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PM 3/95 (1) Inspection of places of production- *Citrus* plants for planting

Specific scope: This Standard describes the procedure for inspection of places of production of citrus plants for planting and includes relevant sampling criteria and the main regulated pests. This Standard covers plants of *Citrus* L. and other genera of the plant family Rutaceae such as *Fortunella* Swingle and *Poncirus* Raf., and their hybrids for fruit production. It focuses on EPPO A1 and A2 pests which infest these plants. Inspections of places of production may provide useful information for export certification, internal country movement of material, contribute to general surveillance or support demonstration of pest freedom (place of production or area).
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1 | INTRODUCTION

Citrus L., and other genera of the plant family Rutaceae such as *Fortunella* Swingle and *Poncirus* Raf., and their hybrids (hereafter collectively referred to as citrus) are widely grown in the Mediterranean area of the EPPO region. EPPO countries produce approximately 12% of the world's total citrus fruit production (FAO, 2021). As the import of citrus plants from outside of the EPPO region is generally prohibited (e.g. into the EU), plant material used for the production of citrus fruit is produced in the EPPO region.

Plants for planting are generally considered to pose a higher pest risk than other regulated articles (FAO, 2012). Such plants are an important pathway for the entry and further spread of pests.

Commercial citrus plants for planting are grafted plants comprising a rootstock and a variety (i.e. scion). Plants for planting for the production of varieties are usually vegetatively propagated, starting from cuttings, in order to preserve the genotype characters. As a result, citrus plants are rarely propagated from seedlings, except within breeding programs. However, plants for planting for the production of rootstock are usually propagated from seeds.

There is a risk of spreading pests through the grafting of cuttings deriving from infested rootstock and scion mother plants. Pests could be further spread through activities, such as pruning, physical contact between different lots and contact with infested plant debris. Pests may also have natural spread capacity or be spread by vectors, wind or rain splash.

Plants for planting produced according to EPPO Standard PM 4/12 *Certification scheme pathogen-tested citrus trees and rootstocks* (EPPO, 1995), or any equivalent phytosanitary certification system, are considered to provide high phytosanitary guarantees which is especially important for certain viruses. The present Standard only covers regulated pests. Nevertheless, some pests are categorized as a quarantine pest in some EPPO countries and Regulated Non-Quarantine Pests (RNQPs) in other EPPO countries, based on a risk-based approach. This is the case for some viruses (e.g. *Citrus exocortis viroid*, *Citrus impietratura agent*, *Ophiovirus citri*), which are RNQPs for the European Union (EU), and quarantine pests for other EPPO countries.

This Standard may be applicable for inspections to maintain place of production freedom or crop freedom for specified pests, as requirements are frequently provided for citrus plants for planting in several countries. It may also provide guidance for export inspection when the requirements of the importing country are similar to those in the country of origin.

2 | PHYTOSANITARY INSPECTIONS

ISPM no. 5 *Glossary of Phytosanitary Terms* (FAO, 2022) defines inspection as 'Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations'.

General background information and more detailed guidance on phytosanitary inspection of places of production is given in EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009).

Young citrus plants for planting may be grown in pots or directly in soil in the field and young plants may be grown in the open field or under physical isolation (for the latter see for example EPPO PM 5/8 *Guidelines on*

the phytosanitary measure 'Plants grown under complete physical isolation' (EPPO, 2016).

It is important to carry out inspections at the most appropriate time, depending on the biology of the pests and the most suitable period to detect symptoms, and to collect suitable samples for testing. Guidance can be found in the relevant EPPO Diagnostic Standards which are mentioned in Appendix 1 and in the references, when available for specific pests.

Inspections should be carried out at the places of production and in different lots of plants at least once a year, in order to ensure that the places of production are free from the relevant pests of quarantine concern. Nevertheless, more frequent inspections may need to be carried out, depending on the crop history, type of materials, origin, and type and distribution of the pest.

As stated in EPPO Standard PM 4/12 *Certification scheme pathogen-tested citrus trees and rootstocks* (EPPO, 1995), importation of citrus plants for planting from outside the EPPO region is generally prohibited (e.g. into the EU). In many EPPO countries, imported material would require a permit and would be subjected to post-entry quarantine (ISPM no. 34 *Design and operation of post-entry quarantine stations for plants*, FAO, 2016).

For pests that are not easily detectable using visual examination (e.g. '*Candidatus* Liberibacter asiaticus'), the inspection procedure may consist of sampling asymptomatic plant material for laboratory testing.

Citrus mother plants can remain in the field for several years and although they are generally produced under physical isolation they may be exposed to pests, such as viruses, bacteria and phytoplasmas.

Inspection may also be carried out for the detection of organisms which are not yet regulated as pests, but which could include potential pests (EPPO, 2009). It should be taken into account that emerging pests could change the phytosanitary status of a country due to the introduction or spread of some pests or vectors (e.g: *Trioza erytreae*, *Diaphorina citri*, *Aphis citricidus*, '*Candidatus* Liberibacter spp.', severe strains of *Citrus tristeza virus*).

3 | TYPES OF MATERIALCONCERNED

This Standard covers all types of propagating material of citrus plants which are used both for the production of rootstock and scion cuttings, and young rooted plants. The main species of these genera that are grown in the EPPO region are listed in Table 1.

Other new taxa such as bergamot (*Citrus bergamia*), limequat (× *Citrofortunella*), variegated lemon (*Citrus limon* Foliis variegatis), buddha's hand (*Citrus limonimedica*), calamondin (× *Citrofortunella mitis*), chinotto (*Citrus myrtifolia*), yuzu (*Citrus Ichangensis* × *Citrus Reticulata* var. austere), etc. are beginning to be propagated for ornamental purpose or even as modern culinary specialties.

TABLE 1 Main *Citrus* species grown in the EPPO region.

Botanical name	Common English name
Citrus aurantiifolia (Christmann & Panzer) Swingle	Key lime
Citrus aurantium Linnaeus	Sour orange
Citrus hystrix de Candolle	Kaffir lime
Citrus latifolia Tanaka	Tahiti lime
Citrus limettioides Tanaka	Palestine sweet lime
Citrus limon (Linnaeus) N. Burman	Lemon
Citrus maxima (Burman) Merrill	Pummelo
Citrus medica Linnaeus	Citron
Citrus paradisi Macfadyen	Grapefruit
Citrus reticulata Blanco	Mandarin
Citrus sinensis (Linnaeus) Osbeck	Sweet orange
Citrus unshiu Markowicz	Satsuma
Fortunella spp. Swingle	Kumquat
Poncirus trifoliata (Linnaeus) Rafinesque	Trifoliate orange

EPPO Standard PM 4/12 *Certification scheme pathogen-tested citrus trees and rootstocks* (EPPO, 1995) provides a scheme for the production of certified citrus plants. The scheme provides separate procedures for the production of grafted citrus tree varieties, rootstocks and seed for rootstocks.

At a place of production different types of citrus plants for planting may be present. The most common types are listed below.

3.1 | 'Mother' trees (Nuclear stock and propagation stock)

These are trees of known variety and health status that are maintained individually in situ for the production of scion wood for grafting or budding onto rootstocks for the production of grafted fruit trees (often known as 'finished' or 'complete' trees).

3.2 | Seed production trees

These are individual trees maintained to produce high-quality seed for the production of rootstocks for later grafting or budding.

3.3 | Seedling rootstocks

Grown directly from seed, seedling rootstocks are grown in the place of production before later budding or grafting to produce grafted fruit trees.

3.4 | Tissue culture

Plants in tissue culture are usually intended for further propagation. Tissue culturing is initially carried out under laboratory conditions during the micropropagation phase. This material is the starting point for large scale multiplication.

3.5 | Grafted trees (certified stock)

These are the final budded or grafted trees grown in the field, greenhouse or a temporary structure, usually for 1 or 2 years, before marketing either as 'bare rooted' plants or in individual pots as finished young trees.

4 | PESTS OF CONCERN FOR THE EPPO REGION

This Standard mainly relates to those organisms affecting citrus plants for planting including pests of fruit (mother trees bearing fruit may be present in the place of production), which are listed in the A1 and A2 EPPO Lists of pests recommended for regulation as quarantine pests. It also considers those pests which are listed in specific EPPO countries, even if not mentioned in the A1 or A2 EPPO lists. For a more comprehensive list of pests associated with citrus species, the EPPO Global Database should be consulted (EPPO, 2023a). For emerging pests, the EPPO Alert List should be consulted, (e.g. *Citrus yellow vein clearing virus* was first listed on the EPPO Alert List in 2022).

Specific pests of citrus (A1 and A2 EPPO lists, and pests listed by specific EPPO countries) are listed in Table 2. Polyphagous pests affecting citrus (A1 and A2 EPPO lists, and pests listed by specific EPPO countries) are described in Table 3. The phytosanitary procedures described in this Standard are primarily aimed at preventing the spread of these pests in the EPPO region or to third countries via exported consignments. The EPPO A1 and A2 Lists, as well as the lists of regulated pests within countries, are subject to additions and deletions. The present list (Tables 2 and 3) will therefore need to be revised whenever relevant new quarantine pests are identified.

Details of all concerned pests can be found in EPPO Global Database (2023a), in EPPO Standards regarding the specific pests or crops, and in relevant scientific references.

5 | IDENTIFICATION OF LOTS

General background information on lot identification is given in EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009).

For non-grafted plants (e.g., rootstock mother plants), the cultivar, and where relevant the clone, are the primary criteria to distinguish lots.

For grafted plants (e.g., scion mother plants), the grafting combination, cultivar and rootstock, and their clones when relevant, are the primary criteria for lot identification.

A lot should include all plants originating from the same propagating material (both rootstock and scion for grafted plants), of the same age and cultivated in one single field or set of plants in the case of potted plants.

6 | SELECTION OF PLANTS FOR INSPECTION AND SAMPLING FOR LABORATORY TESTING

This section contains guidance on inspection of places of production of citrus plants for planting, on the number of growing plants to be inspected (sample size) and on sampling for laboratory testing. Inspections are carried out after checking the location of places of production and assessing the regulations or NPPO requirements for the purpose of the inspection. This may be for monitoring or survey purposes, for the issuance of a Phytosanitary Certificate or for a certificate for internal movement, such as for the issuance of an EU plant passport.

6.1 | Selection of plants for inspection (general aspects)

Inspection of plants at a place of production is covered in general terms by EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009).

For the purposes of this procedure, these principles also apply for different types of plant propagating material, as for rootstock and scion mother plants or rooted cuttings, irrespective of whether they are grafted or own-rooted.

The aim of these inspections is to detect the presence of plant pests by inspection, either alone or in combination with sample collection for laboratory testing to obtain confirmation of the diagnosis.

Depending on the reason for the inspection and the regulations being applied (including the requirements of importing countries), inspection of the whole place of production and the vicinity, the place of production only or only of a consignment of relevant plants may be required.

TABLE 2 Specific pests of citrus.

A1 pests (absent from the EPPO region)	A2 pests (present in the EPPO region)	Other pests regulated by specific EPPO member countries
Bacteria	**Fungi**	**Bacteria**
'*Candidatus* Liberibacter africanus'	*Plenodomus tracheiphilus*	'*Candidatus* Phytoplasma aurantifolia' (EU A1 Quarantine pest (Annex II A))
'*Candidatus* Liberibacter americanus'	**Insects**	*Spiroplasma citri* (EU RNQP, Türkiye A2)
'*Candidatus* Liberibacter asiaticus'	*Trioza erytreae*	**Fungi**
Xanthomonas citri pv. *aurantifolii*	**Viruses and viroids**	*Elsinoë australis* (EU A1 Quarantine pest (ANNEX II A), Israel, Morocco, Tunisia Quarantine pest)
Xanthomonas citri pv. *citri*	*Citrus bark cracking viroid*	*Elsinoë citricola* (EU A1 Quarantine pest (Annex II A))
Fungi	*Citrus tristeza virus*	*Elsinoë fawcettii* (EU A1 Quarantine pest (Annex II A), Israel, Tunisia Quarantine pest)
Phyllosticta citricarpa	*Satsuma dwarf virus*	*Septoria citri* (Morocco quarantine pest)
Pseudocercospora angolensis		**Insects**
Insects		*Aceria sheldoni* (Georgia, Uzbekistan A1 List)
Bactrocera minax		*Aonidiella citrina* (Jordan A1; Israel, Morocco, Tunisia Quarantine pest; Türkiye A2)
Bactrocera tsuneonis		*Brevipalpus californicus* (TürkiyeA1)
Diaphorina citri		*Dialeurodes citri* (Azerbaijan, Georgia, Uzbekistan A2; Belarus Quarantine pest)
Unaspis yanonensis		*Pezothrips kellyanus* (Morocco, Tunisia Quarantine pest)
Viruses and viroids		*Phyllocnistis citrella* (Azerbaijan, Georgia, Uzbekistan A2; Belarus Quarantine pest)
Citrus blight agent		*Prays endocarpa* (Jordan A1; Morocco Quarantine pest)
Citrus leprosis virus		*Unaspis citri* (Belarus, Israel, Morocco, Tunisia Quarantine pest; Azerbaijan, Georgia, Jordan, Türkiye, Ukraine, Uzbekistan A1)
Citrus yellow mosaic virus		**Viruses and viroids**
		Citrus concave gum agent (Jordan A1)
		Citrus dwarfing viroid (Jordan A1)
		Citrus exocortis viroid (EU RNQP, Jordan, United Kingdom A1)
		Citrus impietratura agent (EU, United Kingdom RNQP, Jordan A1)
		Citrus leaf blotch virus (EU, United Kingdom RNQP)
		Citrus variegation virus (EU, Switzerland, United Kingdom RNQP, Jordan A1)
		Citrus vein enation virus (Jordan A1; Türkiye A2; Morocco, Tunisia Quarantine pest)
		Ophiovirus citri (Jordan A1; Türkiye A2; Tunisia Quarantine pest)

6.1.1 | Inspection of the place of production

The number of plants that should be selected for inspection to detect a specified level of infection in a specified lot size is indicated in tables 1, 3 and 4 of ISPM no. 31 *Methodologies for sampling of consignments* (FAO, 2009).[1]

It is up to the NPPO to set the sample size. For example, from a lot of 10 000 plants, 3 689 plants need to be inspected to provide a 99% confidence of detecting symptoms present in 0.1% of the plants, provided the symptoms are uniformly distributed and the plants are randomly selected. To detect symptoms present in 1% of the plants, with a 99% confidence, 448 randomly selected plants should be inspected.

For small lots, the numbers required will often mean that all plants should be inspected.

[1]ISPM 31 provides information on the number of units to be sampled, which is considered useful to determine sample sizes for consignments and can be extrapolated to places of production.

TABLE 3 Polyphagous pests of *Citrus*.

A1 pests (absent from the EPPO region)	A2 pests (present in the EPPO region)	Other pests regulated by specific EPPO member countries
Insects	**Bacteria**	**Fungi**
Aleurocanthus woglumi	*Xylella fastidiosa*	*Neocosmospora ambrosia* (EU A1 Quarantine pest (Annex II A))
Anastrepha fraterculus		*Neocosmospora euwallaceae* (EU A1 Quarantine pest (Annex II A))
Anastrepha ludens	**Insects**	
Apriona rugicollis	*Aleurocanthus spiniferus*	**Insects**
Bactrocera dorsalis	*Anoplophora chinensis*	*Aleurocanthus citriperdus* (EU A1 Quarantine pest (Annex II A))
Bactrocera tryoni	*Aphis citricidus*	*Aleurothrixus floccosus* (Azerbaijan, EU RNQP, Uzbekistan A1)
Ceratitis rosa	*Bactrocera zonata*	*Diaprepes abbreviatus* (Jordan, TürkiyeA1)
Gymnandrosoma aurantianum	*Eutetranychus orientalis*	*Eotetranychus lewisi* (EU Quarantine pest (Annex II A); Jordan, Türkiye, United Kingdom A1; Israel Quarantine)
Naupactus xanthographus	*Euwallacea fornicatus* sensu lato	*Hishimonus phycitis* (vector of *Ca* Phytoplasma sp.) (EU Quarantine pest (Annex II A), Tunisia, Türkiye A1)
Oemona hirta	*Lopholeucaspis japonica*	*Icerya aegyptiaca* (Jordan A1)
Radopholus similis citrus race	*Platynota stultana*	*Icerya purchasi* (Azerbaijan A2; Uzbekistan A1; Belarus, Moldova Quarantine pest)
Scirtothrips aurantii	*Scirtothrips dorsalis*	*Leptoglossus australis* (Israel Quarantine pest)
Scirtothrips citri	*Thaumatotibia leucotreta*	Non-EU *Cicadomorpha* (vectors of *Xylella* spp.) (EU A1)
Spodoptera litura		Non-EU Tephritidae (EU A1)
		Viruses and viroids
		Hop stunt viroid (EU RNQP, Jordan A1)

Each lot should be examined as a separate unit because it will have different visual characteristics, such as size of individual plants and foliar morphology.

Phytosanitary inspection should start with an overall examination of the place of production in order to check the physical condition of the plants. If there is abnormal die-off in a place or lot, or if there are other anomalies within the plants (e.g. abnormal growth, differences in colour, leaf curl), these lots should be checked with particular attention. If no symptoms are seen, a systematic inspection of the of the place of production should be made.

For the inspection of rootstock mother plants and rooted cuttings, where pest symptoms are often latent, hidden or less specific, inspection may be randomly carried out on a set of plants, extrapolating the methodology for consignments included in ISPM 31 (FAO, 2009), and sampling for laboratory testing for detection of latent infestation may be recommended.

Monitoring and sampling for known vectors of pathogens can be a complementary activity when inspecting plants. Traps can be a useful tool for monitoring of flying stages of pests and vectors of pests (for example see EPPO Standard PM 9/27 (EPPO, 2020a) for guidance on traps to detect '*Candidatus* Liberibacter spp.' vectors in citrus orchards). It is also possible to use entomological nets to capture live adult pests for identification. Berlese traps (with citrus leaves) are required to detect Thysanoptera species as their presence is not always detectable by visual examination.

Procedures for the inspection of consignments of plants, plant products and other regulated articles at import and export are described in the ISPM no. 23 *Guidelines for inspection*, which states that inspection can be used to verify the compliance with some phytosanitary regulations (FAO, 2005). Therefore, place of production and pre-export inspections of consignments may be carried out to verify that the exporting lot meets phytosanitary requirements of the importing country.

For export, a common practice to prevent the spread of soil-borne pests is to uproot and remove soil residues from plants using pressure washers. As a result, plants are delivered as bare rooted plants, with a lower pest risk.

6.2 | Sampling for laboratory testing (general aspects)

Following good hygiene procedures is important when collecting samples for the laboratory.

In order not to spread and increase infections, adequate precautions should be taken during inspection and sampling, such as wearing protective clothes (coat, overshoes, gloves, etc.). Gloves should be changed between different lots. All equipment for sampling must be decontaminated between different samples.

Samples should be sent to the laboratory as soon as possible after collection.

6.2.1 | Sampling of symptomatic material

Inspectors should be familiar with the symptoms of the listed pests they may encounter, and if any are observed symptomatic samples should be taken for laboratory testing. Details of the procedures for sampling for the individual pests are given in Appendix 1.

In general, samples should be taken from individual plants, and these should be kept separate in order to aid diagnosis and obtain a measure of the number of plants that are infested. Nevertheless, pooling of items may be acceptable for sampling of certain pests.

When symptoms are detected in the plants, it is recommended to take the parts of these plants that presented those symptoms, including both young and mature plant parts.

If the inspector is confident in the diagnosis and there are large numbers of plants in a lot with similar symptoms, sampling may be limited to a representative number of symptomatic plants.

6.2.2 | Sampling of asymptomatic material

In situations where it is difficult to find symptoms, or to declare a pest-free place of production or determine area freedom, sampling of asymptomatic plants and vectors may be required in order to detect latent or hidden infections of regulated pests. Sample size should be decided when determining the overall confidence level and design prevalence of the survey which will depend on the risk.

In the event of positive results from the laboratory, it is important to be able to trace back all samples to the original plant or plants for eradication purposes. The diagnostic sensitivity of each test to be used should be known in order to organize and perform the sampling protocol according to laboratory needs.

It is also important to keep in mind that sampling can never prove that a pest is truly absent.

6.3 | Selection of plants for inspection and sampling for laboratory testing (specific aspects)

For further details on symptoms, sampling and identification of the relevant pests of citrus plants for planting, see Appendix 1.

6.3.1 | Inspection of citrus plant material

For tissue culture, the majority of potential pests will be excluded due to the confined conditions for micropropagation. This may not be the case with viral or bacterial pathogens, which could go unnoticed during micropropagation. Inspection of plants in tissue culture at the time of sale or export is difficult to perform and unreliable. It is recommended that this material is inspected before it is propagated in tissue culture or after transplanting into growing medium and growth continued to a stage where symptoms could be detected.

In practice, in scion mother plants, symptoms of pests are commonly more frequent and more easily detectable than in rootstock plants. In this case, the inspection of all mother plants may be performed, because lots are generally not very large.

Both rootstock and scion mother plants should be inspected for citrus pests. For the inspection of plantlets and young plants, it should be taken into account that fruit pests such as *Bactrocera* spp. will not be present.

Micro-propagated material is exclusively required to be grown under protected conditions which exclude pests to ensure aseptic conditions and avoid subsequent infestations.

Each lot of young plants (grafted or own-rooted) should be individually inspected because it may have a different origin, grafting combination and specific features, in terms of disease resistance, history, previous treatments and potentially different infestation levels.

The time of year for inspection will vary with the pest species, depending on the optimum time for expression of symptoms for a specific pest survey programme or presence of visible live stages. In general, plants should be in active growth and the spring and summer months are the best times to observe the majority of citrus pest symptoms. However, refer to Appendix 2 for pest specific details.

Visual examination will only detect pests which are apparent (or cause noticeable localized symptoms) on the plants such as insect stages, galls or leafspots, or are systemic but showing symptoms in the foliage or stems, as for phytoplasmas and viruses. Symptoms are best detected when the timing, the climate conditions or variety susceptibility are appropriate.

Inspectors should particularly look for symptoms on the wood or on shoots, leaves and any fruits, if present. Additionally, inspectors should look for vectors that may transmit diseases. Symptoms caused by insects are often generic for most primarily foliage feeding. Sometimes leaf-galls appear. Larval damage is commonly non-specific, especially for those insects which have powerful mouthparts to cut plant tissues. A short procedure for inspectors is detailed in Appendix 3.

Diseases may exhibit different symptoms, which can be apparent via reduced growth, stem grooving, stunting and other deformations. Leaves may be thicker than normal, or may show many other symptoms, such as ring spots, discoloration, pale or reddish areas, clearing

of veinlets, veinal or interveinal necrosis, rolled margins or mild deformation. Shoots may also exhibit several symptoms, with deformed, flexible and drooping aspects, or incomplete lignification. It should be highlighted that an infected plant may not show symptoms systematically, as it may exhibit signs of disease 1 year and no symptoms the following year. If asymptomatic infection is suspected (as for *Citrus Tristeza virus*), or plants are being indexed for possible latent infection, then random samples representative of the whole lot should be collected.

6.4 | Sample preparation

Plant samples should be placed in a plastic bag that is then inflated slightly and sealed, then packed in strong containers such as cardboard or plastic boxes and padded with paper or similar to prevent movement.

Each sample should be individually labelled with nursery name, nursery reference number, date, variety and, if necessary, a way of identifying the individual plant or tree so follow-up action can be taken if needed.

Appendix 1 provides pest specific guidance for sampling and sample preparation.

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APPENDIX 1 - SYMPTOMS AND SAMPLING FOR IDENTIFICATION OF QUARANTINE PESTS OF CITRUS PLANTS

For each of the pests mentioned below basic information on host range, biology, detection and identification can be found in EPPO Global Database (https://gd.eppo.int/), as well as in EPPO Datasheets, EPPO Diagnostic Standards, and EFSA Plant Pest Survey Cards. Illustrations are available on the EPPO Global Database and the EFSA Plant Pest Survey Cards (link). When an EPPO Diagnostic Standard exists, it is mentioned in the text. The fact that there is no EPPO Diagnostic Standard does not mean that no method for diagnosis is available in the scientific literature. In some cases symptoms have been included for information from plants older than those which are usually traded for planting, because symptoms on seedlings are sometimes difficult to spot. As a general rule, symptoms might not be readily visible on very young plants, and they are given in part for illustration purposes. Appendix 1 includes all EPPO A1 and A2 pests specific for citrus listed in Table 2 and selected polyphagous A1 and A2 pests listed in Table 3.

(a) Insects and mites

Aceria sheldoni (Georgia, Uzbekistan A1 List)

Symptoms and description

Aceria sheldoni is one of the main pests of lemon (EPPO, 2004a). It is a minute mite (0.12–0.18 mm) which feeds and reproduces on citrus foliage and moves to buds and young fruit as they become available. Severe attack can contribute to water loss from the fruit, reduction in fruit size and premature fruit drop.

Sampling and identification

EFSA (2008) detail that the species can be identified using morphological characteristics visible under a microscope. Plant material suspected to be infested with *A. sheldoni* can be sent to the laboratory in an air-tight and secure container. Additionally, fruit characteristic deformation may indicate the presence of the pest.

Aleurocanthus spiniferus (EPPO A2 List), *Aleurocanthus woglumi* (EPPO A1 List)

Symptoms description

Adult Aleyrodidae or whiteflies are winged insects of 1–2 mm in length that look like tiny moths (Figure A1a). Their wings are covered with waxy powder. The adults are usually active and feed on leaves of the host. Nymphs are mostly present on the underside of the leaves as are puparia (Figure A1b). In spite of being called whiteflies, *A. spiniferus* has characteristic grey to black coloration. They are most likely to be found on young shoots and leaves of planting material or cut branches but could also be found on fresh fruits. Highly infested leaves can show spots of sticky, transparent honeydew, which can be covered by sooty mould.

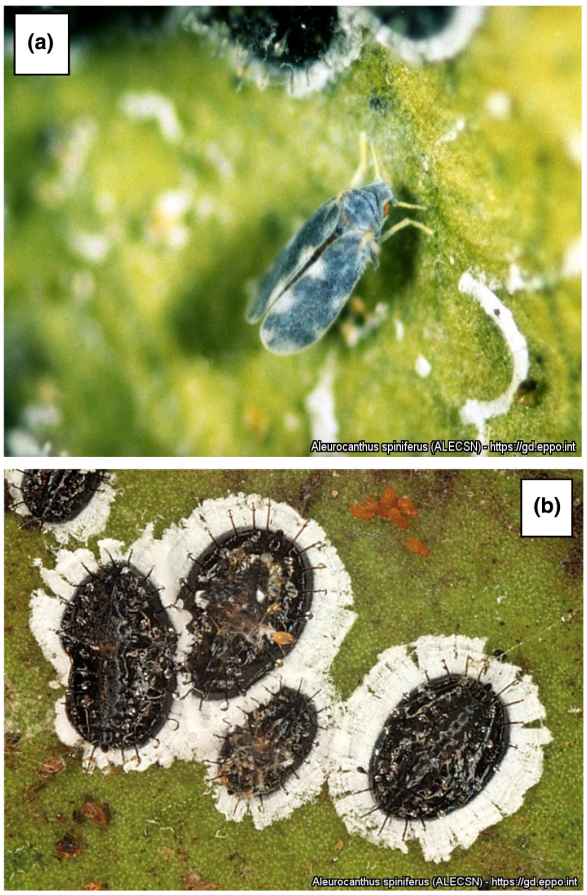


FIGURE A1 (a) Adult and (b) puparia of *A. spiniferus* (Images courtesy of MA van den Berg EPPO Global Database).

Sampling and identification

Collected adults can be transferred to 70% ethanol before being sent to the laboratory for identification. Puparia can be sent alive with host plant material in an air-tight and secure container, except if the pest risk is high. In that case, specimens can be sent with the host plant material in 70% ethanol. Although adult Aleyrodidae may have some identification characters to distinguish them from related species, taxonomy is based on the empty pupal cases and their derm (external surface) morphology. These characters can be adequately seen by microscope study of carefully processed slide-mounted specimens. Identification can be performed according to EPPO Standards EPPO (2022a) PM 7/7(2) *Aleurocanthus citriperdus*, *Aleurocanthus spiniferus* and *Aleurocanthus woglumi*.

Anoplophora chinensis (EPPO A2 List)

Symptom description

EPPO (2013) details plants for planting with a stem or root collar diameter >1 cm can be susceptible to *A. chinensis*.

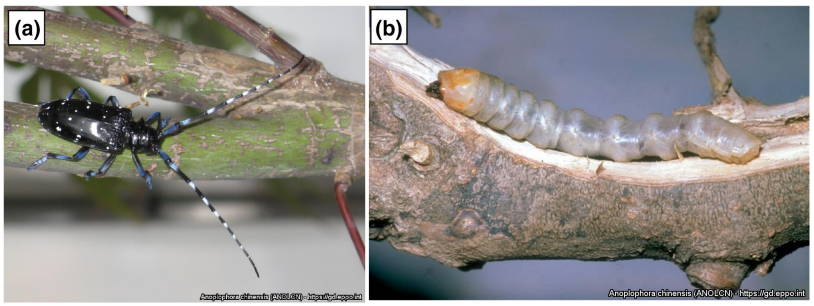


FIGURE A2 (a) Adult *Anoplophora chinensis* (Image courtesy of Wietse den Hartog EPPO Global Database) and (b) larva (Image courtesy of Plant Protection Service, Wageningen, NL EPPO Global Database).

Single eggs are deposited in small 3–4-mm incisions made in the bark just below the surface. Such incisions usually range from the soil surface level to approximately 60 cm above the ground. These oviposition sites are difficult to observe in the field, but in the case of young plants it can cause a swelling of the stem, which can be visible. Signs of chewing by females when they create egg niches may also be visible (EPPO, 2013). The most obvious symptoms of *A. chinensis* damage are adult exit holes which are typically 6–11 mm in diameter and are generally found towards the base of trunks and exposed roots. These holes are circular and on smooth barked trees resemble drilled holes. Symptoms at the crown level (decline or decay of infested trees) can also be observed. August to September is the best period for the observation of symptoms.

Sampling and identification

Adult beetles are large and black with variable white markings (Figure A2a). Their antennae, which are longer than their bodies (between 1.2 and 2 times the body length) are particularly distinctive and are black with white/light blue bands. The adults can be trapped on pheromone traps although traps are not as efficient for *Anoplophora* as they are for some other pests such as Lepidoptera. To take samples of juvenile stages it may be necessary to cut down and split open potentially infested trees. Pulling up the stump of the tree and uprooting major roots can help to detect the pest. The eggs are about 5–7 mm in length, off-white, oblong. Just before hatching, they turn yellowish-brown. The larva are legless grubs up to 50 mm long when fully grown. They are creamy white, with a chitinized brown mark on the prothorax (Figure A2b). Collected adults can be transferred to 70% ethanol before being sent to the laboratory for identification. Larvae can be sent alive with host plant material in airtight and secure containers, or alternatively specimens can be sent in 70% ethanol.

Aonidiella citrina (Jordan A1; Turkey A2; Israel, Morocco, Tunisia Quarantine pest)

Symptom description

Aonidiella citrina usually attacks the leaves and fruit but rarely the bark. Heavy infestations may result in leaf drop, dieback of apical twigs and discoloured, stunted and pitted fruits which fall prematurely or are unmarketable. The small size, pale colour and sessile nature of *A. citrina* (Figure A3) makes it difficult to detect unless present in large numbers. *A. citrina* can easily be confused with *A. aurantii*, which is one of the most commonly intercepted scale insects on imported citrus fruit.

Sampling and identification

Authoritative identification requires detailed microscopic examination of teneral adult females by a scale insect specialist. *A. citrina* is one of a group of six morphologically similar species composed of: *A. aurantii*, *A. comperei*, *A. eremocitri*, *A. inornata* and *A. taxus*. It is closest in appearance to *A. aurantii* and should be distinguished from this species. Adult female *A. aurantii* usually have distinct prevulvar scleroses behind the apophyses which are absent in *A. citrina*. Morphological descriptions, illustrations and keys are provided by Ferris (1938), McKenzie (1937) and Quayle (1938) and, more recently, Longo et al. (1994).

Aphis citricidus (EPPO A2 List)

Symptom description

When infested with *Aphis citricidus*, growth of citrus shoots is greatly impaired, and they become distorted;

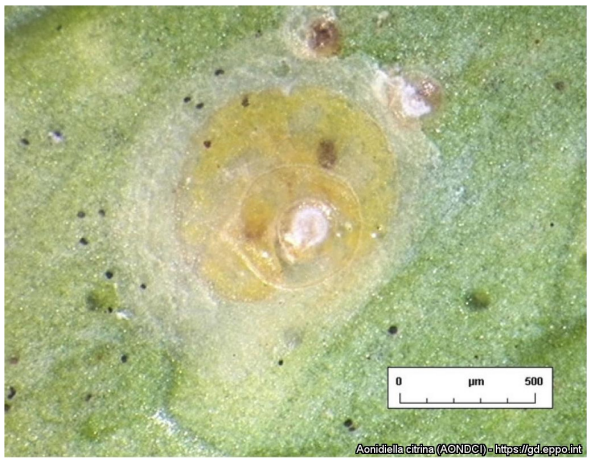


FIGURE A3 *Aonidiella citrina* (habitus; Image courtesy of Jean-François Germain, EPPO Global Database).

leaves become brittle and wrinkled and curl downwards. Attacked flowers fail to open or do so abortively since the ovaries are deformed.

Sampling and identification

Yellow water traps can be used to detect alate viviparous females at the beginning of the vegetative development season. Plants that are transported should be carefully checked whatever their stage of development. Foliage should also be examined for dead, parasitized aphids or mummies, which adhere to the leaves and can be used for identification in the absence of living specimens.

Specimens should be preserved in ethanol. It is adequate to use 70% ethanol to transport the specimens to the laboratory and observe them with the stereomicroscope, to make microscopic preparations, to store them or send them to a specialist. For mitochondrial COII analysis, specimens should be preserved in 96%–99% ethanol. Guidance on the morphological identification of *A. citricidus* can be found in the EPPO diagnostic protocol *PM 7/75 Toxoptera citricidus* (EPPO, 2006a).

Diaphorina citri (EPPO A1 List)

Symptom description

Diaphorina citri is the main vector of '*Candidatus* Liberibacter asiaticus'. It is also a vector of '*Candidatus* Liberibacter americanus' and at least experimentally of '*Candidatus* Liberibacter africanus'. Adults are 2.5 mm long with a yellowish-brown body and greyish-brown legs (Figure A4). Wings are transparent with white spots or light brown with a broad, beige, longitudinal band in the centre. Adults are very active and jump at the slightest disturbance. Symptoms mainly consist of deformations of the shoot and yellowing of leaves and tender shoots. The presence *D. citri* is located mainly on the bottom or underside of the leaves, although with large infestations they also reach the stem. The installation of adhesive chromotropic traps can be useful for inspection, as it allows the capture of adults though it is not compatible with morphological identification. The optimal time for the detection of *D. citri* in most citrus species is the sprouting period, which occurs in spring, late summer and autumn. The lemon tree is a more sensitive species, since its sprouting is continuous throughout the year and consequently examinations can be carried out during the whole year.

Sampling and identification

Adults can be collected in 70% ethanol and sent for identification in a hermetic and solid tube or container in 70% ethanol. Identification involves detailed microscopic examination of adults or last larval instars according to EPPO Standard PM 7/52 *Diaphorina citri* (EPPO, 2005a).

Eutetranychus orientalis (EPPO A2 List)

Symptom description

Mites commence feeding on the upper side of the leaf along the midrib and then spread to the lateral veins, causing the leaves to become chlorotic. Pale-yellow streaks develop along the midrib and veins. Little webbing is produced. In heavier infestations, the mites feed and oviposit over the whole upper surface of the leaf. Very heavy infestations on citrus cause leaf fall and dieback of branches which may result in defoliated trees.

Sampling and identification

Plant material suspected to be infested with *E. orientalis* can be sent to the laboratory in an air-tight and secure container. Identification requires examination of cleared and mounted female specimens by transmitted light microscopy. Diagnostic descriptions are given by Jeppson et al. (1975) and Smith-Meyer (1987).

Euwallacea fornicatus sensu lato (EPPO A2 List)

Symptom description

Euwallacea fornicatus sensu lato is a complex of Ambrosia beetles which associate with symbiotic fungi (EPPO, 2015a). *Citrus limon* and *C. sinensis* are reported as hosts. Each host tree shows different symptoms, mostly depending on the response to the fungus infection. Attack symptoms including staining, sugary exudate, gumming and/or frass may be noticeable before the tiny beetles are observed. Beneath or near these symptoms, the beetle's entry/exit holes may also be seen.

FIGURE A4 (a) *Diaphorina citri* adult and (b) Nymphs (Images courtesy of David G Hall, EPPO Global Database).

The abdomen of the female beetle can sometimes be seen sticking out of the hole (EPPO, 2015a).

Sampling and identification

Adult females are 1.83±0.07 mm × 0.80±0.6 mm. Males are smaller. Eggs are approximately 0.3 mm long. If adults cannot be found it may be necessary to destructively sample host trees by removing bark or cutting through the trunk to reveal galleries and larvae. Samples should be submitted to a laboratory in dry, sealed plastic containers. Collected adults can be transferred to 70% ethanol before being sent to the laboratory for identification. Pupae can be sent alive with host plant material in airtight and secure container except if the pest risk is high. In that case, specimens can be sent with the host plant material in 70% ethanol.

Lopholeucaspis japonica (EPPO A2 List)

Symptom description

Attacks by *L. japonica* result in dieback and premature leaf fall, due to senescence of all infested branches (Figure A5). In the case of light attacks, the scales may be found in cracks in the bark, and are then difficult to detect on superficial examination. The shield of the female is narrow, elongated (1–1.8 mm long), straight or slightly curved and dark. Inspections of *L. japonica* should be performed from late May to early August.

Sampling and identification

The taxonomy of the Coccoidea is almost entirely based on characters of the adult female and a good slide preparation of a teneral female is required for identification to species level. The EPPO diagnostic protocol PM 7/54 *Lopholeucaspis japonica* (EPPO, 2005b) provides a morphological description of the species. Collected specimens can be transferred to 70% ethanol before being sent to the laboratory for identification. Alternatively, sections of infested bark can be sent to the laboratory in sealed containers.

FIGURE A5 *Lopholeucaspis japonica* adults (Image courtesy of Central Science Laboratory, York, (GB), EPPO Global Database).

Naupactus xanthographus (EPPO A1 List)

Symptom description

Above-ground foliage can show feeding damage where the adults feed from the margins of the leaves and create semicircular indentations (EPPO, 2020b). Feeding damage (grooves) caused by larvae may be visible on the thicker roots along with an absence of small roots if the host plant is uprooted. Feeding on the roots may also cause symptoms above ground, i.e. reduced foliage and growth. Plants appear weak and show chlorosis because of the reduced water and nutrient uptake and potassium deficiency.

Sampling and identification

Adults are brown or grey-brown with white or white/yellow stripes on pronotum and elytra (Figure A6a,b). They can be dark brown when they are old. Adults show a high level of sexual dimorphism with female body length 12–16 mm and males being smaller, 11–13 mm in length. Female rostrum is 1–1.5 times as long as wide at the apex. Males are also slenderer than females, with their rostrum being 1.25 times as long as wide. Eggs are oval (ellipsoidal and bluntly rounded at the ends) and yellow and between 1 and 1.2 mm in length (Luppichini et al., 2013). Eggs are often arranged in clusters of 25–45 eggs which adhere to each other with a sticky residue (sticky at egg laying). Collected specimens can be transferred to 70% ethanol before being sent to the

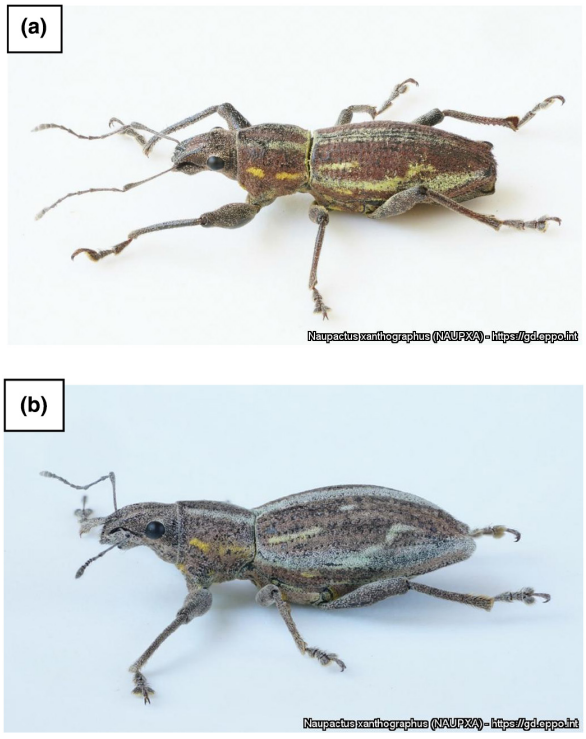


FIGURE A6 (a) Male and (b) female adult *Naupactus xanthographus* (Image courtesy of Renato Ripa (BIOCEA Ltd, EPPO Global Database)).

FIGURE A7 *Pezothrips kellyanus* adults. (Image courtesy of Ferran Garcia-Mari, EPPO Global Database).

laboratory for identification. Alternatively, infested plant material can be sent to the laboratory in sealed containers.

***Pezothrips kellyanus* (Morocco, Tunisia Quarantine pest)**

Symptom description

Heavy infestations can lead to complete scarring of the fruit. Damage to mature fruit is less common but usually more severe, initially showing silvering which leads to thin scarring over most of the fruit surface. Heavy scarring can render fruits unmarketable (EPPO, 2006b). It was observed that fruits were most susceptible to thrips infestations in the period shortly after petal-fall. In Sicilia, Italy *P. kellyanus* is considered as a key pest in citrus orchards.

Sampling and identification

Adults are black and 1.6–2.2 mm long (Figure A7). Life cycle consists of egg 2 larval stages, pre-pupae, pupae and adults. *P. kellyanus* feeds on young tissues (flowers and fruits), particularly near the calyx, producing a circular stem-end scar. Collected specimens can be transferred to 10% ethanol with 0.1% teepol before being sent to the laboratory for identification. Alternatively, infested plant material can be sent to the laboratory in sealed containers.

***Phyllocnistis citrella* (Azerbaijan, Georgia, Uzbekistan A2; Belarus Quarantine pest)**

Symptom description

The small larvae enter the leaf tissue and feed within causing silvery mines that are visible on the leaf (Figure A8). Leaves can become distorted and crinkled and damage can lead to defoliation (Figure A9). A dark thin streak or dotted line that corresponds to the larval frass is visible inside the tunnels and is more conspicuous on the underside of the leaf.

FIGURE A8 Leaf mines caused by *Phyllocnistis citrella* (Image courtesy of Miguel Ángel Fernández, Plant Health Service. Autonomous Community Region of Murcia-Spain).

FIGURE A9 Example of leaf mines in lemon tree caused by *Phyllocnistis citrella* (Image courtesy of Miguel Ángel Fernández, Plant Health Service. Autonomous Community Region of Murcia-Spain).

FIGURE A10 Adult of *Phyllocnistis citrella* captured in a trap (Image courtesy of Miguel Ángel Fernández, Plant Health Service. Autonomous Community Region of Murcia-Spain).

Sampling and identification

P. citrella is a very small, whitish moth, only 2mm in length when at rest (Figure A10). Its wingspan is 4mm.

Markings comprise black and brown lines with an apical black spot, placed so that when at rest it resembles a small insect facing in the opposite direction. The antennae are three-quarters the length of the wing. *P. citrella* larvae are 3mm long when fully grown. They are translucent greenish-yellow. The fifth-instar larva and prepupa have been described by Heppner (1993). Collected specimens can be transferred to 70% ethanol before being sent to the laboratory for identification. Alternatively, infested plant material can be sent to the laboratory in sealed containers.

Platynota stultana (EPPO A2 List)

Symptom description

Evidence of the larval infestation includes folded or adjacent leaves tied together with silk, often with conspicuous pellets of frass (faeces) in the silk webbing. Feeding typically results in holes in leaves or damage along their outer margins.

Sampling and identification

Adults (Figure A11) can be detected by using pheromone traps and larvae by visual examination of leaves, while nests can be found in flower clusters, as well as on leaves and in shoot tips. Further details are available in the EPPO data sheet on *Platynota stultana* (EPPO, 2023b) and other documents available in the EPPO Global Database https://gd.eppo.int/. Collected specimens can be transferred to 70% ethanol before being sent to the laboratory for identification. Alternatively, infested plant material can be sent to the laboratory in sealed containers.

FIGURE A11 Adult of *Platynota stultana* captured in a trap (Image courtesy of Miguel Ángel Fernández, Plant Health Service. Autonomous Community Region of Murcia-Spain).

Scirtothrips aurantii (EPPO A1 List), *S. citri* (EPPO A1 List), *S. dorsalis* (EPPO A2 List)

Symptom description

EPPO (2005c) detail: All stages of *Scirtothrips aurantii*, *S. citri* and *S. dorsalis* feed on epidermal and sometimes palisade cells of young leaves, and on the apex of young fruits especially when concealed under the calyx. They do not feed on mature leaves. These pests could be carried on plants for planting, in particular seedlings or cuttings with young growing leaf buds, and these should be examined carefully. Only young fruits are attacked, so the risk that this species is carried on harvested fruits is low. Symptoms are: silvering of the leaf surface; linear thickenings of the leaf lamina; brown frass markings on the leaves and fruits; grey to black markings on fruits often forming a conspicuous ring of scarred tissue around the apex; ultimately fruit distortion and early senescence of leaves.

Sampling and identification

Considering the small size of thrips (Figure A12), direct inspection and sampling are difficult. Trapping and/or the electric Berlese method can be used for sampling. Shipment to the laboratory can also be performed in 10% ethanol mixed with 0.1% teepol. If intended for preparing permanent microscope slides, thrips should be preserved in AGA (a mixture of 9 parts 60% ethanol, 1 part glycerine and 1 part acetic acid) and sent to a specialist. Identification of the genus *Scirtothrips* is possible at the larval stage by morphological means.

Cleared adult specimens mounted on microscope slides should be identified according to EPPO Standard PM 7/56 *Scirtothrips aurantii*, *Scirtothrips citri*, *Scirtothrips dorsalis* (EPPO, 2005c).

FIGURE A12 Female *Scirtothrips aurantii* adult (Image courtesy of Pablo Alvarado Aldea, EPPO Global Database).

Tephritidae: *Anastrepha fraterculus* (EPPO A1), *Anastrepha ludens* (EPPO A1 List), *Bactrocera* spp. *Bactrocera minax* (EPPO A1 List), *Bactrocera tsuneonis* (EPPO A1 List), *Bactrocera dorsalis* (EPPO A1 List), *Bactrocera tryoni* (EPPO A1 List), *Bactrocera zonata* (EPPO A2 List), *Ceratitis rosa* (EPPO A1 List)

Symptom description

In fruits, Tephritidae species may be detected as eggs or larvae. Infested fruits can show signs of oviposition punctures and the area around these punctures may get necrosed or discoloured. Rotting of the underlying tissue causes a depression on the surface. Damage may occur inside the fruit before external symptoms are seen, often as networks of tunnels accompanied by rotting. Egg detection is very difficult.

Sampling and identification

Infested fruit or fruits suspected to be infested should be cut in half to collect eggs or larvae. The identification of the larvae to family level should be confirmed using a binocular microscope according to Stehr (1991). Identification of Tephritidae based on examination of the third-instar life stage is not sufficient to complete accurate species identification under all circumstances. It is therefore recommended to perform molecular analysis to complete the identification of a larva when the diagnosis is intended to confirm a new record of pest presence. A reliable morphological identification can only be performed on an adult specimen. Therefore, if time allows, rearing under confined conditions can produce adults for morphological identification. Larvae can be sent alive on the host plants in an airtight, secure container. If collected larvae are to be preserved, they should be killed in boiling water for a few seconds (until they become immobile) and then transferred to 70% ethanol (if a molecular test is to be carried out subsequently, 95%–100% ethanol is recommended). Adults can be killed in 70% ethanol. Alternatively, the emergence cage can be placed in a freezer to kill the adults. Adults can be sent for identification in a hermetic tube or container in 70% ethanol. Placing the adults live in a hermetic tube allows for the colour and pattern of the body and wings to appear, which can aid identification. It is recommended to send several adults for identification, ideally at least one male and one female. The EPPO Standard *PM 3/92 Consignment inspection of fresh fruit and vegetables for fruit flies* (EPPO, 2021a).

***Trioza erytreae* (EPPO A2 List)**

Symptom description

Trioza erytreae is the main vector of '*Candidatus* Liberibacter africanus' and also, at least experimentally, a vector of '*Candidatus* Liberibacter asiaticus'. *Trioza erytreae* can cause severe leaf distortion, curling, stunting, galling and chlorosis (Figure A13; EPPO, 2005d).

FIGURE A13 Symptoms of *Trioza erytreae* on citrus plants (Image courtesy of Lidia Duarte, EPPO Global Database).

FIGURE A14 *Trioza erytreae* nymphs (Image courtesy of HD Catling, EPPO Global Database).

Nymphs produce cup-shaped open galls on the abaxial surface (Figure A14; Van der Merwe, 1941). The leaves may be dusted with faecal pellets which appear like minute white eggs and may also be found on the ground beneath heavily infested trees (Van der Merwe, 1941)

Sampling and identification

Adults are winged, pale and delicate initially, later becoming light brown. Males are smaller than females and have a blunt tip to the abdomen, the latter ending in a sharp point in females. Adults can be killed in 70% ethanol and sent for identification in a hermetic tube or container in 70% ethanol. Identification involves detailed microscopic examination of adults, or fifth-instar immatures, according to EPPO Standard PM 7/ 57 *Trioza erytreae* (EPPO, 2005d).

***Unaspis citri* (Belarus, Israel, Morocco, Tunisia Quarantine pest; Azerbaijan, Georgia, Jordan, Turkey, Uzbekistan, Ukraine A1), *Unaspis yanonensis* (EPPO A1 List)**

Symptom description

Infestations of *U. citri* usually occur on the trunk and main limbs of trees under 10 years old. Heavy infestations spread to the twigs, leaves and fruit. This results in yellow spotting on the undersides of leaves which drop

FIGURE A15 *Unaspis citri* adults (Image courtesy of Central Science Laboratory, York GB, EPPO Global Database).

prematurely, dieback of twigs and weakening and eventual killing of branches (Figure A15). Heavily infested bark becomes dark, dull, hard, appears tight and subsequently splits. Weakened limbs and twigs become infected with fungi and may be subsequently attacked by wood-boring insects. The small size (2–2.5 mm), dark colour (brown or brown- black) and sessile nature of the female scales make them difficult to detect unless present in large numbers. In contrast, white masses of male scales are conspicuous.

Sampling and identification

For identification of the species, the body of adult females should be studied, since there are no adequate keys for the separation of species based on nymphs or adult males. Adult females can be sent alive on the host plants in a hermetic container. If the pest risk is high, specimens should be sent with the host plant material in 70% ethanol. Identification involves detailed microscopic examination of teneral adult females according to EPPO Standard PM 7/38 *Unaspis citri* (EPPO, 2004b). A key to *Unaspis* species has recently been published (Niu & Feng, 2019).

(b) Fungi

***Elsinoë australis* (EU A1 Quarantine pest (ANNEX II A), Israel, Morocco, Tunisia Quarantine pest), *Elsinoë citricola* (EU A1 Quarantine pest (Annex II A)), *Elsinoë fawcettii* (EU A1 Quarantine pest (Annex II A); Israel, Tunisia Quarantine pest)**

Symptom description

Lesions vary with age and host plant species (Figure A16). Similar to symptoms caused by *E. fawcettii*, citrus tissues infected with *E. australis* show erumpent scab pustules (Chung, 2011). In contrast with *E. fawcettii* which affects all parts, *E. australis* affects mostly fruit. Fruits are infected in the early stages of their development, i.e. when not more than 20 mm across. They grow misshapen, becoming scarred and distorted, and are subject to premature

FIGURE A16 *Elsinoë australis* symptoms on citrus leaves (Image courtesy of Elizabeth Asteraki CABI SEARC, CABI Compendium).

fall (CABI, 2020). On the rind of developed fruits, raised lesions are formed with different shapes, sizes and colours according to the species and cultivar affected. They appear as scattered protuberances, conical projections or crater-like outgrowths, or they coalesce to give scabby patches or extensive areas of fine eruptions. Scab lesions do not extend into the albedo. *E. australis* forms larger, smoother, more circular scabs than *E. fawcettii* scabs, which are typically irregular, warty and deeply fissured.

Sampling and identification

Samples of infected material showing typical symptoms should be collected and placed in a labelled plastic bag together with a piece of slightly damp absorbent paper, kept in cool conditions and sent to the diagnostic laboratory as soon as possible. There is no international standard available for the detection and identification of these fungal species on citrus fruit, but a diagnostic protocol has been developed by Ahmed et al. (2018).

***Phyllosticta citricarpa* (EPPO A1 List)**

Symptom description

This fungus produces the disease known as citrus black spot. Fruits are where the symptoms of the black spot can best be observed. It produces five main types of symptoms: hard spot, or shot hole spot, early virulent spot, false melanose or speckled blotch, freckle spot, and virulent spot. Therefore, inspections in citrus production areas are aimed at observing fruits for the detection of this type of symptoms, and mainly at the time of ripening of the fruits and as close as possible to the time of harvest. Attention will be paid to those fruits that are exposed to the sun, as well as old trees, and trees which are abandoned or not subjected to treatments. Late sweet orange and lemon trees will be priorities in these inspections.

FIGURE A17 Symptoms of *Phyllosticta citricarpa* on *Citrus limon* leaves (Vladimiro Guarnaccia, EPPO Global Database).

FIGURE A18 First symptoms in a branch of a lemon tree infested by *Plenodomus tracheiphilus* (Image courtesy of Miguel Ángel Fernández, Plant Health Service. Autonomous Community Region of Murcia-Spain).

FIGURE A19 Lemon tree branches infested by *Plenodomus tracheiphilus* (above) comparison with other young branches free of this fungus (bellow).

FIGURE A20 Cut branches with the typical infection in ring spots (Image courtesy of Miguel Ángel Fernández, Plant Health Service. Autonomous Community Region of Murcia-Spain).

Leaf and twigs symptoms rarely occur on sweet orange, mandarin and other commercial citrus species, but they are frequently present on lemons (Figure A17). They appear as round, small, sunken necrotic lesions with a yellow halo (Kotzé, 1981). If symptoms appear on the leaves, they begin as visible spots on both sides, which can increase in size up to 3 mm in diameter. These spots are circular, with the centre grey or light brown surrounded by a dark brown or black border and a yellow halo. Sometimes there may be pycnidia in the centre of the lesions in the leaf bundle. Leaf-like lesions may also appear on small branches, more often in *C. limon* than in other citrus species. The symptoms caused by *P. citricarpa* can easily be confused with lesions caused by other diseases such as Alternaria brown spot (*Alternaria* spp.) or anthracnose (*Colletotrichum* spp.), as well as physical, mechanical or insect damage. It is recommended to carry out the inspection at the time of harvest.

Sampling and identification

Infected plant material should be collected, packaged and sent to the laboratory as described for *Elsinoë* species above. Diagnosis should be performed by laboratory testing according to EPPO (2020c) Standard PM 7/017(3) *Phyllosticta citricarpa* (formerly *Guignardia citricarpa*).

Plenodomus tracheiphilus (EPPO A2 List)

Symptom description

The first symptoms appear as leaf and shoot chlorosis followed by a dieback of twigs and branches (Figure A18). On the affected twigs, raised black points within lead-grey or ash-grey areas of withered twigs indicate the presence of pycnidia. The growth of sprouts from the base of the affected branches and suckers from the rootstock are a very common response of the host to the disease. Gradually the pathogen affects the entire tree which eventually dies. On cutting into the infected twigs, the characteristic salmon-pink or orange-reddish discoloration of the wood can be seen; this internal symptom is associated with gum production within the xylem vessels

(Figures A19 and A20). Finally, part of the old wood in the branches gets a darker colour (brown) in a ring shape. *Citrus limon* is considered the most susceptible species.

Sampling and identification

In the field, samples (twigs and leaves) can be taken at any time of the year. If nursery plants are grafted on a susceptible rootstock, such as sour orange, the rootstock should also be inspected and tested. Infected plant material should be packaged and sent to the laboratory as described for *Elsinoë* species above. Diagnosis should be performed by laboratory testing according to EPPO Standard PM 7/048 *Plenodomus tracheiphilus* (formerly *Phoma tracheiphila*; EPPO, 2015b).

***Pseudocercospora angolensis* (EPPO A1 List)**

Symptom description

Leaf symptoms initially appear as greenish-yellow patches. At maturity the leaf spots are amphigenous, mainly hypophyllous, 4–10 mm or more in diameter, pale-brown to brown or blackish-brown when sporulation is dense, surrounded by a dark-brown margin and a yellow halo, the centre often becoming detached resulting in a shot-hole spot. Generalized foliar necrosis, caused by coalescence of several lesions, can result in defoliation. During wet weather the lesions sporulate and become black. On young fruits, brown necrotic lesions form. These are usually circular, slightly sunken, with a surrounding ring of raised epicarp, giving the fruit a blistered appearance. During wet weather, the lesions sporulate and become black. In young fruits, a generalized necrosis sometimes forms, resulting in premature abscission of the fruit.

Sampling and identification

Infected plant material should be collected, packaged and sent to the laboratory as described for *Elsinoë* species above. There is no international standard available for the detection and identification of this fungus on citrus fruit, but a diagnostic protocol has been developed by Ahmed et al. (2018).

(c) Bacteria (including phytoplasmas)

***'Candidatus* Liberibacter africanus', '*Candidatus* Liberibacter americanus', '*Candidatus* Liberibacter asiaticus' (EPPO A1 List)**

Symptom description

Huanglongbing symptoms are the same for each '*Candidatus* Liberibacter' species and can eventually be observed in most citrus species (Figure A21). Early stages of infection can be identified by the presence of one or several yellow shoots in a tree. On some trees, only one yellow shoot is present, and with time this affected shoot grows into a larger yellow branch. Many leaves show

FIGURE A21 '*Candidatus* Liberibacter asiaticus' symptoms on citrus leaves (Image courtesy of JM Bové, EPPO Global Database).

what is called a 'blotchy mottle' that is not symmetrical on both sides of the leaves. These are the most characteristic symptoms of Huanglongbing wherever the disease occurs and whatever the citrus species affected. This asymmetry distinguishes blotchy mottle from mineral deficiency symptoms that are symmetric. The whole leaf blade may ultimately turn uniformly yellow. The leaves may also become thicker, and leathery, with midribs and lateral veins sometimes enlarged, swollen and corky. In late stages of the disease, the yellow branches take over the canopy of the whole tree, which becomes totally infected. New shoots may show secondary 'rabbit's ear' symptoms (Gomez, 2009). Eventually, defoliation and dieback occur. Green islands on otherwise yellowed leaves have been occasionally observed on sweet orange (Gomez, 2009).

Sampling and identification

Infected plant material should be collected, packaged and sent to the laboratory as described for *Elsinoë* species above. The disease may be latent without any symptom expression for a long period (6 months to 2 years or longer according to the literature; Lee et al., 2015). Diagnosis should be performed by laboratory testing according to EPPO (2021b) Standard PM 7/121(2) '*Candidatus* Liberibacter africanus', '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus'.

***Spiroplasma citri* (EU RNQP; Turkey A2)**

Symptom description

Affected trees are more or less stunted. Leaves are shorter and broader ('little leaf'), cupped, abnormally upright, sometimes mottled or chlorotic. Under very hot conditions, leaves on some shoots may have misshapen, blunted or heart-shaped yellow tips (a highly diagnostic character). Shoots may be abnormally bunched, and development of multiple axillary buds may give rise to witches' brooms. Fruiting tends to be suppressed in infected plants. Fruits may be stunted,

lopsided or acorn shaped (i.e. with thick rind at the base and thin rind at the tip), and may show colour inversion (peduncular end discolours while stylar end remains green). For more details, see Bové et al. (1984) and Bové (1988). The vectors, as such, cause no particular symptoms.

Sampling and identification

Spiroplasma citri can be detected by graft inoculation of indicator plants, of which the most suitable is sweet orange cv. Madame Vinous, kept at 32°C in the day and 27°C at night (Bové, 1988). Other indicators are grapefruit cv. Marsh and Citrus × tangelo cv. Sexton. The best inoculum is young leaf patches including midrib. *S. citri* can fairly reliably be cultured from trees showing symptoms, the best material to use being seeds with various degrees of abortion, the peduncular end of the fruit axis, or mottled summer leaves collected in October (Bové et al., 1984). Since the organism can be cultured, antisera are relatively easy to obtain, and ELISA can be used for detection and/or identification of *S. citri* in extracts from infected plants and insects (Clark et al., 1989; Saillard & Bové, 1983). The latex agglutination technique is also suitable for rapid detection (Fletcher & Slack, 1986). cDNA probes are under development, and are potentially much more sensitive than ELISA (Bové, 1986). Dale (1988) has described a rapid DAPI staining technique applied to crushed leaf midribs, which detects *S. citri* and distinguishes it from phytoplasmas.

Xanthomonas citri pv. *aurantifolii* (EPPO A1 List), *Xanthomonas citri* pv. *citri* (EPPO A1 List)

Symptom description

Lesions on fruits can appear even when they are still small and green. Canker lesions begin as pinpoint oily spots due to water-soaking of the tissue before becoming small, slightly raised pustules or blister-like eruptions. As lesions develop, they increase in size and the epidermis ruptures and the lesions become erumpent, spongy or corky. The pustules then darken and thicken into light tan-brown corky lesions, which are rough to the touch. Eventually, their centre becomes crater-like. On fruits, these lesions tend to have elevated margins and a sunken centre. These craters do not penetrate deep into the rind. Diagnostic symptoms are tissue hyperplasia resulting in cankers with water-soaked margins. Yellow chlorotic halos surrounding the lesions may or may not be present. Canker lesions vary in maximum size from 5 to 10 mm, depending on the susceptibility of the host plant. Young leaves are more susceptible. Trees that present symptoms of the presence of the *Phyllocnistis citrella* are more susceptible to the presence of these bacteria, since this favours their entry. The inspection should preferably be carried out from late summer and early winter.

Sampling and identification

Infected plant material should be sent to the laboratory in a plastic airtight bag. Laboratory testing should be performed according to EPPO Standard PM 7/44 *Xanthomonas axonopodis* subsp. *citri* (EPPO, 2005e) or to ISPM 27, DP 6 *Xanthomonas citri* subsp. *citri*.

Xylella fastidiosa (EPPO A2 List)

Symptom description

The first symptoms of citrus variegated chlorosis (caused by *Xylella fastidiosa*) to appear on leaves are small chlorotic spots on the upper surface that correspond to small gummy brown spots on the underside of the leaf. Symptoms are most obvious on developed leaves independent of plant age and mainly on sweet orange cultivars.

Affected trees show foliar interveinal chlorosis on the upper surface, resembling zinc deficiency (Figures A22 and A23). Sectoring of symptoms can occur in some parts of the canopy on newly infected trees. However, citrus variegated chlorosis generally

FIGURE A22 Citrus variegated chlorosis: typical spots caused on sweet orange (*Citrus* sp.) leaves (Image courtesy of M. Scortichini, Istituto Sperimentale per la Frutticoltura, Rome (IT) EPPO Global Database).

FIGURE A23 Small, raised lesions on the underside of a *Citrus* sp. leaf caused by *X. fastidiosa* infection (Image courtesy USDA).

develops throughout the entire canopy on old infected trees. Affected trees are stunted and the canopy has a thin appearance because of defoliation and dieback of twigs and branches. Blossom and fruit set occur at the same time on healthy and affected trees, but normal fruit thinning does not occur on affected trees and the fruits remain small, have a hard rind and ripen earlier. The plants do not usually die, but the yield and quality of the fruit are severely reduced (Donadio & Moreira, 1998). On affected trees of cv. Pera and other sweet orange cultivars, fruits often occur in clusters of 4–10, resembling clusters of grapes. The growth rate of affected trees is greatly reduced, and twigs and branches may wilt. Trees in nurseries can show symptoms of variegated chlorosis, as do trees older than 10 years. Young trees (1–3 years) become systemically colonized by *X. fastidiosa* faster than older trees. Trees older than 8–10 years are usually not totally affected, but rather have symptoms on the extremities of branches. The infraorder Cicadomorpha (vectors) comprises three superfamilies: Cicadoidea (cicadas), Cercopoidea (cercopids) and Membracoidea (leafhoppers), and have the characteristic that they are phytophagous. In case of suspicion of the presence of any species of Cicadomorpha, an inspection will be made for the collection of these insects by the netting or sweeping system on the treetops or the adjacent vegetation cover.

Sampling and identification

The sample should consist of branches/cuttings representative of the symptoms seen on the plant(s) and containing at least 10–25 leaves depending on leaf size. Symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected from several plants showing similar symptoms. For testing individual asymptomatic plants, the number of branches to be collected is at least 4–10 depending on the host and plant size. Samples should be sent to the laboratory placed in closed containers along with an absorbent component (e.g. plastic sealable bags, etc; EPPO, 2019, 2022b).

(d) Viruses and viroids

Citrus bark cracking viroid (CBCVd; EPPO A1 List)

Symptom description

On citrus plants bark cracking, and green streaks on the cambial face of the bark might be observed on trifoliate orange rootstock and on hybrid rootstock citrange (Figure A24a,b; Murcia et al., 2015). Since similar symptoms to CBCVd infections may be induced by other biotic and abiotic factors, detection and identification requires testing. Because of the incubation period, asymptomatic testing is also crucial for early detection in plants for planting material.

Sampling and identification

For CBCVd detection, different methods have been developed including biological indexing (Duran-Vila & Semancik, 2003), molecular hybridisation (Malfitano et al., 2005; Murcia et al., 2009), polyacrylamide gel electrophoresis (PAGE; Duran-Vila et al., 1988), PCR methods (RT-PCR, RT-real-time PCR; Bernad & Duran-Vila, 2006; Wang et al., 2009, 2013) and next generation sequencing (NGS; Al Rwahnih et al., 2018; Jakše et al., 2015).

Citrus blight agent (EPPO A1 List)

Symptom description

The disease is more noticeable in groves receiving above-average care (high-standard cultural practices). The first

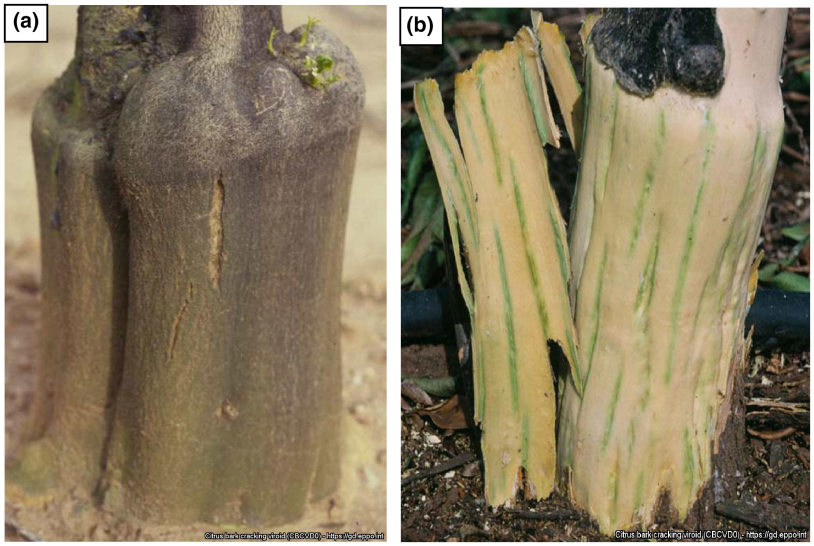


FIGURE A24 (a) CBCVd symptoms on citrus bark cracking on trifoliate orange rootstock and (b) green streaks on the cambial face of the bark of trifoliate orange rootstock (Images courtesy of Nuria Duran-Vila, EPPO Global Database).

FIGURE A25 Zinc deficiency associated with citrus blight (Images courtesy of SM Garnsey, EPPO Global Database).

symptoms appear only on adult trees entering their 4–6th year. The disease generally affects only bearing trees. Once a tree is affected, it does not recover. Blight causes a general decline of the tree canopy with wilt, leaf loss, twig dieback, and poor growth flushes. Symptoms may be confined to one sector of the canopy. While leaves often show zinc deficiency symptoms, zinc accumulates in the bark and outer xylem of the trunk, usually prior to the formation of plugs or to visible symptom development (Figure A25; Albrigo & Young, 1981; Young et al., 1980).

Sampling and identification

No procedures are available for small trees. Blight can be diagnosed by water injection and zinc analysis in field trees (Cohen & Wutscher, 1979). Root grafting could theoretically be used for diagnosis, but the incubation period is long (18–24 months). Once trees begin to be affected, they decline rapidly. The long delay is a serious drawback for use of grafting as a test method. There is an imperative need to develop a more rapid method. Bausher and Sweeney (1991) report use of an antiserum to leaves from blighted citrus as a means of immunological detection of blight; it remains to be proved, however, that the proteins detected are specific to blight.

Citrus concave gum agent (Jordan, A1)

Symptom description

Citrus concave gum agent can be severe to young trees. Leaves can show flecking symptoms which are generally observed in the spring and fall flushes of growth, when outside temperatures are still relatively mild. Symptoms on the young leaves gradually disappear as the leaves mature. Symptoms are seen most readily when the leaf is shaded from the direct sun and viewed against the light of the sky.

Sampling and identification

Disease verification is undertaken by laboratory-based testing techniques. Minutolo et al. (2021) detail molecular identification methods.

Citrus dwarfing viroid (CDVd; Jordan, A1)

Symptom description

Infection of *Citrus sinensis* propagated on *Citrus trifoliata* rootstock has been reported to reduce canopy volume by 50% and apical growth of individual shoots can be reduced by 20% (Dang et al., 2021).

Sampling and identification

Disease verification is undertaken using both biological and laboratory-based testing techniques. Molecular techniques are detailed in Dang et al. (2021).

Citrus exocortis viroid (CEVd; EU RNQP; Jordan, United Kingdom A1)

Symptom description

Trees grown on *P. trifoliata* are the most affected. Symptoms of bark scaling and severe stunting develop when the trees are around 4 years old. When bark scaling occurs, it appears as cracking and peeling of the bark below the bud union. On other sensitive rootstocks, symptoms include tree stunting, yellowing of the canopy and general tree decline and occasional flaking of the bark of the rootstock (NSW DPI, 2008).

Sampling and identification

Disease verification is undertaken using both biological and laboratory based testing techniques. Biological testing involves inserting the budwood from potentially infected trees into an indicator plant ('etrog' citron) grown under high temperatures in a greenhouse. if the budwood is infected with CEVd, the 'etrog' citron will develop symptoms of severe downward leaf curling and stunting and vein browning. Laboratory based testing involves extraction of the viroid from potentially infected plant tissue. this extract is analysed through either gel separation techniques (sPAGE) or amplification of the viroid using molecular methods and then analysis of this product to identify the genetic sequence of the viroid.

Citrus leprosis virus (EPPO A1 List)

Symptom description

All of the viruses causing leprosis disease (Citrus leprosis virus C (CiLV-C), Citrus leprosis virus C2 (CiLV-C2), Hibiscus green spot virus 2 (HGSV-2), the citrus strain of Orchid fleck virus (OFV) and Citrus leprosis virus N (CiLV-N)) produce symptoms including round to elliptical local lesions on fruits, leaves and twigs, the severity of which varies with the type of citrus and the region of origin. Leaf symptoms are usually roundish with a dark-brown central spot about 2–3 mm in diameter, surrounded by a chlorotic halo, in which 1–3 brownish rings frequently appear surrounding the central spot; the overall lesion size varies from 10 to 20 mm, though larger lesions may form

by the fusion of 2 or more adjacent lesions. Occasionally in this type of necrosis they can produce some type of exudation. In green fruits, the affected tissue initially turns yellowish, although over time it becomes dark brown, sometimes depressed, reducing the commercial value of the fruits. On twigs the lesions are protruding, grey, brown or dark red. These can also fuse which can cause the death of the twig. In extreme cases, citrus leprosis can cause severe defoliation and fruit fall.

Citrus leprosis disease is associated with the infestation of false spider mites, species of the genus *Brevipalpus* (Tenuipalpidae) such as: *Brevipalpus inornatus*, *B. obovatus*, *B. californicus* and *B. phoenicis*. Some of these species are widely distributed throughout the EPPO Region. Therefore, especially in leaves, branches and fruits, inspection should look for the presence of these false spider mites that can act as vectors of the virus.

Sampling and identification

For all five viruses, full genomic sequences are available that can be used to develop detection tests (EFSA, 2017).

Citrus tristeza virus (CTV; EPPO A1 List)

Symptom description

CTV infections can induce quick decline, seedling yellows and stem pitting symptoms (Roistacher, 2006). Quick decline is a scion-rootstock incompatibility reaction caused by CTV on trees which have been graft-propagated on sour orange (*C. aurantium*) rootstock (Moreno & Garnsey, 2010). The canopy of infected trees suddenly becomes stunted, wilted, defoliated and dies. In some cases of latent infections, symptoms of inverse pitting or 'honeycombing' could be observed in the inner scion-rootstock interface (Figure A26). Stem pitting is usually associated with severe infections which affect the main trunk, the small branches and the twigs of grapefruit and sweet orange (*C. paradisi* and *C. sinensis*) regardless to the rootstock, by inducing deep pits in the wood under enlarged cheesy bark, accompanied by a general growth cessation of the trees and easy breakage of the twigs (Rocha-Peña et al., 1995; Roistacher, 1991). Seedling yellows is mostly observed in sour orange, lemon, and grapefruit (*C. aurantium*, *C. limon* and *C. paradisi* respectively) seedlings; it is characterized by general stunting, production of small and pale leaves, reduced root system, and sometimes complete cessation of plant growth (Figure A27; Roistacher, 2006).

FIGURE A26 Bud-union of sweet orange CTV-infected tree grafted on sour orange rootstock, and pin holing or honeycombing in the inner face of the bark of the sour orange rootstock below the bud union of the tristeza-infected tree (Images courtesy of L. Navarro and P. Moreno, EPPO Global Database).

FIGURE A27 Mexican lime seedlings (*Citrus aurantiifolia*). Left: healthy; right: inoculated with CTV (Images courtesy of L. Navarro EPPO Global Database).

Sampling and identification

Mexican lime (*C. aurantiifolia*) is still the appropriate universal plant indicator for detecting and distinguishing most of the CTV isolates; whereby, graft inoculation of the virus commonly elicits typical vein clearing and leaf cupping on the new flushes under relatively cool conditions (24–27°C day/18–21°C night). In the presence of severe CTV infections, this woody indicator can also display vein corking. See EPPO diagnostic protocol PM 7/31 Citrus tristeza closterovirus (EPPO, 2004c).

Citrus yellow mosaic virus (CiYMV; EPPO A1 List)

Symptom description

As originally described, citrus mosaic in India involved stunting, chlorosis and uniformly distributed leaf mosaic, followed by mature leaves developing a leathery texture. The characteristic symptoms due to CiYMV in field-infected sweet orange and pummelo are bright-yellow mottling of the leaves and yellow flecking along the veins (Ahlawat et al., 1996). Rather more variable symptoms develop on graft-inoculated *Citrus* spp. in the glasshouse. It is possible that some of the field symptoms earlier described could be due to other causes, or to mixed infections with other viruses (which are quite common in orchard trees in India).

Sampling and identification

CiYMV has been tested by grafting on mandarins cv. Darjeeling orange. More recently, graft transmission to pummelo has been recommended. Mechanical inoculation to pummelo may also provide a test. PCR methods have mainly been used up until now to compare CiYMV with other badnaviruses.

Citrus variegation virus (CVV; EU RNQP; Jordan A1; Switzerland, United Kingdom RNQP)

Symptom description

Citrus medica develops chlorotic leaf symptoms and distortion which persist on the mature foliage. Infected trees may be stunted (EPPO, 1998).

Sampling and identification

EPPO (1998) states: 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. It can also be detected by serological tests (ELISA is preferred).

Citrus vein enation virus (CVEV; Jordan A1; Türkiye A2; Morocco, Tunisia Quarantine pest)

Symptom description

CVEV is transmitted by the aphid vector, *Aphis citricidus*, and is also transmitted by other aphids (*Myzus persicae* and *Aphis gossypii*). CVEV is symptomless in most commercial cultivars. The symptoms for which the disease is named (vein enation, woody gall) are those seen on woody indicators: enations (up to 1 mm) on the leaf veins, cauliflower-like swellings or galls on the bark of the stems, especially associated with thorns or wounds (Garnsey, 1988). Trees grafted on a susceptible rootstock (e.g. rough lemon) may show galling at the graft union.

Sampling and identification

CVEV can be detected by grafting bark chips of suspect material on woody indicators. Enations are seen in 5–8 weeks on the underside of leaves of limes (*Citrus aurantiifolia*), rough lemons (*Citrus jambhiri*) or sour oranges (*Citrus aurantium*). Swellings or galls appear more slowly on stems of *C. jambhiri* or *C. volkameriana*. The virus is not apparently mechanically transmissible, nor have serological methods for its detection been developed.

Ophiovirus citri (CPsV; Jordan A1; Turkey A2; Tunisia Quarantine pest)

Symptom description

CPsV symptoms appear (Frison & Taher, 1991) as large, irregular blotches, or ringspots, on mature

FIGURE A28 Mature leaf symptoms of a Florida isolate of *Ophiovirus citri* (EPPO Global Database).

FIGURE A29 CPsV infected *Citrus sinensis* tree (Images courtesy of SM Garnsey, EPPO Global Database).

leaves (Figure A28), which are frequently gum-impregnated. Some cultivars show shoot necrosis and bark scaling (Figure A29). Fruits may also show ringspot symptoms.

Sampling and identification

CPsV gives shock symptoms when material is grafted on grapefruit or sweet orange. Local lesions are obtained on mechanically inoculated *Chenopodium quinoa*.

Satsuma dwarf virus (SDV; EPPO A1 List)

Symptom description

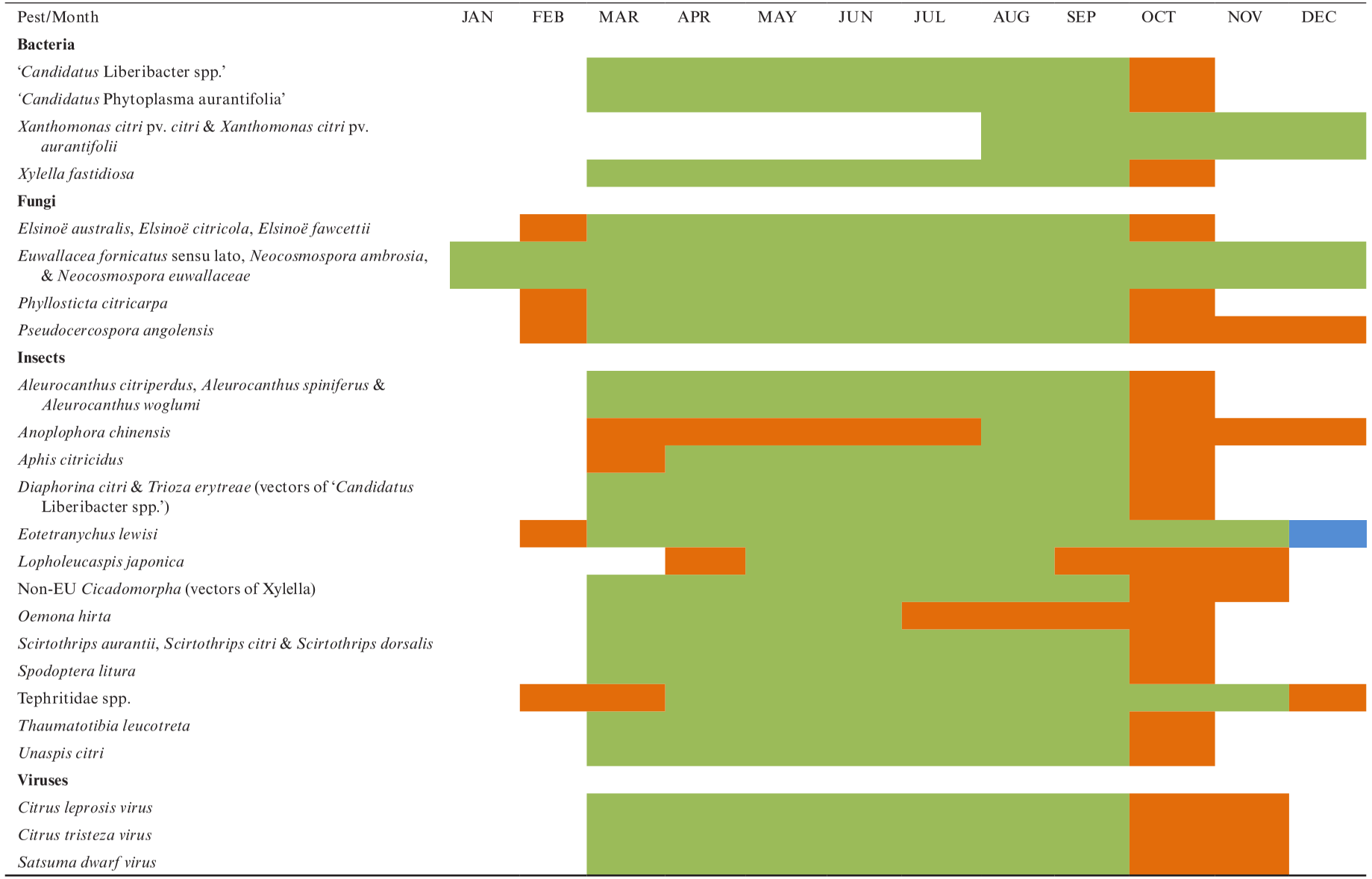
On *Citrus*, SDV typically causes dwarfing and small boat or spoon-shaped leaves (Figure A30). General symptoms are enations, multiple flushing, stunting or dwarfing, reduction in number and size of leaves and shoots, shortened internodes, and small-sized fruits with thick peel.

Sampling and identification

Inspection may allow the detection of symptoms but is not considered reliable since symptoms are not highly specific and are not always obvious in infected plants. White sesame is the best herbaceous indicator plant for detecting SDV through biological assays based on mechanical inoculation of homogenates from *Citrus* plants (EPPO, 1998; Tanaka et al., 1965).

FIGURE A30 Boat and spoon-shaped leaves of satsuma mandarin trees affected with the necrotic strain of satsuma dwarf virus (Images courtesy of T. Azeri, Plant Protection Research Institute, Bornova-Izmir (TR). EPPO Global Database).

APPENDIX 2 - OPTIMAL TIME OF INSPECTION FOR SOME CITRUS PESTS IN SPAIN AND TÜRKIYE (SHADED GREEN) WITH ADDITIONAL TIMES WHERE INSPECTION IS POSSIBLE IN SPAIN (BLUE) AND TÜRKIYE (SHADED ORANGE)



APPENDIX 3 - SHORT PROCEDURE FOR INSPECTORS

TIME OF INSPECTION

The time of year for inspection will vary with the pest species, depending on the optimum time for expression of symptoms for a specific pest survey programme or presence of visible live stages. In general, plants should be in active growth and the spring and summer months are the best times to observe the majority of citrus pest symptoms. Refer to Appendices 1 and 2 for pest specific details.

HYGIENE MEASURES

Following good hygiene procedures is important when inspecting and collecting samples for the laboratory. In order not to spread and increase infections, adequate precautions should be taken during inspection and sampling, such as wearing protective clothes (coat, overshoes, gloves, etc.). Gloves should be changed between different lots. All equipment for sampling must be decontaminated between different samples. Samples should be sent to the laboratory as soon as possible after collection.

LOT IDENTIFICATION

For non-grafted plants (e.g., rootstock mother plants), the cultivar, and where relevant the clone, are the primary criteria to distinguish lots. For grafted plants (e.g., scion mother plants), the grafting combination, cultivar and rootstock, and their clones when relevant, are the primary criteria for lot identification. A lot should include all plants originating from the same propagating material (both rootstock and scion for grafted plants), of the same age and cultivated in one single field or set of plants in the case of potted plants.

INSPECTION

It is up to the NPPO to set the sample size. For example, from a lot of 10 000 plants, 3689 plants need to be inspected to provide a 99% confidence of detecting symptoms present in 0.1% of the plants, provided the symptoms are uniformly distributed and the plants are randomly selected. To detect symptoms present in 1% of the plants, with a 99% confidence, 448 randomly selected plants should be inspected.

For small lots, the numbers required will often mean that all plants should be inspected.

Each lot should be examined as a separate unit because it will have different visual characteristics, such as size of individual plants and foliar morphology.

Phytosanitary inspection should start with an overall examination of the place of production in order to check the physical condition of the plants. If there is abnormal die-off in a place or lot, or if there are other anomalies within the plants (e.g. abnormal growth, differences in colour, leaf curl), these lots should be checked with particular attention. If no symptoms are seen, a systematic inspection of the of the place of production should be made.

For the inspection of rootstock mother plants and rooted cuttings, where pest symptoms are often latent, hidden or less specific, inspection may be randomly carried out on a set of plants, extrapolating the methodology for consignments included in ISPM 31 (FAO, 2009), and sampling for laboratory testing for detection of latent infestation may be recommended.

SAMPLING FOR LABORATORY TESTING

For tissue culture, the majority of potential contaminating pests will be excluded due to the confined conditions for micropropagation. This may not be the case with viral or bacterial pathogens, which could go unnoticed during micropropagation. Inspection of plants in tissue culture at the time of sale or export is difficult to perform and unreliable. It is recommended that this material is inspected before it is propagated in tissue culture or after transplanting into growing medium and growth continued to a stage where symptoms could be detected.

In practice, in scion mother plants, symptoms of pests are commonly more frequent and more easily detectable than in rootstock plants. In this case, the inspection of all mother plants may be performed, because lots are generally not very large.

Both rootstock and scion mother plants should be inspected for citrus pests. For the inspection of plantlets and young plants, it should be taken into account that fruit pests such as *Bactrocera* spp. will not be present.

Micro-propagated material is exclusively required to be grown under protected conditions structure which excludes pests to ensure aseptic conditions and avoid subsequent infestations.

Each lot of young plants (grafted or own-rooted) should be individually inspected because it may have a different origin, grafting combination and specific features, in terms of disease resistance, history, previous treatments and potentially different infestation levels.

Inspectors should particularly look for symptoms on the wood or on shoots, leaves and any fruits, if present. Additionally, inspectors should look for vectors that may transmit diseases. Symptoms caused by insects are often generic for most primarily foliage feeding. Sometimes leaf-galls appear. Larval damage is commonly non-specific, especially for those insects which have powerful mouthparts to cut plant tissues.

Diseases may exhibit different symptoms, which can be apparent via reduced growth, stem grooving, stunting and other deformations. Leaves may be thicker than

normal, or may show many other symptoms, such as ring spots, discoloration, pale or reddish areas, clearing of veinlets, veinal or interveinal necrosis, rolled margins or mild deformation. Shoots may also exhibit several symptoms, with deformed, flexible and drooping aspects, or incomplete lignification. It should be highlighted that an infected plant may not show symptoms systematically, as it may exhibit signs of disease one year and no symptoms the following year. If asymptomatic infection is suspected (as for *Citrus Tristeza virus*), or plants are being indexed for possible latent infection, then random samples representative of the whole lot should be collected.

SAMPLE PREPARATION

Plant samples should be placed in a plastic bag (without any paper) that is then inflated slightly and sealed, then packed in strong containers such as cardboard or plastic boxes and padded with paper or similar to prevent movement.

Each sample should be individually labelled with nursery name, nursery reference number, date, variety and, if necessary, a way of identifying the individual plant or tree so follow-up action can be taken if needed.

Appendix 1 provides pest specific guidance for sampling and sample preparation.