EPPO Datasheet: *Xanthomonas citri pv. aurantifolii*

Last updated: 2024-03-19

This datasheet focusses specifically on less virulent bacterial strains causing citrus canker. These strains have been classified as *Xanthomonas citri* pv. *aurantifolii* pathotypes B and C and the form of canker they cause is called South American citrus canker. The primary more devastating form of citrus canker, so-called Asiatic citrus canker, originating from Asia and caused by more virulent strains, classified as *Xanthomonas citri* pv. *citri* (pathotypes A, A*, A^W) is covered in a separate datasheet. Text between square brackets [ ], on symptoms, morphology and detection, identification and inspection methods, is from the **EPPO datasheet on *X. citri* pv. *citri*** for the reasons explained under the chapter Biology.

IDENTITY

Preferred name: *Xanthomonas citri pv. aurantifolii*
Authority: (Schaad et al.) Constantin et al.
Taxonomic position: Bacteria: Proteobacteria: Gammaproteobacteria: Lysobacterales: Lysobacteraceae
Other scientific names: *Xanthomonas axonopodis pv. aurantifolii* Vauterin et al., *Xanthomonas campestris pv. aurantifolii* Gabriel, Kingsley, Hunter & Gottwald, *Xanthomonas citri f. sp. aurantifolia* Namekata & Oliveira, *Xanthomonas fuscans subsp. aurantifolii* Schaad et al.
Common names: Galician lemon canker (C strains), Mexican lime cancrosis (C strains), South American citrus canker, cancrosis B (B strains), citrus canker, false citrus canker (B strains)
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EPPO Categorization: A1 list
view more categorizations online...
EU Categorization: A1 Quarantine pest (Annex II A)
EPPO Code: XANTAU

Notes on taxonomy and nomenclature

Citrus canker was described for the first time by Stevens (1914) and Wolf & Massey (1914) from the South-Eastern USA, and was considered likely to have introduced with citrus seedlings from Japan (Schoulties *et al.*, 1987; Gottwald *et al.*, 2002; Li *et al.*, 2007). The disease is endemic in China, Japan, Southern Asia, and Oceania, where citrus originated and have long been grown. The causal agent was isolated by Hasse in 1915, who named it *Pseudomonas citri*. Later it was reclassified into the genus *Xanthomonas* as *Xanthomonas citri* by Dowson (1939). In the 1970s, almost all species within the genus *Xanthomonas* were reclassified at pathovar level and the citrus canker pathogen was reclassified in the complex species *Xanthomonas campestris* as *Xanthomonas campestris* pv. *citri* (Dye, 1978). Gabriel *et al.* (1989) determined that the typical citrus canker strains (A or Asiatic strains) deserved species rank and classified them as *X. citri* pv. *citri*. This pathogen aligns with genetic cluster 9.5 of *X. axonopodis* as defined by Vauterin *et al.* (1995) (Rademaker *et al.*, 2000). Revisions in taxonomy, based on Multilocus Sequence Analysis (MLSA), DNA:DNA hybridization, Average Nucleotide Identity (ANI) and whole genome sequence analysis led to its current classification as *X. citri* pv. *citri* (synonyms *X. citri* subsp. *citri*, *X. smithii* subsp. *citri* or *X. axonopodis* pv. *citri*) (Vauterin *et al.*, 1995; Schaad *et al.*, 2005, 2006; Ah-You *et al.*, 2009; Constantin *et al.*, 2016; Ragupathy *et al.*, 2023).

In addition to the classical Asiatic strains of *X. citri* (*X. citri* pv. *citri* pathotypes A, A* and A^W), three other groups of slower growing and less pathogenic strains were isolated in South America, named *X. citri* B, C and D strains, grouping into genetic cluster 9.6 of *X. axonopodis sensu* Vauterin *et al.* (1995). Namekata (1971) first described the C strains and differentiated them from A strains, as *X. citri* forma specialis *aurantifolii*. *X. citri* pv. *citri* A strains (most virulent) were already detected in 1957 and spread to many areas in Brazil (Bitancourt, 1957; Behlau, 2020) The B, C and D strains were reclassified in 2006 as *X. fuscans* subsp. *aurantifolii* (Schaad *et al.*, 2006). However,

subsequent studies refuted *X. fuscans* as a separate species (Young *et al.*, 2008) and these strains were reclassified as *X. citri* pv. *aurantifolii* pathotype B and C (Ah-You *et al.*, 2009; Rodriguez *et al.*, 2012; Constantin, 2016). A recent core genome multilocus sequence typing study has confirmed this classification (Ragupathy *et al*, 2023). Concerning the D strains, the disease called citrus bacteriosis and formerly thought to be caused by *X. campestris* pv. *citri* pathotype D, was finally found to be caused by a fungus, *Alternaria limicola* (Rodriguez *et al.*, 1985; Palm & Civerolo, 1994).

Two closely related bacterial plant pathogens *X. euvesicatoria* pv. *citrumelonis* (Schaad *et al.* 2007; Constantin *et al.*, 2016), causing Citrus bacterial spot in Florida (US), formerly named *X. citri* E strains (syn. *X. axonopodis* pv. *citrumelo, X. alfalfae* subsp. *citromelonis*, in *Xanthomonas* genetic cluster 9.2, see Vauterin *et al.*, 1995) and *X. citri* pv. *bilvae* (Chakravarti *et al*., 1984; Bansal *et al*., 2022) (formerly *X. campestris* pv. *bilvae* or *X. axonopodis* pv. *bilvae*, in *Xanthomonas* genetic cluster 9.5 causing another Citrus bacterial spot disease in India, are not addressed in this datasheet. They are mentioned here, since in the past misnaming/misidentification/nomenclatorial issues have led to confusion, unnecessary and costly quarantine measures and lawsuits in the past (e.g. Gabriel *et al*, 1989; Gottwald *et al*., 1991; Schaad *et al*., 2006; Fonseca *et al*., 2019b). For further details on these pathogens, refer to Graham and Gottwald (1991); Graham *et al*. (2004); Vauterin *et al.* (1995); Rademaker *et al.* (2005); Schaad *et al.* (2006, 2007). The most recent taxonomy and detailed description of bacterial strains causing citrus canker are summarized in Table 1 below.

Table 1 – Summary of pathotypes of *X. citri* pv. *citri* and *X. citri* pv. *aurantifolii*, causing citrus bacterial canker (CBC), described in this datasheet.

Pathovars		*Xanthomonas citri* pv. *citri*		*X. citri* pv. *aurantifolii*	
Disease	Asiatic bacterial canker (CBC-A)			South American Citrus bacterial canker (CBC-B)	South American Citrus bacterial canker (CBC-C)
Pathotypes	A	A*	A_w	B	C Brown-pigmented and non-pigmented strains
Distribution	Asia, Middle East, Americas, Africa, Oceania	Asia and Africa	Indian subcontinent, Arabian Peninsula, USA	Argentina, Paraguay, Uruguay, **not recorded after 1990**	Brazil (São Paulo), **not recorded after 2009**
Host range	wide	narrow	narrow	limited	narrow
Principal natural hosts	*Citrus* spp. and other Rutaceae	Mexican lime	Mexican lime and alemow	Lemon, Mexican lime, Sour orange, grapefruit	Mexican lime
Virulence	high	high	high	low	Low
Growth on nutrient agar and other standard media	+++ fast	+++ fast	+ slow	± slow, needs elective medium	+++ fast
Economic impact	high	high	high	Presently practically nil, not recorded after 1990 Replaced by CBC-A	Presently practically nil, not recorded after 2009 Replaced by CBC-A

HOSTS

***X. citri* pv. *aurantifolii* pathotype B** has a limited host range and predominantly affects *C. x aurantifolia* (Mexican lime). Other natural hosts have also been reported, such as *C. lemon* and *C. maxima* (= *C. grandis*, pummelo) when planted near infected Mexican lime (Schubert *et al.*, 2001) as well as *C. aurantium (*sour orange); *C. limonia* (Rangpur lime); *C. limettioides* (sweet lime). *C. sinensis* (sweet orange) is considered to be a rare host (Rossetti,

1977; EFSA, 2014, 2019). As is the case for pathotypes A* and A^W of *X. citri* pv. *citri* pathotype B does not infect *C. paradisi*.

***X. citri* pv. *aurantifolii* pathotype C** has a narrow host range, mainly affecting *C. x aurantifolia* (Mexican lime). The rootstock, 'Swingle' citrumelo (*Poncirus trifoliata x Citrus paradisi*) is considered to be a rare host (Jaciani *et al.*, 2009; Jaciani, 2012; Fonseca *et al.*, 2019a). Upon artificial inoculation pathotype C strains infected *C. limonia* (Rangpur lime), *C. latifolia* (Persian lime), *C. limon* (lemon), *C. paradisi* (grapefruit), and *C. reshni* (Cleopatra mandarin) (Malavolta *et al.*, 1984a, 1984b, 1987; Jaciani, 2012).

In artificial inoculation studies conducted on many ornamental Rutaceae with *X. citri* pv. *aurantifolii* B (strain JJ59) and C (strain JV596) pathotypes, Licciardello *et al.* (2022) confirmed the pathogenicity of both strains on *Atalantia buxifolia*, *A. ceylanica* and *A. disticha*, *Balsamocitrus dawei*, *Citrus myrtifolia*, *Eremocitrus glauca* and *Citrus* (*Microcitrus*) *australasica*, and of single strains on *Citrus* (*Microcitrus*) *australis* (pathotype B strain) and *Fortunella japonica* (pathotype C strain).

The host range of *X. citri* pv. *aurantifolii* pathotypes B and C is summarized and compared to *X. citri* pv. *citri* pathotypes A, A* and A^W in Table 2 below.

Table 2 – Host range of *X. citri* pv. *citri* pathotypes A, A* and A^W and *X. citri* pv. *aurantifolii* pathotypes B and C. Adapted from Fonseca *et al.*, 2019b and using data from Gottwald *et al.* (1988; 1991), Jaciani *et al.* (2012), Schoulties *et al.* (1987), Schubert *et al.* (2001), Sun *et al.* (2004) and Vernière *et al.* (1998).

Host*	**Pathogen**				
	***Xcc* A**	***Xcc* A***	***Xcc* A^W**	***Xau* B**	***Xau* C**
Citrus x aurantifolia - Mexican lime	+++	+	+	+	+++
C. aurantium - sour orange	+++	-	-	+	-
C. x latifolia - Persian lime	++	-	-	-	+
C. x limon - lemon	++	-	-	++	+
C. x limonia – Rangpur lime	+++	-	-	-	+
C. macrophylla - alemow	+	-	+	-	-
C. maxima - pomelo	+	-	-	+	-
C. x paradisi - grapefruit	+++	-	-	-	-
C. x paradisi x Poncirus trifoliata - citrumelo	+++	-	-	-	++
C. reshni - Cleopatra mandarin (rootstock)	+	-	-	-	+
C. reticulata - mandarin (tangerine ‘Cravo’)	++	-	-	-	+
C. reticulata - mandarin (tangerine ‘Ponkan’)	+	-	-	-	+
C. sinensis – sweet orange	+++	-	-	+	±

+++ highly pathogenic; ++ moderately pathogenic; + weakly pathogenic; ± doubtful.

Host list: *Citroncirus Citrumelo hybrids, Citrus maxima, Citrus reshni, Citrus reticulata, Citrus x aurantiifolia, Citrus x aurantium var. sinensis, Citrus x aurantium, Citrus x latifolia, Citrus x limon, Citrus x limonia*

GEOGRAPHICAL DISTRIBUTION

The geographical distribution of *X. citri* pv. *aurantifolii* has been reported to be restricted to Argentina, Paraguay and Uruguay (pathotype B) and Brazil (pathotype C) (Rossetti, 1977; Behlau *et al.*, 2020) in South America. However, its current distribution is rather uncertain due to the fact that both pathotypes B and C have gradually been replaced

by the more virulent *X. citri* pv. *citri* A pathotypes. *X. citri* pv. *aurantifolii* has not been recorded in the field since 1990 for pathotype B and since 2009 for pathotype C, see below.

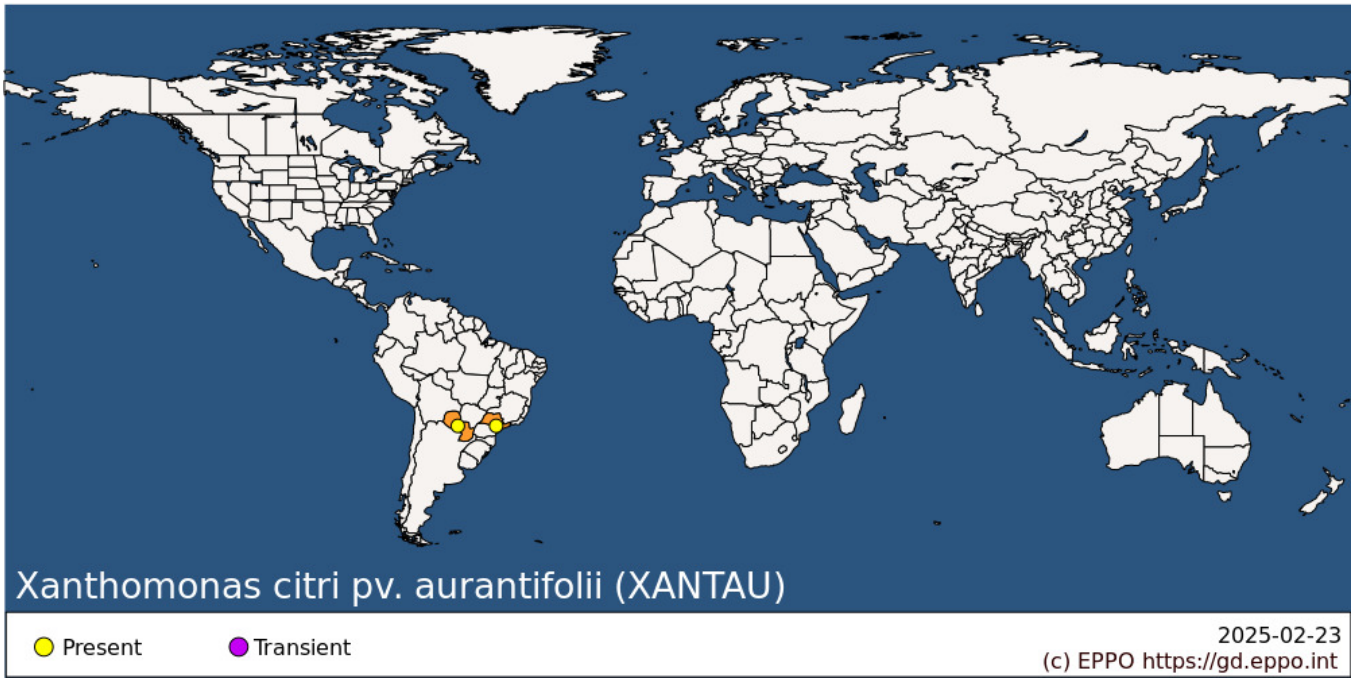
***X. citri* pv. *aurantifolii* pathotype B**

Cancrosis B or South American citrus canker was first observed in North-Eastern Argentina in 1923 in two provinces (Corrientes and Entre Rios), and later in Uruguay in 1936 and Paraguay in 1940 (Fawcett & Bitancourt, 1949; Canteros *et al.*, 1985; Jaciani, 2012; Canteros *et al.*, 2017; Patané *et al.*, 2019). These strains disappeared in Argentina during 1978-90 after the introduction of the more virulent A strains in 1974 (Canteros *et al.*, 1985; Goto *et al.*, 1980). As far as it could be traced, the last B strain isolated in Argentina dates back to 1990 (Fonseca *et al.*, 2019a). A similar situation has been observed in Uruguay and Paraguay (Russi *et al.*, 2013; Licciardello *et al.*, 2022).

***X. citri* pv. *aurantifolii* pathotype C**

Limoneiro gallega (also called Galego acid lime necrosis, Galician lemon canker or cancrosis C) was observed for the first time in 1963 on Mexican lime in São Paulo, Brazil. There are two groups of C strains: brown pigmented, less virulent and non-pigmented, slightly more virulent (Schaad *et al.*, 2006; Jaciani, 2012). A strain of *X. citri* pv. *aurantifolii*, very similar to the original C strains, but only pathogenic to the 'Swingle' citrumelo rootstock (*C. paradisi* × *Poncirus trifoliata*) was described in Severina (São Paulo State). This particular strain induced fewer lesions without erumpent margins, even in young leaves severely infested by the citrus leafminer *Phyllocnistis citrella*, that usually increases incidence (Jaciani *et al.*, 2009; Kapp, 2011; Constantin *et al.*, 2016). Pathotype C strains remained restricted to São Paulo state and were last reported in 2009 on *C. aurantifolia* (Dall'Acqua, 2011; Jaciani, 2012; Fonseca *et al.* 2019a). The fact that C strains have disappeared, or are at least of very limited occurrence, may be also supported by the fact that when citrus canker was observed in Rio Grande do Norte (previously not known to be affected) on *Citrus aurantifolia* (main host of *X. citri* pv. *aurantifolii*) on the cultivar Galego, only *X. citri* pv. *citri* was isolated (Amancio *et al.*, 2021).

The distribution map below is rather uncertain, as no outbreaks of *X. citri* pv. *aurantifolii* have been reported in citrus orchards in South America since the 2000s. Absence has been confirmed in Argentina and Uruguay but not (yet) in São Paulo (Brazil) and Paraguay.



South America: Brazil (Sao Paulo), Paraguay

BIOLOGY

Biological data available in literature predominantly pertains to *X. citri* pv. *citri*, though it is generally considered that the life cycles of both *X. citri* pv. *citri* and *X. citri* pv. *aurantifolii* are largely similar (EFSA, 2014, 2019). Extensive descriptions of the biology (including pathogenesis) of *X. citri* pv. *citri* can be found in Gottwald *et al.* (2002); Ference *et al.* (2018); Caicedo and Villamizar (2021) Naqvi *et al.* (2022) and this has been summarized in the EPPO datasheet on *X. citri* pv.*citri* (EPPO, 2023a). Concerning *X. citri* pv. *aurantifolii*, it can thus be assumed that infection takes place through natural openings (stomata) and wounds created by grove maintenance operations, insects (e.g. *Phyllocnistis citrella*), or adverse climatic conditions (wind, storms). The bacterium most likely survives in canker lesions, which represent the most biologically significant inoculum source. Splash dispersal of the bacterium caused by rain or irrigation occurs over short distances and allows movement of the inoculum between adult trees or between plants in nurseries.

One of the reasons behind the disappearance of the *X. citri* pv. *aurantifolii* pathotype B strains (less virulent) after the introduction of the virulent *X. citri* pv. *citri* A strains into the affected areas, might be linked to the production of inhibitory compounds, as was demonstrated *in vitro*. Such compounds could be bacteriocins, although they have not been identified as such (Gochez, 2014; Canteros *et al.*, 2017).

X. citri pv. *aurantifolii* pathotype C elicits a hypersensitivity response (HR) in specific citrus species, such as sweet orange and lemon (Brunings & Gabriel, 2003; Cernadas *et al.*, 2008). Pathotype C has a narrow host range, unlike pathotype B strains which do not cause this HR and have a broader host range. An avirulence gene, avrGf2, was discovered in a pathotype C strain, responsible for eliciting a HR in grapefruit (*C.* x *paradisi*). This avrGf2 gene is related to avrGf1 found in *X. citri* pv. *citri* pathotype A^W strains, which also cause a HR in grapefruit. *X. citri* pv. *aurantifolii* pathotype B strains contain a transposon in avrGf2, rendering it non-functional. This may explain the broader host range of B strains (Gochez, 2014; Gochez *et al.*, 2008, 2015 and 2017). Additional effector genes that differentiate *X. citri* pv. *citri* pathotype A and *X. citri* pv. *aurantifolii* pathotype B and C strains have been extensively described by Hajri *et al.* (2009); Moreira *et al.* (2010); Escalon *et al.* (2013); Ference *et al.* (2018) and Fonseca *et al.* (2019a). In summary, *X. citri* pv. *aurantifolii* pathotypes B and C lack several key genes important for pathogenesis when compared to *X. citri* pv. *citri A* pathotype. The higher virulence exhibited by *X. citri* pv. *citri* pathotype A strains, as well as their dominance in the field, can be explained by the presence and composition of the Type I and IV Secretion Systems and the Type IV pilus system (Dunger *et al.*, 2014). This in comparison with the lower virulence of the *X. citri* pv. *aurantifolii* pathotype B and C strains, (Fonseca *et al.*, 2019a).

Both pathotypes B and C of *X. citri* pv. *aurantifolii* differed when their genomes were compared with those of *X. citri* pv. *citri* pathotype A strains. For instance, the absence of the *rpf*N gene, critical for biofilm formation, in *X. citri* pv. *aurantifolii* pathotype B might account for its slow growth rate, also related to a low xanthan gum production and its dependence on glutamate in culture media as a carbon source. This is similar to the slow growth of another citrus pathogen, *Xylella fastidiosa*, which also lacks the *rpf*N gene (Moreira *et al.*, 2010).

DETECTION AND IDENTIFICATION

Correct identification of citrus bacterial canker pathogens and related pathogens causing Citrus bacterial spot is critical. Incorrect identification in the USA prompted the removal of thousands of productive citrus trees that were infected only with citrus bacterial spot, a mild disease caused by the related (now largely deregulated) *X. euvesicatoria* subsp. *citrumelonis* (formerly *X. axonopodis* pv. *citrumelo*, *X. alfalfa* pv. *citrumelonis*) Schaad *et al.* (2006).

Extensive description of symptoms on diverse hosts can also be found in Civerolo (1984); Goto (1992); Gottwald *et al.* (2002) and Graham *et al.* (2004).

Symptoms

The symptomatology of *X. citri* pv. *aurantifolii* is similar to that of *X. citri* pv. *citri*. These bacteria causing citrus canker infect all aerial parts of their 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.]

[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].

Symptoms of citrus canker on fruits may be confused with those of citrus scab (*Elsinoe fawcetti*), *Phaeoramularia* leaf and fruit spot disease (*Phaeoramularia angolensis*) or greasy spot (*Mycosphaerella citri*); Civerolo, 1984; Timmer *et al.*, 2000; EFSA, 2014, 2019). Lesions caused by *X. citri* pv. *aurantifolii* appear slower and are generally smaller than those caused by *X. citri* pv. *citri* (A strains) (Goto *et al.*, 1980; EFSA, 2014 and 2019; CABI, 2023).

Morphology

[*X. citri* pv. *aurantifolii* is morphologically similar to *X. citri* pv. *citri*. Bacterial 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].

Strains of *X. citri* pv. *aurantifolii* produce single colonies on agar plates usually after 4-6 days. Occasionally pathotype C strains produce a brown diffusible pigment (Jaciani, 2012). In comparison colonies of *X. citri* pv. *citri* and *X. euvesicatoria* pv. *citrumelonis* grow more rapidly and usually appear after 2-3 days and 1-2 days, respectively (Schaad *et al.*, 2005, 2006).

Detection, identification and inspection methods

Canteros *et al.* (1985) developed an elective medium for isolation and cultivation of *X. citri* pv. *aurantifolii* pathotype B strains. This elective media should contain Difo purified agar as base, as other agars failed to give satisfactory growth.

Serological tests using polyclonal or monoclonal antibodies have been previously developed and can detect both *X. citri* pv. *citri* and *X. citri* pv. *aurantifolii* (Namekata & Oliveira, 1972; Civerolo & Fan, 1982; Alvarez *et al.*, 1991). However, monoclonal antibodies raised against *X. citri* pv. *citri* failed to react with some pathotype A* strains (Vernière *et al.*, 1998) and could cross-react with unrelated xanthomonads (Alvarez *et al.*, 1991). Moreover, enzyme-linked immunosorbent assays (ELISAs) are inadequate for detecting low bacterial populations but could be used for symptomatic material (EFSA, 2014, 2019).

Discriminative physiological and biochemical tests for have been described by Goto *et al.* (1980), Vernière *et al.*

(1991 and 1993) and Schaad *et al.* (2005, 2006). Strains of *X. citri* pv. *citri* are susceptible to bacteriophage CP1 and CP2 whereas those of *X. citri* pv. *aurantifolii* are not (Goto *et al.*, 1980; Schaad *et al.*, 2006, citing the thesis of Namekata, 1971).

Multilocus sequence analysis (MLSA), using e.g., *atpD, dnaK* and *fusA* genes can reliably differentiate between *X. citri* pv. *citri* and *X. citri* pv. *aurantifolii* pathotype B and C (Bui Thi Ngoc *et al.*, 2010; Dall'Acqua, 2011). Conventional PCR and real-time PCR primers that discriminate between *X. citri* pv. *citri* and *X. citri* pv. *aurantifolii* pathotype B and C have been described by Cubero & Graham (2002, 2005); Delcourt *et al.* (2013); Yu *et al.* (2012, 2017); Fonseca *et al.* (2019b); Robène *et al.* (2020); Yasuhara-Bell *et al.* (2023).

Strains of *X. citri* pv. *citri* are susceptible to bacteriophage CP1 and CP2 whereas those of *X. citri* pv. *aurantifolii* are not (Goto *et al.*, 1980; Schaad *et al.*, 2006, citing the thesis of Namekata (1971). Destefano & Rodrigues (2002) found that pigment producing C strains did not differ in 16S-23S intergenic region sequences and in pathogenicity, however in other studies (Nociti *et al.*, 2006; Jaciani *et al.*, 2012) pigment producing strains were less virulent on *C.* x *aurantifolia* than non-pigmented strains and they could be discriminated by ERIC-PCR (Jaciani *et al.*, 2012).

Details about presumptive diagnosis with rapid tests, detection and identification methods (including methods for extraction of bacterial cells and DNA), biochemical, serological and molecular and pathogenicity tests (using inoculation of bean plantlets or hilum injury/seed inoculation) for latent and symptomatic infected material, flow chart, culture media, chemicals and reference material) are provided in the EPPO Standard PM 7/44 *Xanthomonas citri* pv. *citri* and *Xanthomonas citri* pv. *aurantifolii* (EPPO, 2023b) and IPPC Diagnostic protocol DP 6 (IPPC, 2016).

PATHWAYS FOR MOVEMENT

As is the case for *X. citri* pv. *citri, X. citri* pv. *aurantifolii* can be spread by the movement of contaminated plant propagative material, 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, 2019):

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 be very likely to 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*, citrus canker is one of the main phytosanitary threats for citrus industries worldwide. Citrus canker has had and still has serious direct and indirect economic impacts. Direct impacts included alteration of fruit quality and yield (due to early fruit drop), the severity of the effect being influenced by the host species, the bacterial strain and the environmental conditions. Indirect impacts include restricted access to fruit export markets and undesirable

consequences of chemical treatments.

However, due to its generally low virulence and restricted host range, easy control by copper containing bactericides and the replacement of *X. citri* pv. *aurantifolii* by the more virulent strains of *X. citri* pv. *citri* in South America, the current impact of *X. citri* pv. *aurantifolii* in areas where it might still be present is currently very low, if not nil. In Argentina, where B strains occurred for 40 years only in a small area with little impact, disappearing and replaced by pathotype A strains around 1990 (Goto *et al*., 1980; Canteros *et al*., 1985; Jaciani *et al*., 2009; Kapp, 2011; Jaciani, 2012; Canteros *et al*., 2017). Nociti *et al*. (2006) described the occurrence of pathotype C as restricted to a few municipalities in the state of São Paulo, Brazil only, without causing significant economic damage.

Control

As explained in the EPPO datasheet on *X. citri* pv. *citri* (EPPO, 2023a), the control strategy against citrus canker is based on integrated pest management (IPM), which aims to reduce the rate of infection and spread of the disease, and attempt to keep it 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. The integrated approach described above, was and is primarily achieved for *X. citri* pv. *citri* but it would manage and control *X. citri* pv. *aurantifolii* (Leite & Mohan, 1990; Dewdney & Johnson, 2023). B strains can effectively be controlled by copper containing bactericides (Canteros *et al.*, 2017).

For a comprehensive understanding of the various control measures and possibilities in managing citrus canker, recent publications by Gottwald *et al.* (2002), Das (2003), de Carvalho *et al.* (2015), and Ference *et al.* (2018) and EFSA (2014, 2019) offer valuable insights.

Phytosanitary risk

Bacteria associated with citrus canker were estimated to be likely to establish and spread in the European Union if reaching susceptible hosts (EFSA, 2014; 2019). Citrus canker is a risk for the EPPO region 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.

Long-distance spread of citrus canker can occur through the movement of diseased, latently infected or contaminated propagating material (e.g., budwood, rootstock, seedlings and budded trees, and also as its trade is increasing ornamental host plants) and fruits (Graham *et al.*, 2004; Golmohammadi *et al.*, 2007; EFSA, 2014, 2019).

Asiatic bacterial canker, particularly caused by *X. citri* pv. *citri* pathotype A, presents the most significant risk for the European region, primarily concentrated around the Mediterranean Basin. This risk is considerably higher compared to the risk posed by *X. citri* pv. *aurantifolii*. Pathotype B strains have not been observed since 1990 and cause only a mild disease and the pathotype C strains, not observed after 2009, are even of a lesser concern, because *C. aurantifolia* is hardly cultivated in the Mediterranean region (EFSA, 2014, 2019). It is worth noting that the citrus leaf miner *Phyllocnystis citrella*, that can exacerbate the disease and facilitate its spread, is widely distributed in the citrus-producing areas of the Mediterranean Basin. Nonetheless there is at present no citrus bacterial canker reported in the EPPO region, and precautionary phytosanitary measures should be implemented for all forms of *X. citri* as outlined in this datasheet (Timpanaro *et al*., 2020, 2021). This proactive approach aims to prevent and mitigate potential outbreaks of citrus bacterial canker within the EPPO region.

PHYTOSANITARY MEASURES

It has been shown that once transferred to a suitable host, citrus canker can only be controlled with strong phytosanitary measures. Eradication has been attempted against *X. citri* pv. *citri* with different results, it was successful in Australia, unsuccessful in the USA for example (Gottwald *et al*., 2001). Eradication seems a feasible option for the less aggressive *X. citri* pv. *aurantifolii*, should the latter be introduced into new areas. As a general remark, successful eradication requires efficient surveillance systems as well as quick and appropriate management measures on diseased and exposed trees.

Considering the severity of citrus canker, EPPO countries are recommended to prohibit the importation of citrus plants for planting and cut branches from areas or countries where the disease occurs. For the EU, the current phytosanitary measures (EU Regulation 2019/2072, 2019) are targeting both *X. citri* pv. *citri* and *X. citri* pv. *aurantifolii*. In summary, these measures include a prohibition to import plants for planting of *Citrus*, *Fortunella* and *Poncirus* from third countries. Plants for planting of *Citrus*, *Naringi* and *Swinglea* can only be imported from pest-free third countries or pest-free areas. Imports of fruit are also subject to restrictions, such as fruit should be free from peduncles and leaves and should originate pest-free third countries, pest-free areas or pest-free places of production. These measures have been described in more details in the EPPO datasheet on *X. citri* pv. *citri* (EPPO, 2023a).

[National regulatory control systems are recommended to EPPO countries for the surveillance, early detection and eradication of citrus canker, 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 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, 2023b)].

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