

ADOPTED: 21 October 2021 doi: 10.2903/j.efsa.2021.6929

# Pest categorisation of Xanthomonas citri pv. viticola

EFSA Panel on Plant Health (PLH), Claude Bragard, Francesco Di Serio, Paolo Gonthier, Josep Anton Jaques Miret, Annemarie Fejer Justesen, Alan MacLeod, Christer Sven Magnusson, Panagiotis Milonas, Juan A Navas-Cortes, Stephen Parnell, Roel Potting, Hans-Hermann Thulke,

Wopke Van der Werf, Antonio Vicent Civera, Jonathan Yuen, Lucia Zappalà, Quirico Migheli, Emilio Stefani, Irene Vloutoglou, Ewelina Czwienczek, Andrea Maiorano, Franz Streissl and Philippe Lucien Reignault

# Abstract

The EFSA Plant Health Panel performed a pest categorisation of Xanthomonas citri pv. viticola (Nayudu) Dye, a Gram-negative bacterium belonging to the Xanthomonadaceae family. The pathogen is a well-defined taxonomic unit and is the causal agent of the leaf spot and bacterial canker of Vitis vinifera. This bacterium is present in India and Brazil, where it affects table grape cultivation; the same pathogen is able to cause a disease on Azadirachta indica and on some weed species. Reports indicate that the bacterium is present in Thailand as well. The pathogen has never been reported from the EU territory and it is not included in EU Commission Implementing Regulation 2019/2072. The pathogen can be detected on its host plants using direct isolation, serological or PCR-based methods. Its identification is achieved using biochemical and nutritional assays, together with a multilocus sequence analysis based on seven housekeeping genes. The main pathway for the entry of the pathogen into the EU territory is plant propagation material. In the EU, there is large availability of host plants, with grapevine being one of the most important crops in Europe and more specifically in its Mediterranean areas. Since X. citri pv. viticola is only reported in tropical and subtropical areas (BSh and Aw climatic zones according to the Köppen–Geiger classification), there is uncertainty whether the climatic conditions in the EU territory are suitable for its establishment. Nevertheless, due to the great importance of grapevine for the EU agriculture, any disease outbreak may have a high-economic impact. Phytosanitary measures are available to prevent the introduction of the pathogen into the EU. X. citri pv. viticola satisfies the criteria that are within the remit of EFSA to assess for this species to be regarded as a potential Union guarantine pest.

© 2021 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Plant health, Pest risk, Quarantine, Vitis sp., Grapevine, Leaf spot, Bacterial canker

Requestor: European Commission Question number: EFSA-Q-2021-00331 Correspondence: alpha@efsa.europa.eu



**Panel members:** Claude Bragard, Francesco Di Serio, Paolo Gonthier, Josep Anton Jaques Miret, Annemarie Fejer Justesen, Alan MacLeod, Christer Sven Magnusson, Panagiotis Milonas, Juan A. Navas-Cortes, Stephen Parnell, Roel Potting, Philippe L. Reignault, Hans-Hermann Thulke, Wopke Van der Werf, Antonio Vicent Civera, Jonathan Yuen and Lucia Zappalà.

**Declarations of interest:** The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

**Acknowledgements:** EFSA wishes to acknowledge the contribution of Caterina Campese to this opinion.

**Suggested citation:** EFSA PLH Panel (EFSA Panel on Plant Health), Bragard C, Di Serio F, Gonthier P, Jaques Miret JA, Justesen AF, MacLeod A, Magnusson CS, Milonas P, Navas-Cortes JA, Parnell S, Potting R, Thulke H-H, Van der Werf W, Vicent Civera A, Yuen J, Zappalà L, Migheli Q, Stefani E, Vloutoglou I, Czwienczek E, Maiorano A, Streissl F and Reignault PL, 2021. Scientific Opinion on the pest categorisation of *Xanthomonas citri* pv. *viticola*. EFSA Journal 2021;19(12):6929, 25 pp. https://doi.org/10.2903/j.efsa.2021.6929

#### **ISSN:** 1831-4732

© 2021 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the Creative Commons Attribution-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

Reproduction of the images listed below is prohibited and permission must be sought directly from the copyright holder:

Figure 3: © EUROSTAT



The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



# Table of contents

Abstract	t			
1.	Introduction			
1.1.	Background and Terms of Reference as provided by the requestor			
1.1.1.	Background	4		
1.1.2.	Terms of reference	4		
1.2.	Interpretation of the Terms of Reference	4		
2.	Data and methodologies	5		
2.1.	Data	5		
2.1.1.	Literature search	5		
2.1.2.	Database search	5		
2.2.	Methodologies	5		
3.	Pest categorisation	6		
3.1.	Identity and biology of the pest	6		
3.1.1.	Identity and taxonomy	6		
3.1.2.	Biology of the pest	7		
3.1.3.	Host range	8		
3.1.4.	Intraspecific diversity	9		
3.1.5.	Detection and identification of the pest	9		
3.2.	Pest distribution	10		
3.2.1.	Pest distribution outside the EU	10		
3.2.2.	Pest distribution in the EU	11		
3.3.	Regulatory status	11		
3.3.1.	Commission Implementing Regulation 2019/2072			
3.3.2.	Hosts of <i>X. citri</i> pv. viticola that are prohibited from entering the Union from third countries			
3.4.	Entry, establishment and spread in the EU	11		
3.4.1.	Entry	11		
3.4.2.	Establishment	12		
3.4.2.1.	EU distribution of main host plants	13		
3.4.2.2.	Climatic conditions affecting establishment			
3.4.3.	Spread	17		
3.5.	Impacts	17		
3.6.	Available measures and their limitations			
3.6.1.	Identification of potential additional measures	18		
3.6.1.1.	Biological or technical factors limiting the effectiveness of measures to prevent the entry (and			
	spread when applicable) of the pest			
3.7.	Uncertainty			
4.	Conclusions			
	ces			
Abbreviations 22				
	Glossary 23			
Appendix A – Xanthomonas citri pv. viticola host plants 24				
Appendi	Appendix B – Distribution of Xanthomonas citri pv. viticola			



# 1. Introduction

#### **1.1.** Background and Terms of Reference as provided by the requestor

#### **1.1.1. Background**

The new Plant Health Regulation (EU) 2016/2031, on the protective measures against pests of plants, is applying from 14 December 2019. A focus on prevention and risk targeting is amongst the primary objectives of this legislation. Furthermore, conditions are laid down in this legislation for plant pests to qualify for listing as Union quarantine pests, protected zone quarantine pests or Union regulated non-quarantine pests. The lists of the EU regulated plant pests together with the associated import or internal movement requirements of commodities are included in Commission Implementing Regulation (EU) 2019/2072.

In line with the principles of the new plant health law, for a proactive approach, the European Commission with the Member States are discussing monthly the reports of the interceptions, together with data from horizon scanning for plant pests of concern of various sources. As outcome of those discussions, a number of plant pests of concern, not regulated in the EU, are identified, for which a risk assessment is needed to decide on potential EU regulation. *Leucinodes orbonalis* - which was recently spilt into two species *Leucinodes orbonalis* and *Leucinodes pseudorbonalis*, and *Xanthomonas citri* pv. *viticola* are amongst the species identified during these discussions.

In the EU, a number of actions are already in place to mitigate the various multilevel effects of climate change. The aim is to avoid adverse changes to the environment and to ensure food security. As the success of plant pests to establish in an area, depends on various abiotic and biotic parameters, it is anticipated that climate change might affect the risk that certain plant pests pose. Parameters as temperature, humidity,  $CO_2$  concentration and salinity of soil affect the survival and pathogenicity of a number of plant pests, as reported in the scientific literature. Changes in temperature, drought and salinity can affect also the geographic distribution of the hosts of plant pests, and, as a consequence, the plant pests' establishment.

There is therefore a need to develop further the quantitative risk assessment methodology followed for plant pests and consider including the effect of climate change in the assessment of the risk that plant pests pose to the EU.

#### **1.1.2.** Terms of reference

In accordance with Article 29(1) of Regulation (EC) No 178/2002, the Commission asks EFSA to develop further the quantitative risk assessment (phase 1 and phase 2) methodology followed for plant pests, to include in the assessments the effect of climate change for plant pests. Such inclusion of climate change scenarios can benefit of the quantitative methodology with comparison of risk assessment scenarios which has been already developed by the EFSA PLH Panel and included in its Guidance on quantitative pest risk assessment. Examples of abiotic parameters affecting the biology of the pests and their hosts' distribution are given in the background. The aim of this methodological development is to enable risk projections in the future, with models taking into account the relevant critical parameters for spread, establishment and potential impact that are affected in a scenario of 'climate change'.

The risk assessments of *Leucinodes orbonalis*, *Leucinodes pseudorbonalis* and *Xanthomonas citri* pv. *viticola* can be used for the development of the methodology.

#### **1.2.** Interpretation of the Terms of Reference

EFSA PLH Panel has been requested to conduct a risk assessment for *Xanthomonas citri* pv. *viticola*. This document is the phase 1 component (pest categorisation) fulfilling the request. The purpose of the pest categorisation is to determine whether *Xanthomonas citri* pv. *viticola citricitri* fulfils the criteria of a quarantine pest for the area of the EU excluding Ceuta, Melilla and the outermost regions of Member States referred to in Article 355(1) of the Treaty on the Functioning of the European Union (TFEU), other than Madeira and the Azores, and so inform European Commission decision-making as to its appropriateness for potential inclusion in the lists of pests of Commission Implementing Regulation (EU) 2019/2072.

If *Xanthomonas citri* pv. *viticola* fulfils the criteria to be potentially listed as a regulated pest, risk reduction options aimed to prevent entry will be identified. Consideration of climate change is beyond



the scope of pest categorisation, but will follow in a separated phase 2 assessment, if *Xanthomonas citri* pv. *viticola* satisfies the criteria for quarantine pest status.

# 2. Data and methodologies

#### 2.1. Data

#### 2.1.1. Literature search

A literature search on *Xanthomonas citri* pv. *viticola* was conducted at the beginning of the categorisation in the ISI Web of Science bibliographic database, using the scientific names of the pest as search terms. Papers relevant for the pest categorisation were reviewed, and further references and information were obtained from experts, as well as from citations within the references and grey literature.

#### 2.1.2. Database search

Pest information, on host(s) and distribution, was retrieved from the European and Mediterranean Plant Protection Organization (EPPO) Global Database (EPPO, online), the CABI databases and scientific literature databases as referred above in Section 2.1.1.

Data about the import of commodity types that could potentially provide a pathway for the pest to enter the EU and about the area of hosts grown in the EU were obtained from EUROSTAT (Statistical Office of the European Communities).

The Europhyt and TRACES databases were consulted for pest-specific notifications on interceptions and outbreaks. Europhyt was a web-based network run by the Directorate General for Health and Food Safety (DG SANTÉ) of the European Commission as a subproject of PHYSAN (Phyto-Sanitary Controls) specifically concerned with plant health information. TRACES is the European Commission's multilingual online platform for sanitary and phytosanitary certification required for the importation of animals, animal products, food and feed of non-animal origin and plants into the European Union, and the intra-EU trade and EU exports of animals and certain animal products. Up until May 2020, the Europhyt database managed notifications of interceptions of plants or plant products that do not comply with EU legislation, as well as notifications of plant pests detected in the territory of the Member States and the phytosanitary measures taken to eradicate or avoid their spread. The recording of interceptions switched from Europhyt to TRACES in May 2020.

#### 2.2. Methodologies

The Panel performed the pest categorisation for *Xanthomonas citri* pv. *viticola* following guiding principles and steps presented in the EFSA guidance on quantitative pest risk assessment (EFSA PLH Panel, 2018), the EFSA guidance on the use of the weight of evidence approach in scientific assessments (EFSA Scientific Committee, 2017) and the International Standards for Phytosanitary Measures No. 11 (FAO, 2013) and No. 21 (FAO, 2004).

The criteria to be considered when categorising a pest as a potential Union quarantine pest (QP) is given in Regulation (EU) 2016/2031 Article 3 and Annex I, Section 1 to this Regulation. Table 1 presents the Regulation (EU) 2016/2031 pest categorisation criteria on which the Panel bases its conclusions. In judging whether a criterion is met the Panel uses its best professional judgement (EFSA Scientific Committee, 2017) by integrating a range of evidence from a variety of sources (as presented above in Section 2.1) to reach an informed conclusion as to whether or not a criterion is satisfied.

The Panel's conclusions are formulated respecting its remit and particularly with regard to the principle of separation between risk assessment and risk management (EFSA founding regulation (EU) No 178/2002); therefore, instead of determining whether the pest is likely to have an unacceptable impact, deemed to be a risk management decision, the Panel will present a summary of the observed impacts in the areas where the pest occurs, and make a judgement about potential likely impacts in the EU. Whilst the Panel may quote impacts reported from areas where the pest occurs in monetary terms, the Panel will seek to express potential EU impacts in terms of yield and quality losses and not in monetary terms, in agreement with the EFSA guidance on quantitative pest risk assessment (EFSA PLH Panel, 2018) Article 3(d) of Regulation (EU) 2016/2031 refers to unacceptable social impact as a criterion for quarantine pest status. Assessing social impact is outside the remit of the Panel.



**Table 1:** Pest categorisation criteria under evaluation, as defined in Regulation (EU) 2016/2031 on<br/>protective measures against pests of plants (the number of the relevant sections of the<br/>pest categorisation is shown in brackets in the first column)

Criterion of pest categorisation	Criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest (article 3)
Identity of the pest (Section 3.1)	Is the identity of the pest established, or has it been shown to produce consistent symptoms and to be transmissible?
Absence/presence of the pest in the EU territory (Section 3.2)	Is the pest present in the EU territory? If present, is the pest widely distributed within the EU? Describe the pest distribution briefly
Regulatory status (Section 3.3)	If the pest is present in the EU but not widely distributed in the risk assessment area, it should be under official control or expected to be under official control in the near future.
Pest potential for entry, establishment and spread in the EU territory (Section 3.4)	Is the pest able to enter into, become established in, and spread within, the EU territory? If yes, briefly list the pathways
Potential for consequences in the EU territory (Section 3.5)	Would the pests' introduction have an economic or environmental impact on the EU territory?
Available measures (Specific import requirements) (Section 3.6)	Are there measures available to prevent the entry into the EU such that the likelihood of introduction becomes mitigated?
Conclusion of pest categorisation (Section 4)	A statement as to whether (1) all criteria assessed by EFSA above for consideration as a potential quarantine pest were met and (2) if not, which one(s) were not met.

# 3. Pest categorisation

# **3.1.** Identity and biology of the pest

**3.1.1. Identity and taxonomy** 

Is the identity of the pest established, or has it been shown to produce consistent symptoms and/or to be transmissible?

Yes, the identity of the pest is established. The pest has been shown to produce symptoms on its host plants and it is transmissible.

Xanthomonas citri pv. viticola is a Gammaproteobacterium belonging to the Order of Xanthomonadales and Family of Xanthomonadaceae. Its first description dates back to 1972, when it was isolated from cankers affecting grapevine in India (Nayudu, 1972), though Desai et al. (1966) already isolated the pest from Azadirachta indica, without giving it a taxonomic position. Taxonomically, it was initially named Pseudomonas viticola but, later, based on its physiological and biochemical features, it was recognised as a Xanthomonas sp., specifically, Xanthomonas citri pv. viticola (Chand and Kishun, 1990; da Gama et al., 2018). More recently, following a gene sequence analysis of the housekeeping gene gyrase B (gyrB) for over 200 xanthomonads (including 67 poorly characterised pathovars of X. citri), the Indian pathotype strain was grouped in the X. citri subsp. citri clade (Parkinson et al., 2009). Finally, a taxonomic reposition as X. citri pv. viticola comb. nov. was proposed (da Gama et al., 2018) and, in addition, phylogenomic analysis revealed that pv. viticola forms a monophyletic cluster and belongs to one species, X. citri (Bansal et al., 2017). Additional studies are being done on the taxonomic position of X. citri pv. viticola using the in silico taxonomic classification and pan-genome analysis: Such very recent studies raised doubts about the classification of X. citri pathovars, but confirmed the recent reclassification of pv. viticola in the X. citri species (Gomes de Farias, 2020). Three other pathovars affecting Vitis spp. (other than V. vinifera) and taxonomically very close to X. citri pv. viticola have been reported in India in the 1950s and 1960s: pv. vitistrifoliae, pv. vitiscarnosae and pv. vitiswoodrowii. Based on multilocus sequence analysis (MLSA), the four pathovars affecting distinct species in the family Vitaceae are distinct from each other, though pv. vitistrifoliae fells into one clade with pv. viticola (Ferreira et al., 2019). Experimental inoculations of such pvs. on



*V. vinifera*, cv. Sauvignon, confirmed the pathogenicity for pv. *vitistrifoliae* and pv. *vitiscarnosae*, but not for pv. *vitiswoodrowii* (Ferreira et al., 2019).

The EPPO code<sup>1</sup> (Griessinger and Roy, 2015; EPPO, 2019) for this species is: XANTCV (EPPO, online).

#### **3.1.2.** Biology of the pest

The key biological features of *X. citri* pv. *viticola* are included in Table 2.

Disease cycle	Infection process and relation to host	Other relevant information
Overwintering phase of the pathogen	Cankers present on its host plant. As an epiphyte on mango, on infected <i>Azadirachta indica</i> , as an epiphyte on a set of annual plants or weeds.	The pathogen has the potential to survive as an epiphyte on a number of cultivated and wild plant species. This feature is of pivotal importance for the survival of the pathogen in the environment, when its host plants are in the dormant phase or in the off-season.
Primary inoculum	Bacterial exudates from cankers present on host plants. Bacteria populations latently infecting	Bacteria exudating from cankers are easily disseminated through wind-driven raindrops, or heavy rains and showers.
	plant tissues.	Primary inoculum may be disseminated inside the vineyard through pruning. In this casecasecase, bacterial cells that are latently present in the plant tissue may be regarded as primary inoculum as well.
Penetration into the host plant	Pathogen penetration into its host plants occurs through stomata, lenticels, wounds	Tropical showers and rain storms easily cause wounds on leaves and sprouts, thus facilitating the penetration of the inoculum.
Secondary inocula	Evasion of secondary inoculum inocula occurs through lesions on plant tissues where the bacteria grow and ooze out.	The disease is polycyclic. Rains, showers, monsoon, may facilitate dissemination of secondary inoculum inocoulato short and medium distances by means of wind gusts.
Pathogen latency	The pathogen is asymptomatically harboured inside grapevine propagation material in its latent phase.	The presence of the pathogen as an epiphyte on weeds and cultivated plants, other than the specific host, should not be regarded as a latent phase.

Table 2: Important features of the pathogen Xanthomonas citri pv. viticola and its disease cycle

*X. citri* pv. *viticola* is the causal agent of the grapevine bacterial leaf spot and canker and, where the disease occurs, it is considered a major threat in viticulture (Rodrigues Neto et al., 2011; da Gama et al., 2018). Primary inoculum of the pathogen may reside in propagation material, namely in latently infected scions that are grafted onto rootstocks (Rodrigues Neto et al., 2011). Grapevine rootstocks do not appear to be suitable host plants, since they generally belong to *Vitis* spp., or hybrids, other than *V. vinifera*. Wounds and stomata appear to be the main penetration sites for the pathogen; this is also confirmed by pathogenicity tests, where the organ(s) to be infected should be previously wounded (Rodrigues Neto et al., 2011). Under natural conditions, stomata might serve as alternative penetration sites for the pathogen (Lima et al., 1999). There is no indication in the literature that any animal may serve as a vector or vehicle of the pathogen. Symptoms may develop starting from 12 to 14 days after inoculation and they are indicative for a vascular-parenchymatic colonisation of the host tissues (Lima et al., 1999). *X. citri* pv. *viticola* is a systemic pathogen in its major host plant, *V. vinifera*: after its penetration, it colonises the vascular tissue and may infect the grapes and the berries, which may develop necrotic spots or necrotise entirely, up to the seeds (Tostes et al., 2014).

Pathogen evasion from affected host tissues and its dissemination is aided by pruning, when tools become contaminated and support pathogen transmission from infected vines to healthy ones (Lima

<sup>&</sup>lt;sup>1</sup> An EPPO code, formerly known as a Bayer code, is a unique identifier linked to the name of a plant or plant pest important in agriculture and plant protection. Codes are based on genus and species names. However, if a scientific name is changed, the EPPO code remains the same. This provides a harmonised system to facilitate the management of plant and pest names in computerised databases, as well as data exchange between IT systems (Griessinger and Roy, 2015; EPPO, 2019).



et al., 1999). Heavy rainfalls, storms and monsoons also favour pathogen dissemination and penetration, due to leaf ruptures and vine cracks (Nayudu, 1972). Therefore, the pathogen may have two or more cycles during the growing season and it is able to survive during long dry spells that are typical in the areas where the disease is reported. It should be noted that most Indian and Brazilian areas where the disease is reported have a hot semi-arid climate (type BSh, according to the Köppen–Geiger classification). Under that climate, 2–2.5 table grape harvests per year are possible even in the same vineyard (Kok, 2014), and monsoon and tropical rains may favour pathogen survival and dissemination. In Maharashtra, Karnataka and Andhra Pradesh, grapevine cultivation does not follow a seasonal trend as in the EU territory: In the same area, or also side by side, vineyards may contemporarily be in different phenological phases. After crop harvest, plants are pruned to favour the next vines development, contemporarily, other neighbouring vineyards are flowering and other are in the phase of veraison (personal communication Dr. Marisa Fontana, Scarabelli-Ghini Institute of Agriculture, Imola). This kind of continuous grapevine cultivation, common in the subtropics, greatly favours the permanence of sufficient, active inoculum all year round.

From one growing cycle to the next one, pathogen survival occurs in cankers that develop on spurs and canes. *X. citri* pv. *viticola* is a biotrophic and non-sporulating bacterium; therefore, it does not survive long in plant debris or pruning residues, especially if they are chopped and/or mulched. Conversely, as several other xanthomonads, *X. citri* pv. *viticola* may survive epiphytically on suitable cultivated or spontaneous plant species (Nayudu, 1972; Chand and Kishun, 1990).

#### 3.1.3. Host range

The reported host range of X. citri pv. viticola is limited to grapevine (Vitis vinifera L.) as a major host plant; there are indications that hybrids, e.g. Vitis labrusca x vinifera, might be tolerant or immune to the bacterium, since plots of such hybrids located aside a severely affected vineyard did not get any infection (Rodrigues Neto et al., 2011). According to field observations in India and Brazil, X. citri pv. viticola mostly affects seedless table grapes (Chand and Kishun, 1990; Villela et al., 2019); it is so far unclear, if such genotypes are more susceptible than those cultivated for wine production or, conversely, their reported susceptibility is merely due to their cultivation as the main crop in the outbreak areas. Nayudu (1972) reported that cultivated A. indica (neem tree) and the perennial herbaceous species Phyllanthus maderaspatensis, widespread in the tropics and subtropics, are natural hosts of the pathogen, though Chand and Kishun (1990) failed to reproduce symptoms on neem trees after experimental inoculations. Mango (Mangifera indica L.) is reported as asymptomatic inoculum reservoir, ensuring pathogen survival during the off-season (Chand and Kishun, 1990); the same authors, in the same paper, report mango as 'a new collateral host', making this assumption contradictory with the previous one. Experimental inoculations produced symptoms on the following plants: A. indica and P. maderaspatensis (Nayudu, 1972), Mangifera indica (Chand and Kishun, 1990); Citrus limon, Coccinia indica, Euphorbia hirta, E. heterophylla, Lantana camara, Oxalis corniculata, Sida acuta, Solanum nigrum, Taraxacum officinale (Kamble et al., 2019). Possible alternative hosts are Alternanthera tenella, Amaranthus sp., wild Glycine sp. and Senna obtusifolia (Peixoto et al., 2007).

Host plant name	Cultivated or spontaneous	Economic relevance	Reference
Vitis vinifera	Cultivated	Major	Nayudu (1972), Lima et al. (1999)
Azadirachta indica	Cultivated	Minor	Nayudu (1972)
Mangifera indica	Cultivated	Experimental*	Chand and Kishun (1990), Araujo et al. (1999)
Alternanthera tenella	Spontaneous weed	Minor	Peixoto et al. (2007)
Amaranthus sp.	Spontaneous weed	Minor	Peixoto et al. (2007)
Glycine sp.	Spontaneous weed	Minor	Peixoto et al. (2007)
Phyllanthus maderaspatensis	Spontaneous weed	Minor	Nayudu (1972)
Senna obtusifolia	Spontaneous weed and medicinal plant	Minor	Peixoto et al. (2007)

\*: The paper of Chand and Kishun (1990) does not clearly define the role and importance of mango in the epidemiology of *X. citri* pv. *viticola*.



#### 3.1.4. Intraspecific diversity

A collection of isolates from India and Brazil and assigned to the pv. *viticola* of the *Xanthomonas citri* species have been taxonomically studied using MLSA based on seven housekeeping genes and a multilocus variable number of tandem repeat analysis (MLVA) with eight loci (Ferreira et al., 2019). The tight genetic relatedness of all strains has been confirmed. MLSA revealed lack of diversity in all seven genes and grouped grapevine and amaranth strains in a monophyletic group in *X. citri*. Nevertheless, the VNTR (variable number of tandem repeat) typing scheme conducted on 107 strains detected 101 haplotypes. The total number of alleles per locus ranged from 5 to 12. A minimum spanning tree (MST) showed that Brazilian strains were clearly separated from Indian strains, which showed unique alleles at three loci.

#### **3.1.5.** Detection and identification of the pest

Are detection and identification methods available for the pest?

Yes, methods to detect and identify the pest are available.

The detection of *X. citri* pv. *viticola* affecting grapevine is possible during targeted field inspections or surveys, since disease symptoms are easily visible on the canopy (leaves, petioles, vines, sprouts, fruits). Symptoms may appear on leaves, berries and seeds, rachis and peduncle of grape clusters, shoots, vines and canes. Leaves show small necrotic lesions starting as interveinal angular spots, sometimes surrounded by a yellow halo; severely affected leaves turn yellow and fall off. Discoloration and necrosis may also develop along leaf veins and petioles, on pedicels and rachis of grape clusters: Such necrosis will develop to wilting and drying. Severity of symptoms may vary according to grape variety: The very popular 'Red Globe' and the seedless varieties originated from 'Thompson Seedless' appear particularly susceptible (Lima et al., 1999); In general, seedless grape cultivars of *V. vinifera* are far more susceptible than other cultivars (Lima et al., 1999). Common rootstock varieties always failed to develop symptoms following artificial inoculations: e.g. rootstock 420 A (*V. riparia* × *V. berlandieri*), or the tropical rootstock IAC-572 (*V. caribaea* × (*V. riparia* × *V. rupestris* 101-14 Mgt)):

Symptoms quite similar to those described for *X. citri* pv. *viticola* may be caused by another bacterium, *Xylophilus ampelinus*, the causal agent of the bacterial blight of grapevine, a very uncommon disease with restricted distribution that is occasionally observed around the Mediterranean, in Japan and South Africa (EPPO, online). Therefore, a presumptive diagnosis cannot be based on symptoms observation solely, but symptoms description remains a key point during a detection procedure. Pathogen isolation can be easily attempted from cankers using very common agar media, e.g. NA, YDC, Kado 523 and others. On most agar media, *X. citri* pv. *viticola* colonies develop within 2–3 days and, after a purification step, they can be identified using biochemical and nutritional tests, possibly coupled with a PCR assay (Trindade et al., 2005, 2007; Villela et al., 2019). Serological detection methods are also available, based on the use of polyclonal antibodies (Araujo et al., 2005).

*X. citri* pv. *viticola* is a Gram-negative rod with rounded ends, motile by a single polar flagellum and  $0.6 \times 1.2-2.5 \mu m$  in size (Nayudu, 1972). Its isolation is done on common media, and colony growth is observed after 48–72 h at 28–33°C; colonies are round, smooth, mucoid with entire margin and white to creamy-white in colour. On the semi-selective Selective Xanthomonas (SX) agar medium (Schaad et al., 2001) colonies produce a clear starch digestion zone, typical for *X. citri* pathovars. When pure cultures are infiltrated into tobacco leaf panels, the hypersensitive response is negative or weakly positive after 24 h; an intense chlorosis is observed after 48 h from inoculation and tissue hypersensitive necrosis after 3 days (Lima et al., 1999).

A set of tests are used for pest identification: beside starch hydrolysis, additional physiological and biochemical features are common with other xanthomonads and are listed in Chand and Kishun (1990). *X. citri* pv. *viticola* is a non-pigmented xanthomonad; therefore, it does not produce xanthomonadins, the yellow pigments typical of most phytopathogenic xanthomonads (He et al., 2020); this might be the reason why its first taxonomical assignment was as a pseudomonad. Nevertheless, as typical for xanthomonads, the xanthomonadin cluster is present in *X. citri* pv. *viticola* as well, though its arrangement was found to have a frameshift mutation because of a deletion of four nucleotides in the gene coding for phosphotransferase/dehydratase, therefore, giving rise to the formation of a truncated protein (Goel et al., 2002; Midha and Patil, 2014). The same feature is



reported for two others tropical xanthomonads: *X. axonopodis* pv. *manihotis* (Arrieta-Ortiz et al., 2013) and *X. citri* pv. *mangiferaindicae* (Pruvost et al., 1998; Midha and Patil, 2014).

#### **3.2.** Pest distribution

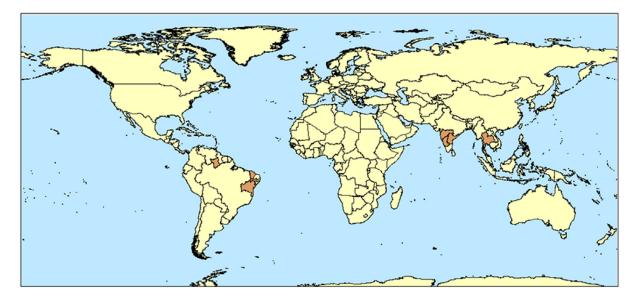
#### **3.2.1.** Pest distribution outside the EU

*X. citri* pv. *viticola* was first reported in India and, later, in Brazil (Figure 1). Midha and Patil (2014) mentioned that the pathogen occurred in Africa in 2005, but they did not give any literature reference, nor the country or region: Therefore, such assumption cannot be confirmed. Currently, its confirmed distribution outside the EU is limited to India and Brazil. In India, the pathogen is present in the states of Andhra Pradesh, Karnataka and Maharashtra (Chand and Kishun, 1990; Jambenal et al., 2011; Kamble et al., 2019).

In Brazil, the disease was reported for the first time in 1998 on table grape cv. Red Globe, in Petrolina, state of Pernambuco (Lima et al., 1999; Malavolta Junior et al., 1999; Naue et al., 2014). Presumably, *X. citri* pv. *viticola* was introduced through cuttings from India (Freire and Oliveira, 2001; Rodrigues Neto et al., 2011). Later, the disease was observed in the states of Bahia and Piauí (Lima et al., 1999; Malavolta Junior et al., 1999; Malavolta Junior et al., 1999; Malavolta Junior et al., 1999) and, more recently, in the states of Ceará (Freire and Oliveira, 2001), Goiás (Junqueira et al., 2006), Minas Gerais (Junqueira et al., 2006), Roraima (Halfeld-Vieira and Nechet, 2006), Paraná (Tomaz et al., 2011) and São Paulo (Rodrigues Neto et al., 2011). Disease eradication was attempted and, currently, the disease is present in the states of Bahia, Ceará, Pernambuco and Roraima (Brasil, 2018).

CABI, citing Kochenko (1993), reported the presence of *X. citri* pv. *viticola* in Ukraine: Such very short note in Russian language did not mention any specific pathogen, but described symptoms on grapevine and some epidemiological aspects of a disease that can be recognised as the crown gall, caused by *Rhizobium* (*Allorhizobium*) *vitis* (formerly *Agrobacterium vitis*). Therefore, this record in the CABI datasheet was not considered.

Finally, an MSc thesis published in Thailand deals with a bacterial necrosis disease of grapevine that is present in the country (Buensanteai, 2004). The causal agent was identified as *X. citri* pv. *viticola* on the basis of metabolic and biochemical features and by ELISA. A PCR assay was not available at that time. This MSc thesis was not followed by any paper published in any peer-reviewed journal: nonetheless, it contains sufficient information to suggest that *X. citri* pv. *viticola* might be present in Thailand.



**Figure 1:** Global distribution of *Xanthomonas citri* pv. *viticola* (Source: literature). Administrative subunits in Brazil and India are shown in the map only where the pathogen is present



#### 3.2.2. Pest distribution in the EU

Is the pest present in the EU territory? If present, is the pest widely distributed within the EU?

*X. citri* pv. *viticola* is not known to be present in the EU territory.

#### **3.3. Regulatory status**

#### 3.3.1. Commission Implementing Regulation 2019/2072

*X. citri pv. viticola* is not listed in Annex II of Commission Implementing Regulation (EU) 2019/2072, the implementing act of Regulation (EU) 2016/2031.

# **3.3.2.** Hosts of *X. citri* pv. *viticola* that are prohibited from entering the Union from third countries

Grapevine (*Vitis L.*) as the major host of *X. citri* pv. *viticola* is a closed pathway, since its introduction into the EU from third countries, other than Switzerland, is prohibited.

**Table 4:**List of plants, plant products and other objects that are Xanthomonas citri pv. viticola<br/>hosts, whose introduction into the Union from certain third countries is prohibited<br/>(Source: Commission Implementing Regulation (EU) 2019/2072, Annex VI)

List of plants, plant products and other objects whose introduction into the Union from certain third countries is prohibited

	Description	CN Code	Third country, group of third countries or specific area of third country
1.	Plants of <i>Vitis</i> L., other than fruits	0602 10 10 0602 20 10 ex 0604 20 90 ex 1404 90 00	Third countries, other than Switzerland

#### 3.4. Entry, establishment and spread in the EU

#### 3.4.1. Entry

Is the pest able to enter into the EU territory? If yes, identify and list the pathways.

Yes, the pest is able to enter into the EU territory and pathways have been identified.

Comments on plants for planting as a pathway.

Plants for planting is a potential pathway which is closed under the current EU legislation.

Despite the introduction into the EU of *Vitis* L. plants from third countries (other than Switzerland) is prohibited, *X. citri* pv. *viticola* is able to enter into the EU territory through the small amount of host plant material introduced according to the Commission Delegated Regulation (EU) 2019/829 of 14 March 2019 that supplements Regulation (EU) 2016/2031, providing for temporary derogations in view of official testing, scientific or educational purposes, trials, varietal selections or breeding. Such material is imported into the EU territory as grapevine cuttings, or *in vitro* plants. Whereas *in vitro* plants appear to be a negligible pathway, due to the nature of such material, grapevine cuttings should be regarded as propagation material of high risk, therefore a major pathway, if not analysed for the presence of latent germs of *X. citri* pv. *viticola*. Additionally, living seeds imported as germplasm for breeding purposes may allow the entry of the pathogen.

The potential pathways for the entry of the pathogen into the EU territory are included in Table 5.



Pathways	Life stage	Relevant mitigations [e.g. prohibitions (Annex VI) or special requirements (Annex VII) within Implementing Regulation 2019/2072]
Description (e.g. host/intended use/source)		
Grapevine ( <i>Vitis</i> L.) propagation material originating from India, Brazil and Thailand.	Bacterial cells latently harbouring in grapevine cuttings, or rootstocks, or rooted cuttings.	Introduction of any plant material, excluding fruits, from third countries (except Switzerland) is prohibited (Annex VI, Commission Implementing regulation 2019/2072).
Grapevine ( <i>Vitis</i> L.) propagation material originating from India, Brazil and Thailand for scientific purpose	Bacterial cells latently harbouring in grapevine cuttings, or rootstocks, or rooted cuttings.	Commission delegated regulation 2019/829 regulates the import for scientific purpose, but <i>X. citri</i> pv <i>viticola</i> is not included.
Living seeds of <i>Vitis</i> L. imported as germplasm for trials, varietal selections or breeding originating from India, Brazil and Thailand.	Bacterial cells associated with seeds	None
Fruits of <i>Vitis</i> L. imported as table grapes originating from India, Brazil and Thailand.	Bacterial cells infecting the pedicle, the rachis and the petioles.	A phytosanitary certificate is required for the import of fresh or chilled grapes (Annex XI, part A of CIR 2019/2072)
Plants for planting of <i>Azadirachta</i> <i>indica</i> originating from India and Thailand.	Bacterial cells, latently harboured in plant tissues or associated with lesions	None

**Table 5:** Potential pathways for the entry of Xanthomonas citri pv. viticola into the EU 27

The major pathway for the entry of *X. citri* pv. *viticola* into the EU territory is by means of any grapevine propagation material of *Vitis* L., i.e. grapevine cuttings (rooted/unrooted), rootstocks, bud chips. Such dormant material may harbour the pathogens in its latent stage, endophytically. This is a closed pathway according to Annex VI of the Implementing Regulation (EU) 2019/2072. Nonetheless, other plant propagation material is imported under the Commission Delegated Regulation (EU) 2019/829 for research purposes: Such material may allow the entry of the pest into the EU territory. Anyway, it should be noted that, following import authorisation, special provisions apply, such as the notification that any activity performed shall be notified and shall be done in quarantine stations or in confined facilities and, ultimately, the material should be destroyed and safely removed or stored under appropriate conditions. Such conditions minimise the possibility of spread of the pathogen to the host plants.

Since *X. citri* pv. *viticola* is a systemic bacterium, it may colonise the plants up to the grape bunches through the vascular bundles. Living bacteria were detected in symptomless grapevine berries and seeds of cv. Red Globe, which is one of the most popular seeded grape variety present on the global markets (Tostes et al., 2014). The pest has also been reported as colonising the petioles, rachis and pedicels of grapes (Tostes et al., 2014). Therefore, it cannot be excluded that grapes are a potential pathway of entry with high uncertainty, due to the lack of information on pathogen survival after harvesting and the longer post-harvest stages, from cold storage to the final markets.

*A. indica* has been reported as a host plant of *X. citri* pv. *viticola* and an internet survey identified several vendors of such species as an ornamental plant. In most cases, such plants originate from India. Although the entry of the pathogen through this route is very likely, uncertainty about *A. indica* plants for planting remains high, due to the fact that there is no data available on the amount of such commodity imported into the EU territory from infested countries. Europhyt notified importation of *A. indica* from Malaysia in 2018.

Notifications of interceptions of harmful organisms began to be compiled in Europhyt in May 1994 and in TRACES in May 2020. As at 20 September 2021, there were no records of interception of *X. citri* pv. *viticola* in the Europhyt and TRACES databases.

#### **3.4.2. Establishment**

Following entry, the pathogen may be transferred from plants for planting to suitable hosts through grafting and pruning. No information is available on the possible transfer from infected grapes to



suitable hosts. The pest can become established, at least locally, since its main host plant is widely distributed throughout the EU territory. Pathogen establishment is possible in those areas where climatic conditions are conducive to disease. Pathogen establishment will be favoured by mechanisation in the vineyards, since mechanical pruning and harvesting produce wounds that might serve as penetration sites into the host plant by the bacterium.

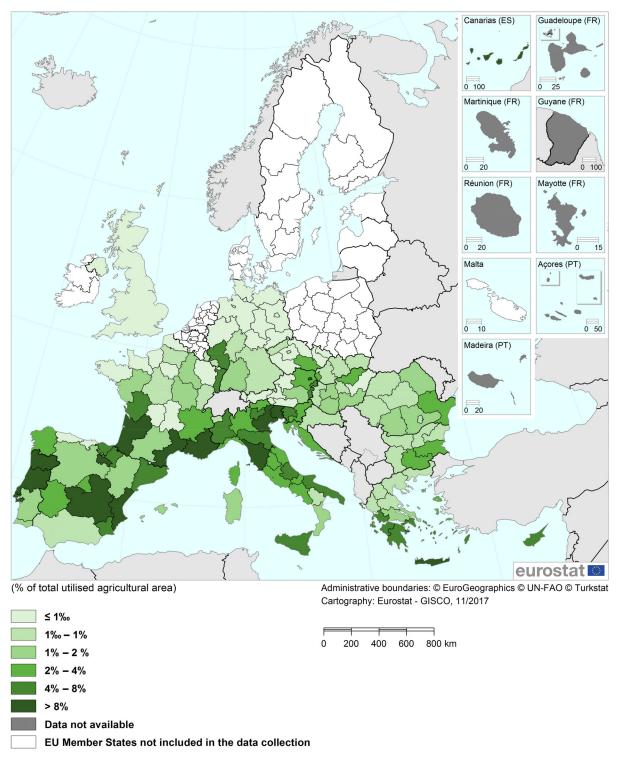
#### Is the pest able to become established in the EU territory?

Yes, the pest is able to be transferred to a host following its entry and become established in the EU territory.

#### **3.4.2.1. EU distribution of main host plants**

The main host plant of *X. citri* pv. *viticola* is *V. vinifera*. Except for Scandinavian and Baltic countries, Poland, Ireland and the Netherlands, grapevine is widely cultivated in the remaining Member States of the EU (Figures 2 and Table 6). In several EU regions (e.g. Aquitaine, Castilla-La Mancha, Crete, PACA, Tuscany), grapevine represents one of the most important crops. In particular, table grapes are intensively cultivated in Apulia, Murcia, Sicily, Thrace, Valencia, etc. (Figure 2).





Note: Structural statistics on vineyards cover the EU Member States having a minimum planted area of 500 hectares of vineyards. Therefore Belgium, Denmark, Estonia, Ireland, Latvia, Lithuania, Malta, the Netherlands, Poland, Finland and Sweden are not included in the data collection. Germany: NUTS level 1. Cyprus, Luxembourg and the United Kingdom: national data. *Source:* Eurostat (online data code: vit\_t1 and agr\_r\_acs)

#### **Figure 2:** Area under grapevines, by NUTS 2 regions, 2015. Source: EUROSTAT, 2017 https:// ec.europa.eu/eurostat/statistics-explained/index.php?title=File:2017-3401aEN\_v2\_EU28.png (accessed 10 October 2021)<sup>2</sup>

<sup>&</sup>lt;sup>2</sup> European Commission, Eurostat. Area under vines, by NUTS 2 regions, 2015 (% of total utilised agricultural area). Accessed on 10 October 2021, https://ec.europa.eu/eurostat/statistics-explained/index.php?title=File:2017-3401aEN\_v2\_EU28.png



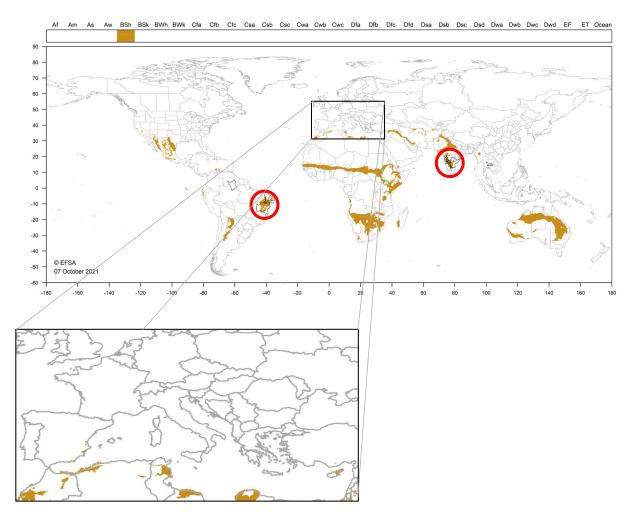
Country	2018/19	2019/20e
Italy	850,000	635,000
Spain	309,870	310,000
Greece	294,183	290,000
Romania	61,400	54,000
France	40,800	45,000
Portugal	17,780	18,000
Bulgaria	11,900	13,000
Sum	1,585,933	1,365,000

**Table 6:** Commercial production of table grapes in the EU (in tons) Source (Source: FAS/EU, available at: https://greekfoodnews.com/increased-exports-for-greek-table-grapes/)

#### 3.4.2.2. Climatic conditions affecting establishment

*X. citri* pv. *viticola* is currently affecting table grapes in tropical and subtropical regions, where rains alternate with long dry spells. Maharashtra (India), for instance, has a subtropical climate, with three distinct seasons: summer (dry) (March–May), monsoon (rainy) (June–September) and winter (dry) (October–February) (Jambenal et al., 2011). In Karnataka (India), grapes are cultivated in the northern and eastern part of the state, where the rainy season is limited (Jambenal et al., 2011). In Andhra Pradesh (India), grapes are extensively cultivated in the districts located in the south-western part of the state (Jambenal et al., 2011). In Brazil, most table grape production is concentrated in the medium–lower part of the São Francisco Valley, states of Bahia, Cearà and Pernambuco (Lima et al., 1999). The Indian and the Brazilian areas where table grapes are cultivated share a subtropical climate, semi-arid with a more or less prolonged rainy season and long dry spells (Figure 3).





**Figure 3:** *Xanthomonas citri* pv. *viticola* climate suitability analysis based on the Köppen–Geiger climate classification. Regions with black borders indicates countries/regions where the pest was observed. Climates not present in EU27 are not mapped. Legend shows the list of Köppen–Geiger climates. Only climates relevant for the pest are coloured. Red circles indicate the areas in India and Brazil where *Xanthomonas citri* pv. *viticola* has been reported and where climate type BSh which is also present in Europe can be found. Climate type BSh occurs in the EU territory, only in small areas in the south of Spain and in Cyprus

Xanthomonads are, in general, thermophilic bacteria and, as most bacteria, they are efficiently disseminated by rains and showers. Therefore, if rains are present during a susceptible phenological phase (e.g. vines development, bloom and fruit set, veraison), more infection cycles are possible, thus favouring the pest establishment. For instance, in the outbreak areas of Andhra Pradesh, temperature ranges from 15 to 40°C and the yearly rainfall is in the range of 500–600 mm (Anonymous, Grape cultivation practices – Andhra Pradesh, available at: https://www.kisansuvidha.com/grape-cultivation/? v=ad4f1670f142): therefore, both temperatures and rainfalls are conducive to disease outbreaks.

It is generally accepted that there are no ecoclimatic limitations for most bacterial diseases establishment, beside those that apply for the host. Table grapes are cultivated in the warmest and driest European regions with Mediterranean climate (Köppen–Geiger: Csa and Csb): Therefore, rainfalls are scarce and, generally, limited to the wintertime. As an example, the grape-growing region of Murcia (Spain) has a monthly average temperature ranging from approx. 10°C in January and 27°C in July, but rainfalls are rather scarce, with a yearly total amount of 293 mm, concentrated in the dormant period. The regions of current distribution of the pest do not have similar climate to that present in most grape-growing areas of the EU. The only areas in the EU territory having a BSh climate type are: Cyprus (the coastal areas between Perivolia, Larnaca and Ormidia; the territory in the municipalities of Pano Lakatamia, Kato Lakatamia, Laxia and Nicosia) and Spain (part of the provinces of Alicante, Elche, Murcia and Cartagena; small spots east and west of Almeria; the area around the



municipality of Motril). Therefore, a high uncertainty is identified whether the pathogen may establish in areas that do not have a subtropical climate similar to that reported in the affected areas (BSh). The districts in Thailand, where the pathogen is reported, do not have the climate type BSh, but Aw (tropical savanna climate, with dry winters). The Brazilian state of Roraima, municipality of Boa Vista, where cases of the disease where recently found has also a climate type Aw. The climate type Aw does not occur in the EU territory.

#### 3.4.3. Spread

Describe how the pest would be able to spread within the EU territory following establishment?

Once X. citri pv. viticola has established in one EU area, plants for planting and, therefore, any propagation material (e.g. cuttings) may latently harbour the pest. The intense internal trade of nursery material may be the most important means responsible for the spread of the pest.

Once established, *X. citri* pv. *viticola* is able to spread within short distance through shears and other pruning tools. Primary and secondary inoculum may disseminate within short to medium distances mainly through rains and storms. In recent years, likely due to the climate change, rains and storms are more and more frequent and intense, especially in the south and south-east of Europe (EEA, 2017): This tropicalisation of climate may favour a rapid spread of the pathogen. An uncertainty is identified whether the combination of suitable climatic conditions and receptive phenological phases of grapevine are contemporary. The presence of suitable spontaneous plants or weeds may support the survival of inoculum as epiphytic populations during its spread (Peixoto et al., 2007). Considering grapes for wine production, in several cases, pruning and harvesting are mechanised in the EU territory; mechanisation may easily spread bacterial inoculum to short–medium distances, as evidenced for *X. citri* pv. *citri* (Gottwald and Graham, 1990). Specific data on the effect of mechanisation are missing, since table grape production in the outbreak areas is not mechanised.

#### 3.5. Impacts

Would the pests' introduction have an economic or environmental impact on the EU territory?

Yes, the pest' introduction into the EU territory is likely to have a considerable economic impact, though uncertainty remains whether the climatic conditions in the EU territory may support severe disease outbreaks in large areas.

Grapevine is one of the most important crops in the EU and even local disease outbreaks may cause high economic losses. Nonetheless, the differences in the climate conditions and the agronomic practices applied in viticulture between the infested area and the EU territory raises uncertainty on the economic impact. The impact of the disease caused by X. citri pv. viticola on grape might be devastating, under conducive conditions: for India, Chand and Kishun (1990) reported yield losses around 60-80% in the outbreak areas, whereas Lima et al. (1999) reported a disease incidence near to 100% and yield losses that, in some cases, were nearly total. Severity of symptoms and yield losses are highest where rainstorms occur (e.g. 100 mm or above, typical for a single tropical rain event) during the vegetative season. Central pivot and overhead sprinkler irrigation incite symptom development and severity, as reported from Brazil (Lima et al., 1999). The environmental conditions in the EU, where grapes are cultivated, are remarkably different, though the global warming appears to tropicalise climate around the Mediterranean. Therefore, considering that tropical rain events and storms are supposed to be very important for the epidemiology of the disease, the economic impact in the EU is likely to be medium to high, with high uncertainty, due to the difference in climate, seasonality, agronomic practices applied. Additionally, grapevine water management in the EU is different than, for instance, in Brazil: European viticulture is mostly rain-feed and where irrigated, preferably uses drip or micro-sprinkler irrigation. Central pivot irrigation is not used and overhead sprinklers are not common. Finally, furrow irrigation may be also anecdotally present, although not suggested by extension services or not allowed in several regions, due to the use of excessive quantity of water.



## 3.6. Available measures and their limitations

Are there measures available to prevent the entry into the EU such that the risk becomes mitigated?

Yes, measures to prevent the entry of the pest into the EU do exist although these measures do not specifically target the pathogen (see Sections 3.3.2 and 3.4.1). Additional measures are also available to mitigate the risk of entry into and spread within the EU of the pathogen (see Section 3.6.1).

Existing measures consider the grapevine (*Vitis* L.) propagation material that has been identified as the major pathway for the entry of *X. citri* pv. *viticola* into the EU territory (Section 3.3.2). Nonetheless, additional measures are needed for an additional potential pathway identified in Section 3.4.1 (host plant material introduced under temporary derogations in view of official testing, scientific or educational purposes, trials, varietal selections or breeding).

Currently, there are no import requirements for seeds of *Vitis* L., grapes and plants for planting of *A. indica* that has been also identified as a pathway for the pest introduction into the EU.

#### **3.6.1.** Identification of potential additional measures

Phytosanitary measures (prohibitions) are currently applied to plants of *Vitis* L., other than fruits (see Section 3.3.2). Additional measures are required for plants of *Vitis* L. imported under the Commission Delegated Regulation (EU) 2019/829 of 14 March 2019. Potential control measures are required for seeds of *Vitis* L., and plants of *A. indica* originating from India.

Potential control measures on hosts that are imported are listed in Table 7.

Special requirements summary (with hyperlink to information sheet if available)	Control measure summary in relation to Xanthomonas citri pv. viticola Used to mitigate likelihood of infestation by specified pest at origin, hence to mitigate entry Plant or plant products come from a country officially free from the pest, or from a pest-free area or from a pest-free place of production.	
Pest freedom		
Growing plants in isolation	Used to mitigate likelihood of infestation by specified pest in vicinity of growing site Growing plants of Vitis L. and Azadirachta indica in isolation may represent an effective control measure.	
Certification of reproductive material (voluntary/official)	Used to mitigate pests that are included in a certification scheme X. citri pv. viticola should be added to the current certification scheme for Vitis L.	
Roguing and pruning	Used to mitigate likelihood of infestation by specified pest (usually a pathogen) at growing site where pest has limited dispersal Growing plants of Vitis L. and Azadirachta indica in a condition of tillage and of strict weed management may present an effective measure.	
Inspections	Used to mitigate likelihood of infestation by specified pest at origin. Inspections of production fields during the vegetative period are an effective measure.	
Chemical treatments on consignments or during processing	<ul> <li>Used to mitigate likelihood of infestation of pests susceptible to chemical treatments</li> <li>Chemical treatments should be used to suppress the possible epiphytic phase of <i>X. citri</i> pv. <i>viticola</i> during cultivation.</li> </ul>	
Heat and cold treatments	Used to mitigate likelihood of infestation of pests susceptible to physical treatments <ul> <li>Dormant grapevine cuttings should be treated with hot water.</li> </ul>	

**Table 7:**Selected control measures (a full list is available in EFSA PLH Panel, 2018) for pest entry<br/>(and spread when applicable) in relation to currently unregulated hosts and pathways



Special requirements summary (with hyperlink to information sheet if available)	Control measure summary in relation to <i>Xanthomonas citri</i> pv. <i>viticola</i>	
Cleaning and disinfection of facilities, tools and machinery	Used to mitigate likelihood of entry or spread of soil borne pests Cleaning, disinfection and disinfestation (sanitation) of equipment and facilities (including premises, storage areas) are good cultural and handling practices employed in the production and marketing of any commodity and may contribute to mitigate likelihood of entry or spread of	
Phytosanitary certificate and plant passport	Used to attest which of the above requirements have been applied Recommended for plant species known as hosts of <i>X</i> . <i>citri</i> pv viticola.	
Post-entry quarantine (PEQ) and other restrictions of movement in the importing country	Plants in PEQ are held in conditions that prevent the escape of pests; they can be carefully inspected and tested to verify they are of sufficient plant health status to be released, or may be treated, re- exported or destroyed. Tests on plants are likely to include laboratory diagnostic assays and bioassays on indicator hosts to check whether the plant material is infected with particular pathogens Recommended for plant species known as hosts of <i>X</i> . citri pv viticola.	

# **3.6.1.1.** Biological or technical factors limiting the effectiveness of measures to prevent the entry (and spread when applicable) of the pest

The effectiveness of measures to prevent the entry of the pest might be limited by the following factors:

- Detection of the pathogen based only on symptomatology, since another bacterium, *Xylophilus ampelinus*, may produce similar symptoms on grapevine plants and create confusion.
- Plants of *A. indica* are also traded on the internet by several vendors (e.g. https://nurserylive. com/products/neem-tree-azadirachta-indica-plant): Such internet trade might limit the effectiveness of measures to prevent the entry of the pest.

### 3.7. Uncertainty

- Uncertainty on the pest distribution in Thailand: apart from an MSc thesis (Buensanteai, 2004) and a few communications in Thai, no data on the possible presence of *X. citri* pv. *viticola* in Thailand were ever published in peer-reviewed journals. Detection and identification of the pest were done with ELISA and never confirmed by another assay, e.g. PCR.
- Uncertainty on the volume and the epidemiological role of grapevine seeds imported by breeders as a pathway for entry of *X. citri* pv. *viticola*.
- Uncertainty on the epidemiological role of fruits as a pathway for entry.
- Uncertainty on the possible role of *Azadirachta indica* as a pathway for entry: very limited data are available on import of this plant that, though cultivated in India (neem tree), it might be considered as an ornamental in the EU.
- Uncertainty on the possible establishment and spread, due to:
  - o the very different climatic conditions occurring in the infested countries as compared to those in the EU territory, except for small areas in Spain and Cyprus, where BSh climate occurs.
  - o the different agronomic practices applied in India and Brazil to grow grapevine in the tropics, e.g.: irrigation, mechanical pruning, pruning time and methods, mechanisation;
  - o no knowledge on whether the pathogen can survive as an epiphyte during the off-season on herbaceous species in Europe.

## 4. Conclusions

*Xanthomonas citri* pv. *viticola* has not been reported to be present in the EU. The pathogen satisfies the criteria that are within the remit of EFSA to assess for this species to be regarded as a potential Union quarantine pest.



**Table 8:**The Panel's conclusions on the pest categorisation criteria defined in Regulation (EU)2016/2031 on protective measures against pests of plants (the number of the relevant sections of the pest categorisation is shown in brackets in the first column)

Criterion of pest categorisation	Panel's conclusions against criterion in Regulation (EU) 2016/2031 regarding Union quarantine pest	Key uncertainties
Identity of the pest (Section 3.1)	The identity of the pest is established and it is shown to produce consistent symptoms on its host and to be transmissible.	None
Absence/presence of the pest in the EU (Section 3.2)	The pest is not known to be present in the EU territory.	None
Regulatory status (Section 3.3)	The pest is not regulated in the EU	None
Pest potential for entry, establishment and spread in the EU (Section 3.4)	<ul> <li>The pest is able to enter into, become established in and spread within the EU territory. Identified pathways are:</li> <li>1) Plants for planting of <i>Vitis</i> L., imported under the Commission Delegated Regulation (EU) 2019/829 of 14 March 2019.</li> <li>2) Plants for planting of <i>Vitis</i> L. other than fruits (closed pathway)</li> </ul>	The uncertainty is high on the amount of imported <i>Azadirachta indica</i> plants and on the ability of the pest to be transmitted to grapevine in the EU agro-climatic conditions. Uncertainty is high on the ability to transfer from infected seeds and fruits to host plants in the EU. No study is available in the current literature to this regard. Uncertainty is high for establishment because of the very different climatic conditions occurring in the infested countries as compared to those in the EU territory, except for small areas in Spain and Cyprus where BSh climate occurs.
Potential for consequences in the EU (Section 3.5)	<i>X. citri</i> pv. <i>viticola</i> has the potential for economic consequences in the EU.	None.
Available measures (Section 3.6)	Although not specifically targeted against <i>X. citri</i> pv <i>viticola</i> , existing phytosanitary measures mitigate the likelihood of the pathogen's entry into the EU territory. Potential additional measures also exist to further mitigate the risk of entry into, establishment and spread of the pathogen within the EU.	None
Conclusion (Section 4)	Xanthomonas citri pv. viticola has not been reported to be present in the EU. The pathogen satisfies the criteria that are within the remit of EFSA to assess for this species to be regarded as a potential Union quarantine pest.	None
Aspects of assessment to focus on/scenarios to address in future if appropriate:	The main gap to fill in the near future is: 1) Develop a consensus protocol for the c	letection and identification of the pest.

## References

Araujo JSP, Robbs CF and Maciel GF, 1999. Novos hospedeiros alternativos de *Xanthomonas citri* pv. *viticola* no Brazil. Summa Phytopathologica, Jaboticabal, 25, 23.

Araujo JSP, Bueno JRF, Cruz GB, Oliveira BC, Robbs CF, Ribeiro RLD and Polidoro JC, 2005. Produção e caracterização de anticorpos policionais contra *Xanthomonas citri* pv. *viticola*. Pesquisa Agropecuária Brasileira, 40, 305–309.



- Arrieta-Ortiz ML, Rodríguez-R LM, Pérez-Quintero ÁL, Poulin L, Díaz AC, Rojas NA, Arias N, Trujillo C, Trujillo C, 2013. Genomic survey of pathogenicity determinants and VNTR markers in the cassava bacterial pathogen *Xanthomonas axonopodis* pv. *manihotis* strain CIO151. PLoS ONE, 8. https://doi.org/10.1371/journal.pone. 0079704
- Bansal K, Midha S, Kumar S and Patil PB, 2017. Ecological and evolutionary insights into *Xanthomonas citri* pathovar diversity. Applied and Environmental Microbiology, 83, e02993–16. https://doi.org/10.1128/AEM. 02993-16
- Brasil, 2018. Ministério da agricultura, pecuária e abastecimento/Secretaria de defesa agropecuária. Instrução Normativa N° 38, de 1° de outubro de 2018. Available online: http://www.in.gov.br/materia/-/asset\_publisher/ Kujrw0TZC2Mb/content/id/43461167/do1-2018-10-02-instrucao-normativa-n-38-de-1-de-outubro-de-2018-43461024> [Accessed: 18 August 2021]
- Buensanteai MN, 2004. Identification, development of detection method and survey of bacterial necrosis disease of grapevine in Thailand. Suranaree University of Technology. Available online: http://sutir.sut.ac.th:8080/sutir/bitstream/123456789/1267/2/natthiya\_fulltext.pdf [Accessed: 18 August 2021].
- Desai SG, Ghandi AB, Patel MK and Kotasthanae WV, 1966. A new bacterial leaf-spot and blight of *Azadirachta indica* A. Juss. Indian Phytopathology, 19, 322–332.
- EEA (European Environment Agency), 2017. Key observed and projected climate change and impacts for the main regions in Europe. Available Online: https://www.eea.europa.eu/data-and-maps/figures/key-past-and-projected-impacts-and-effects-on-sectors-for-the-main-biogeographic-regions-of-europe-5
- EFSA PLH Panel (EFSA Panel on Plant Health), Jeger M, Bragard C, Caffier D, Candresse T, Chatzivassiliou E, Dehnen-Schmutz K, Gregoire J-C, Jaques Miret JA, MacLeod A, Navajas Navarro M, Niere B, Parnell S, Potting R, Rafoss T, Rossi V, Urek G, Van Bruggen A, Van Der Werf W, West J, Winter S, Hart A, Schans J, Schrader G, Suffert M, Kertesz V, Kozelska S, Mannino MR, Mosbach-Schulz O, Pautasso M, Stancanelli G, Tramontini S, Vos S and Gilioli G, 2018. Guidance on quantitative pest risk assessment. EFSA Journal 2018;16(8):5350, 86 pp. https://doi.org/10.2903/j.efsa.2018.5350
- EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Benfenati E, Chaudhry QM, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C, Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A, Hempen M, Valtue~na Martinez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M, 2017. Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi.org/ 10.2903/j.efsa.2017.4971
- EPPO (European and Mediterranean Plant Protection Organization), 2019. EPPO codes. Available online: https:// www.eppo.int/RESOURCES/eppo\_databases/eppo\_codeshttps://www.eppo.int/RESOURCES/eppo\_databases/ eppo\_codes
- EPPO (European and Mediterranean Plant Protection Organization), online. EPPO Global Database. Available online: https://gd.eppo.int [Accessed: 30 June 2021].
- FAO (Food and Agriculture Organization of the United Nations), 2004. ISPM (International Standards for Phytosanitary Measures) 21—Pest risk analysis of regulated non-quarantine pests. FAO, Rome, 30 pp. Available online: https://www.ippc.int/sites/default/files/documents//1323945746\_ISPM\_21\_2004\_En\_2011-11-29\_Refor. pdf
- FAO (Food and Agriculture Organization of the United Nations), 2013. ISPM (International Standards for Phytosanitary Measures) 11—Pest risk analysis for quarantine pests. FAO, Rome, 36 pp. Available online: https://www.ippc.int/sites/default/files/documents/20140512/ispm\_11\_2013\_en\_2014-04-30\_201405121523-494.65%20KB.pdf
- FAO (Food and Agriculture Organization of the United Nations), 2018. International Standards for Phytosanitary Measures. ISPM 5 Glossary of phytosanitary terms. Revised version adopted CPM 13, April 2018. FAO, Rome. Available online: https://www.ippc.int/en/publications/621/
- Ferreira M, Bonneau S, Briand M, Cesbron S, Portier P, Darrasse A, Gama M, Barbosa M, Mariano R, Souza EB and Jacques MA, 2019. *Xanthomonas citri* pv. *viticola* affecting grapevine in Brazil: emergence of a successful monomorphic pathogen. Frontiers Plant Science, 10, 489. https://doi.org/10.3389/fpls.2019.00489
- Freire FCO and Oliveira ADSO, 2001. Ocorrência do cancro bacteriano da videira no Estado do Ceará. Fortaleza: EMBRAPA PA 2. Comunicado Técnico 62.
- da Gama MAS, Mariano RLR, da Silva Júnior WJ, de Farias ARG, Barbosa MAG, Ferreira MÁDSV, Costa Júnior CRL, Santos LA and de Souza EB, 2018. Taxonomic repositioning of Xanthomonas citri pv. viticola (Nayudu 1972) Dye 1978 as Xanthomonas citri pv. viticola (Nayudu 1972) Dye 1978 comb. nov. and emendation of the description of Xanthomonas citri pv. anacardii to include pigmented isolates pathogenic to cashew plant. Phytopathology, 108, 1143–1153. https://doi.org/10.1094/PHYTO-02-18-0037-R. Epub 2018 Aug 20. PMID: 29688131.
- Goel AK, Rajagopal L, Nagesh N and Sonti RV, 2002. Genetic locus encoding functions involved in biosynthesis and outer membrane localization of xanthomonadin in *Xanthomonas oryzae* pv.*oryzae*. Journal of Bacteriology, 184, 3539–3548.



- Gomes de Farias AR, 2020. Análise comparativa e funcional do genoma de Xanthomonas citri pv. viticola: aplicação sobre virulência, patogenicidade e posicionamento taxonômico. PhD thesis, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil. 174 pp.
- Gottwald TR and Graham JH, 1990. Spatial pattern analysis of epidemics of citrus bacterial spot in Florida citrus nurseries. Phytopathology, 80, 181–190.

Griessinger D and Roy A-S, 2015. EPPO codes: a brief description. Available online: https://www.eppo.int/media/ uploaded\_images/RESOURCES/eppo\_databases/A4\_EPPO\_Codes\_2018.pdf

- Halfeld-Vieira BA and Nechet KL, 2006. Bacterial canker of grapevine in Roraima, Brazil. Fitopatologia Brasileira, 31, 604. https://doi.org/10.1590/S0100-41582006000600013
- He YW, Cao XQ and Poplawsky AR, 2020. Chemical structure, biochemical roles, biosynthesis and regulation of the yellow xanthomonadin pigments in the phytopathogen Xanthomonas. Molecular Plant-Microbe Interactions, 33(5), 705–714.
- Jambenal S, Ravikumar MR and Hiremani N, 2011. Evaluation of different chemicals and bioagents against bacterial leaf spot of grapevine and their effect on yield and yield parameters. International Journal of Plant Protection, 4, 377–380.
- Junqueira NTV, Junqueira KP, Braga MF, Vaz CF, Santos EC, Junqueira LP, Souza LS and Lima CA, 2006. Ocorrência do cancro bacteriano da videira na região do entorno do Distrito Federal. Anais do XIX Congresso Brasileiro de Fruticultura, Cabo Frio, 2006, 323.
- Kamble AS, Sawant SD, Sawant I, Ghule S, Patil A and Sujoy S, 2019. Characterization of *Xanthomonas citri* pv. *viticola* causing bacterial leaf spot of grapes in Maharashtra, India. Journal of Environmental Biology, 40, 1145–1150. https://doi.org/10.22438/jeb/40/6/MRN-1071
- Kochenko ZI, 1993. Development of canker tumours. Zashchita Rastenii 7: 42. (In Russian).
- Kok D, 2014. A review on grape growing in tropical regions. Turkish Journal of Agricultural and Natural Sciences, Special Issue, 1, 1236–1241.
- Lima MF, Ferreira MASV, Moreira WA and Dianese JC, 1999. Bacterial canker of grapevine in Brazil. Fitopatologia Brasileira, 24, 440–443.
- Malavolta Junior VA, Almeida IMG, Sugimori MH, Ribeiro IJA, Rodrigues Neto J, Pires EJP and Nogueira EMC, 1999. Occurrence of *Xanthomonas citri* pv. *viticola* on grapevine in Brazil. Summa Phytopathologica, 25, 26–27.
- Midha S and Patil PB, 2014. Genomic insights into the evolutionary origin of *Xanthomonas axonopodis* pv. *citri* and its ecological relatives. Applied and Environmental Microbiology, 80, 6266–6279. https://doi.org/10.1128/AEM. 01654-14
- Naue CR, Costa VSO, Barbosa MAG, Batista DC, Souza EB and Mariano RLR, 2014. Xanthomonas campestris pv. viticola on grapevine cutting tools and water: survival and disinfection. Journal of Plant Pathology, 96, 451–458.
- Nayudu MD, 1972. *Pseudomonas viticola* sp. nov., incitant of a new bacterial disease of grape vine. Journal of Phytopathology, 73, 183–186.
- Parkinson N, Cowie C, Heeney J and Stead D, 2009. Phylogenetic structure of *Xanthomonas* determined by comparison of *gyrB* sequences. International Journal of Systematic and Evolutionary Microbiology, 59, 264–274.
- Peixoto AR, Mariano RLR, Moreira JOT and Viana IO, 2007. Hospedeiros alternativos de *Xanthomonas citri pv. viticola*. Fitopatologia Brasileira, 32, 161–164.
- Pruvost O, Couteau A, Perrier X and Luisetti J, 1998. Phenotypic diversity of *Xanthomonas* sp. *mangiferaeindicae*. Journal of Applied Microbiology, 84, 115–124.
- Rodrigues Neto J, Destéfano SAL, Rodrigues LMR, Sousa Pelloso D and da Cruz Oliveira Jr L, 2011. Grapevine bacterial canker in the State of São Paulo, Brazil: detection and eradication. Tropical Plant Pathology, 36, 42–44.
- Schaad NW, Jones JB and Chun W, 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria, 3rd Edition. APS Press, St. Paul, MN, USA.
- Tomaz R, Scremin RM, Ferreira MASV and Barbosa MAG, 2011. Detecção e erradicação de videiras com sintomas do cancro bacteriano no Estado do Paraná. Tropical Plant Pathology, 36, 896.
- Tostes GO, Araujo JP, Farias ARG, Frade DAR and Olivares FL, 2014. Detection and cellular localization of *Xanthomonas citri* pv. *viticola* in seeds of commercial 'Red Globe' grapes. Tropical Plant Pathology, 39, 134–140.
- Trindade LC, Lima MF and Ferreira MASV, 2005. Molecular characterization of Brazilian strains of *Xanthomonas citri* pv. *viticola* by rep-PCR fingerprinting. Fitopatologia Brasileira, 30, 46–54.
- Trindade LC, Marques E, Lopes DB and Ferreira MASV, 2007. Development of a molecular method for detection and identification of *Xanthomonas citri* pv. *viticola*. Summa Phytopathologica, 33, 16–23. https://doi.org/ 10.1590/S0100-54052007000100002
- Villela JGA, Ritschel P, Barbosa MAG, Baccin KMS, Rossato M, Maia J, Ferreira MASV2019. Detection of *Xanthomonas citri* pv. *viticola* on grapevine by real-time PCR and BIO-PCR using primers designed from pathogenicity and xanthomonadin gene sequences. European Journal of Plant Pathology, 155, 445–459. https://doi.org/10.1007/s10658-019-01779-y

#### Abbreviations

- EPPO European and Mediterranean Plant Protection Organization
- FAO Food and Agriculture Organization



IPPC International Plant Protection Convention

- ISPM International Standards for Phytosanitary Measures
- MS Member State
- PLH EFSA Panel on Plant Health
- PZ Protected Zone
- TFEU Treaty on the Functioning of the European Union
- ToR Terms of Reference

# Glossary

Containment (of a pest)	Application of phytosanitary measures in and around an infested area to prevent spread of a pest (FAO, 2018).
Control (of a pest)	Suppression, containment or eradication of a pest population (FAO, 2018).
Entry (of a pest)	Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO, 2018).
Eradication (of a pest)	Application of phytosanitary measures to eliminate a pest from an area (FAO, 2018).
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO, 2018).
Greenhouse	A walk-in, static, closed place of crop production with a usually translucent outer shell, which allows controlled exchange of material and energy with the surroundings and prevents release of plant protection products (PPPs) into the environment.
Impact (of a pest)	The impact of the pest on the crop output and quality and on the environment in the occupied spatial units.
Introduction (of a pest)	The entry of a pest resulting in its establishment (FAO, 2018).
Pathway	Any means that allows the entry or spread of a pest (FAO, 2018).
Phytosanitary measures	Any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (FAO, 2018).
Quarantine pest	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO, 2018).
Risk reduction option (RRO)	A measure acting on pest introduction and/or pest spread and/or the magnitude of the biological impact of the pest should the pest be present. A RRO may become a phytosanitary measure, action or procedure according to the decision of the risk manager.
Spread (of a pest)	Expansion of the geographical distribution of a pest within an area (FAO, 2018).



Host status	Host name	Plant family	Common name	Reference
Cultivated hosts	Vitis vinifera	Vitaceae	Grapevine	Nayudu (1972), Lima et al. (1999)
	Azadirachta indica	Meliaceae	Neem	Desai et al. (1966), Nayudu (1972)
Wild weed hosts	Phyllantus maderaspatensis	Phyllantaceae		Nayudu (1972)
	Alternanthera tenella	Amaranthaceae		Peixoto et al. (2007)
	<i>Amaranthus</i> sp. <i>Glycine</i> sp.	Amaranthaceae Fabaceae	Amaranth	Peixoto et al. (2007) Peixoto et al. (2007)
	Senna obtusifolia	Fabaceae	American sicklepod	Peixoto et al. (2007)
Artificial/experimental hosts	*Azadirachta indica *Phyllanthus maderaspatensis	Meliaceae Phyllanthaceae	Neem	Nayudu (1972) Nayudu (1972)
	Mangifera indica Anacardium occidentale Spondias dulcis Spondias tuberosa Schinus terebinthifolia Citrus limon Coccinia indica Euphorbia heterophylla Euphorbia hirta Lantana camara Oxalis corniculata Sida acuta Solanum nigrum Taraxacum officinale Dactyloctenium aegyntium	Anacardiaceae Anacaardiaceae Anacardiaceae Anacardiaceae Rutaceae Cucurbitaceae Euphorbiaceae Euphorbiaceae Verbenaceae Oxalidaceae Malvaceae Solanaceae Asteraceae	Mango Cashew tree Ambarella Umbuzeiro Brazilian pepper Lemon Scarlet gourd Wild Poinsettia Asthma plant Lantana Sleeping beauty Wireweed Nightshade Dandelion Crowfoot grass	Chand and Kishun (1990) Araujo et al. (1999) Araujo et al. (1999) Araujo et al. (1999) Araujo et al. (1999) Kamble et al. (2019) Kamble et al. (2019) Kamble et al. (2019) Peixoto et al. (2019) Kamble et al. (2019)
	aegyptium Eragrostis pilosa Pilea sp.	<i>Poaceae Urticaceae</i>	Soft lovegrass	Peixoto et al. (2007) Peixoto et al. (2007)

# Appendix A – Xanthomonas citri pv. viticola host plants

 $\ast:$  Controversial: They are both cited as natural host plants and experimental.



# Appendix B – Distribution of Xanthomonas citri pv. viticola

Distribution records based on literature.

Region	Country	Subnational (e.g. State)	Status	
South America	Brazil	States: Pernambuco, Roraima, Ceará, Bahia (i.e. Vale do Submédio São Francisco)	Present, widespread (Naue et al., 2014)	
		States: Piauí, Goiás, São Paulo, Paraná	Eradicated (Freire and Oliveira, 2001; Halfeld- Vieira and Nechet, 2006; Junqueira et al., 2006; Rodrigues Neto et al., 2011; Tomaz et al., 2011)	
Africa	Unknown	Unknown	Unreliable citation (Midha and Patil, 2014)	
Asia	India	States Karnataka, Andhra Pradesh, Maharashtra	Present (Patil, 1988; Chand and Kishun, 1990; Jambenal et al., 2011)	
	Thailand	Nachon Rachasima Saraburi Rayong Surin Phichit	Present (Buensanteai, 2004). (Note: no published references available in peer- reviewed journals).	