EPPO Datasheet: *Xanthomonas citri pv. citri*

Last updated: 2022-09-01

IDENTITY

Preferred name: *Xanthomonas citri pv. citri*
Authority: (Hasse) Constantin, Cleenwerck, Maes, Baeyen, Van Malderghem, De Vos, Cottyn
Taxonomic position: Bacteria: Proteobacteria: Gammaproteobacteria: Lysobacterales: Lysobacteraceae
Other scientific names: *Xanthomonas axonopodis pv. citri* (Hasse) Vauterin, Hoste, Kersters & Swings, *Xanthomonas campestris pv. citri* (Hasse) Dye, *Xanthomonas citri subsp. citri* (ex Hasse) Gabriel, Kingsley, Hunter & Gottwald
Common names: Asiatic canker, Asiatic citrus canker (A strains), bacterial canker of citrus, cancrosis A (A strains), citrus canker
view more common names online...
EPPO Categorization: A1 list
view more categorizations online...
EU Categorization: A1 Quarantine pest (Annex II A)
EPPO Code: XANTCI


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Notes on taxonomy and nomenclature

Three infra-pathovar groups, which differ in pathogenicity are referred to as pathotypes A, A* and A^W (Vernière *et al.*, 1998; Sun *et al.*, 2004; Rybak *et al.*, 2009).

HOSTS

Pathotype A strains overall have the greatest impact on citrus crops. They induce natural canker infections on a broad range of rutaceous species, including nearly all *Citrus* cultivars, hybrids as well as members of the following genera: *Casimiroa*, *Citropsis*, *Clausena*, *Eremocitrus*, *Fortunella*, *Microcitrus (*now syn. of *Citrus)*, *Naringi* (syn. *Hesperethusa*), *Poncirus*, *Severinia*, *Swinglea* (syn. *Aegle*) and *Zanthoxylum*, but incongruent reports were sometimes produced (Graham *et al.*, 2004, Koizumi, 1978, Koizumi, 1981, Lee, 1918, Reddy, 1997, Stover *et al.*, 2014). Pathotype A* strains are pathogenic to a restricted range of citrus species. Most outbreaks were primarily reported on Mexican lime (*Citrus aurantiifolia*) and to a lesser extent on relatives such as sweet lime (*C. limettioides*) or Tahiti (or Persian) lime (*C. latifolia*). Unique A* strains reported from Iran rarely cause mild natural infections on a few other species (Pruvost *et al.*, 2015). Some strains can produce mild canker-like lesions when artificially inoculated to tested *Citrus* species other than lime (Derso *et al.*, 2009, Escalon *et al.*, 2013, Pruvost *et al.*, 2015, Vernière *et al.*, 1998). Pathotype A^W strains present a host range restricted to Mexican lime and the related alemow (*C. macrophylla*) (Sun *et al.*, 2004, Munoz Bodnar *et al.*, 2017).

When considering natural infections, it is to be noted that all pathotypes affect *Citrus aurantiifolia*. Pathotypes A and A^W affect *C. macrophylla* and pathotypes A and A^* affect *C. latifolia* and C. *limettioides.*

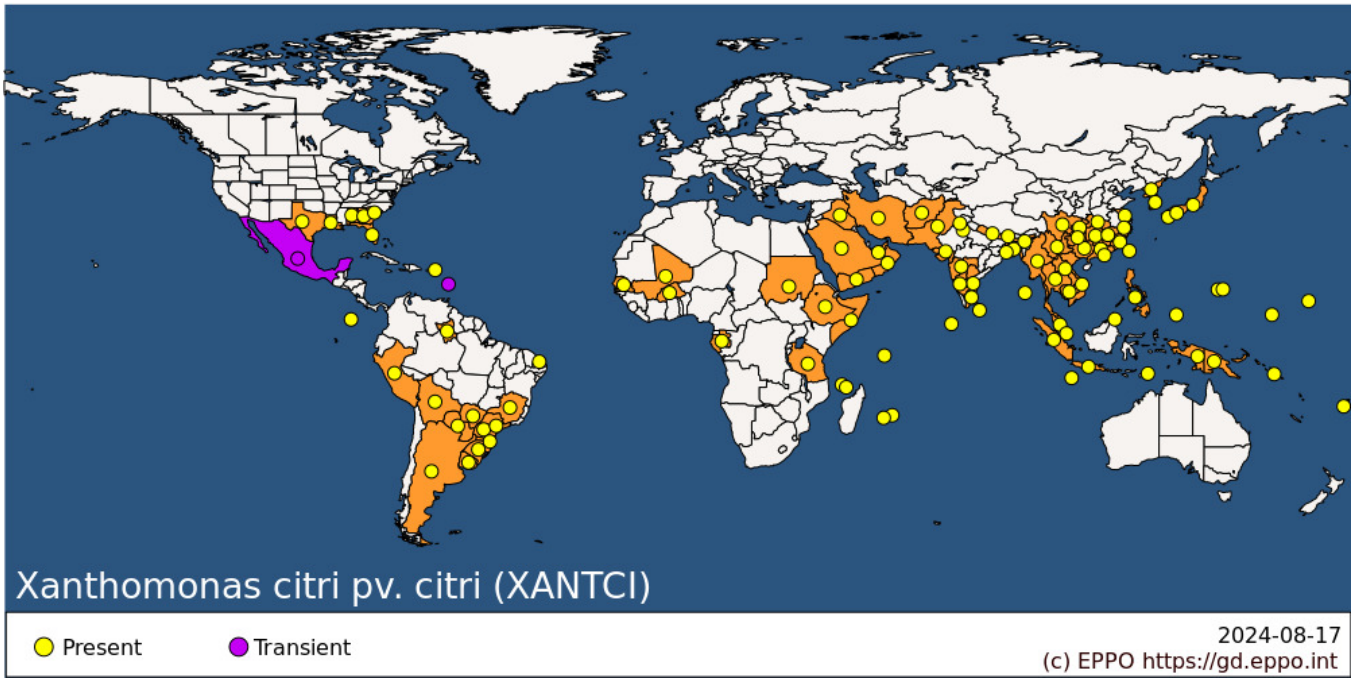
Pathotype A affects *Citrus* lines with different levels of susceptibility, some lines of *C. japonica* seem immune (Khalaf *et al.*, 2007, Chen *et al.*, 2012) whereas others can host natural infections. Pathotype A also affects *Atalantia buxifolia*, *Casimiroa edulis*, *Citropsis daweana*, *Clausena harmandiana*, *Eremocitrus glauca*, *Microcitrus australasica*, *Microcitrus australis*, *Microcitrus inodora*, *Naringi crenulata*, *Swinglea glutinosa*, and *Zanthoxylum ailanthoides* but these species were mostly found to be weakly susceptible.

Host list: *Atalantia buxifolia, Casimiroa edulis, Citropsis daweana, Citrus australasica, Citrus depressa, Citrus glauca, Citrus hystrix, Citrus inodora, Citrus maxima, Citrus medica, Citrus reticulata, Citrus trifoliata, Citrus x aurantiifolia var. macrophylla, Citrus x aurantiifolia, Citrus x aurantium var. paradisi, Citrus x aurantium var. sinensis, Citrus x aurantium var. unshiu, Citrus x aurantium, Citrus x junos, Citrus x latifolia, Citrus x limon var. limettioides*

, Citrus x limon, Citrus x tangelo, Clausena harmandiana, Fortunella, Naringi crenulata, Swinglea glutinosa, Zanthoxylum asiaticum, x Citrofortunella microcarpa

GEOGRAPHICAL DISTRIBUTION

X. citri pv. *citri* is likely to have originated from the Indian subcontinent, as inferred by phylogeographical analyses (Patane *et al.*, 2019), also corresponding to the area from where the oldest diseased herbarium specimens showing canker symptoms were collected (Fawcett & Jenkins, 1933). The disease was first reported in East Asia (Japan) at the end of the 19th century (Kuhara, 1978). Geographical expansion out of Asia was recorded starting in the early 20th century (in the USA, South Africa and Australia) but is very likely to have occurred earlier (Hasse, 1915, Doidge, 1916, Broadbent *et al.*, 1995, Campos *et al.*, 2021). Some of the outbreaks outside Asia were successfully eradicated. The pathogen subsequently emerged in South America (mid-20th century) and Africa (early 21th century). *X. citri* pv. *citri* is now widely distributed across many citrus production basins worldwide. *X. citri* pv. *citri* is currently absent from the EPPO region. Interceptions of citrus diseased fruit or foliage caused by this pest are recurrently reported at the borders of the European Union (more than 300 interceptions were reported over the last 20 years, EPPO Reporting Service).



Africa: Burkina Faso, Comoros, Ethiopia, Gabon, Mali, Mauritius, Mayotte, Reunion, Senegal, Seychelles, Somalia, Sudan, Tanzania
Asia: Afghanistan, Bangladesh, Cambodia, China (Chongqing, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Xianggang (Hong Kong), Yunnan, Zhejiang), Christmas Island, Cocos Islands, East Timor, India (Andaman and Nicobar Islands, Andhra Pradesh, Assam, Gujarat, Haryana, Karnataka, Maharashtra, Punjab, Sikkim, Tamil Nadu, West Bengal), Indonesia (Irian Jaya, Java, Sumatra), Iran, Iraq, Japan (Honshu, Kyushu, Shikoku), Korea Dem. People's Republic, Korea, Republic, Laos, Malaysia (Sabah, West), Maldives, Myanmar, Nepal, Oman, Pakistan, Philippines, Saudi Arabia, Singapore, Sri Lanka, Taiwan, Thailand, United Arab Emirates, Vietnam, Yemen
North America: Mexico, United States of America (Alabama, Florida, Georgia, Louisiana, South Carolina, Texas)
Central America and Caribbean: Martinique, Virgin Islands (British)
South America: Argentina, Bolivia, Brazil (Mato Grosso do Sul, Minas Gerais, Parana, Rio Grande do Norte, Rio Grande do Sul, Roraima, Santa Catarina, Sao Paulo), Paraguay, Peru, Uruguay
Oceania: Fiji, Guam, Marshall Islands, Micronesia, Northern Mariana Islands, Palau, Papua New Guinea, Solomon Islands

BIOLOGY

Infection occurs through natural openings (stomata) and wounds, and variations in susceptibility to infection are related to tissue age for a given citrus cultivar (Vernière *et al.*, 2003). *X. citri* pv. *citri* multiplies in the intercellular spaces of the mesophyll (the bacterium is not intracellular) and induces cell enlargement (hypertrophy) and division (hyperplasia) of host cells, eventually producing canker lesions (Brunings & Gabriel, 2003; Jalan *et al.*, 2014). Green, actively growing fruits and young leaves (one-half to two-thirds expansion) are most susceptible to infection. Wounds created by grove maintenance operations, insects (e.g., the citrus leafminer *Phyllocnistis citrella*), thorns and wind are highly efficient infection sites which allow infection to occur on older organs (Vernière *et al.*, 2003). A single cell of *X. citri* pv. *citri* forced into a stoma may initiate infection (Gottwald & Graham, 1992). When the pathogen is not enforced into stomata by wind pressure and in the absence of wounds, bacterial suspensions containing approximately 10^5 cells/mL^{-1} are typically required to induce lesions from spray inoculation (Goto, 1992). *X. citri* pv. *citri* can produce lesions at temperatures ranging from 12 to 40°C, with an optimum of 25–35°C (Dalla Pria *et al.*, 2006). Temperature also drives the length of the latency period, which can extend over several weeks (Koizumi, 1976).

X. citri pv. *citri* survives efficiently in canker lesions, which represent the most biologically significant inoculum source. Live populations in young leaf lesions typically exceed 10^7 cells per lesion. The decrease of populations sizes while lesions age appears to be environment-dependent with greater decline reported in areas with a definite winter season when compared to tropical situations (Pruvost *et al.*, 2002, Stall *et al.*, 1980). Dynamics of lesion development and *X. citri* pv. *citri* multiplication were found to be related to host resistance (Koizumi, 1979). *X. citri* pv. *citri* was found to be able to survive in twig cankers (and remain culturable – Pruvost, unpublished data) for up to approximately 5 years (Goto, 1970). Survival in lesions on dry leaves on soil or incorporated into soil occurs only at low population sizes (10^2 to 10^3 cells per lesion) for a few months (Goto *et al.*, 1975b; Gottwald *et al.*, 2002). Asymptomatic plant material may host the pathogen either as latent infections or surface populations (Timmer *et al.*, 1996, Gottwald *et al.*, 2009). *X. citri* pv. *citri* was found to be able to form biofilms (Cubero *et al.*, 2011; Rigano *et al.*, 2007). A viable but not culturable (VBNC) state has been suggested for *X. citri* pv. *citri* in response to copper treatments (Del Campo *et al.*, 2009). There is no report of seed transmission of *X. citri* pv. *citri.* The bacterium was found to survive over weeks to months at low population levels in association with weeds (Goto, 1970; Goto *et al.*, 1975a; Leite & Mohan, 1987). The bacterium also survives for short time periods (24–72 hours) on inert surfaces e.g. on agricultural equipment, vehicles, fruit crates, clothes (Graham *et al.*, 2000).

Splash dispersal of *X. citri* pv. *citri* caused by rain or irrigation occurs over short distances and allows movement of the inoculum between adult trees or between plants in nurseries (Pruvost *et al.*, 2002; Serizawa *et al.*, 1969). Although not documented for *X. citri* pv. *citri*, xanthomonads may spread as aerosols over short to medium distances (McInnes *et al.*, 1988). Wind-driven rains spread *X. citri* pv. *citri* over distances that depend on wind speed. Within-grove dispersal among trees is achieved when wind speed reaches or exceeds approximately 8 m s^{-1} (Gottwald *et al.*, 2002). Extreme weather events can spread the pathogen over distances up to approximately 50 km in the case of a major hurricane (Irey *et al.*, 2006).

DETECTION AND IDENTIFICATION

Symptoms

X. citri pv. *citri* infects all aerial parts of its hosts. When the disease is severe, defoliation and early fruit drop can occur but no tree death has been reported.

On leaves, lesions first appear on the lower leaf surface as pin-point oily spots due to water-soaking of the tissue. Later the lesions become visible on both epidermal surfaces as slightly raised pustules or blister-like eruptions. As lesions develop, they increase in size, 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. Diagnostic symptoms are tissue hyperplasia resulting in cankers sometimes with water-soaked margins and yellow halos surrounding the lesions. Lesions with an atypical morphology (flat or blister-like spots) can be sometimes observed, especially in the case of late fruit infections or lesions on some resistant cultivars. In most hosts wilting is a common symptom of infection. The youngest leaves usually wilt first, with symptoms initially appearing at the warmest time of day. Wilting may be visible in only one stem, on one side of a plant or even sectoral in part of a leaf, depending where vascular infections occur (e.g., if they are restricted to sectors of

stems and/or leaf petioles. Leaves may become bronzed or chlorotic and epinasty may occur. Wilting of the whole plant may follow rapidly if environmental conditions are favourable for pathogen growth. As the disease develops, a brown discoloration of the xylem vessels in the stem may be observed above the soil line and adventitious roots may develop. A creamy, slimy mass of bacteria exudes from vascular bundles when the stem is cut. Wilting and collapse of whole plants can lead to rapid death.

On twigs, the symptoms are similar: raised corky lesions initially surrounded by an oily or water-soaked margin. The lesions are generally irregularly shaped and may be sunken. Pustules may coalesce but chlorosis does not typically surround twig lesions. On removal of the corky layer, dark brown lesions are visible in the healthy green bark tissue. On highly susceptible citrus cultivars, diseased twigs can eventually show dieback symptoms.

Lesions on fruits can appear when they are still small and green and are similar to those on leaves, but tend to have more elevated margins and a sunken centre. These craters do not penetrate deep into the rind. Yellow chlorotic halos may or may not be present. Harvestable infected fruit have a reduced value or can be unmarketable depending on the severity of infection.

Morphology

X. citri pv. *citri* cells are Gram-negative rods with a single polar flagellum, non-fluorescent, typically with no diffusible pigment produced on agar media (very rare exceptions of brownish-reddish pigment production occur). After ? 3 days of incubation at 28°C, colonies on agar plates are circular, convex, mucoid, shiny and yellow. Very occasionally, strains altered in xanthomonadin pigment production (and therefore cream-white to pale yellow) can be observed.

Detection and inspection methods

Different molecular tests (conventional PCR, real-time PCR and LAMP) are available for the specific detection of *X. citri* pv. *citri* and have recently been validated in a test performance study (Anthoine *et al.*, 2020). Identification can be based on different tests including conventional or real-time PCR, DNA barcoding, minisatellite-based typing, CRISPR based typing. The use of pathogenicity tests is recommended for critical cases. The EPPO Standard PM 7/44 (EPPO, 2023) provides instructions on sampling and testing plant materials. The procedure for inspection of citrus fruit consignments is reported in EPPO Standard PM 3/90 *Inspection of citrus fruits consignments* (EPPO, 2020).

PATHWAYS FOR MOVEMENT

X. citri pv. *citri* can spread as a consequence of human activities (e.g. movement of contaminated plant propagative material or agricultural equipment or clothes used for grove/nursery maintenance operations) (Graham *et al.*, 2004). Seven significant introduction pathways were identified and evaluated by the EFSA Plant Health Panel (EFSA, 2014):

1. Citrus fruit, commercial trade
2. Citrus fruit and/or leaves import by passenger traffic
3. Citrus plants for planting, commercial trade
4. Citrus plants for planting import by passenger traffic
5. Ornamental rutaceous plants for planting, commercial trade
6. Ornamental rutaceous plants for planting import by passenger traffic
7. Citrus and rutaceous leaves and twigs, commercial trade

Pathways consisting of plants or plant parts for planting have the highest risk for subsequent (and likely) establishment of the pathogen, which would very likely survive during transport and whose probability of transfer to a suitable host is very likely based on the intended use of the material and the large availability of citrus and other rutaceous genera in the EPPO region, either in commercial orchards or in private and public areas. The probability of establishment would be even higher in the case of plants or plant parts illegally imported through the passenger pathway or mail, as they could escape current regulations for official importation of rutaceous plant propagative material or whole plants.

PEST SIGNIFICANCE

Economic impact

Together with '*Candidatus* Liberibacter spp.' (the causal agents of citrus huanglongbing) and *Citrus tristeza virus*, *X. citri* pv. *citri* is one of the main sanitary threats for citrus industries worldwide. Although not the cause of tree death, the pathogen has serious direct and indirect economic impacts. Direct impacts include alteration of fruit quality and yield (due to early fruit drop), the severity of the effect is influenced by the host species, the bacterial strain and the environmental conditions. Reports from Argentina mention disease incidence rates on susceptible grapefruit fruit without chemical control of up to 80 % and early fruit drop reaching 50 % on Hamlin sweet orange (Stall & Seymour, 1983). Direct impacts also include defoliation of heavily infected trees and/or twig dieback, as well as production cost increase due to implemented control measures. The annual cost ascribed to the presence Asiatic citrus canker in Florida only (approximately 0.3 million hectares of commercial citrus at the time of assessment) was estimated as ca. 350 million USD per year (Gottwald *et al.*, 2002). Indirect impacts include restricted access to fruit export markets (e.g. the European Union, Australia) for countries or areas where satisfactory control of the disease cannot be achieved, undesirable consequences of chemical control (e.g. development of copper and antibiotic resistance and risk of transfer to other bacteria in the environment or phytotoxicity of copper compounds accumulating in soils). In Australia, the eradication of a citrus canker outbreak in Queensland in 2004 yielded an estimated potential net benefit of about 70 million AUD. The economic benefits of averting a national outbreak of citrus canker would be 410 million AUD if the estimated cost of an Australian citrus ban for five years would be 2 billion AUD (Alam & Rolfe, 2006, Gambley *et al.*, 2009). The extremely wide geographic distribution of *X. citri* pv. *citri* includes three major world citrus producers (i.e. USA, Brazil and China) as well as many other countries where citrus represents a significant income source for small-scale farmers.

Control

In cases where eradication is not feasible (see section 'Phytosanitary measures'), the alternative strategy is based on integrated pest management (IPM), which aims to reduce the rate of infection and spread of *X. citri* pv. *citri*, and attempt to keep disease below economically damaging levels. IPM combines several control options such as (i) the production of healthy citrus nursery plants for new grove establishment through certified programs, (ii) the recurrent physical elimination of inoculum sources, (iii) the avoidance of grove/nursey maintenance operations when the plant canopy is wet, (iv) the use of cultural practices minimizing infection and spread including general prophylactic measures applied to citrus production sites during grove/nursery maintenance operations, rootstocks controlling high

tree vigour, drip irrigation, efficient windbreaks, preventive application of bactericides timed at host susceptibility peaks (most often using copper-based compounds), disinfection of agricultural equipment and (v) the use of partially resistant citrus lines or molecules inducing plant defence. Chemical control alone cannot adequately control Asiatic citrus canker. Bacteriophage-assisted biological control has been attempted but with limited success. Resistance to copper compounds, first reported in pathotype A strains from Argentina in the mid 1990s (Canteros *et al.*, 2017), was identified more recently in other regions (Huang *et al.*, 2021, Richard *et al.*, 2016, Richard *et al.*, 2017) and in pathotype A* originating from Southeast Asia (Webster *et al.*, 2020). Similarly, streptomycin-resistant strains were reported from South Korea (Hyun *et al.*, 2012).

Postharvest treatments (e.g. sodium orthophenylphenate) are frequently applied in packing lines but they effect is partial, they only decrease population levels of the pathogen but do not eliminate it (Gottwald *et al*., 2009).

Phytosanitary risk

X. citri pv. *citri* has quarantine status in several countries in Africa, America, Asia, Europe and Oceania and for several regional plant protection organizations. Moreover, *X. citri* pv. *citri* is listed as a dual-use organism in the European Union because of its potential use as a biological weapon (Directive 1183/2007 EC) (Young *et al.*, 2008).

The pathogen was estimated to be likely to establish and spread in this European Union if reaching susceptible hosts (EFSA, 2014). Based on the EFSA analysis, this pathogen is a risk for the European Union where citrus is widely commercially cultivated and largely available in public and private non-commercial areas. Once established in a region, its spread would be difficult to control. Therefore, the best risk reduction options to be taken are the ones aiming to maintain its absence.

PHYTOSANITARY MEASURES

Once transferred to a suitable host, *X. citri* pv. *citri* cannot be controlled without strong phytosanitary measures. The effectiveness of strict regulatory control within the European Union has been reviewed (EFSA, 2014). Asiatic citrus canker is a disease for which eradication represents a feasible option, based on (i) its major economic impact, (ii) the biological characteristics of its causal agent and (iii) the successful eradication campaigns achieved in several countries (Australia, New Zealand, South Africa, the USA) (Graham *et al.*, 2004). When an outbreak is observed in a new area, successful eradication requires efficient surveillance systems as well as quick and appropriate management measures on diseased and exposed trees. This strategy represents in this case the most cost-effective option (see ‘Economic impact’ section). Eradication of large outbreaks can become very difficult and costly (for example efforts implemented in Florida over a decade starting in the mid-1990s have cost more than one billion USD) and suffer low acceptability (Gottwald & Irey, 2007, Parnell *et al.*, 2009, Centner & Ferreira, 2012, Cunniffe *et al.*, 2015).

For the European Union, the EFSA Plant Health Panel has reviewed the EU current phytosanitary measures to prevent the introduction and spread of *X. citri* pv. *citri* (EFSA, 2014) and EU Regulation 2019/2072 updated these requirements. Briefly, main current measures include the prohibition of importation of plants of *Citrus*, *Fortunella* and *Poncirus* from third countries. Importation of plants of *Microcitrus*, *Naringi* and *Swinglea* is allowed only from certified pest-free third countries or pest-free areas. and the authorization of fruit (free from peduncles and leaves with a packaging bearing an appropriate origin mark) importation only from certified (1) pest-free third countries, (2) pest-free areas or (3) pest-free place of production established by the national plant protection organisation in the country of origin. Alternatively (4), an official control and examination should ascertain that (i) the site of production and the immediate vicinity are subject to appropriate treatments and cultural practices against *X. citri* pv. *citri*, and (ii) the fruits have been subjected to a treatment with sodium orthophenylphenate, or another effective treatment that should be mentioned on the phytosanitary certificate referred to in Article 71 of EU Regulation 2016/2031, and the treatment method has been communicated in advance in writing to the European Commission by the national plant protection organization of the third country concerned, and (iii) official inspections carried out at appropriate times prior to export have certified that the fruits harvested in the place of production has shown that the fruits are free from symptoms of *X. citri* pv. *citri*, and (iv) information on traceability is included in the phytosanitary certificate. In the case of fruits destined for industrial processing, (i), (iii) and (iv) apply as well as (v) movement, storage and processing takes place under conditions, approved in accordance with the procedure referred to in Article 107 of the EU Regulation, and (vi) the fruits have been transported in individual packages bearing a label, which contains a traceability code and the indication that the fruits are destined for industrial processing. Other EPPO countries have

applied similar phytosanitary measures.

National regulatory control systems are recommended to EPPO countries for the surveillance, early detection and eradication of the pathogen, and for containment measures to prevent spread during eradication. Efficient and regular surveillance actions are recommended as they are key in enabling early detection and prompt implementation of eradication measures. In citrus-growing areas, inspectors, industry experts and workers should be trained to recognize Asiatic citrus canker symptoms and host plants. Countries should have access to laboratories with trained diagnosticians, experienced and competent in the identification of the pathogen according the EPPO PM 7/44 Diagnostic Protocol (EPPO, 2005).

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