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European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 9/27 (1)

National regulatory control systems

PM 9/27 (1) '*Candidatus* Liberibacter' species that are causal agents of Huanglongbing disease of citrus and their vectors: procedures for official control

Scope

This Standard describes procedures for official control with the aim of detecting, containing and eradicating those '*Candidatus* Liberibacter' species which are causal agents of Huanglongbing (also known as citrus greening disease) and their vectors *Trioza erytreae* and *Diaphorina citri*. NPPOs may draw on this guidance when developing national contingency plans for outbreaks of '*Candidatus* Liberibacter' species and their vectors.

Approval and amendment

First approved in 2019-09.

1. Introduction

This Standard presents the basis of a national regulatory control system for the surveillance, detection and eradication of '*Candidatus* Liberibacter' species causing Huanglongbing, also known as citrus greening disease, and their vectors. It also provides guidance on measures to prevent further spread during eradication or from an area of established infestation, as well as a long-term containment strategy in cases where eradication is no longer feasible.

The Euro-Mediterranean citrus industry includes approximately 12% of the world's citrus-growing area and produces approximately 20% of the world's citrus fruit (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, Bosnia and Herzegovina, and Portugal (IPPC, 2017).

Eradication and containment are defined in ISPM 5 (IPPC, 2016) as 'The application of phytosanitary measures to eliminate a pest from an area' and 'The application of phytosanitary measures in and around an infested area to prevent spread of a pest', respectively. Unless it can be achieved quickly, a strategy of eradication has to be accompanied by implementation of measures for containment to avoid spread while the outbreak is eliminated.

1.1. The pathogen

'*Candidatus* Liberibacter' species (Phylum: Proteobacteria, class: Alphaproteobacteria) are fastidious phloem-limited bacteria and most cannot be cultured *in vitro*. The pathogens are Gram-negative bacteria with a double-membrane envelope (Bové, 2006). They are found in plants in the phloem cells and are transmitted from plant to plant by the feeding action of the psyllid vectors (Ammar *et al*., 2011) or by grafting of host material.

Three '*Candidatus* Liberibacter' species (hereafter referred to as 'the pathogen') have been identified as causal agents of Huanglongbing, which can infect all commercial citrus crops (USDA, 2008; EPPO, 2014a) and some related genera in the family Rutaceae: '*Ca*. Liberibacter *africanus*' Jagoueix, Bové & Garnier (EPPO Code: LIBEAF), '*Ca*. Liberibacter *asiaticus*' Jagoueix, Bové & Garnier (EPPO Code: LIBEAS) and '*Ca*. Liberibacter *americanus*' Teixeira, Saillard, Eveillard, Danet, da Costa, Ayres & Bové (EPPO Code: LIBEAM).

At present, neither Huanglongbing nor its causal agents have been reported from the EPPO region (Duran-Vila *et al*., 2014; EPPO, 2019).

All three species are on the EPPO A1 List of pests recommended for regulation as quarantine pests. They are

regulated in several EPPO countries, including those of the EU (EU, 2000). Both '*Ca*. L. *asiaticus*' and '*Ca*. L. *africanus*' are listed as quarantine pests in Israel, and all three species are A1 listed pests in Turkey (EPPO, 2019).

The three identified '*Candidatus* Liberibacter' species have different geographical ranges. '*Ca*. L. *africanus*' (*C*Laf) is found in Africa (including Mauritius and Réunion) and Asia (Saudi Arabia and Yemen) (EPPO, 2019). '*Ca*. L. *asiaticus*' (*C*Las) is found in Africa (Ethiopia, Mauritius and Réunion), East Asia, Iran, Saudi Arabia, South and Central America, and the USA (EPPO, 2019). Finally, '*Ca*. L. *americanus*' (*C*Lam) has been recorded in South America (Brazil in the states of Minas Gerais, Parana and Sao Paulo [EPPO, 2019]). More detail on geographical distribution is provided in the EPPO Global Database (EPPO, 2019).

'*Ca*. Liberibacter' species can be transmitted by propagative plant material and experimentally by *Cuscuta campestris* (dodder) to *Catharanthus roseus* (periwinkle) and other herbaceous plants. The presence of the bacteria has been demonstrated in citrus seeds and seedlings, and seed transmission has been considered as a potential dissemination route for the pathogen but a number of published studies suggest that seeds are not a pathway (Hilf & Lewis, 2016, citing Albrecht & Bowman, 2008; Graham *et al*., 2011; Hartung *et al*., 2010; Hilf, 2011; Shatters, 2008).

1.2. Vectors

The bacteria are known to be transmitted by two psyllid species (hereafter 'the vectors'), namely *Diaphorina citri* Kuwayana (Hemiptera: Liviidae, EPPO Code: DIAACI) and *T. erytreae* (Del Guercio) (Hemiptera: Triozidae, EPPO Code: TRIZER). Both psyllids vectors have been shown to be capable of transmitting '*Ca*. L. *africanus*' and '*Ca*. L. *asiaticus*' species under laboratory conditions. Under natural conditions, *D. citri* can transmit '*Ca*. L. *americanus*' (Teixeira *et al*., 2005) and '*Ca*. L. *asiaticus*' (Bové, 2006), and *T. erytreae* can transmit '*Ca*. L. *africanus*' (Bové, 2006).

In Asia, *C*Las has been detected in *Diaphorina communis* (Donovan *et al*., 2012) and *Cacopsylla citrisuga* (Cen *et al*., 2012). Transmission of the pathogen by *C. citrisuga* in young lemon plants has been shown under experimental conditions (Cen, pers. comm. 2019). However, transmission of the pathogen by the two psyllids has not been reported under natural conditions and therefore the two species are not covered in this Standard.

1.2.1. Diaphorina citri

Diaphorina citri, commonly known as Asian citrus psyllid, is on the EPPO A1 List. It is regulated in several EPPO countries, including those of the EU (EU, 2000), and is listed as a quarantine pest in Israel, Jordan and Uzbekistan and Turkey (EPPO, 2019).

Diaphorina citri is absent from the EPPO region. The species is found throughout Asia from Saudi Arabia to Japan (EPPO, 2019). In Africa, the species is present with a restricted distribution in Kenya and Tanzania and is recorded as present in Mauritius and Réunion (EPPO, 2019). In the Oceania region, the species is present in Guam and the Northern Mariana Islands, and present with a restricted distribution in Papua New Guinea (EPPO, 2019). In North America, it is recorded in Mexico and the USA (EPPO, 2019). It has been recorded in the Caribbean in Puerto Rico, Bahamas, Cayman Islands, Cuba, Jamaica, Dominican Republic, Guadeloupe, the Virgin Islands, Martinique and Dominica. It has been reported from some countries in Central America (Belize, Costa Rica) and South America (Brazil, Colombia, Paraguay, Uruguay, Venezuela)

1.2.2. Trioza erytreae

Trioza erytreae, commonly known as African citrus psyllid, is on the EPPO A2 List. It is regulated in several EPPO countries, including those of the EU (EU, 2000), and is listed as a quarantine pest in Israel and Turkey (EPPO, 2019).

In the EPPO region, *T. erytreae* has been recorded in Portugal, where it was first recorded on the island of Madeira in 1994 (EPPO, 2011). Between 1994 and 1998, the psyllid was contained in part of the island, however, since 1999 the pest spread and since 2008 it has been reported across the whole island. In mainland Portugal, *T. erytreae* was first reported in the region of Porto in 2015 (EPPO, 2015). The situation of *T. erytreae* in Portugal can be described as: present in part of the territory, under containment. It is restricted mainly to coastal areas, particularly in the north and central regions, and in outer areas around Lisbon, and it is not present in the main commercial citrus-growing and propagating regions, and is under official control. Measures are being taken to prevent its spread. In Spain, *T. erytreae* is present with a restricted distribution and under official control. *T. erytreae* was recorded in 2002 in the Canary Islands of Tenerife, La Gomera, La Palma and El Hierro (Spain) (EPPO, 2004, citing Hernández, 2003). *T. erytreae* was also reported by the NPPO of Spain in 2015 with an outbreak detected on the mainland in the province of Pontevedra (Galicia region) in 2014. The species is also present on the continent of Africa (see EPPO Global Database for a full list of countries) and also Madagascar, Mauritius, Saint Helena, Saudi Arabia and Yemen (EPPO, 2019).

1.3. Lifecycle

This section includes an overview of the lifecycle of both the pathogen and the vectors, and it is not intended to be exhaustive. For a more in-depth review of the lifecycle of the vectors refer to Cocuzza Massimino *et al*. (2017) and Hall (2008). For a detailed description of the taxonomy of the vectors see EPPO (2005a,b) and Ministerio de Agricultura (2015, 2016).

1.3.1. The pathogen in the vectors

The bacteria can be acquired at the nymph stage of the psyllid and can be transmitted throughout its lifespan.

Mann *et al*. (2011) indicated that '*Ca*. L. *asiaticus'* can be sexually transmitted from a '*Ca*. L. *asiaticus'* infected male *D. citri* to uninfected females at a low rate (4%) during mating. However, this was not seen where mating was between an infected female and an uninfected male. The pathogen is persistent within the vectors and may multiply within them. Furthermore, there is also evidence of transovarial transmission of '*Ca*. L. *asiaticus'* by *D. citri*, though again at a low rate of 2–6% {Pelz-Stelinski *et al*., 2010).

1.3.2. Diaphorina citri

Diaphorina citri is tolerant to high temperatures (Bové, 2006). The species is multivoltine and has five nymphal stages. Females usually live for 31–32 days (Nava *et al*., 2007) and are highly fecund, producing up to 800 eggs during their lives (Rogers & Stansly, 2007). The time from egg to adult can be between 14 and 48 days. Eggs are laid individually inside the developing buds or in leaf axils or in young tender parts of the tree (Catling, 1970; Pande, 1971, as cited in Australian Government, 2010). YingHong & Tsai (2000) detail that the optimum range of temperatures for population growth is 25–28°C. Adults overwinter and can live for up to 6 months in mild (temperate) climates (Shivankar *et al*., 2000, citing Mangat, 1966).

1.3.3. Trioza erytreae

Trioza erytreae is multivoltine and has five nymphal stages (Cocuzza Massimino *et al*., 2017). Females can lay more than 1300 eggs and can live for 28–48 days (Cocuzza Massimino *et al*., 2017). The time from egg to adult can be between 11 and 43 days. The temperature threshold for nymphal development is approximately 10–12°C and below this range the nymphal development is retarded.

1.4. Symptoms

1.4.1. The pathogen

Huanglongbing symptoms are the same for each '*Candidatus* Liberibacter' species and can eventually be observed in most *Citrus* species. 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). Other genera of Rutaceae may show symptoms after a latent period or may host the pathogen without showing symptoms. Positive test results may be obtained from trees without symptoms of Huanglongbing. However, EPPO (2014b) details that detection in symptomless plants can be very erratic.

The main symptoms are observed in leaves, shoots and fruits. The text below has been updated from the text detailed in EPPO (2014b).

1.4.1.1. Yellow shoots and blotchy mottle leaves. 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 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).

1.4.1.2. Fruit disorders. In diseased trees Huanglongbing induces very characteristic symptoms on fruits. Symptomatic fruits are small, asymmetric and lopsided, with a bent fruit axis. At the time the fruit changes colour, from green to yellow/orange, those from affected shoots show colour inversion: the peduncular end of the fruit turns yellow/ orange, while the stylar end is still green, as opposed to normal fruit colouration. In addition, when the peduncle of a fruit with colour inversion is carefully removed, the resulting circular scar is stained orange, while on a normal fruit the scar is pale green. When fruits are cut in half, perpendicular to the fruit axis, small, brownish/black aborted seeds can be observed, but this symptom can also be present in citrus stubborn disease affected fruits. Cutting a lopsided fruit through the fruit axis reveals its asymmetry and some aborted seeds can also be found. In addition, the vascular bundles within the fruit axis at the peduncular end have a strong brownish stain. The pith (albedo) is sometimes thicker at the peduncular end than at the stylar end. In advanced stages of infection, fruit falls prematurely. The taste of the fruit may also change to a salty or acidic flavour.

Appendix 1 provides images of symptoms of the disease.

1.4.2. Diaphorina citri

Diaphorina citri damages the host by feeding activity. The psyllids can cause leaf distortion, defoliation and die-back, and when a host is heavily infested new shoot growth is abnormal and susceptible to breaking off (EPPO, 2005b; Grafton-Cardwell *et al*., 2006). Honeydew is produced through waxy tubules as a by-product of feeding and covers the aerial parts of the plant, which in turn promotes the growth of sooty mould (Grafton-Cardwell *et al*., 2006).

1.4.3. Trioza erytreae

Trioza erytreae can cause severe leaf distortion, curling, stunting, galling and chlorosis (EPPO, 2015). Nymphs

produce cup-shaped open galls on the abaxial surface (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).

Appendix 2 provides images of the vectors and their symptoms.

1.5. Host plants

All species and cultivars of *Citrus* and some other species of Rutaceae are susceptible to the three Huanglongbing '*Candidatus* Liberibacter' species (see Appendix 3). The family Rutaceae is a major group of angiosperms with over 160 genera. The known hosts of the pathogen should also be combined for survey and inspection purposes with other potential hosts (e.g. other species present in an area which belong to the family Rutaceae). A list of known host plants can be found in EPPO (2019) and EFSA (2019). In addition, users should refer to the current literature (Table 1).

The vectors have varying preference for host plants and the following text details the main host plants for each psyllid species (EPPO, 2019).

Diaphorina citri
Cultivated citrus taxa (*Citrus aurantiifolia*, *Citrus aurantium*, *Citrus limon*, *Citrus medica, Citrus paradise*, *Citrus reticulata*, *Citrus sinensis*, etc.) or intergeneric hybrids (x *Citroncirus,* x *Citrifortunella*), *Fortunella* spp., *Murraya paniculata*, *Murraya koenigii*, *Poncirus trifoliata*, *Serverinia buxifolia* and other species of Rutaceae (see Appendix 3).

Incidental hosts: *Ficus carica*

Trioza erytreae
Cultivated citrus taxa (*Citrus aurantiifolia*, *Citrus limon*) or intergeneric hybrids (x *Citrifortunella*), *Choisya* spp., *Fortunella* spp., *Poncirus* complex, other species of Rutaceae (see Appendix 3).

1.6. Spread

1.6.1. Natural spread
The natural spread of the pathogen is primarily via infectious specimens of the two psyllid vectors *D. citri* and *T. erytreae*. In general, the dispersal behaviour of an insect species comprises two types of behaviour: (a) short-range dispersal, which is very common, and (b) long-range dispersal, which is usually a rare event (e.g. passive movement by strong winds). A typical feature of this stratified dispersal behaviour is that most individuals disperse short distances, while some disperse very long distances (Liebhold & Tobin, 2008). For *D. citri*, it has been demonstrated with recapture studies that most adults do not disperse very far and are captured in the neighbouring trees. However, some adults were captured on traps between 30 and 100 m from citrus trees every month of the year (Hall & Hentz, 2011). In another study in Florida (US), long-distance dispersal was shown where *D. citri* is capable of dispersing at least 2 km within a 12-day period (Lewis-Rosenblum *et al*., 2015). If environmental conditions deteriorate (e.g. no flushing plants are available) psyllids seem to engage more in long-range dispersal (Lewis-Rosenblum *et al*., 2015).

For *T. erytreae,* research carried out in South Africa has shown that the dispersal ability of the adults can be up to 1.5 km within a 7-day period (van den Berg & Deacon, 1988). In this study, where adults were released in an environment without suitable hosts, the long-range dispersal behaviour was hampered by mortality (desiccation).

1.6.2. Human-assisted spread
Long-distance dispersal of the pathogen and the vectors (including infectious vectors) can occur with the movement of host plant material. The vector *D. citri* has been shown to survive up to 20–30 days on picked fruit with stems and leaves attached (Hall & McCollum, 2011). Fruit which has gone through a packing house process in which it has been brushed and washed, and leaves and stems removed is not considered to pose a risk. The illegal introduction of plant material can lead to the spread of infection.

1.7. Definitions

- An orchard is defined as a unit of commercial citrus production under one owner or manager.
- A block is defined as a subdivision of an orchard cultivated as a unit and usually of uniform age and variety.
- The infested area is defined as the area which includes all confirmed infected trees and is delimited based on all available information and expert judgement. In the early stages of an investigation, this may be limited to single trees or groups of trees from which samples have tested positive.
- Host material is defined as any plants for planting of Rutaceae (other than seeds), leaves and branches of Rutaceae, and any citrus fruit which has not been subject to a packing house process in which it has been brushed and washed, and leaves and stems removed.

2. Outline of the system

A national regulatory control system is recommended to all EPPO countries for the surveillance, early detection and eradication of the pathogen and its vectors, and for containment measures to prevent spread during eradication. Consideration should be given to reinforcing import controls and possible prohibition of import of plants for planting and green parts of host plants of the family Rutaceae to prevent the risk of entry.

Elements of this Standard are also relevant to a long-term containment strategy when eradication is no longer a feasible objective.

The described system provides sufficient guarantees to allow movement of some host commodities within and out of the demarcated areas subject to defined conditions.

The Standard describes the surveys which should be carried out on an ongoing basis in high-risk areas for the early detection of new outbreaks of the pathogen or its vectors and for monitoring spread.

All EPPO countries where citrus species are commercially produced and where conditions are suitable for the establishment of the pathogen and its vectors are encouraged to develop and test a contingency plan for outbreaks. NPPOs are advised to draw on the guidance in this Standard and in EPPO Standards PM 9/10 *Generic elements for contingency plans* (EPPO, 2009) and PM 9/18 *Decision-Support Scheme for prioritizing action during outbreaks*, and on recent experience with the pest in the EPPO region and elsewhere.

The contingency plan should take account of the distribution of host plants in commercial citrus orchards and private gardens, climate and weather effects, citrus transportation routes, vectors present in the country, and the commercial practices and socio-economic structure of the citrus-producing industry (California Department of Food and Agriculture, 2018). In larger countries, different contingency plans or adaptations of national plans may be needed for different regions[1] and circumstances. Publicity material and communication plans should be prepared as part of the contingency plan.

Publicity should be carried out to raise awareness of the disease in advance of its arrival among citrus growers and other key stakeholders. This is aimed at educating stakeholders on the identification of the pathogen and its vectors and their symptoms, which will facilitate early detection and reporting.

In the event of a first finding in an area, the communication plan should be activated to explain the risks posed by the pathogen and its vectors to citrus producers and members of the public, and the measures which might have to be taken. In the event of an outbreak of the pest, commercial producers of citrus near the boundary of the demarcated areas should be encouraged to take simple hygiene precautions to avoid inadvertently moving vectors, as well as complying with any measures in place against moving the psyllid on specified commodities.

The Standard recommends, for findings of the pathogen or vectors in a number of different scenarios, increased surveillance, demarcation of infected areas and buffer zones, eradication measures and containment measures to prevent spread. For findings of the vector with or without the pathogen, a demarcated area of at least 3 km radius from the detection point or infested area is recommended. For findings of the pathogen where trapping indicates that the vectors are absent, a radius of at least 500 m is recommended. The 500 m radius is based a scientific evaluation from California (US) where 95% of the subsequent Huanglongbing-infected trees were detected within a 400 m delimitation survey area.

No records of successful eradication of Huanglongbing where vectors were present were identified by the Expert Working Group when compiling this Standard. In addition to surveillance to ensure early detection, three actions are necessary to achieve the possibility of eradicating outbreaks

(a) removal of infected host plants
(b) elimination of vectors
(c) restriction of the movement of host plants within and from the demarcated area.

The suggested radii for the demarcated areas detailed in this Standard are based on the expert opinions of the Expert Working Group. Not all scenarios are detailed; for example the occurrence of *D. citri* in an area where *T. erytreae* is already present. However, the guidance should be readily adapted to such scenarios.

A number of spread models have been developed in recent years. Some spread models may be used to justify variation from the radii set out above for demarcated and infected/infested zones. Any such variation should be technically justified, including a published description of the model and the parameters used. In addition, EPPO is developing a document on buffer zones which can be used to establish limits under various scenarios.

No findings over at least three growing seasons of monitoring and sampling in the regulated area can be considered as evidence of successful eradication of the pathogen. No findings over at least four growing seasons of monitoring and sampling in the regulated area can be considered as evidence of successful eradication of the vector.

3. Surveillance

Effective and regular surveillance for both vectors and pathogen is a key factor in enabling early detection and prompt eradication measures. The approach to surveillance will vary depending on whether or not vectors are known to occur. In citrus-growing areas, inspectors, industry experts and workers should be trained to recognize vectors, symptoms of vector feeding, symptoms of Huanglongbing and host plants which occur in the region. This may be done through materials developed in regions of the world where Huanglongbing occurs. Onsite training in such regions is recommended to ensure that at least a few experts in each citrus-growing country in the EPPO region have field experience of seeing symptoms.

Countries should have access to laboratories with trained diagnosticians, experienced and competent in the identification of the two vector species and the pathogen. EPPO Diagnostic Protocols have been developed for the pathogens and the vectors (EPPO, 2005a,b, 2014b).

A database should be available at national or subnational level to hold and communicate to those who need it within the relevant official services information on survey sites, inspections, samples and laboratory test results. This database should

[1]Subnational regions.

have the capability to reliably receive, store and report information required during an outbreak situation, and this capability should be tested as part of the contingency plan.

Surveillance in an area where neither the pathogen nor vector(s) are known to occur

In an area where the pathogen and the vector(s) have not been detected, surveillance is based on:
- carrying out a specific detection survey (according to ISPM 6) (IPPC, 1997)
- raising awareness.

Surveillance in an area where either the pathogen or vector(s) are known to occur

In an area where the pathogen or vector(s) have been confirmed to occur, surveillance is based on:
- carrying out a specific delimiting survey (according to ISPM 6)
- raising awareness.

3.1. Detection surveys

Early detection of new outbreaks is essential in determining the likelihood of eradication. In areas where citrus plants are grown outside (e.g. commercial citrus orchards), detection surveys should be carried out on an ongoing basis for the presence of the vector and for symptoms on host plants, focussing on high-risk sites and their vicinity (see below). Surveys may be combined with surveys for other pests for best use of resources. The detection surveys should be based on visual inspection of host plants at three or four appropriate times of the year (where surveys are implemented at equal times throughout the year, for example every 3 or 4 months) and on continued visual inspection and trapping for the vectors throughout the year. Laboratory testing of host material showing symptoms should be conducted even in the absence of the vectors.

High-risk sites include sites where host material has been introduced from areas where the vectors or pathogen occur. This may have occurred before implementation of measures to prevent such introduction or as a result of contravention of measures. High-risk sites include sites in or near to:
- areas where either species of vector or the pathogen occur
- sites with a history of imports of ornamental host plants (plants for planting)[2]
- citrus orchards with a history of imported planting material[2]
- urban areas near commercial citrus production areas
- citrus packing houses and processing facilities handling bulk imports[2]
- citrus production nurseries, retail nurseries and garden centres
- airports, ports and ferry ports
- distribution centres managing local citrus production
- farmers' markets and other sites where plants or fruit with leaves may be traded.

For a selected survey site:

Inspection for Huanglongbing symptoms and sample collection

All members of the plant family Rutaceae at the site should be identified (see Appendix 3). All hosts at the site should be inspected for Huanglongbing symptoms (see Section 1.4 and Appendix 1) and/or for symptoms of psyllid feeding (see Sections 1.4.2 and 1.4.3, and Appendix 2). The most common symptom of Huanglongbing is the blotchy mottle on the leaves (which occurs on all host varieties). The symptoms are better observed in the interior part of the canopy where sun is less likely to obscure the symptoms. Symptoms of psyllid feeding are particularly distinctive in the case of *T. erytreae*.

Plant material should be collected from all hosts displaying symptoms of Huanglongbing or of vector feeding. If typical symptoms are present, 5–10 symptomatic leaves should be collected (EPPO, 2014b). If symptoms of feeding by vectors are present, a sample should be sent for laboratory examination, including testing for the pathogen. A sample including suspected vectors should be sealed to prevent any spreading.

Fruit with symptoms should also be collected and sent for analysis.

Inspection for the vector(s) and sample collection

All members of the plant family Rutaceae at the site should be identified (see Appendix 3). At commercial production sites, each host tree selected for visual inspection should be visually inspected for vector(s) by sectioning the tree into quadrants. Each quadrant should be inspected for all vector life stages (adults, nymphs and eggs). For non-commercial hosts, all parts of the plant should be surveyed with a similar systematic method. A sample from all detected life stages should be collected.

Sample of plants should be subjected to a systematic examination in order to detect the presence or signs of pests at commercial production sites. The sample should be selected in accordance with an appropriate statistical method (e.g. ISPM 31[3]). For the purpose of visual inspection, a lot should be defined as a number of plants which are identifiable as being the same variety or clone, with propagating material from the same origin, cultivated in the same field and treated in the same way and at the same time.

Testing vectors for the pathogen from samples caught in sticky traps is not recommended. Vectors should instead be

[2]Where permitted, or where illegal import has been suspected.

[3]Although ISPM 31 is about consignments, the methods can be used for this purpose.

caught by aspirating (or netting or tapping) the adults or by brushing the nymphs. The specimens, both adults and nymphs, should be conserved in alcohol and sent to the laboratory for testing for the pathogen. Live psyllids should not be removed from the infested area or from the demarcated area, either in isolation or on samples of plant material.

Adult psyllids collected from a survey site can be pooled together in a collection tube (since they fly, and a positive detection does not prove infection of a particular tree). Nymphs from different trees should be collected individually since a positive detection in a nymph is good evidence for the presence of the pathogen in the tree from which the nymph was taken.

Inspectors should sample as many vectors as possible. The maximum sub-sample size for laboratory testing is 25 adults or 75 nymphs.

If there is a high population of the vector(s) on host(s), plant material for testing for latent infection should be collected, even in the absence of symptoms.

Trapping of vectors

In citrus orchards and the surrounding area, yellow sticky traps should be placed in host plants at a height of 1–2 m in the upper canopy towards the outer edges. In nurseries, traps should be placed 1 m above the ground near the plants. Traps should be checked every 14 days and replaced when necessary. The traps should be checked by a suitably trained inspector and if the distinctive features of *T. erytreae* or *D. citri* are present (see EPPO 2005a,b), those individuals should be sent to the laboratory for confirmation. Alternatively, all traps may be sent directly to the laboratory for examination. As an intermediate step, images of the suspect insect may be recorded and sent to an entomologist. First detections in a new area should always be confirmed by an entomologist from the official laboratory.

As the vectors do not have a diapause phase (EPPO/CABI, 1996), traps can be placed in the field all year round. Trap monitoring should be intensified in spring and summer months when the psyllid populations would likely reach the highest levels in the EPPO region and specimens are most active during the host flushing.

In an intensive citrus-growing region without additional risk factors (e.g. adjoining areas where the vector is present), the placement and checking every 2 weeks of at least one trap per 200 ha or at least 100 traps for each citrus-growing region (whichever is the larger figure) will provide an acceptable degree of confidence in the absence of the vectors. A higher level of trapping will increase the possibility of early detection of the presence of the vectors.

Traps should be placed along the perimeter of the areas of contiguous citrus production, with a higher level of traps along the edges facing urban areas, transport routes or other high-risk sites (see above).

Urban areas present a particularly high risk if there are host plants of uncontrolled origin in back gardens. Traps may be placed on or near easily accessible host plants in urban areas or sentinel tree sites may be established by identifying trees of highly susceptible varieties (e.g. lemons) which can be inspected regularly. In high-risk urban areas adjacent to commercial citrus production, traps or sentinel sites should be monitored more regularly. Trap density for urban areas should be assessed at a local scale as density of traps will depend on the occurrence of host plants. Higher densities of traps or sentinel plants and higher frequency of inspection will increase the likelihood of early detection.

Because they present an inherent risk of spreading the vector or pathogen, surveillance should be more intensive around nurseries and garden centres with host plants. At least one trap or sentinel plant should be monitored per nursery or garden centre. The staff of these nurseries and garden centres should be appropriately trained for monitoring for presence of the vectors and pathogens, and informed of the risks.

3.2. Detection in a new area

If a finding of the pathogen or a vector is officially confirmed in an area where neither was previously known to occur, immediate action should be taken to prevent spread from the detection site and then to determine which of the following scenarios is faced and the extent of infestation.

The boundaries of the infested area and the buffer zone (which together form the demarcated area) should be established, taking into account the following factors, and should be revised as these factors change:

- the estimated distribution and population level of the pest and its vectors
- the suppression measures being taken
- the distribution of suitable habitat within the area
- natural barriers to the spread of the vectors
- the intensity of surveillance within the buffer zone and outside the buffer zone.

Within the demarcated area, the following measures should be implemented:

- containment measures to prevent spread (see Section 6)
- a delimiting survey (see Section 3.5)
- a communication campaign (see Section 4)
- eradication measures (see Section 5)
- monitoring surveys (Section 3.6).

3.3. Obligation to report findings

An official requirement to report findings along with a communication procedure should be in place so that each finding or suspicion of the pathogen or vectors is immediately reported to the NPPO.

3.4. Public awareness

Adult vectors can be distinguished from other species by some well-informed stakeholders such as growers and

amateur entomologists. The symptoms of *T. erytreae* are also quite distinctive. Raising awareness among these groups may help to ensure early detection of an outbreak.

Awareness activities should especially target those trading plants and plant products, agencies and stakeholders working with high-risk areas (see above). This is very important for improved compliance, early detection and reduced spread. Awareness rising can be achieved, for example, via the internet and mobile apps, and through workshops or local shows involving land managers, growers, municipalities, botanical gardens, arboriculturalists, landscape contractors, gardeners, entomologists, etc.

Fact sheets should be provided to aid the detection and identification of the pathogen and its vectors. Appendices 1 and 2 provides text and pictures which may be used by NPPOs in producing such factsheets.

3.5. Delimiting survey

The area of the delimiting survey may vary depending on the location and nature of the first finding. Factors to consider include the nature of the finding (pathogen, vector or both), the intensity of citrus production in the region, the presence of citrus-producing nurseries, the density of host plants in the region and the presence of residential areas.

Three scenarios are considered for detection in a new area (detailed below).

3.5.1. Scenario 1: Detection of the pathogen in an area where the vector(s) are not known to occur

If the pathogen is detected without the vector(s), steps should be taken to identify the source of the infection, including trace back and trace forward of related host plants. It should be noted that this current scenario (detecting the pathogen without the vector(s)) has not been recorded in the literature. This scenario specifically addresses the potential movement of an infected plant.

If the finding is within a citrus orchard or nursery an evaluation of the risk factors and pathways should be conducted (e.g. the potential of spread due to grafting of plant material). Whether or not the origin can be clearly ascertained a demarcated area comprising the infested or probable infested zone plus a buffer zone of at least 3 km radius should be established.

In this area, inspection should be conducted to detect potential additional infected plants and vectors following the methodology described in Section 3.2. If no symptoms of the pathogen are seen, samples should be selected in accordance with an appropriate statistical method (e.g. ISPM 31).

In addition, to confirm the absence of the vector, a wider survey should be conducted within a 3 km radius of the infested area and this should continue for at least 1 year and include the full growing period of the host plants.

If no vectors are found, the demarcated area may be reduced to 500 m.

If the origin of the infection with planting material can be clearly identified, and intensive trapping confirms absence of the vector, the demarcated area can be lifted as soon as the infected plants are destroyed.

If the finding is in a host plant other than in a citrus orchard or nursery, a demarcated zone of at least 500 m radius should be established around the infested area. In this zone, all host plants should be inspected and tested.

In any scenario, when the pathogen is found the intensity of trapping should be increased to provide more confidence that a vector is not present.

3.5.2. Scenario 2: Detection of the vector(s) without the detection of the pathogen

Trace back and trace forward should be carried out in case the vector has recently arrived with an associated plant.

A survey should be conducted (see Section 3.1) and the following should be recorded where there is likely to be a locally established population of the vectors, and if the populations are localized or scattered. The NPPO should demarcate a zone comprising the infested or probable infested zone plus a buffer zone of at least 3 km radius. An intensive survey (where all known host plants are surveyed for adult and nymphs) should be conducted within a 1 km radius of the infested zone. In commercial citrus orchards and in urban areas 10 traps per square kilometre should be used for delimitation of the infested area.

In many areas of the world, the first detection of vectors has been followed after a few years by detection of the pathogen. Surveillance for the pathogen should therefore be intensified in the demarcated area, including trapping and testing of the vectors. Testing of vectors from sticky traps is not recommended. Vectors should instead be caught by aspirating (or netting or tapping) of adults or brushing of nymphs, conserved in alcohol and then sent to the laboratory for testing for the pathogen. If nymphs test positive for the pathogen, the host on which they were collected should be considered infected. Thus, careful recording of samples collected should be maintained from the field to the laboratory. Infected plants should be tagged, and their position should be recorded (using GPS) so infected plants can be revisited and treated. Live psyllids should not be removed from the infested area or from the demarcated area, either in isolation or on samples of plant material.

In areas neighbouring the demarcated area, surveillance should be increased in response to the finding, in line with the relevant risk factors described above.

3.5.3. Scenario 3: Detection of the pathogen and the vector(s)

If both the disease and the vector(s) are detected, the delimiting survey should combine the measures in Scenarios 1 and 2.

If nymphs test positive for the pathogen, the host on which they were collected should be considered infected. Thus, careful recording of samples collected should be

maintained from the field to the laboratory. Infected plants should be tagged, and their position should be recorded (using GPS) so infected plants can be revisited and destroyed.

If the only findings of the pathogen are in adult vectors, the origin of the infection should be sought through intensive surveillance of host plants within a radius of at least 1 km. If no infected plants can be found, the infected area should be demarcated based on the location at which the vector was trapped with a buffer zone of 3 km.

3.6. Monitoring surveys within the infested area to assess effectiveness of measures

Monitoring surveys based on trapping and visual inspection for the vectors and inspections of host plants for symptoms of the pathogen or of vector feeding should be conducted within the infested area to determine the distribution and population levels of the pest. This information should also be used to target control measures and monitor their effectiveness.

4. Communications campaign in case of an outbreak

As soon as there is a finding of the pathogen or vectors in a new area, a communications campaign should be launched to explain the finding to industry stakeholders and the public, encourage reporting of symptoms and encourage compliance with the measures which need to be taken. (An EPPO Standard on raising public awareness is under development and may be adopted in 2019.) Communications professionals should be involved in the planning and execution of this plan and should be the initial contact point for media enquiries. Basic facts about the disease and the outbreak situation should be available in a question and answer format and should be regularly updated. An agreed situation report should be issued on a regular basis (daily, weekly or monthly depending on the circumstances).

All statements of a technical nature about the pathogen and vectors should be validated by relevant experts before publication. Selected senior technical experts should be given media training (preferably in advance, as part of contingency preparation) and these experts should be used for interviews and other media contacts.

Any uncertainties about the situation should be communicated clearly but should not delay the provision of information about what is known.

Within urban areas, publicity materials should be appropriate to the audience, which may have little understanding of plants and plant disease. Municipalities, schools, community groups and gardening societies may be able to offer advice and assistance on the best style, language and channels of communication. Statements from citrus growers explaining the possible impact of Huanglongbing on their livelihood may be used to reinforce the messages from official services. Public meetings in affected areas should be considered at an early stage.

5. Eradication measures

On suspicion that the pathogen or its vectors are present in an area previously recorded as being free, immediate action should be taken, pending laboratory confirmation. Both eradication and containment measures (see Section 6) should be conducted in combination to reduce the risk of spread. This should include covering of infested plants with nets, treatment of vectors and access restrictions to the outbreak site. Movement of host material should be prohibited on a provisional basis from the vicinity of the suspect finding and from an appropriate provisional buffer zone based on a rapid analysis of the potential distribution of the pest. The buffer zone should be at least 3 km if vectors are known to be present and at least 500 m if vectors are not known to occur.

Laboratory testing according to EPPO PM 7 (Diagnostic Protocols) should be performed urgently, and the following actions taken if presence of the pathogen or vectors is confirmed.

The relevant contingency plan (which may have been adopted at national, regional (subnational) or local level) should be put into action in accordance with EPPO Standard PM 9/10. The contingency plan should have allocated responsibilities and indicate who will provide which resources for the eradication campaign. Based on the contingency plan, an action plan should be drawn up to rapidly implement appropriate specific management structures, resources and communication plans for handling the emergency. To prioritise action during an outbreak, the NPPO should follow EPPO Standard PM 9/18 *Decision-support scheme for prioritizing action during outbreaks*.

The infested area and buffer zone (together forming the demarcated area) should be defined if this has not already been done. These should be adjusted in response to any new findings. Following the official confirmation of the pathogen or its vector(s) or both in an area, a combination of measures should be taken to reduce the infestation with a view to eradication.

5.1. Scenario 1: Detection of the pathogen in an area where the vector(s) were previously not known to occur

Action taken should depend on the results from the trace back and trace forward exercise carried out. Plants with direct clonal links or common origin to the infected tree should be tested for infection.

5.1.1. Nursery

In the case of a confirmed finding of the pathogen within a nursery, all plants in the lot and any clonally related lots should be destroyed. Other lots in the same place of production should be sampled and tested before release. The

sample should be selected in accordance with an appropriate statistical method (e.g. ISPM 31).

5.1.2. Orchards
Infected plants should be destroyed. If more than 20% of the total plants in the block are infected, all plants in the block should be destroyed (Spanish Royal Decree 23/2016).

5.1.3. Urban areas
In urban areas, including private gardens, all infected trees should be destroyed and removed.

5.1.4. Treatment and disposal of infected plants
For both nurseries and orchards, even in the absence of vectors, prior to removal of any plants authorized foliar insecticides should be applied to infected plants (see Appendix 4) and all host plants within 100 m radius of the confirmed positive. All infected plants should be destroyed. Where there may be a risk of root grafts, adjacent plants should also be destroyed.

After removal, infected plants should be disposed of by shredding and drying to make them unattractive to vectors, by burning or by deep burial on site. In all cases, national regulations on waste disposal should be observed. Strict containment practices should be in place if infected material is taken off site for destruction under official control. Infected plant material should be double-bagged and sealed prior to removal.

5.2. Scenario 2: Detection of the vector(s) without the detection of the pathogen

Following the identification of the vector(s) at either a nursery or an orchard, or in an urban area, eradication measures should be taken, including the application of authorized insecticides and the removal of infested material. At least two consecutive treatments should be carried out at an interval of 2–3 weeks, with alternating active ingredients. This will maximize the mortality of different life stages of the vector(s).

In a demarcated area, plants for planting must be grown in a vector-proof structure officially approved and regularly inspected by the NPPO (see Appendix 5).

Options for the control of vectors are presented in Appendix 4.

5.3. Scenario 3: Detection of the pathogen and the vector(s)

Following the detection of both '*Candidatus* Liberibacter' species and the vector(s) a combination of eradication measures should be implemented (see Scenarios 1 and 2).

5.3.1. Action to be implemented within a demarcated area
5.3.1.1. Nursery:
5.3.1.1.1. Outdoor host plants—Outdoors host plants should either be destroyed or moved into a vector-proof structure officially approved and regularly inspected by the NPPO. These plants should then be treated with an approved insecticide, and inspected and tested every month for infection following statistical sampling methods described in ISPM 31 or other appropriate methods. After a 2-year period, the plants may then be approved for movement and planting within the demarcated area only.

5.3.1.1.2. Plants in a vector-proof structure—Plants already growing within a vector-proof structure officially approved and regularly inspected by the NPPO and where the pathogen and the vectors have not been detected may be exempted from destruction. These plants should not be mixed with any plants which were previously growing outside and for which an exemption from destruction is applied under the previous paragraph. For these plants there would be no restriction on movement.

5.3.1.2. Citrus orchards. In a citrus orchard, if <2% of the total plants in a block are infected, all infected plants should be destroyed. If more than 2% of the total plants in a block are infested, consideration should be given to destroying all plants in the block (whether infested or not).

Declaration of eradication—Eradication may be declared and a pest-free area may be re-established if:
- for the pathogen: no findings over at least three growing seasons of monitoring and sampling in the regulated area can be considered as evidence of successful eradication.
- for the vector: no findings over at least four growing seasons of monitoring and sampling in the regulated area can be considered as evidence of successful eradication.
- if both of the above conditions are met, a pest-free area can be declared for both the pathogen and the vector following no findings over at least four growing seasons.

When the pathogen or vector is declared eradicated from part of a previously demarcated area, the infested area and buffer zone should be reviewed and amended accordingly.

Where the pathogen alone is found, its origin in imported planting material is clearly demonstrated, trapping indicates that the vectors are not present, eradication can be declared when all infested plants have been destroyed and a survey has shown that the area is free.

6. Containment[4] measures to prevent spread

When the presence of the pathogen is confirmed in an area with the presence of the vectors, an evaluation of pathways should be carried out in order to determine whether other areas have already been put at risk from those pathways and what appropriate measures should be taken to reduce the risk of further spread. Steps should be taken to identify the source of the infection, including trace back and trace forward of

[4]Application of phytosanitary measures in and around an infested area to prevent spread of a pest [IPPC, 2016].

related host plants. A survey should be carried out in any area which has been put at risk. If the area is in another country, the NPPO of that country should be informed. Containment measures should be taken in all cases to reduce the risk of spread, whether the strategy being followed is one of eradication or containment. Areas with high population densities of the pest should be identified within the entire infested area and specific monitoring and containment measures put in place to reduce the risk of passive dispersal (e.g. the movement of vectors on vehicles).

Measures taken within the demarcated zone to reduce the risk of spread should include:

- reduction of the population of vectors in infested areas (see Appendix 4)
- prohibition or restriction of the movement of host material from the demarcated area as a whole, and from the infested area into the buffer zone, with the following exceptions:
- plants for planting which have been grown throughout their lifetime in a vector-proof structure officially approved and regularly inspected by the NPPO and moved in sealed containers
- fruit (without leaves or petioles) from the demarcated area may be moved in sealed containers under official control for packing and processing outside the demarcated area. All of these activities should be conducted under official control.

7. Long-term containment strategy

If eradication is no longer considered to be feasible, a decision to move to a strategy of long-term containment may be taken by the NPPO and this may include increasing the size of the buffer zone, taking into account scientific and technical advice. Neighbouring countries and other stakeholders should be consulted or at least informed of this change of strategy. Containment measures should then continue to be applied as part of a long-term strategy for management of the pest risk across the EPPO region. These containment measures should include suppression of population levels within the infested/infected area to reduce the risk of active and passive spread, and demarcation and monitoring of a buffer zone. Measures to prevent human-assisted movement out of the infested area should be reinforced. Hygiene measures should be promoted to reduce risks from passive movement of vectors on vehicles, particularly those associated with the citrus industry. Prohibition on movement of host material out of the infected area, other than from pest-free places of production (see Appendix 5) or through appropriate quarantine facilities, should continue to be enforced.

8. Enquiries

Enquiries may be addressed to the EPPO Secretariat, 21 Boulevard Richard Lenoir, Paris 75011 (FR) or hq@eppo.int.

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References

Albrecht U & Bowman KD (2008) Gene expression in *Citrus sinensis* (L.) Osbeck following infection with the bacterial pathogen '*Candidatus* Liberibacter asiaticus' causing huanglongbing in Florida. *Plant Science* **175**, 291–306.

Ammar E, Shatters RG & Hall DG (2011) Localization of Candidatus Liberibacter asiaticus, associated with citrus Huanglongbing, in its psyllid vector using fluorescence in situ hybridization. *Journal of Phytopathology* **159**, 726–734.

Aubert B, Bové JM & Etienne J (1980) La lutte contre la maladie du greening des agrumes à l'ile de la Réunion. Résultats et perspectives. *Fruits* **35**, 605–624.

Australian Government (2010) *Draft Pest Risk Analysis Report for 'Candidatus Liberibacter species' and their Vectors Associated with Rutaceous Hosts*. Plant Biosecurity, Canberra (AU).

van den Berg MA & Deacon VE (1988) Dispersal of the citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae), in the absence of its host plant. *Phytophylactica* **20**, 361–368.

Bové JM (2006) Huanglongbing: a destructive, newly emerging, century-old disease of citrus. *Journal of Plant Pathology* **88**, 7–37.

California Department of Food and Agriculture (2018) Action plan for Asian citrus psyllid and Huanglongbing (Citrus greening) in California.

Catling HD (1970) Distribution of the psyllid vectors of citrus greening disease with notes on the biology and bionomics of *Diaphorina citri*. *FAO Plant Protection Bulletin* **18**, 8–15.

Cen Y, Zhang L, Xia Y, Guo J, Deng X, Zhou W *et al*. (2012) Detection of 'Candidatus Liberibacter Asiaticus' in Cacopsylla (Psylla) citrisuga (Hemiptera: Psyllidae). *Florida Entomologist* **95**, 304–311.

Cocuzza Massimino G, Urbaneja A, Hernandez E, Siverio F, Di Silvestro S, Tena A *et al*. (2017) A review of *Trioza erytreae* (African citrus psyllid), now in mainland Europe, and its potential risk as vector of huanglongbing (HLB) in citrus. *Journal of Pest Science* **90**, 1–17.

Donovan NJ, Beattie GAC, Chambers GA, Holford P, Englezou A, Hardy S *et al*. (2012) First report of Candidatus Liberibacter asiaticus in Diaphorina communis. *Australasian Plant Disease Notes* **7**, 1–4.

Duran-Vila N, Janse JD, Foissac X, Melgarejo P & Bové JM (2014) Addressing the threat of hunglongbing in the Mediterranean region: a challenge to save the citrus industry. *Journal of Plant Pathology* **96**, S.43–S.48.

EFSA (2019) Pest survey card on Huanglongbing and its vectors. *EFSA Supporting Publications* **16**, https://doi.org/10.2903/sp.efsa.2019.en-1574

EPPO (2004) First report of Trioza erytreae in Islas Canarias (Spain) EPPO Reporting Service no. 04 – 2004 Num. article: 2004/059.

EPPO (2005a) PM 7/57(1) *Trioza erytreae*. *EPPO Bulletin* **35**, 357–360.

EPPO (2005b) *Diaphorina citri*. *EPPO Bulletin* **35**, 331–333.

EPPO (2009) PM 9/10 (1) Generic elements for contingency plans. *EPPO Bulletin* **39**, 471–474.

EPPO (2011) Situation of Trioza erytreae in Madeira (PT) EPPO Reporting Service no. 06 – 2011 Num. Article: 2011/139.

EPPO (2014a) *PM 9/18 (1)* Decision-Support Scheme for prioritizing action during outbreaks. *EPPO Bulletin* **44**, 443–456.

EPPO (2014b) PM 7/121 (1) 'Candidatus *Liberibacter africanus*', 'Candidatus *Liberibacter americanus*' and 'Candidatus *Liberibacter asiaticus*'. *EPPO Bulletin* **44**, 376–389.

EPPO (2015) Updated situation of *Trioza erytreae* in Spain, EPPO Reporting Service no. 11 – 2015. Num. article: 2015/205.

EPPO (2019) EPPO Global Database. https://gd.eppo.int/ [accessed on 1 November 2019]

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

Etienne J, Qullici S, Marival D & Franck A (2001) Biological control of *Diaphorina citri* (Hemiptera: Psyllidae) in Guadeloupe by imported *Tamarixia radiata* (Hymenoptera: Eulophidae). *Fruits* **56**, 307–315.

EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Union* **L169** (10.07.2000, consolidated version of 30.06.2014), 1–181.

EU (2016) Final report of an audit carried out in Spain from 17 October 2016 to 28 October 2016 in order to evaluate the situation and control for Trioza erytreae and Tecia solanivora. Final report of an audit carried out in Spain, DG (SNTA) 2016-8797-MR

Ghosh SK, Giannotti J & Luis C (1977) Multiplication antibodies against the bacterial-like organism associated with citrus greening disease. *Annales de I Institut Pasteur/Microbiologies* **138**, 639–650.

Gomez H (2009) Screening aid for Huanglongbing (HLB) or citrus greening disease symptoms. http://download.ceris.purdue.edu/file/526 [accessed on 1 October 2018]

Grafton-Cardwell EE, Godfrey KE, Rogers ME, Childers CC & Stabsly PA (2006) *Publication 8205*. University of California, Division of Agriculture and Natural Resources, Exotic and Invasive Pests and Diseases Research Program.

Graham J, Johnson EG, Bright DB & Irey MS (2011) Lack of development of huanglongbing in seedlings from citrus seed. *Proceedings of the Florida State Horticultural Society* **124**, 65–68.

Hall DG (2008) *Biological control of Diaphorina citri*. Hermosillo, Sonora (MX).

Hall DG & Hentz MG (2011) Seasonal flight activity by the Asian citrus psyllid in east central Florida. *Entomologia* **139**, 75–85.

Hall DG & McCollum G (2011) Survival of adult Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), on harvested citrus fruit and leaves. *Florida Entomologist* **94**, 1094–1096.

Hartung JS, Halbert SE, Pelz-Stelinksi K, Brlansky RH, Chen C & Gmitter FG (2010) Lack of evidence for transmission of 'Candidatus Liberibacter asiaticus' through citrus seed taken from affected fruit. *Plant Disease.* **94**, 1200–1205.

Hernández AG (2003) Trioza erytreae (Del Guercio 1918): nueva plaga de los cítricos en Canarias. *Phytoma España: La revista profesional de sanidad vegetal*, **153**, 112–118.

Hilf ME (2011) Colonization of citrus seed coats by 'Candidatus Liberibacter asiaticus': implications for seed transmission of the bacterium. *Phytopathology* **101**, 1242–1250.

Hilf ME & Lewis RS (2016) Transmission and propagation of 'Candidatus Liberibacter asiaticus' by grafting with individual citrus leaves. *Bacteriology* **5**, 452–458.

IPPC (1997) *ISPM 6 Guidelines for Surveillance*. FAO, Rome (IT).

IPPC (1999) *ISPM 10 Requirements for the Establishment of Pest Free Places of Production and Pest Free Production Sites*. FAO, Rome (IT).

IPPC (2016) *ISPM 5 Glossary of Phytosanitary Terms*. FAO, Rome (IT).

IPPC (2017) FAO Citrus fruit. Fresh and processed, statistical Bulletin, 2016. http://www.fao.org/3/a-i8092e.pdf [accessed on 24 May 2018]

Lee JA, Halbert SE, Dawson WO, Robertson C, Keesling JE & Singer BH (2015) Asymptomatic spread of huanglongbing and implications for disease control. *Proceedings of the National Academy of Sciences USA* **112**, 7605–7610.

Lewis-Rosenblum H, Martini X, Tiwari S & Stenlinski LL (2015) Seasonal movement patterns and long-range dispersal of Asian citrus psyllid in Florida citrus. *Journal of Economic Entomology* **108**, 3–10.

Liebhold AM & Tobin PC (2008) Population ecology of insect invasions and their management. *Annual Review of Entomology* **53**, 387–408.

Mann RS, Pelz-Stellnski P, Hermann SL, Tiwari S & Stellinski LL (2011) Sexual transmission of a plant pathogenic bacterium, Candidatus Liberibacter asiaticus, between conspecific insect vectors during mating. *PLoS ONE* **6**, e29197.

Mangat BS (1966) Biology and control of citrus psylla *Diaphorina citri* Kuw. *PIant Protection Bulletin, New Delhi* **18**, 18–20.

Ministerio de agricultura (2015) Plan de contingencia de Diaphorina citru Kuwayana. https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/pnc_diaphorina_julio_2015_tcm30-512380.pdf. [accessed 25 May 2018].

Ministerio de agricultura (2016) Plan de contingencia de Trioza erytreae (Del Guercio). https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/pnctrioza-octubre2016_tcm30-512379.pdf [accessed 25 May 2018].

Nava DE, Torres MLG, Rodrigues MDL, Bento JMS & Parra JRP (2007) Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. *Journal of Applied Entomology* **131**, 709–7015.

Pande YD (1971) Biology of citrus psylla, *Diaphorina citri* Kuw. (Hemiptera: Psyllidae). *Israel Journal of Entomology* **6**, 307–311.

Pelz-Stelinski KS, Brlansky RH, Ebert TA & Rogers ME (2010) Transmission parameters for Candidatus Liberibacter asiaticus by Asian Citrus psyllid (Hemiptera: Psyllidae). *Journal of Economic Entomology* **103**, 1531–1541.

Rogers ME & Stansly PA (2007) Biology and management of the Asian citrus psyllid, Diaphorina citri Kuwayama, in Florida citrus. University of Florida, IFAS Extension, ENY-739.

Shatters RG (2008) Detection of 'Candidatus Liberibacter asiaticus' in citrus seedlings germinated from Florida seed, p. 198. In: Proceedings of the International Research Conference on Huanglongbing. Gottwald TR & Graham JH, eds. http://www.plantmanagementnetwork.org/proceedings/irchlb/2008/ [accessed on 31 July 2018]

Shivankar VJ, Rao CN & Singh S (2000) Studies on citrus Psylla *Diaphorina citri* Kuwayama: a review. *Agricultural Review* **21**, 199–204.

Siverio F, Marco-Noales E, Bertolini E, Teresani GR, Peñalver J, Mansilla P *et al*. (2017) Survey of huanglongbing associated with '*Candidatus* Liberibacter'species in Spain: analyses of citrus plants and *Trioza erytreae*. *Phytopathologia Mediterranea* **56**, 98–110.

Teixeira DC, Saillard C, Eveillard S, Danet JL, da Costa PI, Ayres AJ *et al*. (2005) 'Candidatus Liberibacter americanus', associated with citrus huanglongbing (greening disease) in Sao Paulo State, Brazil. *International Journal of Systematic and Evolutionary Microbiology.* **55**, 1857–1862.

Thomas DB & De Leon JH (2011) Is the old world fig, *Ficus carica* L. (Moraceae), an alternative host for the asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Homoptera: Psyllidae)? *Florida Entomology* **94**, 1081–1083.

USDA (2008) New pest response guidelines. Citrus greening disease. https://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/cg-nprg.pdf [accessed on 24 May 2018]

Van der Merwe CP (1941) The citrus psylla (Spanioza erytreae, del G.). *Science Bulletin 233, Entomology Series 8*, p. 12. Department of Agriculture and Forestry, Union of South Africa.

YingHong L & Tsai JH (2000) Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). *Annals of Applied Biology* **137**, 201–206.

Appendix 1 – Characteristics of the pathogen

All images are available on the EPPO Global Database and are available for use for non-commercial purposes.

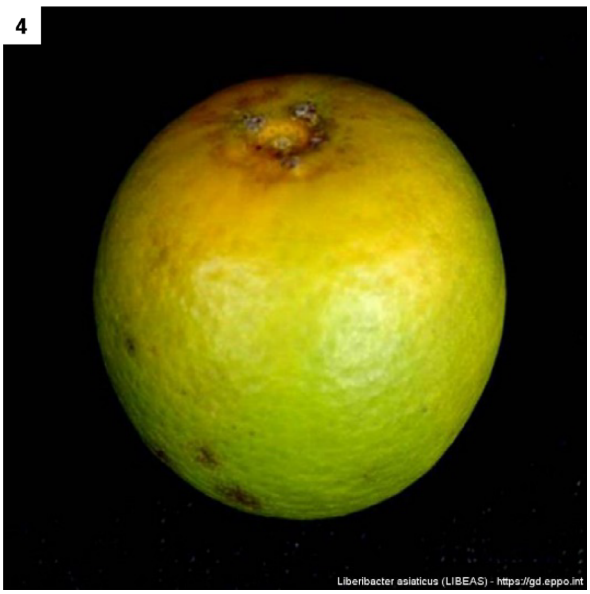
Huanglongbing (citrus greening) blotchy mottle symptoms and yellowing of central vein

See Figs 1 and 2.

Figs 1 and 2 Huanglongbing (citrus greening) symptoms on *Citrus* sp. in Central America: blotchy mottle and yellowing of central vein. Courtesy: Xavier Isaac Funez Euceda (OIRSA) (SV)

Huanglongbing (citrus greening) deformation and discoloration of the fruit

See Figs 3 and 4.



Figs 3 and 4 Huanglongbing (citrus greening) symptoms on *Citrus sp.* Fig. 3 shows fruit with small and brownish aborted seeds and asymmetric lopsided with bent fruit axis sweet. Fig. 4 shows colour inversion symptoms where the peduncular end of the fruit turns yellow/orange while the stylar end is still green. Courtesy: JM Bove´ INRA, Bordeaux (FR).

Huanglongbing (citrus greening) rabbit ear symptoms

See Figs 5 and 6.

Figs 5 and 6 Huanglongbing (citrus greening) symptoms on *Citrus sp.* in Central America: new shoots showing rabbit's ear symptoms. Courtesy: Xavier Isaac Funez Euceda (OIRSA) (SV).

Huanglongbing (citrus greening) yellow vein symptoms

See Figs 7 and 8.

Figs 7 and 8 Huanglongbing (citrus greening) symptoms on *Citrus sp*. in Central America showing yellow vein symptoms. Courtesy: Xavier Isaac Funez Euceda (OIRSA) (SV).

Huanglongbing (citrus greening) Green island symptoms

See Figs 9 and 10.

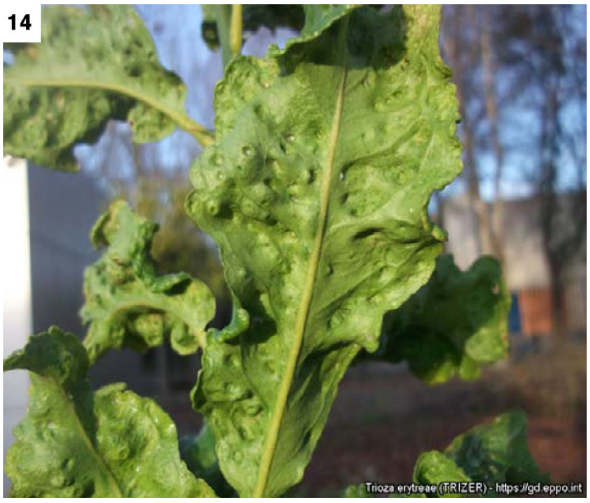
Figs 9 and 10 Huanglongbing (citrus greening) symptoms on *Citrus* sp. showing green islands symptoms. Courtesy: Xavier Isaac Funez Euceda (OIRSA) (SV).

Appendix 2 – Characteristics of the vectors

All images are available on the EPPO global database and are available for use for non-commercial purposes.

Trioza erytreae

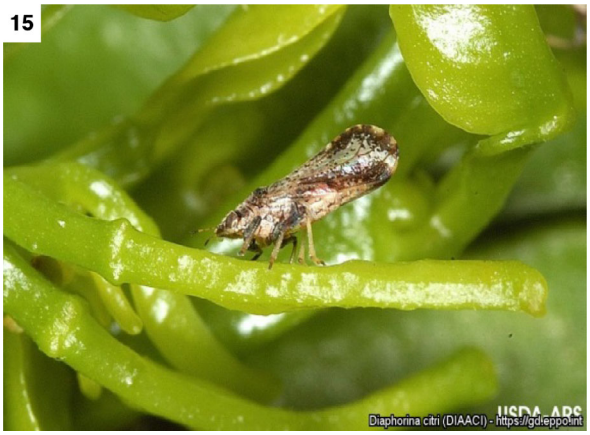
Damage caused by *Diaphorina citri*.
See Figs 11–14.



Figs 11–14 *Trioza erytreae* adult (courtesy: SP van Vuuren, Citrus Research International, Bugwood.org), nymphs (courtesy: HDCatling) and symptoms in citrus (courtesy: Carlos Alberto Coutinho Conceição).

Diaphorina citri

See Figs 15–17.



Figs 15–17 *Diaphorina citri* adult (courtesy: DG Hall USDA ARC Florida US), nymphs (courtesy: DG Hall USDA ARC Florida US) and symptoms in citrus (courtesy: INRA, Bordeaux FR).

Appendix 3 – Guidance to select relevant host plants

The available lists of host plants for Huanglongbing (citrus greening) and its vectors focus mainly on *Citrus* species, close *Citrus* relatives and tropical and subtropical Rutaceae.

For the EPPO region, known hosts must be combined with potential hosts (e.g. species from the family Rutaceae) and adapted to plants adapted to climates that are mainly Mediterranean or temperate.

Potential host species can be categorized into four types.

Type 1: Native host plants in EPPO region

The flora of EPPO countries includes indigenous plants of the Rutaceae family and some of them are common in many countries cultivating citrus crops (e.g. plants from the genus *Ruta* or *Cneorum*). There is no data available concerning the fact that the plants of these genera are hosts of the pathogen or the vectors.

Knowledge of the occurrence of these species and their surveillance is nevertheless important in case of an outbreak to organize surveillance and control.

Recommended actions: Contact botanists in the region for confirming the presence of these plants and for preparing training/information for plant health staff in order to include the surveillance of those plants in an overall surveillance scheme dedicated to Huanglongbing and its vectors.

Type 2: Exotic (and sometimes native) plants of the Rutaceae family used for ornamental purposes

Some plants are well known and have been cultivated on a large scale for a long time (e.g. *Choisya* and *Skimmia*)

in many gardens of the EPPO countries, others are gaining popularity (e.g. *Dictamnus*, *Tetradium* and *Zanthoxylum*) and some are restricted to collections, greenhouses or sub-tropical parts of EPPO region. Some of these plants are already known host plants (a) of the pathogen or the vectors (b), but some were not studied for this factor.

Recommended actions: Contact the horticultural industry and horticultural and botanist societies for data collection on trade, exchanges of plants and preparing training/information for staff (e.g. California field guide).

(a) Known host plants of the pathogen beside citrus plants are mainly tropical or sub-tropical Rutaceae.
(b) *Choisya ternata* and *C. arizonica* are known host plants of *Triozea erytraea* (there are hybrids cultivars between these *Choisya* species).

Type 3 *Citrus/Fortunella/Poncirus* complex

Besides fruit production, *Citrus* is also an important ornamental production in the EPPO region and plants from the *Citrus/Fortunella/Poncirus* complex are often propagated mainly for ornamental or small-scale fruit production. The cultivars are easily hybridized, providing plants with different aspects.

Particular attention might be given to species and hybrids of the Australian citrus group, formerly named *Microcitrus* and *Eremocitrus*, due to an overall aspect that is different from the classical orange or lemon tree.

Some of the trifoliate plants (*Poncirus* and *Poncirus* hybrids, either deciduous or evergreen) may also need a trained look to be recognized, but they are usually better known and documented due to their common use as rootstock. This use is often responsible for the presence of these plants in gardens in places too cold for the most common citrus fruits.

Type 4: Host plants not belonging to the Rutaceae family

A few plants outside the Rutaceae family are mentioned as Huanglongbing hosts in the literature.

The dodder (*Cuscuta* sp.) was identified in the first studies about Huanglongbing as a host plant that can allow a high presence of the organism, reaching higher titers than in *Citrus* (Ghosh *et al*.,1977). Different species of *Cuscuta* have been used experimentally to transmit the pathogen between *Citrus*, or from *Citrus* to other plants.

The observation of infested dodder in field has never been recorded, but some dodder species have been observed in *Citrus* fields.

Recommend actions: Check for the presence of dodder (*Cuscuta* sp.) in *Citrus* orchards before removing plants during eradication process. If present, destroy the dodder and do not grow host plants in the same field if there is a risk of re-sprouting of dodder.

Specifics for vectors

Concerning vectors, the observation of *D. citri* nymphs on *Ficus carica* in Texas (and the rearing of this psyllid on this new host plant) is the only documented non-Rutaceous plant on which *D. citri* has been found to breed (Thomas & De Leon, 2011). Some data are also available on *Artocarpus* sp., but this tree is strictly tropical.

Recommend actions: In presence of *D. citri*, check the presence of the vector on *Ficus carica*. In the presence of *D. citri* and the pathogen, consider including *F. carica* in the host plant list for eradication and containment measures.

Table 1. List of some genus of *Rutaceae* growing in Mediterranean and temperate climates

Genus	Habitat/information for ornamental	Status	Type of plant
Choisya	Ornamental/Mexican orange	Exotic	Evergreen shrub
Cneorum	Dry matorral (shrubland, thicket or bushes)	Indigenous	Evergreen small shrub
Coleonema	Ornamental/Australian origin	Exotic	Evergreen small shrub
Correa	Ornamental/Australian Fuchsia	Exotic	Evergreen shrub
Dictamnus	Dry forest in sub-Mediterranean climate, small-scale ornamental	Indigenous	Herbaceous
Eriostemon	Ornamental	Exotic	Evergreen small shrub
Haplophyllum	Dry and degraded matorral in North Africa, Spain, eastern Mediterranean countries and West Asia – close to *Ruta*	Indigenous	Evergreen small shrub
Murraya	Ornamental	Exotic	Evergreen shrub
Neochamaelea	Close to Cneorum, endemic to the Canaries Islands	Indigenous	Evergreen small shrub
Phellodendron	Ornamental/cork tree	Exotic	Deciduous tree
Poncirus	Ornamental and citrus rootstock – cold hardiest rootstock/trifoliate orange	Exotic	Deciduous small tree
Ptelea	Ornamental (small scale)/hope tree	Exotic	Small tree
Ruta	Dry matorral, common	Indigenous	Evergreen small shrub
Skimmia	Ornamental	Exotic	Shrub
Tetradium	Ornamental, recommended for beekeeping purposes	Exotic	Tree
Zanthoxylum	Ornamental/Sichuan pepper	Exotic	Shrub

Appendix 4 – Control of vectors

No single method of controlling the vectors is likely to be fully effective. Eradication will only be possible through rigorous removal of infested host plants and vector control. This is needed as in these circumstances one cannot be completely sure the pathogen is not present, and it may continue to spread in the presence of low populations of the vector.

Chemical control

No cases of successful eradication are known using chemical control options. Eradication using chemical options will only be possible in the case of early detection, where a low population is present in a very limited area, and should be used in combination with severe pruning and removal and destruction of infested parts of the host plants.

In Spain plant protection products based on the following active ingredients have been approved for use against *T. erytreae*: Tiametoxam 25% WG and Clorpirifos 48% (EU, 2016).

In Portugal, only one active ingredient is used: Acetamiprida with two treatments (M. Serra, pers. comm. 2019).

It should be highlighted that the availability of products for chemical control will vary nationally and other products may be available and effective. Products should be used according to plant protection product national regulations.

Biological control

Two biological control agents have been identified for *D. citri*, both parasitic wasps (*Tamarixia radiata* and *Diphorencyrtus aligarhensis*) (Hall, 2008). *T. radiata* was released and successfully established in the USA but parasitism rates have been variable, particularly where ants protect the psyllids. *T. radiata* was also released for psyllid control in Taiwan and Guadeloupe. Success in reducing populations of *D. citri* was achieved following releases and establishment of *T. radiata* in Réunion Island. Good levels of biological control were reported in Guadeloupe after the introduction of this parasitoid. As for *T. erytreae*, the biological control of *D. citri* by natural enemies has to be considered insufficient in reducing the incidence and spread of the disease.

At the time of writing, biological control has not been implemented against *T. erytreae* in the EPPO region. In Réunion Island, biological control has been applied in the control of *T. erytreae* using the Hymenoptera parasitoid *Tamarixia dryi*, which is native to Africa (Aubert *et al*., 1980; Etienne *et al*., 2001) and promising results have been obtained in the Canary Islands (B. Martinez, pers. comm. 2019). Different insect species which are known to be common in citrus orchards of the EPPO region have been shown to act as predators or parasites of *T. erytreae*, but they would not be sufficiently specific or numerous to control the potential field populations of the psyllid.

Appendix 5 – Specific requirements for pest-free places of production of *Citrus* and other Rutaceae host plants against Huanglongbing vectors

The EPPO Standard PM 5/8 (1) *Guidelines on the phytosanitary measure 'Plants grown under complete physical isolation'* provides general guidance on the type of physical isolation and associated phytosanitary measures that are required to enable plants to be produced free of a particular pest in an area where the pest is present.

In particular, specific requirements for pest-free places of production of *Citrus* and other Rutaceae host plants against Huanglongbing vectors include the combination of the following measures and should be established in accordance with ISPM 10 (IPPC, 1999). The procedure should be followed for *Citrus* and other Rutaceae including mother plants and final plants.

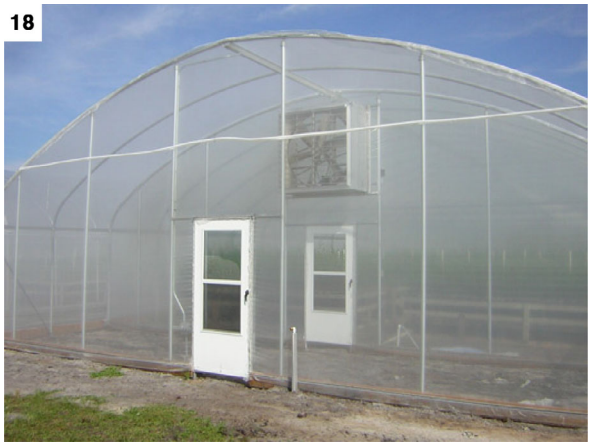
1. Establishment of a 200 m radius around the place of production which is free from the pest as confirmed by at least two official inspections at appropriate times during the last vegetative cycle. Preferably away from commercial citrus crops.
2. At least two annual inspections in the place of production.
3. Possible windbreaks around the place (artificial or natural material).
4. Within 200 m placement of chromo tropic yellow sticky traps in a quantity depending on the risk. Monitoring and replacement of the traps should be conducted every 2–3 weeks and any psyllids should be sent for identification.
5. Regular inspection of the protective structures.

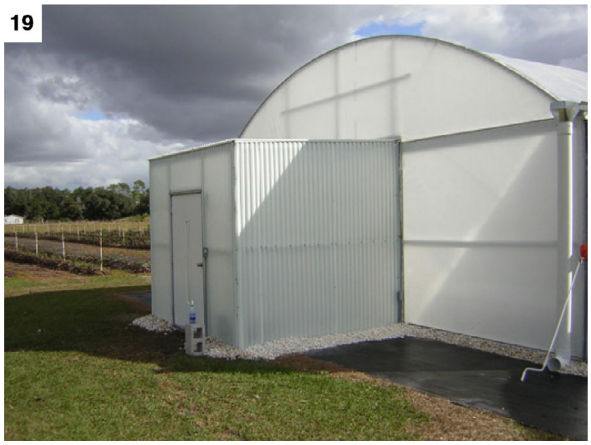
6. Treatments at appropriated times with authorized products against vectors inside and in the 200 m radius in host plants.
7. Records should be kept on the vectors caught in traps and chemical treatments applied.
8. Requirements of the physical protective unit:
   - Metal structure covered with specific net screen mesh size 45–50 mesh (or rectangular hole with max 0.3 mm in one of the dimensions) or other rigid material (polystyrene).
   - Double-door entrance with air barrier (air speed should be adequate for entrance size) in a first door or positive pressure system (forced air blows out) to expel insects from entering. Activated automatically. The double doors may be sliding doors or open to the outside.
   - The double-door entry area must be adequate for loading and unloading plants.
   - Any other opening windows with natural or forced ventilation should have net screens of the same mesh size referred to above.
   - The laterals of the structure should be sealed at ground level.
   - Periodic cleaning of the screens.
   - Placement of chromo tropic yellow sticky traps inside the double-door chamber and inside the unit close to windows.

The physical protective structure should be monitored at regular intervals throughout the year where all material is checked for any damage. For example, the net screens should be inspected for damage and required maintenance at regular intervals.

Personal should be trained to follow hygiene protocols before entering and working in the physical protective unit and access should be strictly limited to trained personal.

See Figs 18 and 19.





Figs 18 and 19 show examples of double-door entry system for physical protective unit.