard PM 3/25(1). Phytosanitary procedure for *Clavibacter michiganensis* subsp. *sepedonicus*. *Bulletin OEPP/EPPO Bulletin* **20**, 235-254.

OEPP/EPPO (1990b) EPPO Standard PM 3/26(1). Phytosanitary procedure for *Pseudomonas solanacearum*. *Bulletin OEPP/EPPO Bulletin* **20**, 255-262.

OEPP/EPPO (1991) EPPO Standard PM 3/30(1). Phytosanitary procedure for *Globodera pallida* and *Globodera rostochiensis*. *Bulletin OEPP/EPPO Bulletin* **21**, 233-240.

Pérombelon MCM & Burnett EM (1991) Two modified crystal violet pectate (CVP) media for the detection, isolation and enumeration of soft rot erwinias. *Potato Research* **34**, 79-85.

Pérombelon MCM, Bertheau Y, Cambra M, Fréchon D, Lopez MM, Niepold F, Persson P, Sletten A, Toth IK, van Vuurde JWL & van der Wolf JM (1998) Microbiological, immunological and molecular methods suitable for commercial detection and quantification of the blackleg pathogen, *Erwinia carotovora* subsp. *atroseptica*, on potato seed tubers: a review. *Bulletin OEPP/EPPO Bulletin* **28**, 141-156.

Schroeder M & Weidemann HL (1989) Simplified application of return gel electrophoresis for the routine detection of potato spindle tuber viroid. *Bulletin OEPP/EPPO Bulletin* **19**, 661-665.

Shamloul AM, Hadidi A, Zhu SF, Singh RP & Sagredo B (1997) Sensitive detection of potato spindle tuber viroid using RT-PCR and identification of a viroid variant naturally infecting pepino plants. *Canadian Journal of Plant Pathology* **19**, 89-96.

Toth IK, Hyman LJ & Wood JR (1999) A one-step PCR-based method for the detection of economically important soft rot *Erwinia* species on micropropagated potato plants. *Journal of Applied Microbiology* **87**, 158-166.

UN/ECE (1994) *Standard S1 - Seed potatoes. Revised Standard for Seed Potatoes recommended by the Working Party on Standardization of Perishable Produce and Quality Development of the United Nations Economic Commission for Europe*. United Nations, Genève (CH).

Weidemann HL & Buchta U (1998) A simple and rapid method for the detection of potato spindle tuber viroid (PSTVd) by RT-PCR. *Potato Research* **41**, 1-8.

Table 1. Tuber-borne potato pests occurring in the EPPO region to be tested for in the scheme

Pests	Spread in potato fields by
Viruses	
Potato A potyvirus (PVA)	Aphids
Potato M carlavirus (PVM)	Aphids
Potato S carlavirus (PVS)	Aphids, contact*
Potato X potexvirus (PVX)	Contact
Potato Y potyvirus (PVY)	Aphids
Potato leafroll polerovirus (PLRV)	Aphids
Alfalfa mosaic alfamovirus (AMV)	Aphids
Cucumber mosaic cucumovirus (CMV)	Aphids
Potato aucuba mosaic potexvirus (PAMV)	Aphids
Potato mop-top pomovirus (PMTV)	*Spongospora subterranea*
Potato V potyvirus (PVV)	Aphids
Tobacco mosaic tobamovirus (TMV)	Contact
Tobacco necrosis necrovirus (TNV)	*Olpidium brassicae*
Tobacco rattle tobravirus (TRV)	Nematodes (certain species *Paratrichodorus* and *Trichodorus* spp.)
Tomato black ring nepovirus (TBRV)	Nematodes (*Longidorus elongatus, L. attenuatus*)
Tomato mosaic tobamovirus (ToMV)	Contact
Tomato spotted wilt tospovirus (TSWV)	Thrips
Viroid	
Potato spindle tuber viroid (PSTVd)	Contact
Bacteria	
Clavibacter michiganensis subsp. *sepedonicus*	Contact
Ralstonia solanacearum	Contact, water
Erwinia spp.	Contact, water
Nematodes	
Globodera pallida	Soil
G. rostochiensis	Soil

* Contact here means contact between plants or with machinery.

Table 2. Test methods for potato viruses. The range of indicator plants given here is an example of the use of a limited number of species to detect all potato viruses (apart from PLRV); other suitable combinations may also be used.

		Indicator plants				
	ELISA	*Chenopodium quinoa*	*Nicotiana benthamiana*	*N. glutinosa*	*N. tabacum* cv. White Burley	*Phaseolus vulgaris* cv. The Prince
Potato A potyvirus (PVA)	•				•	
Potato M carlavirus (PVM)	•					•
Potato S carlavirus (PVS)	•	•*				
Potato X potexvirus (PVX)	•		•	•	•	
Potato Y potyvirus (PVY)	•		•		•	
Potato leafroll polerovirus (PLRV)	•					
Alfalfa mosaic alfamovirus (AMV)		•				•
Cucumber mosaic cucumovirus (CMV)		•				
Potato aucuba mosaic potexvirus (PAMV)				•		
Potato mop-top pomovirus (PMTV)		•	•			
Potato V potyvirus (PVV)	•		•			
Tobacco mosaic tobamovirus (TMV)				•		
Tobacco necrosis necrovirus (TNV)		•			•	
Tobacco rattle tobravirus (TRV)		•				•
Tomato black ring nepovirus (TBRV)		•			•	
Tomato mosaic tobamovirus (ToMV)				•	•	
Tomato spotted wilt tospovirus (TSWV)			•	•	•	

* Note that for PVS, *Chenopodium murale* can detect more isolates, but it can only detect PVS. Moreover, ELISA should allow the detection of all isolates

Table 3. Suggested minimum tolerances for seed potato pests at Propagation stock I, II, III and IV

			UN/ECE standards	
	Propagation stock I (minitubers)	Propagation stock II	Propagation stock III	Propagation stock IV
	Prebasic seed-TC	Prebasic seed	Basic seed	Certified seed
Growing crop inspection / Inspection pendant la période de végétation	As a % of plants			
Virus	0	0.1	Not specified	Not specified
Blackleg (*Erwinia*)	0	0.01	1	2
Off-types[a]	-	0.01	Not specified	Not specified
Direct progeny[b]	As a % of plants			
Virus	0	0.5	4	10[c]
Off-types[a]	0	0.01	0.25	0.5
Tuber diseases and defects[d]	As a % of weight			
Blight (*Phytophthora infestans*)	0	0.1		
Dry rot/wet rot	0	0.1 } 0.2	} 1[e]	} 1[e]
Frost	0	0.1		
Black scurf (*Rhizoctonia solani*)	0	1 (sa[f] > 10 %)	5 (sa > 10 %)[e]	5 (sa > 10 %)[e]
Common scab (*Streptomyces scabie*s)	0	5 (sa > 33 %)	5 (sa > 33 %)[e]	5 (sa > 33 %)[e]
Powdery scab (*Spongospora subterranea*)	0	1 (sa > 10 %)	Not specified	Not specified
Defects e.g. misshapen, cracks	3	3	3[e]	3[e]
Dirt	1	1	2[e]	2[e]
Visual necrosis[g]	0	1[h]	Not specified	Not specified

a. Including malformation caused by plant protection products.

b. The direct progeny tolerances also apply to post-harvest tuber tests.

c. Severe virus symptoms only.

d. Only applies when tubers are to be marketed.

e. For all marked with e, total tolerance is 6 %.

f. sa, surface area affected.

g. "Visual necrosis" means necrotic spots, arcs or rings within or on the surface of the tuber. These symptoms are often (but not exclusively) caused by virus infection.

h. Provided not more than 0.5% surface necrosis.

Fig. 1. Diagram of the stages in the potato certification scheme