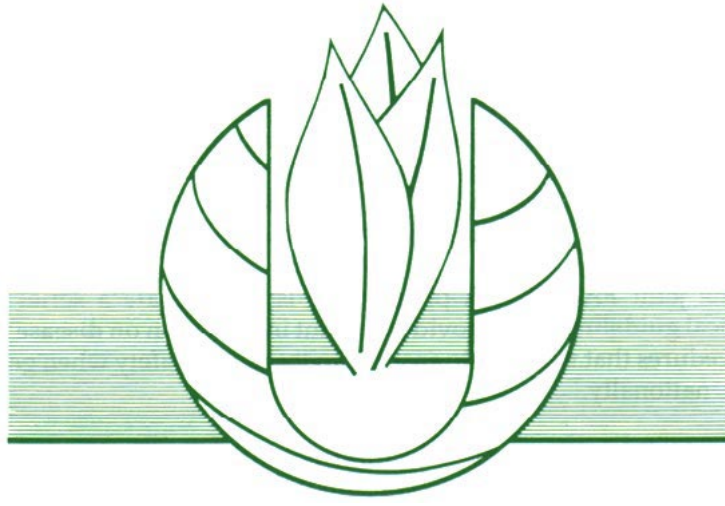
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS

INTERNATIONAL BOARD FOR
PLANT GENETIC RESOURCES

FAO/IBPGR TECHNICAL GUIDELINES
FOR THE

SAFE MOVEMENT OF CITRUS GERMPLASM



Edited by
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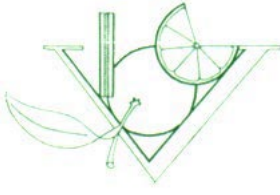

INTERNATIONAL ORGANIZATION
OF CITRUS VIROLOGISTS

INTRODUCTION

Collecting, conservation and utilization of plant genetic resources and their global distribution are essential components of international crop improvement programmes.

Inevitably, the movement of germplasm involves a risk of accidentally introducing plant quarantine pests* along with the host plant material; in particular, pathogens that are often symptomless, such as viruses, pose a special risk. In order to minimize this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of pests that are of quarantine concern.

The ever-increasing volume of germplasm exchanged internationally, coupled with recent rapid advances in biotechnology, has created a pressing need for crop-specific overviews of the existing knowledge in all disciplines relating to the phytosanitary safety of germplasm transfer. This has prompted FAO and IBPGR to launch a collaborative programme for the safe and expeditious movement of germplasm, reflecting the complementarity of their mandates with regard to the safe movement of germplasm. FAO has a long-standing mandate to assist its member governments to strengthen their Plant Quarantine Services, while IBPGR's mandate - *inter alia* - is to further the collecting, conservation and use of the genetic diversity of useful plants for the benefit of people throughout the world.

The aim of the joint FAO/IBPGR programme is to generate a series of crop-specific technical guidelines that provide relevant information on disease indexing and other procedures that will help to ensure phytosanitary safety when germplasm is moved internationally.

The technical guidelines are produced by meetings of panels of experts on the crop concerned, who have been selected in consultation with the relevant specialized institutions and research centres. The experts contribute to the elaboration of the guidelines in their private capacity and do not represent the organizations to which they belong. FAO, IBPGR and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines. By their nature, they reflect the consensus of the crop specialists who attended the meeting, based on the best scientific knowledge available at the time of the meeting.

* The word 'pest' is used in this document as it is defined in the revised edition of the International Plant Protection Convention. It encompasses all harmful biotic agents ranging from viroids to weeds.

The technical guidelines are written in a short, direct, sometimes 'telegraphic' style, in order to keep the volume of the document to a minimum and to facilitate updating. The guidelines are divided into two parts: The first part makes recommendations on how best to move germplasm of the crop concerned and is divided into general, technical and practical recommendations. Institutions recovering and maintaining healthy citrus germplasm are listed at the end of this first part. The second part gives descriptions of the most important pests that could be of quarantine concern.

The information given on a particular pest does not pretend to be exhaustive but concentrates on those aspects that are most relevant to quarantine. A selected list of references is given, referring mainly to methods of therapy and indexing.

It should be realized that information on the geographical distribution of pests is strongly influenced by the intensity-of research carried out in a given country or region and should therefore be considered as relative.

The present guidelines were developed at a meeting held in Riverside, California, USA from 18 to 21 November 1989. The meeting was hosted by the Plant Pathology Department of the University of California, Riverside, and organized in collaboration with the International Organization of Citrus Virologists (IOCV).

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GENERAL RECOMMENDATIONS

- Material should be collected, processed and shipped with the necessary precautions to avoid accidental movement of pests.
- Under no circumstances should germplasm be moved as rooted plant material.
- Where possible, all germplasm should be moved as seed.
- *Citrus*, *Fortunella* and *Poncirus* can be moved as vegetative material only if proper therapy and indexing procedures are followed.
- All germplasm should be collected from healthy looking trees.
- When available, accessions or cultivars should be obtained from a 'healthy collection'. Otherwise, material should be obtained from the lowest risk area possible and should undergo the therapy and indexing procedures described in the following pages.
- Whenever possible, germplasm should not be collected from high risk areas which are defined by the following criteria:

    1. Areas with diseases for which no indexing procedures exist.
    2. Areas where background information on donor trees is lacking or is very limited.
    3. Areas with diseases of high economic threat.
    4. Areas with diseases for which vector transmission is known or suspected.

- If material is to be introduced from an area with diseases for which no indexing methods exist or where little or no background information on source trees is available, it should undergo an additional cycle of therapy as described below, and should be maintained under observation in containment.
- If material is obtained from a centre where it has undergone the therapy and indexing procedures described below, it should be kept under containment or isolation for at least three growth flushes before being released.

TECHNICAL RECOMMENDATIONS

A. Collecting and movement of seed

- Collect sound fruit from parts of the trees more than 1 metre above the ground.
- Extract seed and wash thoroughly with soapy water to free seed from pulp.
- Surface-sterilize seed with 0.5% sodium hypochlorite with 0.1% wetting agent for 10 min and then rinse thoroughly with water.
- Treat seed with hot water for 10 min at 52°C and immediately immerse in ambient temperature water.
- Surface-dry seed under shaded conditions. The seed may be treated with a 3 min dip in a 1% solution of 8-hydroxyquinoline or after drying with a powdered fungicide such as thiram.
- Follow the practical recommendations given below for collecting, documentation, packing and shipment.
- Before germination, rinse seed with water to remove fungicide.
- Remove seed coat and surface-sterilize seed by immersion for 1 min in 0.5% sodium hypochlorite with 0.1% wetting agent.
- Wash seed 3 times with sterile distilled water and germinate aseptically.
- Once seed has germinated, it may be transplanted in to sterilized soil mix in an insect-free containment facility.
- When the seedling has reached sufficient size it should be indexed.

B. Collecting and movement of budwood

- Sterilize collecting tools (clippers, knives, etc.) by dipping in a 0.5-1.0% sodium hypochlorite solution.
- Collect budwood from healthy-looking trees, as free of pests as possible, which have been evaluated for freedom of symptoms on fruit, foliage, limbs and trunk. Bark patches and exposed trunk should be observed at the bud-union (where applicable) and on twigs.

- The budwood should be collected from the last fully mature growth flushes from the exterior canopy of the tree, preferably near true-to-type fruit. Avoid all suckers.

- Remove leaves and thorns, but leave petioles attached, label, pack in a plastic bag and maintain at 25°C or lower, preferably in an ice chest.

- Collected budwood should be thoroughly washed with a mild detergent and water using a soft brush.

- The budwood should be rinsed well with tap water, dipped for 1 min in a solution of 0.5% sodium hypochlorite with 0.1% wetting agent, rinsed again thoroughly with water and air- or towel-dried.

- At this point, the budstick ends or the entire budstick may be dipped in melted, low temperature paraffin wax.

- Follow the practical recommendations given below for collecting, documentation, packing and shipment.

- As soon as possible after receipt, shoot-tip grafting should be done. If shoot-tip grafting cannot be done immediately, buds should be propagated in a containment facility and then shoot-tip grafted as soon as possible.

- Follow the recommendations given under ‘Therapy procedures’ for shoot-tip grafting.

- When budwood is received from an area where there may be diseases for which no indexing methods are known, the primary shoot-tip grafted propagation should be subjected to thermotherapy and then shoot-tip grafted again.

- After applying the recommended therapy procedures, indexing for bud transmitted diseases should be performed as described in following pages.

- If found free of all known diseases the germplasm material can be released; if not found free, repeat therapy or destroy the material by autoclaving.

C. Therapy procedures

Therapy procedures that are applicable to all pests are described below.

1. Introduction into citrus -growing areas

- **Preliminary inspection:** Budwood should be inspected without opening the bag. If found abnormal, or heavily contaminated or infested with living pests, the entire package should be sterilised by autoclaving and destroyed.

- **Budwood disinfection:** Budwood should be disinfected by immersion for 10-15 min in a 0.5% sodium hypochlorite solution containing 0.1% wetting agent and rinsed three times with sterile distilled water.

- **Budwood culture *in vitro:*** Bud sticks, about 15 cm long and containing 6-8 buds, can be cultured in 38 x 200 mm test tubes containing 50 ml of the Murashige and Skoog salts solution solidified with 1.2% Bacto agar. Cultures are kept in an incubator at constant 32°C and 16 h daylight with a minimum illumination of 45 $\mu E\ s^{-1}m^{-2}$(about 1000 1ux). Most buds will produce a flush in 7 to 14 days.

- **Shoot disinfection:** New shoot flushes produced *in vitro* are removed from the bud sticks under aseptic conditions with the aid of long forceps. They are immediately disinfected by immersion for 5 min in a 0.25% sodium hypochlorite solution containing 0.1% wetting agent, and then rinsed three times with sterile distilled water

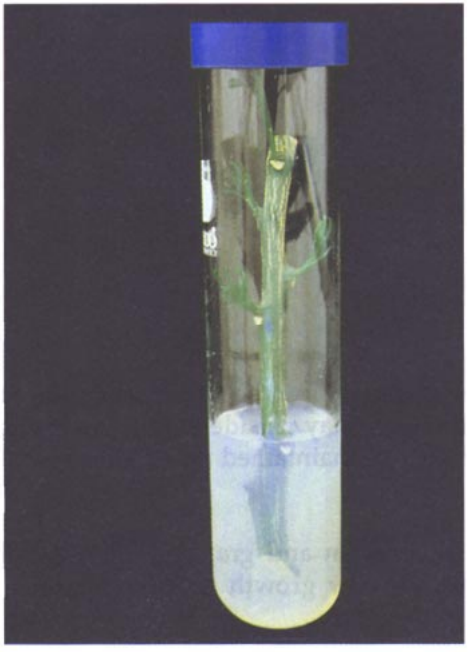


Fig. 1. Budstick of Washington navel cultured *in vitro* showing flushes produced after ten days in culture. (Dr L. Navarro, Instituto Valenciano de Investigationes Agrarias, Valencia)

- **Shoot-tip excision:** Shoot-tips containing the apical meristem and a maximum of three leaf primordia (measuring 0.1-0.2 mm) are excised from the new shoot flushes.

- **Shoot-tip grafting:** Excised shoot-tips are grafted *in vitro* on to 12-16 day-old rootstocks obtained by *in vitro* seed germination according to the standard procedures of shoot-tip grafting *in vitro*. Grafted plants are grown in a liquid medium in a culture room at 26-27°C and exposed to a 16 h day of 45 $\mu E\ s^{-1}\ m^{-2}$ (about 1000 lux) illumination.

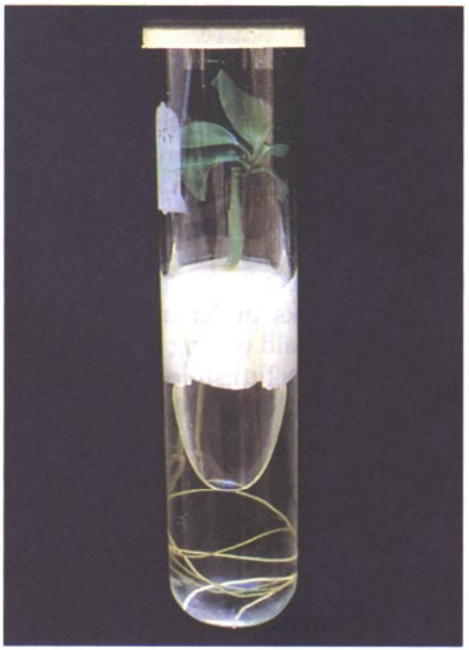


Fig. 2. Shoot-tip grafted plantlet of Washington navel on Troyer citrange after four weeks in culture.
(Dr L. Navarro, Instituto Valenciano de Investigationes Agrarias, Valencia)

- **Transplanting to soil.** Successfully grafted plants are transplanted directly to pots containing a sterilized artificial soil mix or may be side-grafted to young vigorous rootstock seedlings. Plants should be maintained in an insect-proof greenhouse.

- **Tissue destruction.** Following shoot-tip isolation and grafting, the original budwood and all remaining tissue from the new growth flushes should be destroyed by autoclaving.

- When sufficient donor material is available, the micrografted plants should be indexed.

- If, following indexing, the plants are determined to be infected or show obvious disease symptoms, the potted shoot-tip grafted plants should be subjected to thermotherapy for 2 weeks under the following conditions: 16 h daylight at 35-40°C and 8 h darkness at 30°C.

- Prior to thermotherapy, remove all leaves from plants to induce new growth.

- At the end of the incubation period, viable shoots are removed and shoot-tip grafted as before and indexed again.

- If still found to be infected, the material must once again be subjected to therapy.

2. Introduction into a non-citrus growing area

- Buds from introduced budsticks should be propagated in an isolated area.

- The newly propagated plants can be subjected to thermotherapy and shoot-tip grafting as described under section A above.

- When sufficient donor tissue is available, the material which has gone through therapy should be indexed. If found to be still infected with bud-transmissible disease the therapy procedure must be repeated until indexing indicates that the plants are free of infection.

D. Indexing strategy

Indexing after therapy is absolutely indispensable, since even the most reliable therapy methods do not work perfectly.

Detection of graft-transmitted diseases of citrus is largely based on biological indexing, either by graft transmission to specific indicator plants or by mechanical transmission to herbaceous plants. Laboratory tests that complement these biological assays include: ELISA, sequential polyacrylamide gel electrophoresis (sPAGE), culturing (for *Spiroplasma citri)*, dsRNA analysis, immunosorbent electron microscopy (ISEM) and dot blot nucleic acid hybridization.

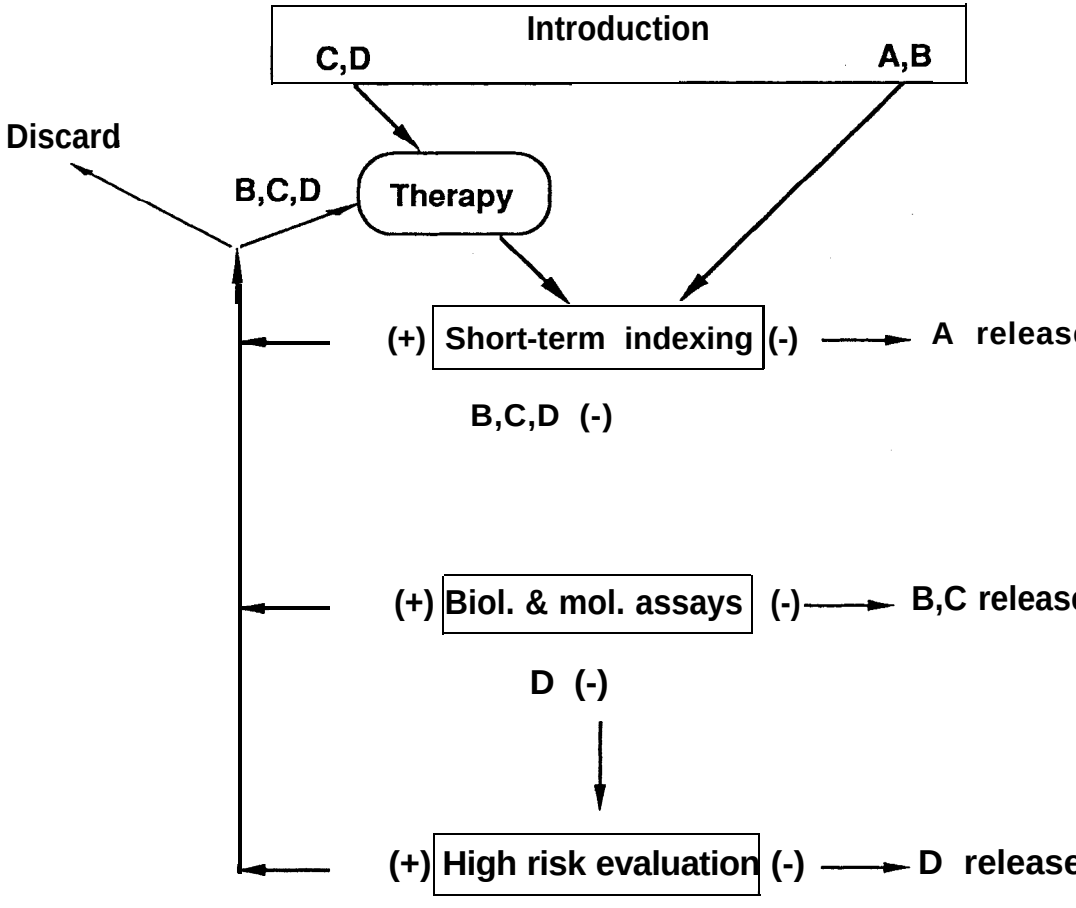
Insect-proof greenhouse facilities with efficient light and temperature control are required. They should preferably include 3 sections: a cool section for cool temperature viruses (tristeza, vein enation, infectious variegation, satsuma dwarf, etc.) and a warm section for viroid pathogens such as exocortis, cachexia and the citrus viroids, and the stubborn pathogen. An intermediate, relatively warm section is helpful for growing the indicator plants.

Further details about greenhouse facilities, as well as recommendations and procedures for operating these facilities and the indexing laboratory, are given in the 'Detection and Diagnosis of Graft-transmissible Diseases of Citrus' (Roistacher, in press).

Most commonly, indicator seedlings are bud-, chip- or blind bud graft-inoculated. Inoculation by two buds is a recommended standard, except for detection of stubborn or greening where multiple side grafts or leaf grafts are preferred. Adequate positive and negative controls must be included for each test.

The recommended indexing strategy is given in Fig. 1, and recommended indexing methods for detection of most graft-transmissible diseases of citrus are given in Table 1.

Fig. 1. Indexing strategy



A: Seed - Non-graft compatible with *Citrus*
B: Seed - Graft compatible with *Citrus*
C: Budwood - Low risk
D: Budwood - High risk

Terms used in Fig. 1.

1. Short-term indexing

   Observe symptoms on seedlings or growing plants
   Mechanical inoculation to herbaceous hosts
   ELISA
   Culturing (Stubborn)

2. Molecular indexing

   sPAGE - for viroids
   dsRNA
   Hybridization (optional)

3. Biological indexing on *Citrus*

   Basic

Sweet orange (Madam Vinous or Pineapple)	Cool
Mandarin (King or Dweet tangor)	Cool
Mexican lime	Cool
Citron (861-S-1)	Cool
Citron (861-S-1)	Warm

   Optional

Parson's Special mandarin	Warm
Orlando tangelo	Field
Rusk or Troyer citrange	Cool
Citrus excelsa	Cool
Lemon	Cool

4. High risk additional evaluation.

   The protocol is specific for each disease.

Table 1. Indexing methods for detection of graft-transmitted diseases of citrus

Diseases	Biological indicators						Laboratory methods		Minimum incubat.
	SwO	Mex.L.	Mand.	Citron	Other	Herb.	Serology	Other	
Cachexia					Pars/RoLe			*sPage	3 mo (1) 2yr (2)
Citrus viroids				Citron				*sPage	2-3 mo
Citrus leaf rugose		Mex.L..			*Le		*ELISA		2-4 mo (Le)
Citrus mosaic			*Mand.						2-4 mo
Concave gum	SwO		*Mand.		*Dweet				2-3 mo
Cristacortis	SwO		*Mand.		*Dweet				2-3 mo
Exocortis				*Citron				sPage	2-4 mo (Citron)
Gummy bark									6-15 yr
Greening	*SwO		Mand.		Gft			*EM	6-12mo
Impietratura	SwO		*Mand.		*Dweet				2-3 mo
Infectious variegation	SwO		Mand.	*Citron	*Le	Cowpea	*ELISA		2-4 wk (3) 4 mo (2)
Leathery leaf			Mand.			*Gomph			2-4 wk (3)
Leprosis	SwO								2-4 wk
Psorosis-A	*SwO	Mex.L.	Mand.	Citron	Dweet, Le				2-3 mo
Ringspot psorosis	*SwO	Mex.L.	Mand.	Citron	Gft, Le	*Chq			2-3 mo
Rubbery wood	SwO				*Le			* E M	2-4 mo
Satsuma dwarf					Satsuma	*Sesame	*ELISA		2-4 wk (3)
Stubborn	SwO						ELISA	*Culture	2-4 wk (4) 4 mo (2)
Tatterleaf					*Rusk	Chq	ELISA		2-4 wk (3) 4 mo (2)
Tristeza		*Mex.L.		Citron			*ELISA	dsRNA	2-6 mo
Vein enation		*Mex.L.			RoLe				2-4 mo
Witches' broom		*Mex.L.					*IF		6-12 mo
Unknown pathogens								dsRNA	

* Preferred indexing methods

SwO = Sweet orange
Dweet = Dweet tangor
Le = Lemon
EM = Electron microscopy
(1) sPAGE (2) Index (3) Herbaceous (4) Culture

Mex.L = Mexican lime
Gft = Grapefruiut
Mand = Mandarin
IF = Immunofluorescence

Gomph = *Gomphrena globosa*
Rusk = Rusk citrange
Pars = Parson's special mandarin on rough lemon rootstock
sPAGE = sequential polyacrylamide gel electrophoresis

RoLe = rough lemon
Chq = *Chenopodium quinoa*

PRACTICAL RECOMMENDATIONS

- When possible, source trees should be identified to allow future observation, if required.

- Information on the prevalence of pests in the area of collection should be recorded and included with the passport data.

- Seed and budwood should be packed in a polyethylene plastic bag together with a lasting label. The bag should be wrapped tightly around the material to avoid excessive air spaces and should be placed in a second bag. This bag should be sealed or tightly closed with a twist tie or rubber band and be labelled again.

- All accessions should be placed together in a larger plastic bag which should be sealed or tightly closed.

- Germplasm material should be packed in a firm cardboard box with insulation material to protect against extremes of temperature during shipment.

- Packing should be carried out on a clean bench in a closed building.

- Plant material should be dispatched as soon as possible, using the most rapid mode of transportation. Clear instructions should be given to the shipper to avoid exposure to extremes of temperature.

- Material can be stored temporarily in a household refrigerator prior to shipment.

- The parcel should contain all required documents and the passport data for all accessions. It should be properly addressed and, to avoid delays, the receiver should be notified in advance of all dispatching details.

- Upon receipt, material should be unpacked as soon as possible in a closed room, preferably with a double door system. If any suspect pests are observed in the parcel, the room should be fumigated.

- Packing material and any discarded plant material should be destroyed by incineration or autoclaving.

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Virus and virus-like diseases

1. Algerian navel orange virus

Cause
A virus with flexuous rod particles of about 780 nm long.

Symptoms
The virus has not been associated with any symptoms in citrus.

Host range
Old and nucellar lines of sweet orange, grapefruit, some tangerines and tangerine hybrids.

Geographical distribution
Has been isolated in Florida from budwood sources originating in Algeria.

Transmission
It is mechanically transmitted to *Chenopodium quinoa* and from herbaceous hosts to citrus.

Therapy
No data available. The disease should be eliminated by standard thermotherapy and shoot-tip grafting procedures.

Indexing
Mechanical inoculation on *Chenopodium quinoa.*

2. Citrus leaf rugose

Cause
Citrus leaf rugose virus (CLRV) is an ilarvirus with isometric particles which vary in size from 25 to 32 nm. Serologically related to citrus variegation and several other ilar viruses.

Symptoms
Mexican lime develops leaf puckering, Eureka lemon develops pinpoint chlorotic spotting and Duncan grapefuit shows stunting and chlorosis.

Host range
CLRV infects a broad range of citrus hosts.

Geographical distribution
CLRV was discovered in Florida, but is present in several countries, including Argentina and Japan.

Transmission
CLRV is easily transmitted experimentally by grafting or mechanical inoculation of citrus by leaf abrasion or stem-slash methods. Local spread in the field and in experimental plots has been observed, but no vector is known. The virus can also be transmitted mechanically to an extensive range of noncitrus plants, most of which are local lesion hosts or support a latent systemic infection.

Therapy
No data available. Similar ilarviruses such as citrus variegation virus (CVV) are readily eliminated by shoot-tip grafting and thermotherapy.

Indexing
CLRV can be detected by indexing to Eureka lemon or herbaceous hosts and by serological assays. Bush bean (cultivar Red Kidney) is a useful diagnostic host to distinguish CLRV from CVV.

3. Citrus leathery leaf

Cause
Citrus leathery leaf virus.

Symptoms
Thickening of the new leaves. Apical bud on the main trunk or branches is killed. Suppression of normal growth. Dieback.

Host range
Mandarin, sweet orange, Rangpur lime, lemons. Experimentally transmitted to herbaceous hosts.

Geographical distribution
India.

Transmission
Transmitted by grafting, mechanical inoculation and *Aphis gossypii*.

Therapy
No data available.

Indexing
Grafting on Rangpur lime. Mechanical inoculations to *Gomphrena globosa*, beans and sesame.

4. Citrus mosaic

Cause
Citrus mosaic virus (CiMV). Spherical virus.

Symptoms
Stunting, chlorosis and uniformly distributed mosaic.

Host range
Mandarin, sweet orange, limes, lemons, kumquats.

Geographical distribution
India. A citrus mosaic is also reported from Japan (a form of satsuma dwarf) and it is not clear if it is related to the CiMV described in India.

Transmission
Transmitted by grafting, mechanical inoculation and by *Myzus persicae* Sultz. and *Aphis craccivora* Koch.

Therapy
Shoot-tip grafting.

Indexing
Grafting on mandarin cv. Darjeeling orange.

5. Citrus ringspot

Cause
Mechanically transmitted virus-like agent. Two components are required for infectivity. Particle morphology apparently filamentous, but not confirmed. Citrus necrotic ringspot, naturally spread psorosis and psorosis-B are synonyms.

Symptoms
Ringspots or large, irregular chlorotic patterns on mature leaves which are frequently gum-impregnated. Fruit may also have ringspot symptoms. Shock reaction and twig dieback in some cultivars and frequently severe bark scaling.

Host range
Can infect most citrus cultivars and hybrids. Can also be transmitted experimentally to herbaceous hosts including *Chenopodium* spp., *Gomphrenaglobosa* and *Nicotiana* spp.

Geographical distribution
Argentina, Australia, France, Greece, India, Iran, Italy, Spain, Turkey, Uruguay and USA.

Transmission
Transmitted by grafting and by mechanical inoculation to citrus and herbaceous hosts. Natural spread by unknown means is common in Argentina and Uruguay.

Fig. 3. Mature leaf symptoms of citrus ringspot in Navel orange.
(Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

Therapy
Shoot-tip grafting and thermotherapy.

Indexing
Shock symptoms in grapefruit and sweet orange. Variable leaf patterns, including mature leaf symptoms. Local lesions on mechanically inoculated *Chenopodium quinoa*.

6. Citrus variegation virus

Cause
Citrus variegation virus (CVV) is an ilarvirus whose spherical-shaped particles vary in size. Several strains have been described. There is a serological relationship to citrus leaf rugose and several other ilarviruses.

Symptoms
Etrog citron and grapefruit usually develop chlorotic leaf symptoms and distortion, which persist on the mature foliage. Infected trees may be stunted and some fruit may be distorted or have chlorotic patterns. Severity differs among isolates. Citrus crinkly leaf is a mild form of CVV.

Fig. 4. Severe (top) and mild (bottom) isolates of CVV in Etrog citron. (Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

Host range
Has a broad host range including lemon, sour orange, Etrog citron and grapefruit. Experimentally transmitted to herbaceous hosts.

Geographical distribution
Algeria, Argentina, Australia, India, Israel, Italy, Spain, Uruguay, USA (California, Florida) and other locations.

Transmission
Transmitted by grafting and readily by mechanical inoculation. No vector has been reported.

Therapy
Thermotherapy and shoot-tip grafting.

Indexing
Grafting to lemon or Etrog citron. Sap inoculation of red kidney beans produces a brilliant systemic vein banding and vein clearing on trifoliate leaves. Sap inoculated cowpea show chlorotic/necrotic local lesions on primary leaves.

7. Concave gum

Cause
Unknown, presumably virus-like.

Symptoms
Broad concavities in trunk and limbs with gum that may exude through cracks in the bark. Gum-filled wood layers in concentric rings are observed in cross-sections of trunk and branches. Affected trees show oak-leaf patterns in young leaves.

Host range
The disease affects mandarins, sweet oranges and tangelos.

Geographical distribution
Most citrus-growing areas in older varieties.

Transmission
Readily graft-transmitted. Seed transmission of an infectious agent that induces oak-leaf pattern, and that may be associated with the concave gum disease, has been reported in trifoliate orange and Carrizo and Troyer citranges.

Therapy
Shoot-tip grafting and thermotherapy.

Indexing
Seedlings of sweet orange and mandarin incubated in a cool greenhouse (18-25 C) will show the typical oak-leaf symptoms.

8. Cristacortis

Cause
Unknown, presumably a virus-like pathogen.

Symptoms
Induces vertical depressions (large pits) in trunk. Sometimes gum-like materials stain the bottom of the pit and top of the peg. The depression, even when severe, can disappear as a consequence of radial growth. Traces of these depressions can be observed by cross-sections of trunk and limbs. Pits are often observed in both scion and rootstock. Affected trees show oak-leaf patterns on young leaves.

Host range
Susceptible species are tangelo, tangor, sour orange, sweet orange, mandarins, grapefruit and rough lemon.

Geographical distribution
Mediterranean citrus-growing areas.

Transmission
Readily graft-transmitted. No natural or mechanical transmission has been reported.

Therapy
Shoot-tip grafting and thermotherapy.

Indexing
Seedlings of mandarin and sweet orange incubated in cool greenhouse (18-23 C) will show oak-leaf patterns. Orlando tangelo seedlings growing in the field will show typical depressions in one to five years.

9. Impietratura

Cause
Unknown. Presumably virus-like.

Symptoms
Fruit of affected trees have gum pockets in the albedo under flat, sunken or raised areas of the rind. These areas do not de-green normally as fruit ripens and remain green during the period of colour change. Gum deposits may be observed on the columella of severely affected fruits. Affected fruits are often smaller than normal ones and become hardened. The disease induces an abnormally high small-fruit drop, reducing the crop. Affected trees also show oak-leaf pattern symptoms.

Host range
The disease mainly affects grapefruit and sweet orange, but also induces symptoms on clementines, sour orange, tangelo and *Citrus volkameriana.*

Geographical distribution
It is restricted to Mediterranean citrus-growing areas and India, but has been found in Venezuela.

Transmission
Readily transmitted by grafting. No natural or mechanical transmission has been reported.

Therapy
Shoot-tip grafting and thermotherapy.

Indexing
Sweet orange and mandarin seedlings will show oak-leaf patterns under cool conditions. Inoculation on twigs of field trees of *Citrus volkameriana* or grapefruit carrying flowers will induce the typical symptoms on fruits when they develop.

10. Leprosis

Cause
The causal agent of leprosis is presumed to be a mite-vectored bacilliform virus. Virus particles have been observed in the nuclei of cells from lesioned areas of leprosis-infected plants.

Symptoms
Leprosis lesions are first chlorotic, with or without a necrotic centre. Later they develop in to flat, or some what raised necrotic areas on leaves and twigs and flat or depressed areas on fruit. The lesions may contain concentric patterns and be gum-impregnated. They are usually surrounded by a chlorotic zone. Leaves and fruit abscise when lesions are abundant. Extensive lesion development on twigs causes dieback. Bark lesions may coalesce on larger limbs and resemble psorosis-induced bark scaling.

Host range
Leprosis is observed primarily on sweet orange. Sour orange and mandarins are also reactive, but other citrus cultivars do not normally show conspicuous symptoms.

Geographical distribution
Leprosis has been reported in USA (Florida), where it is now rare, and in some South American countries, particularly in Brazil, where it is a very serious problem.

Transmission
In Brazil, leprosis has been readily transmitted by larvae of *Brevipalpus phoenicis.* Nymphs and adults are less efficient. Vectoring is apparently strain-specific. Leprosis is associated in Florida with *B. californicus* and in Argentina and Venezuela with *B. obovatus.*

Therapy
No data available.

Indexing
Leprosis has been graft-transmitted, but only with difficulty. The best transmission results have been obtained with tip grafts of infected shoots. Symptoms on receptor plants remain localized near the graft union.

11. Psorosis-A

Cause
The causal agent of psorosis-A is presumed to be virus-like. Psorosis-B is described under citrus ringspot.

Symptoms
Classical psorosis-A produces scaling and flaking of the bark on the trunk and limbs of sweet orange, grapefuit and mandarin trees. Bark scaling does not occur on most other cultivars. Wood becomes impregnated with gum, which forms an irregular

circular pattern in the trunk viewed in cross-section. When twig bark from a tree affected with psorosis-A is grafted to sweet orange seedlings, a necrotic shock reaction occurs in new shoots and leaves under cool conditions. Subsequent growth flushes develop the characteristic young leaf flecking without necrosis, usually under cool conditions.

Host range
Most citrus species and their hybrids and some citrus relatives.

Geographical distribution
The disease is present in most citrus-growing areas.

Transmission
The psorosis disease agent can be readily graft-transmitted. Some isolates are mechanichally transmitted and are similar to citrus ringspot. Some hosts develop few symptoms, if any, whereas other are highly reactive. The causal agent of psorosis-A is usually widely distributed within infected plants. The method of natural spread of a psorosis-like disease in Argentina has not yet been determined, but aphids have been implicated as possible vectors.

Therapy
Shoot-tip grafting and thermotherapy.

Indexing
Seedlings of Pineapple and Madam Vinous sweet oranges, mandarins, and Dweet tangor are commonly used as indicators. The identification of psorosis-A is generally made by graft inoculation of seedlings and observation of new growth for leaf-flecking symptoms. These symptoms develop most reliably under cool conditions. Shock reactions are typically observed, under cool conditions, in sweet orange seedlings inoculated with materials from trees affected with bark-scaling forms of psorosis, and some or all of the first new flush may die.

12. Satsuma dwarf

Cause
Satsuma dwarf virus (SDV). The virus has isometric particles, about 26 nm in diameter and contains two RNA species. Citrus mosaic virus (CiMV), natsudaidai dwarf virus (NDV) and navel orange infectious mottling virus (NiMV) are also similar in morphology and are serologically related to SDV. The relationship with CiMV described in India is not known.

Symptoms
Symptoms caused by SDV in satsuma mandarin include stunting and narrow, boat- or spoon-shaped leaves, resulting in lower yields. Trees are dwarfed or stunted. In other cultivars, the symptoms are mild or absent. NDV causes vein clearing, mottling and curling of new leaves of natsudaidai. CiMV causes spotting and blotching of fruit rind in some cultivars. NiMV produces chlorotic spotting in sweet orange; the symptoms persist in mature leaves.

Host range
SDV, NiMV, NDV and CiMV can infect a wide range of citrus species and cultivars, and have been mechanically transmitted to many herbaceous plants including sesame, *Chenopodium quinoa* and cowpea.

Geographical distribution
SDV has been found in many areas of Japan and has been reported from China, Korea and Turkey in infected budwood. CiMV, NiMV and NDV have been found in small restricted areas in Japan.

Transmission
The viruses are readily graft-transmitted between citrus plants and can be mechanically transmitted to citrus and non citrus hosts. Seed transmission was reported in bush bean but has not been observed in other hosts. Local spread, apparently by soilborne vector, has been observed. Symptomless infection occurs in *Viburunum odoratissimum.* High rates of SDV have been observed near infected *Viburunum.*

Therapy
Shoot-tip grafting. Thermotherapy (40°C/30°C day/night for 6 weeks) can also eliminate SDV.

Indexing
Plants should be maintained under cool temperatures. ELISA, using anti-SDV serum, is recommended to index for SDV, CiMV, NDV, and NiMV. Mechanical inoculation to white sesame is also recommended.

13. Tatter leaf

Cause
Tatter leaf virus (TLV). Flexible rod-shaped particles approximately 650 nm in length. Also called ‘yellow ring’ of sweet orange on trifoliate orange rootstock in China. Serologically related to apple stem grooving virus and a lily symptomless virus from Japan.

Symptoms

Often symptomless. Chlorotic leaf symptoms are produced in *Citrus excelsa,* Rusk and Troyer citranges, and other trifoliate orange hybrids. Leaves of *C. excelsa* may be deformed, but infected plants often recover after the initial reaction. Stems of citrange plants may be deformed and have a zigzag growth pattern associated with chlorotic areas on the stem. Citrange and citrange hybrids are often pitted. Trifoliate orange is immune or highly resistant to TLV. However, when infected latent hosts are grafted on rootstocks of trifoliate orange or trifoliate orange hybrids, a bud-union crease can develop, and affected plants are stunted and chlorotic. Over blooming and early maturing of fruit are observed for a few years and the trees may die within 5-6 years after grafting.

Host range

Almost all citrus plants are hosts. *Chenopodium quinoa,* cowpea and some *Nicotiana* spp. can be infected by mechanical inoculation and develop symptoms. Periwinkle and petunia are symptomless hosts.

Geographical distribution

Widespread in China and Taiwan. Reported in Japan, Korea, Republic of South Africa and USA in cultivars imported from China. Also present wherever older 'Meyer' lemon trees are found since most are infected with both tatter leaf and a severe form of CTV.

Fig. 5. *Chenopodium quinoa,* systemically infected by CTLV. (Dr M. Koizumi, Fruit Tree Research Station, Okitsu)

Transmission
TLV is easily graft-transmitted from systemically infected latent hosts and from symptomatic tissues of reactive hosts. It is also readily mechanically transmitted by sap inoculation and by stem slashing. There is little evidence of natural spread of the virus.

Therapy
TLV can not be eliminated by shoot-tip grafting alone. Heat treatment for 30 days at 35-40°C/30°C (day/night) followed by shoot-tip grafting can be an effective therapy. Incubation of budsticks on medium *in vitro* for 10-14 days at 32°C, followed by shoot-tip grafting can also produce TLV-free plants with 30-50% success. Long term heat treatment of affected plants for 120 or more days at 40°C/30°C (day/night) can eliminate the virus.

Indexing
Seedlings of *Citrus excelsa* or Rusk citrange are recommended. Mechanical inoculation to *Chenopodium quinoa* or cowpea is also effective. Indexing temperatures should be controlled at 22-25°C. ELISA can index the virus rapidly if anti-TLV serum is available. In PAGE, 2 typical dsRNA bands are observed.

14. Tristeza

Cause
Citrus tristeza virus (CTV) is a closterovirus with flexuous rod-shaped particles of 12x2000 nm. Quick decline, Hassaku dwarf and seedling yellows are synonyms.

Symptoms
Stunting, stem pitting, chlorosis, and reduced fruit size are common symptoms. Decline in trees on sour orange rootstock. Leaf cupping and vein clearing are observed on indicator plants. Symptom severity is highly variable between isolates.

Host range
CTV infects most species, cultivars, and intergeneric hybrids of citrus and some citrus relatives. Trifoliate orange, some trifoliate orange hybrids, *Severinia* and *Swinglea* are resistant. The only known non-rutaceous host is *Passiflora.*

Geographical distribution
All citrus-growing areas. However, no natural spread has been reported from Cuba and the Mediterranean (excluding Spain and Israel).

Fig. 6. From left to right: healthy, mild symptoms and severe symptoms of CTV vein clearing in Mexican lime. (Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

Fig. 7. CTV vein clearing in Etrog citron. (Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

Transmission
Important aphid vectors are *Aphis citricola, Aphis gossypii* and *Toxoptera citricidus.* Graft-transmitted and mechanically transmitted experimentally by stem slash inoculation.

Therapy
Shoot-tip grafting and thermotherapy.

Indexing
Mexican lime under cool temperatures, ELISA. Grapefruit, sour orange or Madam Vinous sweet orange for detection of severe seedling yellows or stem pitting isolates under cool conditions.

15. Vein enation/woody gall

Cause
A spherical virus-like particle, 27 nm in diameter, is suspected but has not been purified or characterized.

Symptoms
Symptomless in most commercial varieties. When indexed under cool conditions, enations are seen on underside of leaves of Mexican lime, sour orange, and rough lemon. Swelling or galls on stems of rough or Volkamer lemon.

Host range
Mexican lime, sour orange, and rough lemon are reactive hosts.

Geographical distribution
Australia, India, Japan, Peru, Republic of South Africa, Spain, Turkey and USA.

Transmission
Graft-transmitted. Transmitted by *Myzus persicae, Toxoptera citricidus* and *Aphis gossypii.*

Therapy
Shoot-tip grafting, thermotherapy.

Indexing
Mexican lime, sour orange seedlings, under cool temperatures.

Viroid diseases

1. Cachexia

Cause
Citrus cachexia viroid (CCaV). It is an infectious, circular, single-stranded RNA molecule with around 300 nucleotides which is highly base-paired and forms a stable rod-like structure. Nucleic acid hybridization studies indicate that it is distinct from citrus exocortis viroid (CEV) and highly homologous with CV-II viroids and hop stunt. CCaV belongs to the CV-II group and has also been listed as CV-IIb and CV-IIc.

Symptoms
Discoloration and gum impregnation of the bark. The inner bark shows numerous pegs which fit into pits in the wood. Cachexia mainly affects some mandarins, mandarin hybrids such as tangelos, and *Citrus macrophylla.* Most other commercial citrus species are apparently symptomless unless grafted on sensitive rootstocks.

Host range
Possibly found in all citrus cultivars.

Geographical distribution
Worldwide.

Transmission
Readily graft-transmitted, primarily through bud-propagation from symptomless trees. It may also be mechanically transmitted as a contaminant on cutting and pruning tools.

Therapy
Shoot-tip grafting.

Indexing
Nucleic acid extraction from inoculated citron and sequential polyacrylamide gel electrophoresis (sPAGE) has recently been recommended as a fast and reliable test for CCaV. Biological indexing on Parson's special mandarin grown on a vigorous rootstock. Gum deposits develop within 6-18 months on plants maintained at 35-40°C.

2. Citrus viroids

Cause
At least 10 different viroids, distinct from CEV and CCaV, have been identified on citrons inoculated with different field sources. Based on the physical and biological properties, these viroids have been classified in to four different groups (I, II, III, IV). CV-II viroids are highly homologous to CCaV.

Symptoms
These viroids occur naturally as mixtures of 2-6 different viroids including CEV and CCaV. Although their specific role as disease-causing agents has not been fully demonstrated, they are believed to be responsible for mild forms of the exocortis disease. Recent studies show that CV-IIa causes bark cracking on *Poncirus trifoliata.* The viroids included in each viroid group induce specific symptoms on Etrog citron 861-S1.

Host range
Possibly found in all citrus cultivars.

Geographical distribution
Worldwide.

Transmission
Readily graft-transmitted, primarily through bud propagation from symptomless trees. Also mechanically transmitted by contaminated cutting and pruning tools.

Therapy
Shoot-tip grafting.

Indexing
Nucleic acid extraction and sPAGE of inoculated citrons provides the most reliable test for citrus viroids. Some CV-II-infected sources can only be detected after sPAGE. Biological indexing by graft inoculation of Etrog citron 861-S1. Inoculated citrons develop specific symptoms within 2-6 months when incubated at 35-40°C. CV-I viroids induce very mild stunting and mild epinasty as a result of point necrosis in the midrib. Some CV-II viroids (CV-IIa) induce very mild necrosis of the petiole and leaf tip burning, only when the indicator plants are incubated in very precise environmental conditions. CV-III and CV-IV viroids induce moderate stunting and necrosis of the petiole, resulting in moderate epinasty. Biological indexing of field sources results in a variety of symptoms, ranging from symptomless to moderately severe.

Fig. 8. Mixture of mild viroids in Etrog citron. (Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

Fig. 9. Petiole browning caused by mild citrus viroids. (Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

3. Exocortis

Cause
Citrus exocortis viroid (CEV). It is an infectious, circular, single-stranded RNA molecule with 371 nucleotides which is highly base-paired, forming a stable rod-like structure.

Symptoms
Bark scaling, tree stunting and stem blotching on trifoliate orange *(Poncirus trifoliata),* Palestine sweet lime, Rangpur lime and some citrons and lemons. In most commercial citrus species, CEV is apparently symptomless, unless grafted on sensitive rootstocks

Host range
Possibly found in all citrus cultivars.

Geographical distribution
Worldwide.

Transmission
Readily graft-transmitted, primarily through bud propagation from symptomless trees. Also transmitted mechanically as a contaminant on cutting and pruning tools. Seed and vector transmission have been suspected in several instances but have not been confirmed experimentally.

Therapy
Shoot-tip grafting.

Indexing
Biological indexing by graft inoculation to sensitive clones of Etrog citron. Etrog citron 861-Sl is highly recommended for indexing of CEV and other citrus viroids. It develops severe stunting, epinasty, vein and petiole necrosis within 2-3 months in plants incubated at 35-40°C. Biological indexing on Etrog citron is very reliable. However, the sPAGE described for other viroids will also detect CEV even before symptom development.

Fig. 10. From left to right: CEV, mixture of mild citrus viroids and healthy Etrog citron. (Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

Fig. 11. CEV in Etrog citron. (Dr S.M. Garnsey, US Horticultural Research Laboratory, Orlando)

Procaryotic diseases

1. Greening

Cause
Fastidious phloem-limited, gram negative bacterium.
Also called huanglongbing, likubin, leaf mottling and vein phloem degeneration.

Symptoms
Leaf mottle, zinc deficiency-like symptoms and yellowing. Twig dieback. Lopsided fruit, aborted seeds, inversion of fruit colouration. Asiatic isolates express symptoms under warm conditions. South African isolates express symptoms best under relatively cool conditions. Greening symptoms may be confused with severe CTV symptoms in some cultivars. Coinfection is common in Asia.

Host range
All citrus species.

Geographical distribution
China, eastern and southern Africa, India, Madagascar, Mauritius, Reunion, Saudi Arabian peninsula, southeast Asia.

Transmission
Transmitted by psyllids, *Diaphorina citri* in southeast Asia, India and Saudi Arabia (Asian greening), *Trioza erytreae* in Africa and Yemen (African greening). The disease is graft-transmitted.

Therapy
Thermotherapy and shoot-tip grafting.

Indexing
Sweet orange, mandarin, grapefruit. Electron microscopy. Monoclonal antibodies have been obtained for the Puna serotype from India and the Fujian serotype from China. Inoculation to mandarin is useful for distinguishing greening from CTV. Greening causes symptoms in mandarin while CTV does not.

Fig. 12. Graft-inoculated sweet orange seedling showing stunting and leaf yellowing (Indian greening). (Dr M. Garnier, Institut National de la Recherche Agronomique, Bordeaux)

Fig. 13. Graft-inoculated sweet orange seedling showing leaf mottling (African greening). (Dr M. Garnier, Institut National de la Recherche Agronomique, Bordeaux)

2. Citrus rubbery wood

Cause
Mycoplasma-like organism (MLO).

Symptoms
Thin and linear shoots. Unusual flexibility or elasticity of branches. Stunting. Chlorotic and smaller leaves. Dieback. Affected trees produce no flowers or fruit.

Host range
Limes, lemons, mandarin and sweet oranges.

Geographical distribution
India.

Transmission
Graft-transmitted. Natural vector suspected.

Therapy
Shoot-tip grafting.

Indexing
Inoculate lemon *(Citrus limon)*, kagzi lime *(Citrus aurantifolia)* or sweet orange.

3. Stubborn (little leaf)

Cause
Spiroplasma citri.

Symptoms
Severe stunting of young trees, shortened internodes and dense foliage. Cupped, abnormally thick leaves with variable chlorotic patterns. Small, lopsided fruit with aborted seeds. Rarely fatal. Symptoms are expressed in warm weather.

Host range
Most citrus species and cultivars and a wide range of non-citrus plants.

Geographical distribution
Eastern Mediterranean, Middle East, North Africa and western USA. Not a problem in cool areas or areas with warm, humid climate.

Transmission
Graft-transmitted. Vectors are the leafhoppers *Scaphytopius nitridus* and *Circulifer tenellus* in California and *Neoaliturus haemoceps* and *N. tenellus* in Mediterranean and Middle East countries.

Therapy
Shoot-tip grafting.

Indexing
Culturing or inoculation using young leaf pieces or side grafts to Marsh grapefruit or Madam Vinous sweet orange seedlings in a warm greenhouse. ELISA and DNA hybridization are also available. Pathogen concentration may be low and make detection by ELISA or by probes difficult.

4. Witches' broom disease of lime

Cause
Mycoplasma-like organisms (MLO).

Symptoms
Witches' broom with small leaves and twig dieback. No flowers or fruits are produced. Quick death of the plant.

Host range
Small fruited acid lime. Has been transmitted experimentally to Troyer citrange.

Geographical distribution
Sultanate of Oman, United Arab Emirates.

Transmission
Graft-transmitted. Natural transmission unknown. Insect vector suspected.

Therapy
Possibly shoot-tip grafting.

Indexing
Immunofluorescence. Electron microscopy. Mexican lime.

Other grafted transmitted diseases

1. Australian citrus dieback

This disease, possibly caused by a mycoplasma-like organism and showing similarities with greening, has been described in eastern Australia. It mainly affects grapefruit and sour orange, but orange is also a host. The disease is graft-transmitted at low rates.

2. Bahia Psorosis

Cause
Unknown, possibly a transmissible pathogen.

Symptoms
Initially small areas of raised scaling bark on trunk (popcorn symptoms) followed by psorosis-like bark scaling symptoms. With time, tree will deteriorate. Chlorotic patterns may appear in mature leaves.

Host range
Old and nucellar lines of sweet orange, grapefruit, some tangerines and tangerine hybrids.

Geographical distribution
So far only described in the state of Bahia in Brazil.

Transmission
Unknown, possibly transmitted by insects. Graft-transmission not confirmed.

Therapy
No data available.

Indexing
No data available.

3. Blight

Cause
Causal agent is unknown. Also called declinio and young tree decline.

Symptoms
Early symptom of blight is wilting of the canopy. Blighted trees may also show zinc deficiency symptoms. As the decline progresses, leaf loss and twig dieback occur. Blighted trees rarely die, but never recover from chronic decline. Blight does not appear in young trees and has only been observed in field trees, usually 6 years old or more. Symptoms of blight can be easily confused with those caused by other diseases such as greening, tristeza and spreading decline caused by burrowing nematodes. Water transport in the xylem of blighted trees is impaired, and zinc accumulates in the bark and outer wood. These symptoms are not associated with other citrus declines.

Host range
Blight occurs most commonly in sweet orange, grapefruit and mandarin trees grafted on rough lemon, Rangpur lime and trifoliate orange, but can occur on any rootstock. Trees on sweet orange, sour orange and mandarin rootstock are affected at an older age and at a lower incidence, but symptoms are similar in severity when they occur. Blight may occur in seedling trees.

Geographical distribution
Blight has been reported in Australia (Queensland), Central and Latin America (including Cuba), Republic of South Africa and USA (Florida and Hawaii).

Transmission
Blight has been transmitted experimentally by approach grafting roots between blighted and healthy trees and by grafting root pieces from blighted trees to healthy trees. It has not been transmitted by grafting from the scion portion of affected trees or propagated from affected trees. No vector has yet been implicated. Movement may be soil-associated.

Therapy
No data available.

Indexing
No procedures are available for small trees. Blight can be diagnosed by water injection and zinc analysis in field trees. The incubation period in root graft-inoculated trees has been 18-24 months and once trees are affected they decline rapidly. If budwood is moved from areas with blight, propagations should be held under containment for at least 24 months, and the donor tree kept under surveillance for the same period.

4. Bud-union crease on rough lemon

Cause
Not known; possibly virus-like.

Symptoms
Varies from moderate bud union indentation or crease to severe indentation and discolouration at the bud union, with swelling and occasional gumming under the bark.

Host range
Mostly on sweet orange or rough lemon rootstocks.

Geographical distribution
Wherever rough lemon is used as a rootstock, primarily the Republic of South Africa and USA (Florida).

Transmission
By graft inoculation or bud propagation.

Therapy
Shoot- tip grafting.

Indexing
None available or known.

5. Citrus yellow mottle

A graft-transmitted agent associated with vein clearing and symptoms like those of ringspot has been reported in two satsuma mandarin trees in Japan, causing a disease described as citrus yellow mottle. It can be graft-transmitted to a wide range of cultivars and is associated with a rod-shaped particle of 12-14 x 690-740 nm. The virus can be eliminated through shoot-tip grafting.

6. Fovea

Fovea was originally described as a disease causing decline and death of Murcott tangor trees in Florida. The affected trees also had inverse stem pitting. Later, it was shown that inverse stem pitting in Murcott could also be induced by inoculation with tissue from cachexia-affected plants. The causal relationship between inverse pitting and tree decline in Murcott has not been clarified, partly because tree decline from periodic overcropping is also common in this cultivar. The relationship between fovea and cachexia is still unclear.

7. Gummy bark

Uncharacterized, graft-transmitted disease (possibly caused by a viroid). The symptoms are reddish-brown gum in lightly scraped bark of sweet orange scion above the bud union. Severe pits may be present in sweet orange scion. The disease is found in the Near East, North Africa and Turkey. Sweet orange on sour orange is used for indexing; symptoms occur in 6 to 8 years.

8. Gum pocket and gummy pitting

Graft-transmitted, uncharacterized disease, possibly caused by a viroid.
Symptoms are pockets of gum in the bark and woody tissues of the trifoliate orange rootstock, followed by necrosis of the bark in severely affected areas. Trees are frequently stunted. The disease is found in Argentina, Australia and Republic of South Africa.

9. Kassala disease

Graft-transmitted disease (possibly caused by a viroid) similar to gummy bark (gum impregnation of the bark). It affects grapefruit in the the Sudan.

Yellow vein is a virus-like disease discovered in several limequat trees in California. It can be experimentally graft-transmitted to a range of citrus cultivars, but there is no evidence of natural spread. A synergistic interaction of the agents causing yellow vein and vein enation has been reported.

11. Zonate chlorosis

Zonate chlorosis is a disease, somewhat similar to leprosis, found in the coastal areas of Brazil. Zonate chlorosis causes chlorotic spots on leaves, twigs, and fruits, but the spots do not become necrotic. Affected trees frequently have psorosis-like bark lesions and old limbs. It affects sweet orange, grapefruit, tangerines and limes. Zonate chlorosis is transmitted by *Brevipalpus phoenicis* and is graft-transmitted with difficulty.

Appendix: Some characteristics of the major Aurantoideae genera

	Graft Compatible to *Citrus*	True-to-type from seed	Propagation from cuttings	Shoot-tip grafting	Monoembryony
Aegle	+	+	+	+	+
Aeglopsis	+	+	+	+	+
Afraegle	+[1]	+	+	N/A	+
Atalantia	+	+	+	+	±
Balsamocitrus	+[1]	+	+[1]	N/A	+
Burkillanthus	-	+	+[1]	N/A	+
Citropsis	+	+	+	+	+
Citrus	+	±	±	+	±
Clausena	±	+	+	N/A	+
Clymenia	+	+[2]	+[1]	N/A	-
Eremocitrus	+	+[2]	+[1]	+	+
Feronia	+[1]	+	+	N/A	
Feroniella	±[1]	+	+[1]	N/A	+
Fortunella	+	+	+	+	+
Glycosmis	-	+	+	N/A	+
Hesperethusa	+	+	+[1]	+	±
Limnocitrus	-	+	+[1]	N/A	+
Luvunga	-	+	N/A	N/A	+
Merope	-	+	+[1]	N/A	+
Merrilla	-	+	+[1]	N/A	+
Microcitrus	±	+	+[1]	+	+
Micromelum	-	+	+	N/A	+
Monanthocitrus	-	+	N/A	N/A	+
Murraya	±	+	+	N/A	+
Oxanthera	-	+	N/A	N/A	+
Pamburus	-	+	+[1]	-	+
Paramignya	-	+	+	N/A	+
Pleiospermium	+[1]	+	+[1]	N/A	
Poncirus	+	+	+	+	+
Severinia	+	+	+	+	+
Swinglea	+	+	+	+	+
Triphasia	+[1]	+	+	N/A	±
Wenzalia	-	+	+	N/A	+

[1] low success rate / requires special techniques
[2] under controlled self-pollinations
N/A data not available
± variable by species

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