◆ EPPO Standards ◆

CERTIFICATION SCHEMES

PATHOGEN-TESTED CITRUS TREES
AND ROOTSTOCKS

PM 4/12(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 4/12(1) English

Certification scheme

PATHOGEN-TESTED CITRUS TREES AND ROOTSTOCKS

Specific scope

This standard describes the production of certified pathogen-tested material of citrus trees and rootstocks.

Specific approval and amendment

First approved in September 1995.
Edited as an EPPO Standard in 1998.

For the production of certified pathogen-tested trees and rootstocks of *Citrus*, *Poncirus*, *Fortunella* and their hybrids, 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. The scheme provides separate indications for the production of grafted citrus tree varieties, rootstocks and seed for rootstocks. 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 especially for varieties.

1. Selection of material

Varieties

Select, in different orchards and/or pomological trial fields, a number of productive trees, with the typical characters of each variety to be taken into the scheme (i.e. true to type). Select trees with no apparent symptoms. It may be useful to perform rapid serological tests on material taken from these trees.

Trees for production of seeds for seedling rootstocks

Select, in different orchards or plantations, vigorous and productive trees of each rootstock type to be taken into the scheme. For species in which seed-transmissible viruses are known, select trees with no apparent symptoms or affected as little as possible by these seed-transmissible diseases. The selected trees should be known to produce true-to-type progeny with uniform growth, or else this should be checked.

2. Production of nuclear stock

General procedure

Collect propagation material from the pomologically selected trees. Bud or graft this material onto virus-free rootstocks. Keep these plants (candidate material for nuclear stock status) in an isolated, suitably designed, insect-proof house, separately from the nuclear stock (in quarantine) during the testing period. The plants should be grown in sterilized growing medium, in containers isolated from the soil to avoid any type of contamination. The plants should be tested for the viruses, phytoplasmas and diseases specified in Table 1, by the methods of Appendix I[1].

If any of the selected material gives a negative test result, the corresponding candidate nuclear stock plant can be promoted to nuclear stock and transplanted to the nuclear stock plot, or nuclear stock material can be propagated from it.

Elimination of pathogens

For plants of which none of the selected material gave a negative result, or for any material which is suspected to be infected, production of nucellar clones, thermotherapy and shoot-tip grafting can be used as sanitation procedures (Appendix II).

Inspection for other pests

All material (varieties, trees for seed production) should, besides the diseases and pathogens mentioned above, also be checked for the presence of other pests which can be transmitted on propagation material. In

[1] The pathogens covered are only those occurring in the EPPO region. Importation of citrus from outside the EPPO region is generally prohibited. Any material imported would enter with a permit, and be subject to post-entry quarantine under the authority of the National Plant Protection Organization. An EPPO Phytosanitary Procedure is in preparation on tests for non-European viruses and virus-like organisms of citrus. Material under quarantine may also be submitted to shoot-tip grafting as a sanitation procedure.

particular this should be done for: *Aleurothrixus floccosus* and *Parabemisia myricae*; *Deuterophoma tracheiphila*, *Phytophthora citrophthora* and *P. parasitica*.

3. Maintenance of nuclear stock

Maintain the nuclear stock plants in a repository under conditions ensuring freedom from (re)infection. Plants are grown in containers with sterilized growing medium, isolated from the soil, and in a suitably designed insect-proof house. If possible, store some material of each source of each variety or rootstock type *in vitro*.

Maintain a limited number of nuclear stock plants (at least 2) of each source of each variety or tree for seed production taken into the scheme and check that it remains true to type. Initially, maintain all the sources of each variety or tree for seed production. However, the number of sources may be reduced when the pomological comparisons have been made and the best sources are known.

In the repository, each plant should be tested every 3 years for viroids and every 6 years for virus and virus-like diseases (Table 1). The plants should be tested every 1-2 years for citrus tristeza closterovirus by serological testing. They should be maintained free from *D. tracheiphila*. They should also be visually inspected every year for possible mutations or back mutations.

4. Production of propagation stock

Multiply the nuclear stock in as few steps as possible to obtain the required quantity of propagation stock. This multiplication may be done *in vitro*. The propagation material is kept in an insect-proof house, or else in fields separated by at least 100 m from non-certified citrus material and reasonably isolated from sources of infection. For example, a surrounding zone 2 m wide around the plot should be kept free from any vegetation. Note that irrigation water may be a source of infection by pathogens and their vectors.

In countries where *D. tracheiphila* occurs, propagation stock of *Citrus limon* (lemon), *C. aurantifolia* (lime) and *C. bergamia* (bergamot) should be kept on fields on which none of those species have been grown for at least 5 years. Mother plants of those species should be covered with net (white plastic net normally used to protect trees from wind and hail damage) to avoid contamination by *D. tracheiphila*.

Propagation stock should be kept under continuous surveillance and sprayed regularly with appropriate plant protection products, to control the normal pests of citrus. The variety trees should not be kept for more than 30 years and the trees for production of seeds for seedling production should not be kept for more than 40 years.

Visually check the propagation stock each year for virus symptoms (at the appropriate time when symptoms are likely to be most visible) and other pests (e.g. *A. floccosus* and *Parabemisia myricae*), as well as for possible mutations or back mutations. Plants should be tested for virus and virus-like diseases so that, after 10 years, all plants have been tested at least once. In countries where citrus tristeza closterovirus is present, the plants should be tested by ELISA at least once a year for this virus.

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

Distribute the material from propagation stock 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.

Where there is a risk of infection by *Phytophthora citrophthora*, rootstocks should be grafted at not less than 40 cm from the collar. If, exceptionally, it is necessary to regraft (with material of the same cultivar, origin and sanitary status), the distance should be 35 cm from the collar.

Certified stock is kept in nurseries found free from *Pratylenchus vulnus* and substantially free from *Tylenchulus semipenetrans*, *Phytophthora citrophthora* and *P. parasitica* (or which have been fumigated and retested to ensure freedom), separated by at least 15 m from non-certified citrus material, and reasonably isolated from infected sources. For example, a surrounding zone 2 m wide around the plot should be kept free from any vegetation. Note that irrigation water may be a source of infection by pathogens and their vectors.

In countries where *D. tracheiphila* occurs, lemon plants should be protected with net (white plastic net normally used to protect trees from wind and hail damage) to avoid contamination by *D. tracheiphila*.

6. Control on the use and status of the 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 virus-free plants on the basis of field inspections and of the records and documents presented by the nurserymen. During production of certified stock in nurseries, some random tests on virus status should also be applied if short-time testing methods (e.g. ELISA test) for the detection of citrus viruses are available.

The nursery plant protection programme and the check inspections should also take account of fungal and bacterial diseases, and animal pests (e.g. *Aleurothrixus floccosus* and *Parabemisia myricae*), so that the certified stock delivered to the fruit grower is substantially free from these pests.

7. Certification

The certifying authority issues the nurseryman with certificates on the basis of points 4, 5 and 6. For many purposes (but this depends on the certification and production systems of each country), labelling of individual trees provides an excellent manner of certification. The correct number of labels is issued and their use is officially checked.

Certified citrus 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

Guidelines on testing procedures

1. Testing on citrus indicators

The use of citrus indicators is still a compulsory step in any citrus certification programme. It cannot be excluded because there are several diseases, some of major importance, which cannot be identified except on woody differential hosts. The method consists of graft-inoculating indicator plants with budwood from candidate or suspected plants and observing the new growth on the sensitive plants for symptoms, which are normally specific and highly diagnostic for many diseases. Testing should preferably be conducted in a glasshouse, with heat and cooling facilities provided for specific sections to ensure the correct temperatures for symptom expression. Two grafting techniques can be used:

(a) grafting of chip buds, blind buds or other inoculum directly on seedlings of the indicator plant (used in most cases);

(b) support method, by which a rootstock is inoculated with blind buds and then budded with the indicator (recommended specially for cachexia-xyloporosis).

The main indicators for virus and virus-like diseases of citrus occurring in the EPPO region are shown in Table 2.

2. Inoculation to herbaceous hosts

The use of herbaceous indicators allows detection of mechanically transmissible viruses, including those of minor importance. Inoculation to herbaceous hosts is regarded as a complement to, but not as a substitute for, other diagnostic procedures. It may be useful, for example, for preliminary screening or for random testing.

3. Serological testing

The ELISA method allows large-scale testing for citrus viruses for which antisera are available. However, there are certain limitations in any antibody technique, such as the fact that citrus viruses usually exist in very low concentrations in the tree, may be irregularly distributed or be temporarily non-detectable during periods of warm summer temperatures. Immunoblotting is an alternative serological technique, with the same limitations as ELISA, which can easily be adapted to large-scale routine use.

4. PAGE electrophoresis

More recently, citrus viroids including exocortis and cachexia have been detected by isolation of the viroid molecule using gel electrophoresis, opening up the possibility of actually identifying specific strains.

5. Detection of individual diseases

Citrus tristeza closterovirus

Indicator:	Mexican lime *(Citrus aurantifolia)*
No. plants per test:	3-5 seedlings
Inoculum:	3 blind buds for each seedling
Temperature:	18-26°C
Symptoms:	Symptoms appear within 3-4 weeks in the form of vein-clearing areas, followed by the development of wood pits in the young new branches. The severity of the stem pitting indicates the severity of the tristeza virus strain present in the inoculum
Other tests:	Serology (ELISA, ISEM).

Citrus ringspot virus and other members of the psorosis complex

Indicator:	Sweet orange (*Citrus sinensis)* cvs Pineapple, Madam Vinous, Hamlin, Dweet tangor
No. plants per test:	3-5 seedlings
Inoculum:	3 blind buds
Temperature:	18-26°C
Symptoms:	Symptoms appear within 1-4 months in the form of clear flecks along the main vein, giving an oakleaf pattern, or along the veinlets, sometimes ring-like patterns on mature leaves, rapid dieback of new growth usually followed by recovery. Plants should be kept for at least 6 months. Symptoms are often transitory which means that periodic inspection of new growth is essential. Spring and autumn flushes are more favourable for symptom expression in the indicator plants. When testing for certification purposes, it is not essential to determine which type of psorosis is present.

Citrus exocortis viroid

Indicator:	Etrog citron (*Citrus medica*) (60-13, 861-S1 or other selections)
No. plants per test:	2-4 plants
Inoculum:	Since Etrog is normally mono-embryonic, seedling plants should not be used. Etrog is budded into any vigorous seedling. Inoculation is done with 2 blind buds per plant
Temperature:	26-35°C
Symptoms:	Symptoms appear after the first new flush of growth, about 4-6 weeks after inoculation. Leaf epinasty is the most striking symptom. Mild strains may cause only some cracking of the lower side of the mid-veins and petiole plus some browning
Other tests:	PAGE electrophoresis 3-4 months after inoculation to Etrog citron.

Citrus cachexia-xyloporosis viroid

Indicator:	Parsons special mandarin (*Citrus reticulata*), Etrog citron 861-S1
No. plants per test:	4-5 plants
Inoculum:	The support method is recommended, using *Citrus jambhiri* (rough lemon) or any other vigorous seedling as the rootstock. Seedlings are grafted with buds of Parsons special mandarin and simultaneously inoculated with 3 buds taken from the candidate tree
Temperature:	26-35°C
Symptoms:	Gum in the bark and bud union, and pits in the wood
Other tests:	PAGE electrophoresis, 3 months after inoculation on Etrog citron.

Spiroplasma citri

Indicator:	Sweet orange cvs Pineapple, Madam Vinous, Dweet tangor
No. plants per test:	3-5 seedlings
Inoculum:	The best inoculum is young leaf patches including midrib
Temperature:	26-34°C
Symptoms:	Symptoms appear within 1-6 months after inoculation. Positive reactions include general stunting, short leaf internodes and small, often upright cupped and mottled leaves. Testing is considered definitely positive when inoculated test plants develop a green mottle near the tip of the leaf. In some countries, stubborn disease can be detected on the field tree, but this is not usually the case in Europe
In vitro culture:	Young leaves and stem tips are finely chopped and placed in a medium containing various nutrients. The organism is identified by dark-field microscopy or by electron microscopy that both reveal tiny spinning spirals, or by electron microscopy of pellets from liquid medium. Growth of *S. citri* also changes the red broth to amber and creates light turbidity in the cultures in 1-4 weeks.

Impietratura

Indicator:	Sweet orange cvs Pineapple, Madam Vinous, Hamlin, Dweet tangor
No. plants per test:	3-5 seedlings
Inoculum	3 blind buds for each seedling
Temperature:	18-26°C
Symptoms:	Symptoms appear after one year or more in the form of hard

	gummy deposits in rind and core of fruit. Psorosis-like leaf symptoms may occur but are not sufficient for diagnosis.

Cristacortis

Indicator:	Sweet orange cvs Pineapple, Madam Vinous, Hamlin, Dweet tangor
No. plants per test:	3-5 seedlings
Inoculum:	3 blind buds for each seedling
Temperature:	18-26°C
Symptoms:	Symptoms appear after 10 months or more. Concave gum or psorosis-like leaf symptoms may appear, but are not diagnostic. Disorganization of the cambium may appear in the form of depressions in the wood.

Citrus leaf rugose ilarvirus

Indicator:	Mexican lime, Eureka lemon, Duncan pomelo
No. plants per test:	4-5 seedlings
Inoculum:	A minimum of two blind buds or chip buds for each indicator
Temperature:	24-27°C
Symptoms:	Variable rugosity of leaves of Mexican lime, pinpoint chlorotic flecks on expanding leaves of lemon cv. Eureka without leaf distortion, and severe stunting and chlorosis on seedlings of pomelo cv. Duncan
Other tests:	CLRV can be detected by mechanical inoculation to herbaceous hosts and by serological assays (ELISA is preferred).

Citrus infectious variegation ilarvirus

Indicator:	Lemon or Etrog citron
No. plants per test:	4-5 seedlings
Inoculum:	A minimum of two blind buds or chip buds for each indicator
Temperature:	24-30°C
Symptoms:	Etrog citron develops chlorotic leaf symptoms and distortion, which persist on the mature foliage. Infected trees may be stunted and some fruit may bedistorted or have chlorotic patterns. Severity differs among isolates
Other tests:	Sap inoculation of *Phaseolus* vulgaris cv. Red Kidney produces a brilliant systemic vein banding and vein clearing on trifoliate leaves. Sap inoculated *Vigna sinensis* shows chlorotic/necrotic lesions on primary leaves. CIVV can also be detected by serological assays (ELISA is preferred).

Citrus vein enation

Indicator:	Mexican lime, rough lemon
No. plants per test:	4-5 plants
Inoculum:	Blind buds
Temperature:	18-26°C
Symptoms:	Enations appears on the underside of leaves. Swelling or galls develop on stems of rough lemon.

Satsuma dwarf virus

Indicator:	*Citrus natsudaidai*, citron, sour lemon, Dweet tangor, mandarin or satsuma are used as seedlings
No. plants per test:	4-5 seedlings
Inoculum:	A minimum of two blind buds or chip buds are grafted to the lower part of the indicator seedlings
Temperature:	Cool temperatures, not exceeding 26°C maximum by day or 12-18°C minimum at night
Symptoms:	In general, the symptoms induced in indicator plants are similar to those induced by CIVV. Young leaves show psorosis-like leaf patterns of flecking, mottle, chlorosis. Mature leaves may show leaf curl and crinkle. Line patterns and misshapen leaves are common
Other tests:	ELISA, using anti-SDV serum, is recommended to test for SDV. Mechanical inoculation to white sesame is also recommended.

APPENDIX II

Guidelines on sanitation procedures

Methods for elimination of pathogens from citrus include the production of nucellar clones, heat therapy and shoot-tip grafting. Testing of the treated material for assessment of its health status must follow.

Nucellar clones

The production and selection of nucellar lines is a standard method for obtaining varieties free from intracellular pathogens. Most citrus varieties present the phenomenon of nucellar polyembryony, i.e. the formation of nucellar embryos within the seed. Nucellar embryos result in true-to-type seedlings, free from pathogens that may infect the parent tree. In their early years, nucellars show juvenile characteristics: vegetative invigoration, thorniness, upright growth, slowness in fruiting and early alternate bearing. The juvenile conditions tend to decrease with time, as the ageing process is associated with repeated cell division rather than with the age of the nucellar clone *per se*. Generally, 10-15 years, and sometimes more, are necessary before the ageing process allows commercial propagation of nucellar seedlings. In the process of production of nucellar clones, pollination is controlled by using *Poncirus trifoliata* as male parent, since the character of trifoliate leaves is determined by a dominant gene, making it easy to differentiate at an early age between the nucellar and sexual seedlings.

Heat therapy

Thermotherapy or heat therapy has proved an efficient method for eliminating citrus tristeza closterovirus, psorosis and impietratura. It was found to be inefficient for elimination of citrus cachexia-xyloporosis and exocortis viroids, and *Spiroplasma citri*. More recently this method has been partially abandoned with the development of shoot-tip grafting technique. However the two methods can be combined for better results in the process of virus elimination.

When thermotherapy is used, pre-conditioning of plants prior to their use is vital to success. Plants should be grown (pre-conditioned) at warm temperatures (32-40°C by day and 26-30°C at night) for 1-3 months prior to budwood treatment. Buds cut from pre-conditioned plants are then grafted onto heat-tolerant rootstocks. The budded plants are placed in a chamber with high humidity and at an initial temperature of 38-39°C for 16 h under lights and 8 h at 30°C in the dark. After one week, the temperature is raised to 40-50°C. The budded plants are kept at this temperature for 8-12 weeks after which they are moved to a glasshouse and the buds forced. The new sprouts are allowed to grow and then tested to verify absence of viruses.

Shoot-tip grafting

The technique of shoot-tip grafting *in vitro* is performed with special tools under aseptic conditions and involves the following steps. Rootstock seedlings are grown in nutrient agar in the dark. Shoots of the material to be regenerated, approximately 1 cm long, are collected and disinfected in the laboratory, the immature leaves are removed and then, under the microscope, a very small shoot tip (about 0.1-0.2 mm) consisting of the meristem and 2-3 leaf primordia is excised. The tip is carefully inserted into a T-cut made near the top of a decapitated rootstock seedling. The young grafted plant is grown in nutrient medium for at least 5 weeks under lights (1000 lux Growlux illumination) and then transplanted into soil or grafted on *C. jambhiri* (rough lemon) or *C. volkameriana*. The plants are then tested as usual for viruses and virus-like diseases of citrus.

Soil test for Phytophthora spp.

The inoculum density (ID) of *Phytophthora* spp. in the soil can be determined by the method of plate dilution, using the selective substrate PDA + BNPRAH (Masago *et al*., 1977). Soil samples (10-20 per ha, 500 g each) are air-dried for 24 h and screened with a 2-mm sieve. Aliquots of 10 g of soil are collected from each sample and suspended in 0.1% water agar in a ratio 1:10 (w/v). The resulting suspension is pipetted into Petri dishes (1 ml per dish) containing PDA + BNPRAH (potato dextrose agar containing (mg litre^{-1}) benomyl 10, nystatin 25, pentachloronitrobenzene (PCNB) 25, rifampicin 10, ampicillin 500, hymexazole 25-50) at melting temperature (43-45°C). Plates are incubated in the dark for 4-6 days at 20 ± 2°C. For each sample, the test is replicated three times, with 10 dishes per replicate. *Phytophthora* spp. are identified after having transferred, under sterile conditions, the isolates obtained from soil to Petri dishes of CMA (cornmeal agar). The average number of colonies per plate, divided by the dry weight obtained, represents the ID of the soil, expressed as number of propagules per g of soil.

Sampling of soil for nematode analysis

Before sampling, visually divide the orchard into sampling blocks representing differences in soil texture, drainage pattern or cropping history. In an established planting, collect soil and root samples at the drip line or around emitters where feeder roots are most abundant. The soil should not be too dry or too wet. Sample fallow land at any time of year. The best time to sample an established nursery is March/April. In loamy soil, sample down to 60 cm; in sandy soils, sample down to 90 cm. Use a soil auger, Viehmeyer tube or shovel. A soil auger (7.5 cm diameter) is convenient for depths to 60 cm in sandy soils. To sample deeper than 60 cm, a Viehmeyer tube is recommended to reduce the soil volume taken. The tube can easily be hammered down to 1.2 m; the

amount of roots collected will be much smaller than with a soil auger.

From each sampling block, collect 10 or 20 cores or sub-samples. Combine the sub-samples, mix thoroughly and pour the soil and roots into durable plastic bags or other moisture-proof containers. Seal tightly and place bags in the shade until last sample has been taken. Attach labels providing name and address, location of the orchard, sample block, soil texture, cropping history, notable symptoms and, if possible, rootstock and soil and air temperature. This information is critical for a meaningful analysis. Send or deliver the samples to the laboratory as soon as possible. Ship them in a cardboard box insulated with newspaper, or in a styrofoam ice chest. If any delay occurs, keep samples in a cool place (5-10°C).

Extract nematodes from soil samples using elutriation/flotation method followed by the Baermann funnel. Nematodes found should be identified at high magnification by an experienced nematologist. Report the method used and the extraction efficiency together with the results.

References

MASAGO, H., YOSHIKAWA, M., FUKADA, M. & NAKANISHI, N. (1977) Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soils and plants. *Phytopathology* **67**, 425-428.

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. Virus and virus-like diseases of citrus occurring in the EPPO region and covered by the scheme

Disease	Geographical distribution	Transmission
Viruses		
Citrus tristeza closterovirus(CTV)	Worldwide (scattered records in many parts of Mediterranean area, where it is everywhere officially controlled)	*Toxoptera citricida, T.aurantii, A. spiraecola, A. crassivora, Macrosiphum euphorbiae, Myzus persicae*
Citrus infectious variegation ilarvirus (CIVV)	Algeria, France (Corse), Italy, Greece, Lebanon, Morocco, Spain	Seeds?
Citrus ringspot virus and other members of the psorosis complex (psorosis A, concave gum ,blind pocket)	Worldwide	
Satsuma dwarf virus (SDV)	Iran, Japan, Turkey	Via sap on cutting instruments
Citrus leaf rugose ilarvirus	Italy, Argentina, Japan, USA (Florida)	?
Viroids		
Citrus exocortis viroid	Worldwide	Via sap on cutting instruments, seeds of orange cv. Baianinha Navel
Citrus cachexia-xyloporosis viroid	Worldwide	No insect vector is known
Phytoplasmas		
Spiroplasma citri	Worldwide	Leafhoppers: *Scaphytopius nitridus, S. acutus, Circulifer tenellus, Neoaliturus haematoceps*
Virus-like diseases		
Impietratura	Cyprus, Greece, Israel, Italy, Lebanon, Morocco, Spain, Turkey	No insect vector is known
Cristacortis	Mediterranean area	Pollen transmission
Citrus vein enation/woody gall	Spain, Australia, India, Iran, Japan, Peru, South Africa, Turkey, USA	Grafting, *Aphis gossypii, Myzus persicae, Toxoptera citricida*

Table 2. Main indicators for virus and virus-like diseases of citrus occurring in the EPPO region

Indicator	Diseases identified
1. Mexican lime (*C. aurantifolia*)	Tristeza, rugose leaf, vein enation/woody gall
2. Sweet orange (*C. sinensis*) cvs Pineapple, Madam Vinous, Hamlin	Psorosis complex, stubborn, impietratura, cristacortis
3. Etrog citron (*C. medica*) clones 861-S1, 60-13	Exocortis, infectious variegation and other citrus viroids
4. Parsons special mandarin (*C. reticulata*)	Cachexia-xyloporosis
5. Dweet tangor (*C. reticulata* × *C. sinensis*)	Satsuma dwarf, psorosis, cristacortis, impietratura

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