Bulletin OEPP/EPPO Bulletin (2020) **50** (3), 383–400 ISSN 0250-8052. DOI: 10.1111/epp.12684

Phytosanitary procedures

PM 3/90 (1) Inspection of citrus fruits consignments

Specific scope

This Standard describes the procedure by which consignments of citrus fruits are subjected to import control, including sampling and identification. This Standard covers fruits of *Citrus* L. and other genera of the plant family Rutaceae such as *Fortunella* Swingle, *Poncirus* Raf., *Microcitrus* Swingle, *Naringi* Adans, *Swinglea* Merr. and their hybrids. This Standard only covers the inspection of citrus fruit consignments without leaves and peduncles. Import of leaves or peduncles, including when in association with fruits, is prohibited in many EPPO countries, as they present a higher risk from a wider range of pests than fruits. The Standard also provides guidance that may be relevant to exports.

Specific approval

First approved in 2020-09.

1. Introduction

Citrus fruits are imported into the EPPO region (about 3 million tonnes per year) from other parts of the world, including countries in the southern hemisphere. Imported consignments of citrus fruits may carry pests that are included in the EPPO A1 and A2 lists of pests (EPPO, 2020) or regulated by EPPO member countries [e.g. those listed in Annex II of Commission Implementing Regulation (EU) 2019/2072]. These pests can be specifically associated with citrus fruits, such as *Phyllosticta citricarpa* or *Xanthomonas citri* pv. *citri*, or they can be polyphagous pests.

The entry of pests associated with citrus imports into the EPPO region could result in economic impact due to yield and/or quality losses and costly control measures. The Euro-Mediterranean citrus industry covers approximately 12% of the world's citrus growing area and produces approximately 20% of the world's citrus fruits (Siverio *et al.*, 2017; faostat.fao.org). Around 70% of the area of citrus fruit production is concentrated in four countries: Spain (27%), Italy (16%), Egypt (15%) and Turkey (10%) (Siverio *et al.*, 2017). Citrus is also produced in the following EPPO countries (listed in descending order of citrus production): Morocco, Algeria, Greece, Israel, Tunisia, Portugal, Cyprus, Jordan, Georgia, Croatia, Albania, France, Azerbaijan and Bosnia and Herzegovina (FAO, 2017). The entry of pests that can affect citrus production in the EPPO region could also have negative social impacts in areas where citrus is a major crop.

Based on pest risk analysis, many EPPO countries have established phytosanitary import requirements for citrus fruits. Some of them are general and apply to all imported citrus fruits irrespective of their origin (e.g. citrus fruit consignments should be free from leaves and peduncles). Other requirements are more specific (depending on the citrus species, the origin of the fruit and according to the regulated pests) and may include the need for inspections at the site of production during the growing season, the application of appropriate treatments in the field against pests or treatment either prior to export or during transport (such as cold treatments for Tephritidae) or inspections of consignments prior to export.

At import, consignment freedom of pests is usually verified by inspection and, where appropriate, by testing before the consignment is released. Similar procedures may be applied in the exporting country before dispatching the consignment if the importing country requires consignment freedom or verification of the efficacy of other phytosanitary measures (e.g. treatment).

2. Phytosanitary inspections

In ISPM 5 *Glossary of phytosanitary terms* (IPPC, 2019), inspection is defined 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'.

The general background for carrying out import inspections is included in ISPM 20 *Guidelines for a phytosanitary*

import regulatory system (IPPC, 2017) and ISPM 23 *Guidelines for inspection* (IPPC, 2016).

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

The procedures described in this Standard mainly concern the inspection of consignments at a point of entry in an EPPO country, but they may also be applicable for export inspection to check compliance with the phytosanitary requirements of the importing country.

Inspections at import (including document and identity checks) aim to verify compliance with phytosanitary import requirements such as the absence of pests and compliance with specific phytosanitary measures (e.g. treatment or originating in a pest-free area). Inspection may also be carried out for the detection of organisms for which the phytosanitary risk has not yet been determined.

Inspections of consignments of citrus fruits in the importing country may be carried out at the point of entry or at the place of destination. In the case, this will depend on the possibility of carrying out efficient inspections at the place of destination and provided that the fruits remain under official control. In case of inspection at the place of destination, the risk of quarantine pests escaping from the consignment should be minimized during transport and from the time that the consignment has arrived at its destination until the inspection has verified that the consignment is compliant.

When a sample has been taken from the consignment because the presence of a pest is suspected, the citrus fruits should remain under official control and should not be released until the final laboratory result confirms that no quarantine pests were detected.

It is important to pay extra attention to consignments that are most likely to carry pests (e.g. the most susceptible varieties, small air-freighted consignments, with place of origin of the fruits where particular pests of concern occur and from areas/producers with previous instances of noncompliance of consignments). All documents that are associated with a consignment (e.g. phytosanitary certificate, bill of loading, consignment invoice) should be examined before initiating the visual examination of the fruits. These documents can provide useful information (such as the country of origin, place of production, citrus variety, port and date of loading, etc.) that can be used to focus visual examinations on pests that the imported consignments may carry and to rule out others that are known not to occur in the country of origin.

Information on the presence of regulated pests of citrus fruits in a country of origin may be found in the EPPO Global Database (EPPO, 2020). Users can search for a genus (e.g. *Citrus*) or species (*Citrus sinensis*) and filter by commodity (fruit or vegetables) and country to produce a country-specific list of pests of concern. Europhyt reports of interceptions are a useful tool to check notifications of noncompliance related to the presence of citrus pests (European Commission, 2018).

2.1. Commodities concerned

Common citrus species that produce citrus fruits are listed in Table 1.

Citrus fruits imported into EPPO countries are usually traded in large lots and transported by sea in palletized boxes, ship holds or containers, or by air freight.

Imported citrus fruits are either intended for consumption as fresh fruit or destined for industrial processing (predominantly juice making). Fruits destined for juice production are usually packed in big boxes or bins, while fruit for the fresh market is usually in smaller boxes. Phytosanitary import requirements may differ for citrus for consumption compared to citrus destined for processing. However, regardless of the end use, there should be no differences in the inspection of the commodity.

Table 1. Main citrus species cultivated worldwide (amended from EFSA, 2014a)

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

2.2. Pests of concern for the EPPO region

This Standard mainly relates to those organisms affecting citrus fruits, which are listed in the EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests. It also considers those pests that are regulated in specific EPPO countries. The phytosanitary procedures described in the Standard are primarily aimed at preventing the introduction of these pests into the EPPO region via imported consignments of fruits. Details on all these pests can be found in EPPO/CABI (1997) and in species-specific EPPO datasheets available via EPPO (2020). For additional up-to-date information, the respective scientific literature should be consulted.

A list of pests that could be introduced with citrus fruit trade and may present a risk to cultivated species or varieties of citrus in the EU was established by the DROPSA consortium in 2016. Not all of these pests have been specifically mentioned in this Standard, but they could be looked for during import inspections (Suffert *et al.,* 2018).

EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests are subject to annual additions and deletions. It is recommended to check that the list of species in Tables 2 and 3 is up to date when using the Standard by referring to the EPPO Global Database.

The Standard does not cover pests that are not known to be transmitted by fruit.

Table 2. Specific pests of citrus fruits

A1 pests (absent from the EPPO region	A2 pests (present in the EPPO region)	Other pests regulated by specific EPPO member countries
Insects		
*Diaphorina citri**	*Trioza erytreae**	*Aceria sheldoni* (Uzbekistan, A1, 2008), *Aonidiella aurantii* (Moldova, A1, 2006; Uzbekistan, A1, 2008)
		Prays endocarpa (Jordan, A1, 2013; Morocco, Quarantine pest, 2018)
		Unaspis yanonensis (Azerbaijan, A1, 2007; Belarus Quarantine 1994; Israel Quarantine Pest, 2009; Morocco, Quarantine pest, 2018; Turkey A1, 2016; Uzbekistan A1, 2008)
Bacteria		
Xanthomonas citri pv. *aurantifolii*		
Xanthomonas citri pv. *citri*		
Fungi		
Phyllosticta citricarpa	*Plenodomus tracheiphilus*†	*Elsinoe australis* (EU Annex II/A1; Israel Quarantine Pest, 2009; Morocco Quarantine Pest, 2018) *Elsinoë citricola* (EU Annex II/ A1 2019) *Elsinoe fawcettii* (EU Annex II/ A1; Azerbaijan A1 List, 2007; Israel Quarantine Pest, 2009)
Pseudocercospora angolensis		*Zasmidium citri-griseum* (Morocco Quarantine Pest, 2018; Israel Quarantine Pest, 2009)

*Fruits that have gone through a packing house process in which they have been brushed and washed, and leaves and stems removed, are not considered to pose a risk regarding the introduction of these pests (EPPO, 2019a). However, interception data shows that those vectors can probably move on fresh and unprocessed citrus fruits (Halbert and Nunez, 2004).

†Fruit and seeds of susceptible citrus species may become infected but there is no evidence that the pathogen can spread via infected citrus fruit or seed (EFSA, 2014a citing CABI, 2010 and Ippolito *et al.*, 1987, 1992).

Table 3. Polyphagous pests affecting citrus fruits

A1 pests (absent from the EPPO region	A2 pests (present in the EPPO region)	Other pests regulated by specific EPPO member country
Insects/Acarida		
Aleurocanthus woglumi	*Aleurocanthus spiniferus*	*Aleurodicus dispersus* (Israel Quarantine Pest, 2009; Jordan A1 2013; Morocco Quarantine Pest, 2018)
Anastrepha fraterculus	*Bactrocera zonata*	
Anastrepha ludens	*Ceratitis capitata*	*Ceroplastes japonicus* (Azerbaijan A2, 2007; Belarus Quarantine Pest. 1994; Kazakhstan A1, 2017; Uzbekistan A1, 2008)
Anastrepha obliqua	*Scirtothrips dorsalis*	
Bactrocera caryeae	*Thaumatotibia leucotreta*	*Deudorix isocrates* (Morocco Quarantine Pest, 2018; Turkey A2, 2016)
Bactrocera dorsalis complex		*Diaprepes abbreviatus* (Jordan A1, 2013; Turkey A1, 2016)
Bactrocera minax		*Eotetranychus lewisi* (EU Annex II/A1, Israel Quarantine Pest, 2009; Jordan A1, 2013; Turkey A1, 2016)
Bactrocera tryoni		
Bactrocera tsuneonis		*Eotetranychus sexmaculatus* (Israel Quarantine Pest, 2009; Morocco Quarantine Pest, 2018)
Ceratitis rosa		
Gymnandrosoma aurantianum		*Ferrisia virgata* (Israel Quarantine Pest, 2009)
Homalodisca vitripennis		*Nipaecoccus viridis* (Turkey A1, 2006)
Naupactus xanthographus		Non-European Tephritidae (EU)
Scirtothrips aurantii		
Scirtothrips citri		
Unaspis citri		
Fungi		
		Ceratocystis fimbriata (Israel Quarantine Pest, 2009; Russia A1, 2014)

2.3. Possible contaminating pests

Some citrus pests have a low probability of being associated with the fruits, but could be intercepted. Examples of such species include *Maconellicoccus hirsutus* (EPPO A2, 2003), *Eutetranychus orientalis* (EPPO A2), *Eutetranychus africanus* (Israel Quarantine Pest, 2009), *Helicoverpa armigera* (EPPO A2), *Frankliniella insularis* (Israel Quarantine Pest, 2009; Kazakhstan A1 List, 2017), and *Icerya aegyptiaca* (Jordan A1 List, 2013).

Inspections should also be carried out for the detection of pests for which the phytosanitary risk has not yet been determined. When an unfamiliar pest is detected, the procedures specified in EPPO (2002a) Standard PM 5/2 *Pest risk analysis on detection of a pest in an imported consignment* should be followed to allow the NPPO to decide the phytosanitary action to take.

For an indication of the regulatory status and the distribution of the pest, the EPPO Global Database (EPPO, 2020) may be consulted.

2.4. Lot identification

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

According to ISPM 5, a lot is 'a number of units of a single commodity, identifiable by its homogeneity of composition, origin etc., forming part of a consignment' (IPPC, 2019). Criteria for lot identification for citrus consignments should include the species and the country of origin. Variety, area of production, grower, packaging, distinguishing marks (e.g. commercial brand or lot number) and exporter may also be considered. Lots identified on the phytosanitary certificate and declared separately to customs should be the starting point for planning the inspection. When a consignment comprises more than one lot, the inspection to determine compliance should consist of multiple separate visual examinations, and each lot should be sampled separately. Packaging normally contains an indication of the country of origin and additional information that may be used to identify individual lots.

2.5. Sampling

This section contains guidance on sampling for visual examination (general and specific aspects) and laboratory testing of consignments of citrus fruits.

2.5.1. Sampling for visual examination (general aspect)
Visual examination of imported consignments of citrus fruit is usually adequate to detect the presence or absence of regulated pests or their symptoms. However, a magnifying lens (at least 10×) or binocular microscope (35×) should be used to detect small pests.

An adequate proportion of fruits from each lot should be subjected to a systematic examination to detect the presence or signs of pests.

If sampling is undertaken to provide information about the general phytosanitary condition of a consignment, to detect pests or to verify compliance with phytosanitary import requirements, as in the case of inspection of citrus fruits consignments, statistically based methods are appropriate. The sample (as the minimum number of individuals selected from the lot or consignment to be examined) should be determined based on lots, taking into account the statistical background provided in ISPM 31 *Methodologies for sampling of consignments* (IPPC, 2008).

Sampling first involves the identification of the appropriate unit for sampling (a fruit, a unit of weight or a box).

For citrus fruit consignments which are usually large lots sufficiently mixed, and for which the sample size is less than 5% of the lot size, the sample size can be calculated using either the binomial or Poisson distribution (IPPC, 2008). A confidence level of 95% should normally be used for citrus fruits, as for other fruits and vegetables.

It is up to the NPPO to set the sample size. For example, from a consignment consisting of a container of citrus fruits (where a single citrus fruit is the sampling unit), 300 fruits should be inspected to provide a 95% confidence of detecting symptoms present in 1% of fruits, provided the symptoms are uniformly distributed and the fruits are randomly selected. However, to detect 5% of the fruits with symptoms with 95% confidence, 60 fruits should be inspected provided the symptoms are uniformly distributed and the fruits are randomly selected. For other levels of confidences or other percentages of symptoms consult ISPM 31.

If the inspector suspects the presence of a quarantine pest, the lot or consignment should be detained under official control.

2.5.2. Sampling for visual examination (specific aspects)
During visual examination, inspectors should be alert to the possible presence of all regulated pests but also of unfamiliar pests.

For visual examination of citrus fruit consignments, plant health inspectors should be equipped with a torch, a knife and a magnifying lens (10×). The place where the inspection is conducted should be well lit. The visual examination should begin with an overall examination of the consignment. Visual examination of the container, packaging and means of conveyance can provide indications of adverse conditions during transport (e.g. adverse temperatures or signs of damp or wetness) which may affect the physical condition of the fruits. Visual examination can also be carried out to look for living or dead insect life stages.

The sampling unit commonly used for citrus fruits is an individual fruit. Samples should be representative of the lot. The fruit should be selected from across the lot, for example if in boxes a minimum of 10 boxes or 1% of the boxes in the lot, whichever is more.

The necessary number of fruits should be selected from different places and depths within each box.

If the facilities and equipment allow, each box to be sampled should be emptied in such a way that all fruits in the box can be checked, for example on a specific inspection table (Fig. 1). The empty box should be inspected for any signs of pests (e.g. frass, pupal cases, different life stages etc.).

Any wrapping on individual fruits should be removed. After examining the overall condition of the fruits (e.g. the absence of leaves and peduncles, rotten fruits and/or superficial lesions), each fruit should be examined over its whole surface looking for lesions and oviposition marks or scales. Each fruit should be gently pressed to detect soft areas of the fruit indicative of Tephritidae or Lepidoptera larvae such as *Thaumatotibia leucotreta* or *Gymnandrosoma aurantianum*.

Some symptoms or lesions are characteristics of certain pests. These symptoms should be looked for during visual examination of the fruits, such as (see also Appendix 1):

- Ring-shaped scars around the calyx or fruit bottom or clear yellow thrips ≤1 mm (*Scirtothrips aurantii*, *Scirtothrips citri*, *Scirtothrips dorsalis*);
- Oviposition holes (nearly imperceptible, particularly in the case of *Anastrepha*) with little discoloration on the fruit surface or rotten fruits (Tephritidae);
- Pink to grayish-brown scab pustules (*Elsinoe* spp.);
- Brownish, slightly erumpent, crater-like lesions with a corky texture (*Xanthomonas citri* pv. *citri* and *Xanthomonas citri* pv. *aurantifolii*);
- Black or dark spots (lesions on fruit are very diverse) (*Phyllosticta citricarpa*);
- Circular to irregular spots up to 10 mm in diameter (*Pseudocercospora angolensis*);
- Dark or soft patch on the skin with an entrance hole (*Thaumatotibia leucotreta*, *Gymnandrosoma aurantianum*).

Fruits with any symptom or characteristic lesion or with any anomaly should be removed for further thorough examination and, if necessary, sent to the laboratory for pest identification.

It would be unusual to find Tephritidae or *Thaumatotibia leucotreta* adults while inspecting consignments of citrus fruit. For these species, the visual examination should focus on detecting the larval stage or eggs. When Tephritidae or *Thaumatotibia leucotreta* symptoms are found on the fruit surface, the fruit should be carefully opened with a sharp blade and examined for eggs or larvae.

Since thrips, mites and scale insects can be found under the calyx. This part of the fruit should be removed during inspection and thoroughly examined using a hand lens or microscope. A Berlese funnel trap (Fig. 2) could also be used where there is a high risk of thrips being present. The trap is also very effective at collecting mites, which move away from the light source and are caught in a preservation tube.

Fig. 1 Example of specific inspection table used for citrus inspection in Spain (Courtesy: Vañó García).

Fig. 2 Berlese funnel trap used to capture small invertebrates (Courtesy: SPPS Switzerland).

When citrus fruit varieties have a navel, this part of the fruit should be examined to confirm the absence of any pest.

2.5.3. Destructive sampling

Given that oviposition marks can be difficult to detect by visual inspection, an appropriate number of asymptomatic fruits should be randomly selected and cut in half to look for larvae of Tephritidae or *Thaumatotibia leucotreta*. For example, from the sample taken for visual inspection, random sampling of a minimum of 30 fruits gives a 95% confidence level of detecting the pest at a 10% infestation level. Sixty fruits need to be sampled to achieve this level of confidence at a 5% infestation level.

3. Sampling for laboratory testing

If citrus fruits show symptoms of pests, or in the case of detection of a larvae or adult insect, a sample should be taken and sent to the laboratory to confirm the identity of the pest if needed. If pests or symptoms of the pest (or species suspected to be a pest or an unfamiliar species), these should be submitted to a laboratory for identification.

The fruits should be wrapped in paper to prevent proliferation of saprotrophic bacteria and fungi. The fruits should then be placed in a sealed airtight box or plastic bag and sent directly to the laboratory. Living larvae may be packed with a section of fruit or whole fruit. Alternatively, larvae or adults can be preserved in sealed tubes containing an alcoholic solution (see specific procedure in Appendix 1).

Appendix 2 includes a short procedure for inspectors.

Acknowledgements

This Standard was first drafted by Ms C Trontin (FR) and Ms T Vañó García (ES). It was reviewed by the Panel on Phytosanitary Inspections.

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Appendix 1 – Specific procedures

Symptoms and sampling for identification of pests of *Citrus* that might be present in fruit consignments

For each of the pests of concern mentioned below, basic information on host range, biology, detection and identification can be found in EPPO/CABI, (1997) and EPPO (2020). Information on the current distribution of relevant pests and photographs of symptoms can be found in the EPPO Global Database at https://gd.eppo.int/ (EPPO, 2020).

Further information can be found in relevant EPPO Standards and in named scientific references. When an EPPO Diagnostic protocol exists, it is mentioned in the text. However, the fact that there is no EPPO Diagnostic protocol does not mean that there is no method for diagnostic available in the scientific literature.

A) Insects

Aceria sheldoni (citrus bud mite) (A1 List Uzbekistan)

Symptoms and description

Aceria sheldoni belongs to the family Eriophyidae and is one of the main pests of lemon, which is its preferred host (EPPO, 2003). It is a minute mite (0.12–0.18 mm). *A. sheldoni* feeds and reproduces on citrus foliage and moves to buds and young fruit as they become available. EPPO (2003) states: 'the mite population builds up throughout the period of fruit development. The mites puncture rind cells and extract the cell contents. Cells damaged by repeated puncturing discolour, and the result is a russeting of the fruit surface. Severe attack can contribute to water loss from the fruit, reduction in fruit size and premature fruit drop. One generation is completed in 15 days in summer and 30 days in winter. The optimal conditions for development are relative humidity close to 100%'.

Sampling and identification

EFSA (2008) detail that the species can be identified using morphological characteristics visible under a microscope. However, due to the small size of the species, it is not easy to detect in the absence of symptoms.

Aleurocanthus spp.: *Aleurocanthus woglumi* (citrus blackfly) (EPPO A1 List), *Aleurocanthus spiniferus* (citrus mealywing) (EPPO A2 List)

Symptoms description

Aleurocanthus belongs to the family Aleyrodidae. Adult *Aleyrodidae* or whiteflies are winged insects of 1-2 mm in length that look like tiny moths (Figs 3 and 4). Their wings are covered with waxy powder. The adults are usually active and feed on leaves of the host. They are most likely to be found on the leaves of planting material or cut branches but could also be found on fresh fruits. Highly infested fruits can show spots of sticky, transparent honeydew, which can be covered by sooty mold (Fig. 5). It should be noted, however, that honeydew can also be produced by other hemipterans active on the fruits. Consequently, the presence of honeydew does not necessarily imply the presence of *Aleurocanthus* sp.

Sampling and identification

Collected adults can be killed and transferred to 70% ethanol before being sent to the laboratory for identification. Pupae can be sent alive with host plant material in an airtight and secure container, except if the phytosanitary 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 (2002b) PM 7/7(1) *Aleurocanthus spiniferus* (under revision) and EPPO (2002c) PM 7/8(1) *Aleurocanthus woglumi* (EPPO, 2002c) (under revision).

Aonidiella aurantii (California red scale) (A1 List Moldova and Uzbekistan)

Symptoms description

Aonidiella aurantii belongs to the family Diaspididae and is thought to be native to South-East Asia. The insect causes distortion of the rind on young fruits. There is no distortion on mature fruits but the scale adheres strongly to the fruit.

Sampling and identification

Females of *A. aurantii* are reddish-brown, scales are orange-red (Figs 6 and 7). EPPO (2005b) highlights that *A. citrina* and *A. aurantii* are morphologically similar and provides images detailing the morphological differences between the two species. The prevulvar scleroses and apophyses are present for *A. aurantia.* In *A. aurantii*, each basal apophysis is accompanied by two submedian transversal scleroses.

Identification can be performed according to EPPO Standard EPPO (2005b).

Fig. 3 Adult *Aleurocanthus woglumi* and larvae (Courtesy: Bayer Pflanzenschutz, Germany., EPPO Global Database).

Fig. 4 Adult *Aleurocanthus spiniferus* with eggs (Courtesy: Francesco Porcelli, Università di Bari (IT), EPPO Global Database).

Fig. 5 Sooty mould on citrus fruit (Courtesy: M.A. van den Berg, ITSC, Nelspruit, South Africa. EPPO Global Database).

Diaphorina citri (Asian citrus psyllid) (EPPO A1 List)

Symptoms description

Diaphorina citri is the main vector of '*Candidatus* Liberibacter asiaticus', but is also a vector for '*Candidatus* Liberibacter americanus' and at least experimentally for '*Candidatus* Liberibacter africanus'. Adults are 2.5 mm long with a yellowish-brown body and greyish-brown legs (Fig. 8). 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.

Fig. 6 *Aonidiella aurantii* close up [Courtesy: Jean-François Germain, LNPV Entomologie, Montpellier (FR) (EPPO Global Database)].

Fig. 7 *Aonidiella aurantia* [Courtesy: Ilya Mityushev, Department of Plant protection of the Russian Timiryazev State Agrarian University (EPPO Global Database)].

Sampling and identification

Adults can be killed in 70% ethanol and sent for identification in a hermetic and solid tube or container in 70% ethanol. Identification involves detailed microscopic examination of teneral adult females or advanced larval instars by a competent taxonomist according to EPPO Standard PM 7/52 *Diaphorina citri* (EPPO, 2005c).

Gymnandrosoma aurantianum **(citrus fruit borer) (EPPO A1 List)**

Symptom description

Gymnandrosoma aurantianum (Lepidoptera: Tortricidae) is a pest of citrus and other fruit crops in tropical regions of the Americas. Eggs can be laid on the surface of the fruit and the first-instar larvae make an entry hole approximately 4.5 cm from the hatching point. Within 3-4 days of entry excrements appears on the rind of the fruit around the entrance hole. On citrus fruit there may be abnormal coloration of the fruit and a necrotic area around the entry hole. Frass may be visible extruding from the entry hole and sticking to the rind of the fruit.

Fig. 8 *Diaphorina citri* adult (Courtesy: D.G. Hall (USDA), EPPO Global Database).

Sampling and identification

Eggs are ca. 1.2 mm long, flattened, circular to ovoid in shape, pale white, darkening to reddish-brown. Larvae are eruciform, with a cream/pale yellow body (Fig. 9). The head is pale yellow to pale orange with a red-brown patch. The last larval instar measures ca. 15-19 mm (Adamski & Brown, 2001). Newly hatched (neonatal) larvae measure 5 mm. Pupae are fusiform, 9-12 mm long and 2.5-3 mm wide, rounded at the anterior and posterior ends (Adamski & Brown, 2001). Newly formed pupae are pale yellow, later becoming brown. Morphological identification of adults and larvae is possible (pupae of different species of *Gymnandrosoma* cannot be distinguished) (Adamski & Brown, 2001). Collected larvae can be transferred to 70% ethanol.

Fig. 9 *Gymnandrosoma aurantianum* larvae.

Homalodisca vitripennis **(glassy-winged sharpshooter) (EPPO A1 List)**

Symptoms description

Homalodisca vitripennis (Cicadellidae) (Fig. 10) is an efficient vector of *Xylella fastidiosa*. It is a stem feeder, which leaves no visible symptoms other than white, powdery, dried excrements that can cover the stems, foliage and fruit when the insects are abundant. When populations are abundant, egg masses can be laid into the rind of immature fruits of crops such as citrus. Old hatched egg masses appear as grey or tan scars on the surface of the rind (Blua *et al.*, 1999). The sausage-shaped eggs are laid side by side in masses averaging 10–11 eggs. The egg masses appear as greenish water blisters beneath the leaf. They are elongate, with the individual eggs running transversely across the mass.

Sampling and identification
Adults can be placed in 70% ethanol and sent for identification in a hermetic secure tube or container in 70% ethanol. Larvae can be killed in boiling water for a few minutes and then transferred to 70% ethanol. Identification involves detailed microscopic examination. Eggs can only be identified using molecular methods.

Fig. 10 Adult *Homalodisca vitripennis* (Courtesy: J.N. Dell, Bugwood).

Naupactus xanthographus **(South American Fruit tree weevil) (EPPO A1 List)**

Symptoms description
Apart from faeces attached to the fruit there is unlikely to be any other symptoms on citrus fruits. The mouthparts of the weevil are not suited to feeding on the fruits (mandibles are lost following emergence from the soil). Adult weevils are the most likely stage to be associated with fruits though eggs may be present if a gravid female is present.

Sampling and identification
Adults are brown or grey-brown with white or white/yellow stripes on pronotum and elytra (Fig. 11). 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 (R. Ripa, personal communication, 2019). 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).

Fig. 11 Adult *Naupactus xanthographus* (male) (Courtesy: R. Ripa (BIOCEA: EPPO Global Database).

Prays endocarpa **(citrus rind borer) (Jordan and Morocco A1 List)**

Symptoms description
Prays endocarpa eggs can occur on the fruit with larvae feeding on the fruit rind although they never enter the endocarp (Baker *et al.*, 2008). Both external and internal feeding symptoms may be visible on the fruit, and feeding can cause gall-like swellings (lignified galls) around the site of larval activity (EPPO, 2016).

Larvae exit the fruit through an exit hole before pupating on the outer surface of the fruit, leaves or twigs (Baker *et al.*, 2008).

Sampling and identification
Baker *et al.* (2008) consider that the species can be reliably identified by experts based on the genitalia and wing markings of adult specimens. Live larvae should be collected with a piece of fruit, contained in a sealable secure plastic box and sent to the laboratory for identification. If adults are collected, they can be preserved in hermetic containers before being sent to the laboratory for identification. Care must be taken to preserve the wings, which are very fragile.

Scirtothrips aurantii **(South African citrus thrips) (EPPO A1 List),** ***S. citri*** **(California citrus thrips) (EPPO A1 List),** ***S. dorsalis*** **(yellow tea thrips) (EPPO A2 List)**

Symptoms description
All stages of *Scirtothrips aurantii*, *S. citri* and *S. dorsalis* feed on the apex of young fruits. Adults can be found occasionally on mature fruits. Symptoms on fruits include brown frass markings, grey to black markings often forming a conspicuous ring of scarred tissue around the apex and ultimately fruit distortion.

Eggs of *S. aurantii*, *S. citri* and *S. dorsalis* are bean-shaped and minute (less than 0.2 mm). The two feeding larval stages are yellow to orange, have an elongated shape

and are just visible to the naked eye. The adult thrips is yellowish-orange, less than 1 mm long (Fig. 12).

Sampling and identification

Considering the small size of thrips, direct visual inspection and sampling are difficult. The electric Berlese method should be used to sample thrips efficiently. Specimens detected in imported consignments can be preserved in 70% ethanol for 24–48 h. Sampling using the Berlese method has to be performed in a 10% ethanol solution mixed with 0.1% teepol. The sample has to be maintained in this solution for at least 24 h before being transferred to 70% ethanol for preservation or shipment to the laboratory. 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 *Scirtothrips* species in the larval stage is not possible. However, 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, 2005d).

Fig. 12 *Scirtothrips citri* adults [Courtesy: J. Morse (University of California), EPPO Global Database].

Tephritidae

***Anastrepha fraterculus* (South American fruit fly) (EPPO A1 List), *A. ludens* (Mexican fruit fly) (EPPO A1 list), *A. obliqua* (Antillean fruit fly) (EPPO A1 List) *Bactrocera caryeae* (EPPO A1 List), *B. dorsalis* (oriental fruit fly) (EPPO A1 List), *B. minax* (Chinese citrus fly) (EPPO A1 List), *B. tryoni* (Queensland fruit fly) (EPPO A1 List), *B. tsuneonis* (Japanese orange fruit fly) (EPPO A1 List), *B. zonata* (guava fruit fly) (EPPO A2 List), *Ceratitis capitata* (Mediterranean fruit fly) (EPPO A2 List), *C. rosa* (natal fruit fly) (EPPO A1 List)**

Symptoms description

In fruits, Tephritidae species (Figs 13 and 14) 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. Sometimes there may be some tissue decay or secondary rot around these marks. Rotting of the underlying tissue causes a depression on the surface. However, these, or any other symptoms of damage, are often difficult to detect in the early stages of infestation. 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 suspicious fruits 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). Sometimes identification is possible at the genus level with stage 3 larva. A reliable identification can only be performed on an adult specimen. Therefore, if time allows, rearing under quarantine containment conditions can produce adults for identification.

Larvae can be sent alive on the host plants in an airtight, secure container, except if the phytosanitary risk is high. 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.

Morphological identification with a binocular microscope can be performed according to White & Elson-Harris (1994) or ISPM 27 Diagnostic protocols for regulated pests (IPPC, 2006), DP 5: DP 09: Genus *Anastrepha* Schiner or available EPPO Standards PM 7/114 *Bactrocera zonata* (EPPO, 2013) and PM 7/104 *Ceratitis capitata* (EPPO, 2011).

Fig. 13 *Ceratitis capitata* adults on orange (Courtesy: M. Muñiz, Centro de Ciencias Medioambientales, EPPO Global Database).

Fig. 14 *Anastrepha ludens* adults (Courtesy: EPPO Global Database).

Thaumatotibia leucotreta (false codling moth) (EPPO A2 list)

Symptom description

Thaumatotibia leucotreta (Fig. 15) may be associated with fruits in the form of eggs or larvae. Eggs (whitish, translucent, about 0.9 mm long) are laid on the fruit surface, singly or in small numbers. After hatching, larvae enter the fruit and feed internally. On citrus, larvae bore into the albedo and usually feed just below the fruit surface. The rind around the point of infestation turns yellowish-brown as the tissue decays and collapses (Fig. 16). Feeding damage can also lead to the development of secondary infections by fungi or bacteria. Larvae prefer the navel end of the fruit but can burrow into any part of the fruit.

Symptoms vary according to host. On oranges there is sometimes a scar on the fruit surface (brown or discolored patch on the skin, usually with evidence of a hole bored in the centre, sometimes with dark brown frass exuding). On most other host species, the habit of internal feeding leaves few symptoms. Young larvae are whitish with a dark brown head, and usually develop through five instars. Mature larvae are about 15 mm long, pinkish-red with a brown head. There may be one to three larvae per citrus fruit. It is unusual to find *T. leucotreta* adults while inspecting.

Sampling and identification

Morphological identification of larvae and adults can be performed with a binocular microscope according to PM 7/137 *Thaumatotibia leucotreta* (EPPO, 2019b).

Collected larvae can be placed in boiling water for a few minutes and then transferred to 70% ethanol. If a molecular test needs to be carried out, the larva should not be boiled and should instead be placed in 95–100% ethanol. If adults are collected, they can be conditioned in hermetic containers before being sent to the laboratory. Care must be taken to preserve wings that are very fragile.

Fig. 15 Adult *Thaumatotibia leucotreta* (dorsal view) (Courtesy: Marja van der Straten, National Reference Centre, National Plant Protection Organization, EPPO Global Database).

Fig. 16 Damage caused by larva of *Thaumatotibia leucotreta* on *Citrus sinensis*. (Courtesy: Marja van der Straten, National Reference Centre, National Plant Protection Organization, EPPO Global Database).

Trioza erytreae (citrus psyllid) (EPPO A2 List)

Symptoms description

Trioza erytreae is the main vector of '*Candidatus* Liberibacter africanus' but also, at least experimentally, of '*Candidatus* Liberibacter asiaticus'. Adults, which are the most likely to be associated with fruits, are about 4 mm in length. They are winged, pale and delicate initially, later becoming light brown (Fig. 17). Males are smaller than females and have a blunt tip to the abdomen, the latter ending in a sharp point in females. They fly well, and often jump and fly when disturbed.

Sampling and identification

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 one adult male, or fifth-instar nymph and adult female, by a competent taxonomist according to EPPO Standard PM 7/57 *Trioza erytreae* (EPPO, 2005e).

Fig. 17 *Trioza erytreae* adult (Courtesy: S.P. van Vuuren, Citrus Research International, Bugwood.org).

Unaspis citri (citrus snow scale) (EPPO A1 List) and *U. yanonensis* (oriental citrus scale) (A1 List Azerbaijan, Georgia, Turkey, Uzbekistan; Quarantine pest Morocco, Israel, Belarus)

Unaspis citri can be found on fruits of all citrus, especially on oranges (*C. sinensis*) as well as on fruits of *Fortunella* and *Poncirus trifoliata*.

Symptoms description

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 (Fig. 18). On citrus fruit, the female scales can be confused with the common *Lepidosaphes* spp. or easily overlooked as dirt particles.

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

Fig. 18 *Unaspis yanonensis* (Courtesy: Jean-François Germain, Plant Health Laboratory, Montpellier (FR), EPPO Global Database).

Fig. 19 Symptoms of *Xanthomonas citri* pv. *citri* on lemon rind (Courtesy: Dalia Del Nista, EPPO Global Database).

hermetic container. If the phytosanitary risk is high, specimens should be sent with the host plant material in 70% ethanol. Identification involves detailed microscopic examination of teneral adult females by a competent taxonomist according to EPPO Standard PM 7/38 *Unaspis citri* (EPPO, 2004).

B) Bacteria

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

Symptoms 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 (Figs 19 and 20). 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.

Symptoms of citrus canker may be confused with citrus scab caused by *Elsinoe fawcettii* on *Citrus sinensis* and *Citrus lemon* fruits. There are some similarities also with anthracnose (*Glomerella cingulata*) symptoms on fruits.

Sampling and identification

The whole fruits 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, 2005a) or to ISPM 27, DP 6 *Xanthomonas citri* subsp. *citri*.

Fig. 20 Symptoms of *Xanthomonas citri* pv. *citri* on oranges (Courtesy: Dalia Del Nista, EPPO Global Database).

C) Fungi

Elsinoe fawcettii (scab of citrus) (Quarantine pest for Israel, A1 List Azerbaijan), *E. citricola* (EU Annex II/ A1 2019) and *E. australis* (scab of sweet orange) (Quarantine pest for Israel and Morocco)

Symptoms description

Fruits are infected in the early stages of their development and deformed growth can occur. These fruits are subject to premature drop. On the rind of developed fruits, raised lesions are formed with different shape, size and colour

Fig. 21 Raised lesions, scabs, caused by *Elsinoe fawcettii* on citrus fruit [Courtesy: Central Science Laboratory, York (GB), British Crown, EPPO Global Database].

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 (Fig. 21). Scab lesions do not extend into the albedo. *E. australis* scabs are larger, smoother and more circular than *E. fawcettii* scabs, which are typically irregular, warty and deeply fissured.

Sampling and identification

The whole fruits should be sent to the laboratory for analysis in a hermetically closed plastic bag. 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).

***Zasmidium citri-griseum* (*Mycosphaerella citri*) (greasy spot of citrus) (Quarantine pest for Morocco and Israel)**

Symptoms description

The pathogen can invade the stomata and surrounding cells forming minute black spots. The surrounding cells maintain their chlorophyll as the uninfected part of the fruit colours and green blemishes form on the surface of the fruit with small black spots (Fig. 22).

Fig. 22 Minute black spots caused by *Zasmidium citri-griseum* on *Citrus* sp. (Courtesy: C. Calderon, Invasive.org).

Sampling and identification

The whole fruits should be sent to the laboratory in a hermetically closed plastic bag. There is no international diagnostic protocol available for this fungus.

***Phyllosticta citricarpa* (black spot of citrus) (EPPO A1 List)**

Symptoms description

Several different types of overlapping symptoms occur on citrus fruits. Four main types, generally referred to as hard or shot hole spot, false melanose or speckled blotch, freckle spot and virulent spot, exist (Figs 23 and 24).

Hard spot is the most typical black spot symptom. It generally occurs when fruit starts maturing or on mature fruit. The crater-like spots (3–10 mm in diameter) have a light centre, a dark-brown to black rim and often a green halo on mature orange fruit. Pycnidia are often apparent in these lesions as tiny and slightly elevated black dots.

False melanose (or speckled blotch) usually appears on green fruit as small, raised, dark brown to black lesions often surrounded by dark specks that may coalesce. No pycnidia form on these lesions and it is difficult to isolate the pathogen.

Freckle spots are orange to red, slightly depressed, 1–3 mm in diameter and occur on mature fruits, usually after harvest. The spots turn brown with age. Often, but not always, they have a dark-red or brown rim. Pycnidia are only incidentally present in freckle spot lesions. Freckle spots often occur as satellite spots around hard spot lesions and many intermediates occur between these spots and the previous type.

Freckle spots may coalesce to form virulent spots. Those spots are large, slightly sunken, develop a leathery texture and spread irregularly over large areas of the mature fruit. The pathogen is most readily isolated from these types of lesions and pycnidia may eventually form. A magnifying

Fig. 23 *Phyllosticta citricarpa* symptoms on lemon fruit ,Courtesy: Jean Michel Mei, Benjamin Vigier, Reynaud Pascal, Border inspection post (BIP) of Marseille (FR), EPPO Global Database].

Fig. 24 Symptoms caused by *Phyllosticta citricarpa* on Valencia sweet orange (EPPO Global Database).

glass or dissecting microscope is needed to clearly observe pycnidia.

Fruit lesions caused by *P. citricarpa* can be confused with those caused by other citrus fungal pathogens, such as *Phyllosticta* spp., *Alternaria alternata* pv. *citri*, *Colletotrichum* spp., *Diaporthe citri*, *Zasmidium citrigriseum*, *Septoria* spp., or by mechanical, insect or cold damage (EPPO, 2009).

Sampling and identification

The whole fruits should be sent to the laboratory for analysis in an airtight plastic bag. Diagnosis should be performed by laboratory testing according to EPPO Standard PM 7/17 (under revision) *Guignardia citricarpa* (EPPO, 2009b) or ISPM 27, DP 5 *Phyllosticta citricarpa* (McAlpine) Aa on fruit (IPPC, 2006).

Plenodomus tracheiphilus **(dieback of citrus) (EPPO A2 List)**

Symptoms description

The principle host of *Plenodomus tracheiphilus* is lemon but the fungus has also been reported on many other *Citrus* species as well as on species in the genera *Fortunella*, *Poncirus* and *Severinia* (EPPO, 2015). On fruits, browning of vascular bundles can be observed in the area of insertion of the peduncle (EPPO, 2015). Although fruits can be infected, they mostly drop prematurely.

Sampling and identification

The whole fruits should be sent to the laboratory in a plastic sealable bag. Diagnosis should be performed by laboratory testing according to EPPO Standard PM 7/048 *Plenodomus tracheiphilus* (formerly *Phoma tracheiphila*) (EPPO, 2015).

Pseudocercospora angolensis **(leaf spot of citrus) (EPPO A1 List)**

Symptoms description

On young fruits, symptoms consist of brown necrotic lesions (Fig. 25). EPPO/CABI (1997) detail that these lesions are usually circular and slightly sunken with a surrounding ring of raised epicarp that can give the infected fruit a blistered appearance (see also Seif & Hillocks, 1993; Kuate, 1998).

Sampling and identification

The whole fruits should be sent to the laboratory for analysis in a hermetically closed plastic bag. 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).

Fig. 25 Symptoms caused by *Pseudocercospora angolensis* (Courtesy: Jacqueline Hubert, EPPO Global Database).

Appendix 2 – Short procedure for inspectors

General

For visual examination of citrus fruit consignments, plant health inspectors should be equipped with a torch, a knife and a magnifying lens (10×). The place where the inspection is conducted should be well lit. The visual examination should begin with an overall examination of the consignment. Visual examination of the container, packaging and means of conveyance can provide indications of adverse conditions during transport (e.g. adverse temperatures or signs of damp or wetness) which may affect the physical condition of the fruits. Visual examination can also be carried out to look for live or dead insect life stages.

Hygiene measures

Inspections and sampling can themselves be a pathway for spreading pests, therefore inspectors should take appropriate precautions during inspection and sampling, such as wearing gloves and disinfecting of hands and tools. Good hygiene procedures when collecting samples for the laboratory should be followed by decontaminating tools and hands. Applicable rules relating to food hygiene should be followed.

Sample size

The sampling unit commonly used for citrus fruits is an individual fruit. Samples should be representative of the lot. The fruit should be selected from across the lot, for example if in boxes a minimum of 10 boxes or 1% of the boxes in the lot, whichever is more.

The number of individual units that have to be inspected should be determined on the basis of lots, taking into account the statistical background provided in ISPM 31 *Methodologies for sampling of consignment* (IPPC, 2008).

It is up to the NPPO to set sample size. For example, from a consignment consisting of a container of citrus fruits (where a single citrus fruit is the sampling unit), 300 fruits should be inspected to provide a 95% confidence of detecting symptoms present in 1% of fruits, provided the symptoms are uniformly distributed and the fruits are randomly selected. However, to detect 5% of the fruits with symptoms with 95% confidence, 60 fruits should be inspected provided the symptoms are uniformly distributed and the fruits are randomly selected. For other levels of confidences or other percentages of symptoms consult ISPM 31.

The necessary number of fruits should be selected from different places and depths within each box.

If the facilities and equipment allow, each box to be sampled should be emptied in such a way that all fruits in the box can be checked, for example on a specific inspection table (Fig. 1). The empty box should be inspected for any signs of pests (e.g. frass, pupal cases, different life stages etc.).

Visual examination

Any wrapping on individual fruits should be removed. After examining the overall condition of the fruits (e.g. the absence of leaves and peduncles, rotten fruits and/or superficial lesions), each fruit should be examined over its whole surface looking for lesions and oviposition marks or scales. Each fruit should be gently pressed to detect soft areas of the fruit indicative of Tephritidae or Lepidoptera larvae such as *Thaumatotibia leucotreta* or *Gymnandrosoma aurantianum*.

Some symptoms or lesions are characteristics of certain pests. These symptoms should be looked for during visual examination of the fruits, such as (see also Appendix 1):

- Ring-shaped scars around the calyx or fruit bottom or clear yellow thrips ≤1 mm (*Scirtothrips aurantii*, *Scirtothrips citri*, *Scirtothrips dorsalis*);
- Oviposition holes (nearly imperceptible particularly in case of *Anastrepha*) with small discoloration on the fruit surface or rotten fruits (Tephritidae);
- Pink to grayish-brown scab pustules (*Elsinoe* spp.);
- Brownish, slightly erumpent, crater-like lesions with a corky texture (*Xanthomonas citri* pv. *citri* and *Xanthomonas citri* pv. *aurantifolii*);
- Black or dark spots (lesions on fruit are very diverse) (*Phyllosticta citricarpa*);
- Circular to irregular spots up to 10 mm in diameter (*Pseudocercospora angolensis)*;
- Dark or soft patch on the skin with an entrance hole (*Thaumatotibia leucotreta*, *Gymnandrosoma aurantianum*).

Fruits with any symptoms or characteristic lesions, or with any anomaly should be removed for further thorough examination and, if necessary, sent to the laboratory for pest identification.

The calyx of the fruit should be removed during the inspection and thoroughly examined for the presence of different live stages (larvae, adult) using a lens or microscope. Another method, for consignments where there is considered to be a high risk of thrips being present, is the use of a Berlese funnel. This trap is also very effective to detect mites.

Regarding varieties with 'navel', this part of the fruit should be examined to discard presence of any pest. During visual examination, inspectors should be alert to the possible presence of all regulated pests but also of unfamiliar pests or pests on the EPPO Alert List.

Destructive sampling

Given that oviposition marks can be difficult to detect by visual inspection, an appropriate number of asymptomatic fruits can be randomly selected and cut in half to look for the presence of larvae of Tephritidae or *Thaumatotibia leucotreta*.

For example, from the sample taken for visual inspection, random sampling of a minimum of 30 fruits gives a 95% confidence level of detecting the pest at a 10% infection level. Sixty fruits need to be sampled to achieve this level of confidence at a 5% infestation level.

Sampling for laboratory testing

If citrus fruits show symptoms of regulated pests or in case of detection of a larvae or adult insect, a sample should be taken and sent to the laboratory to confirm the identity of the pests. If pests or symptoms of the pest (or species suspected to be a pest or an unfamiliar species), these should be submitted to a laboratory for identification.

The fruits should be wrapped in paper to prevent proliferation of saprotrophic bacteria and fungi. They should then be placed in a sealed airtight box or plastic bag and sent directly to the laboratory. Live larvae shall be packed with a section of fruit. Alternatively, larvae or adults can be preserved in sealed tubes containing an alcoholic solution (see specific procedure in Appendix 1).