SCIENTIFIC OPINION

Scientific Opinion on the pest categorisation of *Citrus tristeza virus*[1]

EFSA Panel on Plant Health (PLH)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Plant Health performed a pest categorisation of European isolates of *Citrus tristeza virus* (CTV) for the European Union (EU) territory. European CTV isolates are listed in Annexes IIAII and IIB of Directive 2000/29/EC. CTV is a well-defined and easily diagnosed *Closterovirus* species transmitted by the vegetative multiplication of infected hosts and through the activity of aphid vectors. *Toxoptera citricida* is the most efficient vector but *Aphis gossypii* is the most important in Europe. European isolates of CTV have been reported in seven of the eight EU Member States (MSs) with significant citrus production. The natural host range of CTV is restricted to citrus species and to a few, related genera, such as *Fortunella* and *Poncirus*. CTV is unlikely to be affected by ecoclimatic conditions in regions where its host plants are grown and has the potential to establish in southern regions of the EU territory. The majority of European CTV isolates cause severe decline symptoms (tristeza disease) in several citrus species, in particular sweet orange and mandarin grafted on susceptible sour orange or lemon rootstocks, which are commonly used in many EU MSs with the exception of Spain and, to a lesser extent, France. Symptoms of the severe stem pitting disease (SP) have not been reported by any EU MSs, despite the identification of isolates closely related to non-European isolates that cause SP in other regions of the world. The observed impact of CTV is on citrus industries still heavily reliant on susceptible rootstocks. Replacing those with CTV-tolerant rootstocks, as was done in Spain, virtually eliminates the impact of CTV in the absence of SP. Comprehensive certification systems can also reduce CTV spread and impact. The most critical area of uncertainty concerns the potential ability of some European isolates to cause SP in sweet orange.

KEY WORDS

tristeza, quick decline, stem pitting, seedling yellows, *Aphis gossypii*, *Toxoptera citricida*, pest categorisation

---

[1] On request from the European Commission, Question No EFSA-Q-2014-00283, adopted on 27 November 2014.

[2] Panel members: Richard Baker, Claude Bragard, Thierry Candresse, Gianni Gilioli, Jean-Claude Grégoire, Imre Holb, Michael John Jeger, Olia Evtimova Karadjova, Christer Magnusson, David Makowski, Charles Manceau, Maria Navajas, Trond Rafoss, Vittorio Rossi, Jan Schans, Gritta Schrader, Gregor Urek, Irene Vloutoglou, Stephan Winter and Wopke van der Werf. Correspondence: alpha@efsa.europa.eu

[3] The Panel wishes to thank the members of the Working Group on Directive 2000/29 Viruses: Thierry Candresse, Stephan Winter and Mariano Cambra, as well as the visiting scientist Francesco di Serio, for the preparatory work on this scientific opinion, and EFSA staff member Virág Kertész for the support provided.

Suggested citation: EFSA PLH Panel (EFSA Panel on Plant Health), 2014. Scientific Opinion on the pest categorisation of *Citrus tristeza virus*. EFSA Journal 2014;12(12):3923, 32 pp. doi:10.2903/j.efsa.2014.3923

Available online: www.efsa.europa.eu/efsajournal

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p. 1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union, the list of harmful organisms whose introduction into or spread within the Union is prohibited and the control measures to be carried out at the outer border of the Union on arrival of plants and plant products.

The Commission is currently carrying out a revision of the regulatory status of organisms listed in the Annexes of Directive 2000/29/EC. This revision targets mainly organisms which are already locally present in the EU territory and that in many cases are regulated in the EU since a long time. Therefore it is considered to be appropriate to evaluate whether these organisms still deserve to remain regulated under Council Directive 2000/29/EC, or whether, if appropriate, they should be regulated in the context of the marketing of plant propagation material, or be deregulated. The revision of the regulatory status of these organisms is also in line with the outcome of the recent evaluation of the EU Plant Health Regime, which called for a modernisation of the system through more focus on prevention and better risk targeting (prioritisation).

In order to carry out this evaluation, a recent pest risk analysis is needed which takes into account the latest scientific and technical knowledge on these organisms, including data on their agronomic and environmental impact, as well as their present distribution in the EU territory. In this context, EFSA has already been asked to prepare risk assessments for some organisms listed in Annex IIAII. The current request concerns 23 additional organisms listed in Annex II, Part A, Section II as well as five organisms listed in Annex I, Part A, Section I, one listed in Annex I, Part A, Section II and nine organisms listed in Annex II, Part A, Section I of Council Directive 2000/29/EC. The organisms in question are the following:

Organisms listed in Annex II, Part A, Section II:

- *Ditylenchus destructor* Thorne
- *Circulifer haematoceps*
- *Circulifer tenellus*
- *Helicoverpa armigera* (Hübner)
- *Radopholus similis* (Cobb) Thorne (could be addressed together with the IIAI organism *Radopholus citrophilus* Huettel, Dickson and Kaplan)
- *Paysandisia archon* (Burmeister)
- *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al*.
- *Erwinia amylovora* (Burr.) Winsl. *et al*. (also listed in Annex IIB)
- *Pseudomonas syringae* pv. *persicae* (Prunier *et al*.) Young *et al*.
- *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye
- *Xanthomonas campestris* pv. *pruni* (Smith) Dye
- *Xylophilus ampelinus* (Panagopoulos) Willems *et al*.
- *Ceratocystis fimbriata* f. sp. *platani* Walter (also listed in Annex IIB)
- *Cryphonectria parasitica* (Murrill) Barr (also listed in Annex IIB)
- *Phoma tracheiphila* (Petri) Kanchaveli and Gikashvili
- *Verticillium albo-atrum* Reinke and Berthold
- *Verticillium dahliae* Klebahn
- Beet leaf curl virus
- Citrus tristeza virus (European isolates) (also listed in Annex IIB)
- Grapevine flavescence dorée MLO (also listed in Annex IIB)
- Potato stolbur mycoplasma

- *Spiroplasma citri* Saglio *et al*.
- Tomato yellow leaf curl virus

Organisms listed in Annex I, Part A, Section I:

- *Rhagoletis cingulata* (Loew)
- *Rhagoletis ribicola* Doane
- Strawberry vein banding virus
- Strawberry latent C virus
- Elm phloem necrosis mycoplasm

Organisms listed in Annex I, Part A, Section II:

- *Spodoptera littoralis* (Boisd.)

Organisms listed in Annex II, Part A, Section I:

- *Aculops fuchsiae* Keifer
- *Aonidiella citrina* Coquillet
- Prunus necrotic ringspot virus
- Cherry leafroll virus
- *Radopholus citrophilus* Huettel, Dickson and Kaplan (could be addressed together with IIAII organism *Radopholus similis* (Cobb) Thorne)
- *Scirtothrips dorsalis* Hendel
- *Atropellis* spp.
- *Eotetranychus lewisi* McGregor
- *Diaporthe vaccinii* Shear.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a pest risk assessment of *Ditylenchus destructor* Thorne, *Circulifer haematoceps*, *Circulifer tenellus*, *Helicoverpa armigera* (Hübner), *Radopholus similis* (Cobb) Thorne, *Paysandisia archon* (Burmeister), *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al*., *Erwinia amylovora* (Burr.) Winsl. *et al*., *Pseudomonas syringae pv*. *persicae* (Prunier *et al.)* Young *et al*. *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, *Xanthomonas campestris* pv. *pruni* (Smith) Dye, *Xylophilus ampelinus* (Panagopoulos) Willems *et al*., *Ceratocystis fimbriata* f. sp. *platani* Walter, *Cryphonectria parasitica* (Murrill) Barr, *Phoma tracheiphila* (Petri) Kanchaveli and Gikashvili, *Verticillium albo-atrum* Reinke and Berthold, *Verticillium dahliae* Klebahn, Beet leaf curl virus, Citrus tristeza virus (European isolates), Grapevine flavescence dorée MLO, Potato stolbur mycoplasma, *Spiroplasma citri* Saglio *et al*., Tomato yellow leaf curl virus, *Rhagoletis cingulata* (Loew), *Rhagoletis ribicola* Doane, Strawberry vein banding virus, Strawberry latent C virus, Elm phloem necrosis mycoplasma, *Spodoptera littoralis* (Boisd.), *Aculops fuchsiae* Keifer, *Aonidiella citrina* Coquillet, Prunus necrotic ringspot virus, Cherry leafroll virus, *Radopholus citrophilus* Huettel Dickson and Kaplan (to address with the IIAII *Radopholus similis* (Cobb) Thorne), *Scirtothrips dorsalis* Hendel, *Atropellis* spp., *Eotetranychus lewisi* McGregor and *Diaporthe vaccinii* Shaer., for the EU territory.

In line with the experience gained with the previous two batches of pest risk assessments of organisms listed in Annex II, Part A, Section II, requested to EFSA, and in order to further streamline the preparation of risk assessments for regulated pests, the work should be split in two stages, each with a specific output. EFSA is requested to prepare and deliver first a pest categorisation for each of these 38 regulated pests (step 1). Upon receipt and analysis of this output, the Commission will inform EFSA for which organisms it is necessary to complete the pest risk assessment, to identify risk reduction options and to provide an assessment of the effectiveness of current EU phytosanitary requirements (step 2). *Clavibacter michiganensis* spp. *michiganensis* (Smith) Davis *et al*. and *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, from the second batch of risk assessment requests for Annex IIAII organisms requested to EFSA (ARES(2012)880155), could be used as pilot cases for this approach, given that the working group for the preparation of their pest risk assessments has been constituted and it is currently dealing with the step 1 "pest categorisation". This proposed modification of previous request would allow a rapid delivery by EFSA by May 2014 of the first two outputs for step 1 "pest categorisation", that could be used as pilot case for this request and obtain a prompt feedback on its fitness for purpose from the risk manager's point of view.

As indicated in previous requests of risk assessments for regulated pests, in order to target its level of detail to the needs of the risk manager, and thereby to rationalise the resources used for their preparation and to speed up their delivery, for the preparation of the pest categorisations EFSA is requested, in order to define the potential for establishment, spread and impact in the risk assessment area, to concentrate in particular on the analysis of the present distribution of the organism in comparison with the distribution of the main hosts and on the analysis of the observed impacts of the organism in the risk assessment area.

ASSESSMENT

1. Introduction

1.1. Purpose

This document presents a pest categorisation prepared by the EFSA Scientific Panel on Plant Health (hereinafter referred to as the Panel) for the species *Citrus tristeza virus* (CTV) in response to a request from the European Commission.

1.2. Scope

This pest categorisation covers European isolates of CTV, which are defined by their geographical origin in the European continent. As such, CTV isolates occurring in the European Union (hereinafter referred to as EU), in non-EU European countries and in the part of the Turkish territory on the European continent, are considered as European isolates of CTV. On the other hand, a plant infected with CTV originating in a non-European country is considered infected with a non-European CTV isolate. Non-European CTV isolates are not covered by the present pest categorisation, unless necessitated for a better understanding. In this case, the extension of coverage to non-European isolates is explicitly stated.

The pest categorisation area is the territory of the EU with 28 Member States (hereinafter referred to as MSs), restricted to the area of application of Council Directive 2000/29/EC, which excludes Ceuta and Melilla, the Canary Islands and the French overseas departments.

2. Methodology and data

2.1. Methodology

The Panel performed the pest categorisation for CTV following guiding principles and steps presented in the EFSA Guidance on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and as defined in the International Standards for Phytosanitary Measures (ISPM) No 11 (FAO, 2013) and ISPM No 21 (FAO, 2004).

In accordance with the Guidance on a harmonised framework for pest risk assessment in the EU (EFSA PLH Panel, 2010), this work is initiated as result of the review or revision of phytosanitary policies and priorities. As explained in the background of the European Commission request, the objective of this mandate is to provide updated scientific advice to the European risk managers for their evaluation of whether these organisms listed in the Annexes of the Directive 2000/29/EC still deserve to remain regulated under Council Directive 2000/29/EC, or whether they should be regulated in the context of the marketing of plant propagation material, or be deregulated. Therefore, to facilitate the decision making process, in the conclusions of the pest categorisation, the Panel addresses explicitly each criterion for quarantine pest according to ISPM 11 (FAO, 2013) but also for regulated non-quarantine pest according to ISPM 21 (FAO, 2004) and includes additional information required as per the specific terms of reference received by the European Commission. In addition, for each conclusion the Panel provides a short description of its associated uncertainty.

Table 1 presents the ISPM 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria against which the Panel provides its conclusions. It should be noted that the Panel's conclusions are formulated respecting its remit and particularly with regards to the principle of separation between risk assessment and risk management (EFSA founding regulation[4]), therefore, instead of determining whether the pest is likely to have an unacceptable impact, the Panel will present a summary of the observed pest impacts. Economic impacts are expressed in terms of yield and quality losses and not in

[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31/1, 1.2.2002, p. 1–24.

monetary terms, in agreement with the Guidance on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010).

Table 1: International Standards for Phytosanitary Measures ISPM 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria under evaluation

Pest categorisation criteria	ISPM 11 for being a potential quarantine pest	ISPM 21 for being a potential regulated non-quarantine pest
Identity of the pest	The identity of the pest should be clearly defined to ensure that the assessment is being performed on a distinct organism, and that biological and other information used in the assessment is relevant to the organism in question. If this is not possible because the causal agent of particular symptoms has not yet been fully identified, then it should have been shown to produce consistent symptoms and to be transmissible	The identity of the pest is clearly defined
Presence (ISPM 11) or absence (ISPM 21) in the PRA area	The pest should be **absent from all or a defined part of the PRA area**	The pest is **present** in the PRA area
Regulatory status	If the pest is present but not widely distributed in the PRA area, it should be under official control or expected to be under official control in the near future	The pest is under official control (or being considered for official control) in the PRA area with respect to the specified plants for planting
Potential for establishment and spread in the PRA area	The PRA area should have ecological/climatic conditions including those in protected conditions suitable for the establishment and spread of the pest and, where relevant, host species (or near relatives), alternate hosts and vectors should be present in the PRA area	–
Association of the pest with the plants for planting and the effect on their intended use	–	Plants for planting are a pathway for introduction and spread of this pest
Potential for consequences (including environmental consequences) in the PRA area	There should be clear indications that the pest is likely to have an unacceptable economic impact (including environmental impact) in the PRA area	–
Indication of impact(s) of the pest on the intended use of the plants for planting	–	The pest may cause severe economic impact on the intended use of the plants for planting
Conclusion	If it has been determined that the pest has the potential to be a quarantine pest, the PRA process should continue. If a pest does not fulfil all of the criteria for a quarantine pest, the PRA process for that pest may stop. In the absence of sufficient information, the uncertainties should be identified and the PRA process should continue	If a pest does not fulfil all the criteria for an regulated non-quarantine pest, the PRA process may stop

In addition, in order to reply to the specific questions listed in the terms of reference, three issues are specifically discussed only for pests already present in the EU: the analysis of the present EU distribution of the organism in comparison with the EU distribution of the main hosts, the analysis of the observed impacts of the organism in the EU and the pest control and cultural measures currently implemented in the EU.

The Panel will not indicate in its conclusions of the pest categorisation whether to continue the PRA process as it is clearly stated in the terms of reference that at the end the pest categorisation the European Commission will indicate if further risk assessment work is required following their analysis of the Panel's scientific opinion.

2.2. Data

2.2.1. Literature search

A literature search on CTV was conducted at the beginning of the mandate. The search was conducted for the scientific name of the pest together with the most frequently used common names on the ISI Web of Knowledge database. Further references and information were obtained from experts, from citations within the references as well as from grey literature.

2.2.2. Data collection

To complement the information concerning the current situation of the pest provided by the literature and online databases on pest distribution, damage and management, the PLH Panel sent a short questionnaire on the current situation at country level, based on the information available in the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Retrieval (PQR) system, to the National Plant Protection Organisation (NPPO) contacts of the 28 EU MSs, and of Iceland and Norway. Iceland and Norway are part of the European Free Trade Association (EFTA) and are contributing to EFSA data collection activities, as part of the agreements EFSA has with these two countries. A summary of the pest status based on EPPO PQR and NPPO replies is presented in Table 2.

Information on distribution of the main host plants were obtained from the EUROSTAT database

3. Pest categorisation

3.1. Identity and biology of *Citrus tristeza virus*

3.1.1. Taxonomy

CTV is a member of the genus *Closterovirus*, in the family *Closteroviridae* (Martelli et al., 2012; Karasev and Bar-Joseph et al., 2010), which includes viruses with flexible and elongated particles and monopartite or divided genomes composed of linear, positive sense, single-stranded RNA. *Closteroviridae* are transmitted in nature by insects (aphids, mealybugs or whiteflies) in a semi-persistent manner, and generally have a specific tissue tropism (mostly phloem-limited). CTV has a monopartite RNA genome of about 19.3 kb, containing two untranslated regions of 107 and 273 nucleotides at the 5′ and 3′ termini, respectively, and 12 open reading frames (ORFs), which potentially encode more than 17 proteins (Moreno et al., 2008). CTV gene expression is controlled by a combination of three different strategies including proteolytic processing, ribosomal frameshifting and the generation of a set of 3′-coterminal subgenomic RNAs (for review or references, see Moreno et al., 2008).

The complete or partial genome sequences of numerous CTV isolates have been determined and, at present, 47 complete sequences of CTV isolates are available from the INSDC (International Nucleotide Sequence Database Collaboration). Overall, CTV is a well-characterised virus of clear identity and taxonomic status.

3.1.2. Pest biology

CTV is a phloem-associated virus that replicates in the cytoplasm of companion or phloem parenchyma cells. It is a graft-transmissible agent which, as other plant viruses, is transmitted through the vegetative multiplication of infected host plants. In addition, as with other members of the genus *Closterovirus*, CTV is transmitted by aphids in a semi-persistent manner (Yokomi et al., 1989). It is not known to be seed- (McClean, 1957) or pollen-transmitted in any of its hosts (Moreno et al., 2008). Experimental transmission of the virus to *Citrus* hosts is possible through wounding (slash-inoculation of partially purified particles, Garnsey et al., 1977; Garnsey and Muller, 1988; Müller and Garnsey, 1984) or by dodder (Weathers and Hartung, 1964).

In nature, the virus is transmitted by several aphid species (Moreno et al., 2008; Michaud, 1998) which acquire the virus during feeding on infected trees. Feeding for between five minutes and a few hours is sufficient for virus uptake. The aphid vectors can transmit the virus without any latency period but, because virus multiplication or circulation do not occur in the aphid, the aphid remains viruliferous for only about 24 hours, and infectivity is completely lost within 48 hours of virus acquisition (Raccah et al., 1976, also cited by Moreno et al., 2008). *Toxoptera citricida* (Kirkaldy) is the most efficient vector of CTV (Gottwald, 2010; Moreno et al., 2008; Michaud et al., 1998). *Aphis gossypii* (Glover), although less efficient than *T. citricida*, is also an effective vector (Yokomi et al., 1994, cited by Gottwald 2010 and Moreno et al., 2008). *A. spiraecola* (Patch, formerly *A. citricola* van der Goot) and *T. aurantii* (Boyer de Fonscolombe) can transmit CTV under experimental conditions (Hermoso de Mendoza et al., 1984; Yokomi and Garnsey, 1987) but are generally considered less efficient and less important vectors than the aforementioned two species. Transmission efficiency can also vary between virus isolates.

In nature, the host range of CTV is restricted to plant species of the genera *Citrus*, *Poncirus* and *Fortunella* (subfamily *Aurantioidae*, family *Rutaceae*, Moreno et al., 2008). In these host plants, CTV isolates can cause a variety of symptoms depending on the host species, the cultivar and the particular CTV isolate involved.

The properties described above apply to all CTV isolates, and there is no information to suggest that European CTV isolates differ from non-European ones in these respects. However, different CTV isolates can cause considerably different symptoms in citrus and can differ in their vector transmission properties.

3.1.3. Intraspecific diversity

3.1.3.1. Serological and molecular diversity

There is ample evidence for serological diversity, and monoclonal antibodies have been generated that react against either a broad spectrum of CTV isolates or with very specific isolates. The antibody MCA13 reacts only with severe CTV isolates (Permar et al., 1989) and is used to discriminate between mild (non-decline- and non-stem pitting disease (SP)-inducing) and severe (decline- or SP-inducing) isolates. The molecular diversity of CTV was evident from analyses of partial genome sequences (Ayllón et al., 2001), but when a comprehensive dataset of full genome sequences became available, a more complete definition of CTV strains was possible. Following the most recent review of current knowledge on CTV, virus isolates of this species have been grouped into strains (Harper, 2013). A strain is typified by a specific genotype (isolate) for which the complete genome sequence is available from the INSDC: T36 strain (isolate T36, U16304), T3 strain (isolate T3, KC525952), T30 strain (isolate T30, AY260651), resistance-breaking (RB) strain (isolate NZRB-TH28, FJ525433), T68 strain (isolate T68-1, JQ965169) and the VT strain, which has an Asian (T318A, DQ151548) and a Western (FS701, KC517494) subtype. The recombinant isolate HA16-5 (GQ454870) might represent a new strain (Harper, 2013).

It should however be noted that the term "strain" has been very loosely used in the literature in the past, sometimes as a synonym for "isolate" and sometimes to regroup isolates based on their biological properties, or on a combination of the molecular and predicted biological properties. As a consequence of this loose and inconsistent use of terminology, the literature is frequently confusing.

Because recombination was shown to have contributed significantly to the evolutionary history of some isolates or strains of CTV (Vives et al., 2005; Melzer et al., 2010; Harper, 2013), the entire genome sequence is currently taken into account for the taxonomic assignation of isolates to CTV strains. For strain demarcation, the complete genome sequence has to differ by > 7.5 % (and the sequence of either ORF1a or the encoded protein by > 8 %). Recombination analyses of representatives of the recognised strains are also required (Harper, 2013). However, for practical reasons, assignation of an isolate to a particular strain has been (and often still is) frequently based on short genome sequence fragments obtained following polymerase chain reaction (PCR) amplification.

3.1.3.2. Biological diversity

Three major syndromes are associated with CTV infections in citrus: tristeza, SP and seedling yellows (SY, Moreno and Garnsey, 2010; Dawson et al., 2013). Tristeza is a decline syndrome caused by the vast majority of CTV isolates in different citrus species such as sweet orange (*Citrus sinensis*), mandarins (*C. reticulata*), grapefruits (*C. paradisi* Macfadyen), kumquats (*Fortunella* sp.) and limes (*C. aurantifolia* (Christm.) Swingle) when grafted on rootstocks of sour orange (*C. aurantium*) or lemon (*C. limon*). Tristeza is therefore a bud union disease that develops only in susceptible rootstocks/scion combinations. The observed decline can be extremely rapid (“quick decline”), with wilting and death of trees occurring within a few days or weeks, or it can be a slower process, occurring over months or even years.

SP is the second type of syndrome associated with CTV infection. It occurs in susceptible species regardless of the rootstock used, and can affect both rootstock and grafted varieties (Moreno et al., 2008). It is characterised by the development of pits in the trunk and stem resulting from cambium malfunctioning. SP symptoms are associated with decreased tree vigour, dwarfing of plants and reduced fruit yield and quality.

SY is a CTV-induced syndrome observed in young plants, most notably under greenhouse conditions. It is characterised by a general yellowing and stunting of affected seedlings and is mostly observed in sour orange, lemons and grapefruit (Moreno et al., 2008).

There is biological variability in the ability of CTV isolates to cause these three types of syndromes in susceptible hosts (Moreno et al., 2008) and, consequently, CTV isolates have been grouped into pathogenic categories (Garnsey et al., 2005). Within the limits of the assays, symptom differences can be attributed to properties of the infecting CTV isolate. When sour orange is used as a rootstock, the majority of CTV isolates are able to cause tristeza decline symptoms; however, some isolates, such as the T385 Spanish isolate, do not appear to cause decline and are therefore often referred to as “mild isolates” (Vives et al., 1999; Moreno et al., 2008). This term is also commonly used to refer to isolates unable to cause SP or SY symptoms, adding confusion to the literature. Similarly, the term “severe isolates” is used to describe decline-inducing isolates (in particular in quick decline situations) but, confusingly, is also used to describe isolates causing SP or SY.

CTV isolates also show variability in their ability to overcome the CTV resistance observed in trifoliate orange (*Poncirus trifoliata*). *P. trifoliata* is used as a rootstock, albeit not extensively, in Europe. While the majority of virus isolates cannot infect trifoliate orange, a few recombinant RB isolates have been described (Harper et al., 2010) that can overcome this resistance, and are able to replicate in and systemically invade resistant plants.

3.1.3.3. Correlation between molecular and biological diversity

By combining host response, serological and molecular data, efforts were made to establish clear and reproducible correlations between molecular variability of virus isolates/strains and their biological (pathogenic) properties. Genome sequences of reference isolates with experimentally well-characterised pathogenicities (mild isolate T30 from Florida, severe isolate T36 from Florida (decline- and SY-inducing), SP-inducing isolates T3 and VT from Florida and Israel (Garnsey et al., 2005)) were determined. This provided a framework of CTV reference isolates to which sequences, biological properties and virulence of newly characterised isolates could be compared. This showed that, to a certain extent, biological properties correlated with those of the most closely related reference (Moreno et al., 2008; Roy and Brlansky, 2009).

However, growing evidence from sequencing and biological assays demonstrates that CTV isolates assigned to a particular strain can differ remarkably in their abilities to induce particular symptoms; therefore, the notion of a tight correlation between CTV strains and the symptoms induced is no longer valid (Harper, 2013). As with other viruses, slight differences in sequence can lead to important changes in the phenotype of the disease induced (Harper, 2013; Moreno et al., 2008); as a result, CTV strains cannot be considered to be a homogenous ensemble of isolates sharing identical pathogenicity profiles. Similarly, the monoclonal antibody CTV MCA13 (Permar et al., 1989), commonly used to identify severe (tristeza- and SP-inducing) isolates, can sometimes react with mild isolates (Hilf and Garnsey, 2002), which, as shown by complete genome sequencing (Varveri et al., 2014), is probably caused by mutations in the region where the neotope for MCA13 is localised.

The analysis of CTV infections in citrus has also revealed that, as with other RNA viruses, infected plants may contain a pool of sequence variants that may belong to a single strain or even to several strains (Rubio et al., 2001). Thus, CTV isolates often comprise mixed virus populations (Harper, 2013), further complicating the analysis of the symptoms caused by individual variants/strains. There is essentially no understanding of how combinations of virus genotypes affect disease symptoms and severity, further complicating any efforts to establish a connection between virus genotype and disease phenotype (Harper, 2013).

Unfortunately, much confusion in the literature has resulted from initial attempts to ascribe specific pathogenic properties to CTV strains and, later, from attempts to dispell the underlying hypothesis.

3.1.3.4. Diversity of European CTV isolates

Partial or complete genome sequences of a number of European CTV isolates are available, and these demonstrate the presence of several CTV strains (Rubio et al., 2001). Several CTV isolates/strains (e.g. RB isolates) are not known to occur in Europe. From a biological perspective, both tristeza decline-inducing isolates and mild isolates, unable to induce decline in susceptible rootstock/scion combinations, are known in Europe (Varveri et al., 2014). CTV isolates causing severe SY symptoms in citrus have also been reported (Ferretti et al., 2014). Although sequence variants genetically similar to those of the SP-inducing non-European CTV isolates have been detected in the EU (Ruiz-Ruiz et al., 2006), and have even been implicated in outbreaks with severe tristeza decline symptoms (Owen et al., 2014), SP symptoms in sweet orange have not been observed in field surveys and only rarely occurring, inconspicuous symptoms were induced in indicator plants in the greenhouse (Ballester-Olmos et al., 1993; Pedro Moreno, Valencian Institute for Agricultural Research, personal communication, 2014). RB isolates which can overcome *P. trifoliata* resistance have been found in New Zealand (Harper et al., 2010), and sequence variants similar to those of the RB isolates have been reported in a few additional countries outside of Europe but not in the EU (Mariano Cambra, Valencian Institute for Agricultural Research, personal communication, 2014).

Overall, European CTV isolates appear to represent only a fraction of the biological and molecular diversity present in CTV isolates throughout the world. Given that, aside from the pathogenic properties of virus isolates characterised on a limited set of indicator hosts, the biological properties of European CTV populations are incompletely understood, this general evaluation is associated with significant uncertainties.

3.1.4. Detection and identification of *Citrus tristeza virus*

CTV can be detected by bioassays such as graft-inoculation of indicator seedlings of Mexican lime, sour orange, Madam Vinous sweet orange and Duncan grapefruit seedlings, or plants of sweet orange budded on sour orange rootstocks (Wallace and Drake, 1951; Garnsey et al., 2005; Pina et al., 2005). While biological methods are time consuming and can be applied only to a limited number of samples, they are the sole method for conclusive assessment of the pathogenic features of CTV isolates. The availability of polyclonal and monoclonal antibodies (Cambra et al., 2000a) and of highly efficient serological methods, including double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Garnsey and Cambra, 1991) and tissue print ELISA (Garnsey et al., 1993; Cambra et al., 2000b), has greatly improved the efficiency and sensitivity of CTV detection. Highly sensitive and specific molecular tests based on reverse transcription PCR (RT-PCR), frequently used in combination with immunocapture (Nolasco et al., 1993) or print-capture of virus particles (Olmos et al., 1996), have also been developed, and standard protocols allowing the unequivocal identification of CTV are available (EPPO, 2004). An update of this CTV standard is in progress and will probably include real-time RT-PCR (Bertolini et al., 2008) as a detection method. In fact, tissue-print ELISA (using the specific monoclonal antibodies 3DF1 and 3CA5) and real-time RT-PCR are the techniques of choice for CTV detection (Vidal et al., 2012).

Methods based on single and multiplex RT-PCR have been developed to discriminate between CTV genotypes. These molecular methods, sometimes in combination with immunocapture or single-strand conformation polymorphism analysis, have increased our ability to differentiate genotypes (Ayilon et al., 2001; Sambade et al., 2003; Roy et al., 2009; Nolasco et al., 2009; Rubio et al., 1996), and even to identify RB genotypes (Roy et al., 2013).

Sequence analysis of an informative portion of the CTV sequence or the entire genome, and a comparison with reference isolates, may help identify molecular and phylogenetic correlations between CTV genotypes (Harper et al., 2013). However, in the absence of appropriate biological assays (Garnsey et al. 2005; Wang et al., 2013), these methods appear of limited value for the prediction of pathogenic properties of CTV isolates (Bar-Joseph et al., 2010; Harper, 2010). Therefore, a combination of biological, molecular and, possibly, serological data are needed for a conclusive characterisation of the genetic and pathogenic features of a CTV isolate.

3.2. Current distribution of *Citrus tristeza virus*

3.2.1. Global distribution of *Citrus tristeza virus*

CTV is originally a pathogen of non-European origin. CTV has been recorded in most citrus-growing areas of all five continents (Figure 1). In general, country reports do not specify the presence of particular CTV isolates/strains or of the biological properties of the isolates; however, RB isolates have been specifically reported from New Zealand (Harper et al., 2010) and, more recently, from Puerto Rico, where they have most likely been present since 1992 (Roy et al., 2010). In addition, outside of Europe, in the main citrus-producing countries of the world, CTV isolates causing SP appear to be present and prevalent, and in some citrus-producing industries cross-protection against these CTV isolates is necessary for economic production (Moreno et al., 2008).

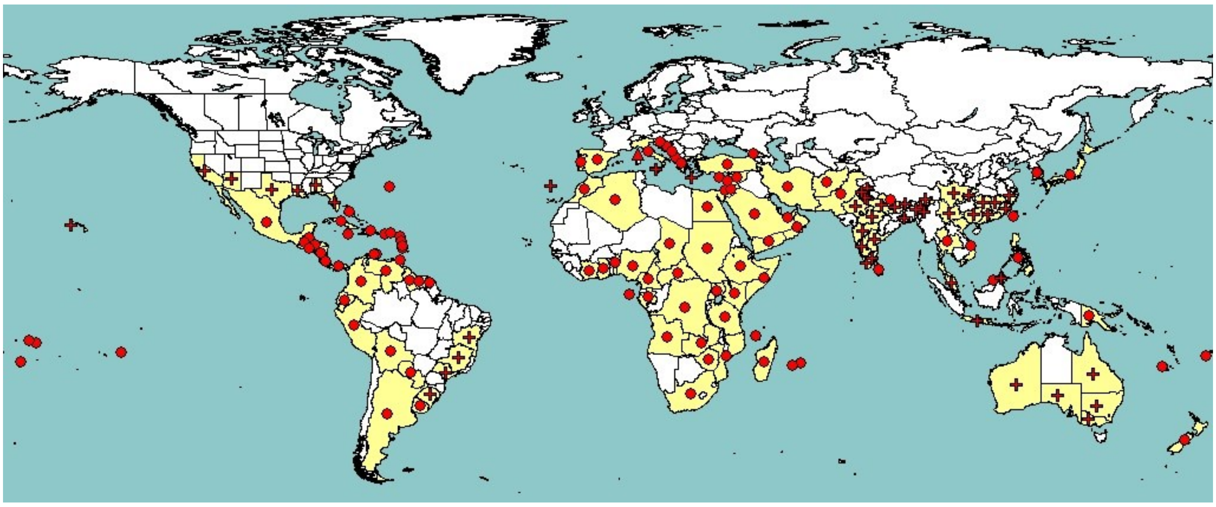

Figure 1: Global distribution map for *Citrus tristeza virus* (extracted from EPPO PQR, 2012, version 5.3.1, accessed in June 2014). Red circles represent national records of pest presence and red crosses represent sub-national records of pest presence (note that this figure combines information from different dates, some of which could be out of date)

3.2.2. Distribution in the EU of *Citrus tristeza virus*

Based on MSs' answers to the EFSA questionnaire, CTV is present in seven out of the eight EU MSs (Table 2) with significant citrus production (according to the Eurostat database, see Table 8). In Malta, where virus surveys are continuously conducted (Attard et al., 2009), occasional findings of CTV have been followed by eradication efforts, and CTV is now considered to be eradicated here (Table 2). For other MSs, CTV is considered transient, under eradication (France), present with few occurrences (Greece) or with restricted distribution (Cyprus, Italy), or present but with parts of the country still unaffected (Portugal). CTV is present and widespread in Spain and Croatia. With regards to France, the protected zone status of Corsica has recently been removed (Commission Implementing Directive 214/78/EU[5]). The most recent reports of CTV interception are from Italy, France and Portugal and concern CTV found in sweet orange (*C. sinensis*) and mandarin (*C. reticulata*) plants imported from Spain.

In general, CTV infections in Europe in citrus species grafted on sour orange rootstocks are characterised by typical tristeza rapid decline symptoms, ranging in severity, or by no symptoms at all (Ballester-Olmos, 1993; Moreno et al., 2008), the latter situation corresponding to mild isolates unable to cause decline (Varveri et al., 2014). Irrespective of the rootstock/scion combination, symptoms of SP have not yet been observed on sweet orange in the field in Europe. Despite this, CTV genotypes closely related to isolates found in other parts of the world, and associated with severe SP symptoms, have been reported in Sicily (Davino et al., 2005; Rizza et al., 2007), Spain (Ruiz-Ruiz et al., 2006), Crete (Owen et al., 2014), Greece (Malandraki et al., 2011) and the east Adriatic region (mainly Croatia and Montenegro, Cerni et al., 2009). CTV genotypes representing the RB strain, able to "break" the resistance of *P. trifoliata*, are not known to occur Europe.

There are uncertainties about the reason(s) for the apparent inability of CTV isolates, closely related to SP-inducing isolates, to cause SP symptoms in sweet orange orchards in Europe, and about the potential mid- and long-term evolution of this situation.

[5] Commission Implementing Directive 2014/78/EU of 17 June 2014 amending Annexes I, II, III, IV and V to Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 183, 24.6.2014, p. 23–48.

Another area of uncertainty concerns the extremely limited information available on the prevalence and biological properties of CTV isolates that may be present in ornamental citrus such as kumquats (*Fortunella* sp.) and calamondin (*Citrofortunella microcarpa*) in Europe.

Table 2: Current distribution of *Citrus tristeza virus* (European isolates) in the 28 Member States, Iceland and Norway, based on the answers received via email from the NPPOs or, in absence of reply, on information from EPPO PQR

Member State	*Citrus tristeza virus*	Comments of MS
Austria	**Absent**, no pest records	
Belgium	**Absent**, no pest records	No findings since 2007
Bulgaria	**Absent**	
Croatia	**Present**, widespread	It is planned to conduct a survey in 2014
Cyprus	**Present**, restricted distribution	
Czech Republic	**Absent**, no pest records	
Denmark	Known not to occur	
Estonia	–	
Finland	**Absent**, no pest records	
France	**Transient**, under eradication	
Germany	**Absent**, no pest records	
Greece [(a)]	**Present**, few occurrences	
Hungary	**Absent**, no pest records	
Ireland	**Absent**, no pest records	
Italy	**Present**, restricted distribution **Absent**, no longer present in Sardinia	Present in southern Italy
Latvia [(a)]	–	
Lithuania [(a)]	–	
Luxembourg [(a)]	–	
Malta	**Absent**, pest eradicated	
Netherlands	**Absent**, no pest records	
Poland	**Absent**, no pest records	
Portugal	**Present** in the Algarve and Madeira **Absent** in the rest of the territory as confirmed by survey	
Romania [(a)]	–	
Slovakia	**Absent**, no pest records	
Slovenia	**Absent**, no pest records on *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf.	
Spain	**Present**, widespread	
Sweden	**Absent**	
United Kingdom	**Absent**	
Iceland [(a)]	–	
Norway [(a)]	–	

(a): When no information was made available to EFSA, the pest status in the EPPO PQR (2012) was used.
–, no information available; EPPO PQR, European and Mediterranean Plant Protection Organization Plant Quarantine Data Retrieval system; NPPO, National Plant Protection Organisation.

3.2.3. Vectors and their distribution in the EU

The most efficient vector for CTV, *T. citricida*, is a regulated pest listed in Annex IIAI of Council Directive 2000/29/EC. There is only one interception report for *T. citricida* in the Europhyt database. It has been reported in the EU in only Portugal and Spain, where it was found on isolated trees far from areas relevant to commercial citrus production (Moreno et al., 2008); however, in Madeira, it is reported as being widespread. The other known CTV vectors, *A. spiraecola*, *T. aurantii* and *A gossypii*, are present in Europe. In particular, *A. gossypii*, the second most efficient vector, is widespread (Table 3). The efficiency by which CTV isolates are transmitted by *A. gossypii* varies with the particular virus isolate, but is generally greater than 50 % and thus, with its high population sizes, *A. gossypii* plays a major role in epidemics of CTV in Spain (Cambra et al., 2000a) and across Europe. Overall, and with minimal uncertainty, aphid vectors, with the potential to contribute to CTV spread, can be considered to be widely available in the EU.

Table 3: Current distribution of *Citrus tristeza virus* vectors *Toxoptera citricida* and *Aphis gossypii* in the risk assessment area, based information from EPPO PQR, 2012 and CABI Crop Protection Compendium

Member State	*Toxoptera citricida*	*Aphis gossypii*
Austria	–	**Present**
Belgium	–	**Present**
Bulgaria	–	**Present**
Croatia	–	–
Cyprus	**Absent**, invalid record	**Present**
Czech Republic	–	**Present**
Denmark	–	**Present**
Estonia	–	–
Finland	–	–
France	–	**Present**
Germany	–	**Present**
Greece	–	**Present**
Hungary	–	**Present**
Ireland	–	–
Italy	**Absent**, invalid record	**Present**
Latvia	–	–
Lithuania	–	–
Luxembourg	–	–
Malta	**Absent**, invalid record	**Present**
Netherlands	**Absent**, confirmed by survey	**Present**
Poland	–	**Present**
Portugal	**Present,** restricted distribution; **Present**, widespread in Madeira	**Present**
Romania	–	**Present**
Slovakia	–	–
Slovenia	–	**Present**
Spain	**Present,** restricted distribution	**Present**
Sweden	–	**Present**
United Kingdom	–	**Present**
Iceland	–	–
Norway	–	**Present**

–, no information available; EPPO PQR, European and Mediterranean Plant Protection Organisation Plant Quarantine Data Retrieval system.

3.3. Regulatory status of CTV European isolates

3.3.1. Council Directive 2000/29/EC

3.3.1.1. Harmful organism

European isolates of CTV are regulated harmful organisms in the EU and are currently listed in Annex IIAII and IIB of Council Directive 2000/29/EC (Table 4).

Table 4: *Citrus tristeza virus* in Council Directive 2000/29/EC

Annex II, Part A	Harmful organisms whose introduction into, and spread within, all Member States shall be banned if they are present on certain plants or plant products
Section II	Harmful organisms known to occur in the community and relevant for the entire community

(d)	Viruses and virus-like organisms		
	Species	Subject of contamination	
4	Citrus tristeza virus (European isolates)	Plants of *Citrus L*., *Fortunella* Swingle, *Poncirus Raf*., and their hybrids, other than fruit and seeds	
Annex II, Part B	Harmful organisms whose introduction into, and whose spread within, certain protected zones shall be banned if they are present on certain plants or plant products		
(d)	Viruses and virus-like organisms		
	Species	Subject of contamination	Protected zone(s)
	Citrus tristeza virus (European isolates)	Fruits of *Citrus L*., *Fortunella Swingle*, *Poncirus Raf*., and their hybrids, with leaves and peduncles	EL (except the Regional Units of Argolida and Chania), M, P (except Algarve and Madeira)

3.3.1.2. Regulated vectors of *Citrus tristeza virus*

Table 5: *Toxoptera citricida*, the vector of *Citrus tristeza virus*, in Council Directive 2000/29/EC

Annex II, Part A	Harmful organisms whose introduction into, and spread within, all Member States shall be banned if they are present on certain plants or plant products	
Section I	Harmful organisms not known to occur in the community and relevant for the entire community	
(a)	Insects, mites and nematodes, at all stages of their development	
	Species	Subject of contamination
30	*Toxoptera citricida* Kirk	Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds

3.3.1.3. Regulated hosts of *Citrus tristeza virus*

Below, specific requirements of Annex III, Annex IV and Annex V of Council Directive 2000/29/EC are presented for the host plants and commodities regulated for *Citrus tristeza virus* in Annex IIAII.

Table 6: *Citrus tristeza virus* host plants in Council Directive 2000/29/EC

Annex III, Part A	Plants, plant products and other objects the introduction of which shall be prohibited in all Member States	
16	Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus Raf*., and their hybrids, other than fruit and seeds	Third countries
Annex IV, Part A	Special requirements which must be laid down by all Member States for the introduction and movement of plants, plant products and other objects into and within all Member States	
Section I	Plants, plant products and other objects originating outside the Community	
	Plants, plant products and other objects	Special requirements
16.1	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, originating in third countries	The fruits shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark.

Section II	Plants, plant products and other objects originating in the Community		
	Plants, plant products and other objects	Special requirements	
10	Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds	Official statement that: (a) the plants originate in areas known to be free from […] *Citrus tristeza* virus (European strains); (b) the plants derive from a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and has been subjected to official individual testing for, at least, *Citrus tristeza* virus (European strains) […], using appropriate indicators or equivalent methods, approved in accordance with the procedure referred to in Article 18(2), and have been growing permanently in an insectproof glasshouse or in an isolated cage on which no symptoms of […] *Citrus tristeza* virus (European strains) […] have been observed; (c) the plants : — have been derived from a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and has been subjected to official individual testing for, at least […] *Citrus tristeza virus* (European strains), using appropriate indicators or equivalent methods, approved in accordance with the procedure referred to in Article 18(2), and has been found in these tests, free from *Citrus tristeza* virus (European strains), and certified free from at least *Citrus tristeza* virus (European strains) in official individuals tests carried out according to the methods mentioned in this indent, and — have been inspected and no symptoms of […] *Citrus tristeza* virus have been observed since the beginning of the last complete cycle of vegetation	
30.1	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids	The packaging shall bear an appropriate origin mark	
Part B	Special requirements which shall be laid down by all Member States for the introduction and movement of plants, plant products and other objects into and within certain protected zones		
	Plants, plant products and other objects	Special requirements	Protected zone(s)
31	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids originating in BG, HR, SI, EL (Regional Units of Argolida and Chania), P (Algarve and Madeira) E, F, CY and I	Without prejudice to the requirement in Annex IV, Part A, Section II, point 30.1 that packaging should bear an origin mark: (a) the fruits shall be free from leaves and peduncles; or (b) in the case of fruits with leaves or peduncles, official statement that the fruits are packed in closed containers which have been officially sealed and shall remain sealed during their transport through a protected zone, recognised for these fruits, and shall bear a distinguishing mark to be reported on the passport.	EL(except the Regional Units of Argolida and Chania) M, P (except Algarve and Madeira)
Annex V	Plants, plant products and other objects which must be subject to a plant health inspection (at the place of production if originating in the Community, before being moved within the Community—in the country of origin or the consignor country, if originating outside the Community) before being permitted to enter the Community		

Part A	Plants, plant products and other objects originating in the Community
Section I	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community and which must be accompanied by a plant passport
1	Plants and plant products
1.4	Plants of *Fortunella* Swingle, *Poncirus* Raf., and their hybrids […], other than fruit and seeds
1.5	Without prejudice to point 1.6, plants of *Citrus* L. and their hybrids other than fruit and seeds
1.6	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf. and their hybrids with leaves and peduncles
Part B	Plants, plant products and other objects originating in territories, other than those territories referred to in Part A
Section I	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community
3	Fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids […]

3.3.2. Marketing directives

Host plants of CTV that are regulated in Annex II of Council Directive 2000/29/EC are explicitly mentioned in the following Marketing Directive:

- Council Directive 2008/90/EC[6].

3.4. Elements to assess the potential for establishment and spread in the EU

3.4.1. Host range

CTV has a restricted host range, and plants of *Citrus* spp., including lemon, lime, sweet and sour orange, tangerine, mandarin, grapefruit; *Fortunella* spp., a genus comprising several kumquat species (Moreno et al., 2008); and *Poncirus* spp. are the only known natural hosts. Citrus species are widely cultivated in the Mediterranean part of the EU (Table 7), while kumquats and some other citrus species, such as calamondin, are cultivated mainly as ornamental trees and have a more limited commercial importance.

Several plant species belonging to other genera within the subfamily *Aurantioideae* (*Aegle*, *Aeglopsis*, *Afraegle*, *Atalantia*, *Citropsis*, *Clausena*, *Eremocitrus*, *Hesperethusa*, *Merrillia*, *Microcitrus*, *Pamburus*, *Pleiospermium* and *Swinglea*) have been shown to be experimental hosts of CTV (Moreno et al. 2008). CTV has also been experimentally transmitted to *Passiflora gracilis* and *P. caerulea* (family *Passifloraceae* (Müller et al., 1974; Kitajima et al., 1974; Roistacher and Bar-Joseph, 1987)). However, experimental hosts of CTV, outside of the *Rutaceae* family, are unlikely to have any practical significance. Uncertainties exist on the status of *Rutaceae* other than *Citrus*, *Fortunella* and *Poncirus* as natural hosts for CTV, especially those that are used as ornamentals, and about their potential significance for virus dissemination and CTV epidemiology.

Table 7: Area of citrus production (in 1 000 ha) in Europe in 2007 according to the Eurostat database (Crops products—annual data [apro_cpp_crop], extracted on 21 February 2013)

Member State	Orange varieties	Lemon varieties
Croatia	0.2	0.1
Cyprus	1.554	0.665
France	0.028	0.022
Greece	32.439	5.180

[6] Council Directive 2008/90/EC of 29 September 2008 on the marketing of fruit plant propagating material and fruit plants intended for fruit production. OJ L 267, 08/10/2008, p. 8–22.

Member State	Orange varieties	Lemon varieties
Malta [(a)]	0.095	0.038
Italy	73.785	16.633
Portugal	12.416	0.494
Spain	158.824	39.859
European Union	279.048	62.854

(a): Data for the citrus production area in Malta are provided according to FAOSTAT (online) for the year 2011.

3.4.2. Analysis of the potential pest distribution in the EU

CTV is a pathogen that systemically invades its citrus hosts so that the virus is present in all parts of the infected plants, albeit only in phloem tissues. Thus dispersal of CTV over long distances is by trade and by the use of infected plants for planting or of infected budwood used for graft propagation. The ecoclimatic requirements of CTV are similar to those of its host plants and therefore it is not expected to be limited by ecoclimatic conditions in areas where its hosts are able to develop. Citrus cultivation occurs in the warmer regions of Europe, where citrus plants are widely grown in orchards (see EFSA PLH Panel, 2014). In addition, citrus plants are widely grown in Europe as ornamental species, either in the open-field or under protected cultivation. Several isolates of CTV are already present and established in most EU MSs where citrus plants are grown (Table 2); the area of potential CTV distribution coincides with areas of citrus cultivation in the EU.

3.4.3. Spread capacity

The rate of CTV transmission in the field is influenced by many factors, including the composition and density of aphid populations, environmental conditions and the susceptibility of citrus species and varieties present (Moreno et al., 2008). Studies on the spatial and temporal spread of CTV conducted in citrus orchards in different parts of the world (Gottwald et al., 2002) provide evidence suggesting that a long time may elapse between the introduction of a primary source of CTV inoculum and the development of a tristeza disease epidemic (Garnsey and Lee, 1988).

In Europe, given the restricted presence of the very efficient *T. citricida* vector, *A. gossypii* is the most relevant vector for CTV spread, and disease epidemics are associated with this vector (Gottwald et al., 1997; Cambra et al., 2000a; Davino et al., 2005). Recent evidence from virus/vector studies under laboratory conditions highlights the important role played by *A. gossypii* in CTV disease outbreaks in Calabria (Campolo et al., 2014). Single *A. gossypii* insects acquired local CTV isolates after a 30-minute feeding acquisition period and transmitted the virus, in a semi-persistent transmission mode, after a 60-minute feeding transmission period (Campolo et al., 2014). Only four aphids per plant were needed to reach a 50 % CTV transmission probability, thereby demonstrating the ability of local *A. gossypii* populations to efficiently spread CTV. *A. gossypii* is prevalent throughout the risk assessment area (Table 3).

Recent studies conducted in various countries (Davino et al., 2005, 2013; Ferretti et al., 2014; Cambra et al., 2000a; Gottwald et al., 1995; Owen et al., 2014) show that spread of CTV in orchards can be rapid and including also isolates closely related to non-European isolates able to cause SP symptoms. Spread is associated with aphid vectors, but also with the movement of vegetatively propagated plants for planting, including ornamental citrus such as calamondin and kumquats (Chatzivassiliou and Nolasco, 2014).

Despite a limited number of interception reports (Europhyt database) linking intra-EU trade of plants for planting with CTV movement, existing citrus certification systems constitute a strong limitation to the CTV spread through the plants for planting pathway.

3.5. Elements to assess the potential for consequences in the EU

3.5.1. Potential pest effects

CTV causes two very serious diseases of citrus, tristeza decline and SP, and has had a serious impact in all major citrus-growing regions of the world. Almost 100 million trees grafted on susceptible rootstocks have died worldwide from tristeza decline, the affected species being mainly sweet orange (*C. sinsensis*) and mandarin (*C. reticulata*) (Bar-Joseph et al., 1989). Affected trees commonly show decline symptoms including foliage yellowing and shedding, twig dieback, progressive reduction of root systems, size decrease and discoloration of fruits, which are eventually followed by plant death. In its most dramatic manifestation, citrus tristeza disease causes a quick decline characterised by the sudden appearance of rapidly progressing symptoms eventually resulting in collapse and death of the tree within days or weeks from symptom onset. Tristeza decline can also be slow, which results in plant deterioration over longer periods of up to several years, sometimes with a latency period of up to 20 years, during which time CTV infection causes only mild symptoms or no symptoms at all (Garnsey and Lee, 1988).

In contrast to tristeza decline, SP affects mostly lime, grapefruit, and sweet orange (*C. sinensis* (L.) Osbeck), regardless of the rootstock on which these species are grafted. Symptoms of SP consist of irregular radial growth of the tree or its stems caused by the disruption of meristematic activity at localised parts of the cambium. This generates depressions in the wood that may assume a ropy, channelled, porous or spongy appearance. SP can be accompanied by stunting, yellowing and size reduction of leaves. It affects tree vigour and is associated with a considerable reduction in fruit yield and quality (Bar-Joseph and Dawson, 2008; Moreno and Garnsey, 2010). However, there is no deterioration or death of affected trees. Despite the fact that European isolates closely related to non-European, SP-inducing isolates have been detected in several EU MSs, SP symptoms have not been observed in sweet orange groves of the EU. There is uncertainty regarding the reasons underlying this observation and concerning possible future developments.

SY consists of stunting, small, pale or yellow leaves, and reduced root systems appearing in sour orange, grapefruit or lemon seedlings. The syndrome is sometimes transitory and followed by recovery of affected plants, which may resume normal growth. SY is generally not considered a major constraint and is mostly observed in greenhouse-grown plants (Moreno et al., 2008).

Overall, CTV causes very severe diseases of citrus and can have a very considerable impact on the citrus industry, especially when sour orange is used as the predominant rootstock, which is the case in most of the EU MSs, with the exception of Spain and, to a lesser extent, of Corsica (France).

3.5.2. Observed pest impact in the EU

From its introduction in the early 1930s and severe outbreaks in the late 1950s and in subsequent years, CTV outbreaks, with the greatest impacts, were recorded in Spain, where tree decline caused by CTV resulted in the serious destruction of citrus trees, leading to the replacement of tens of millions of trees in Spain until the year 2000 only (Cambra et al., 2000a; Moreno et al., 2008). This was because susceptible sour orange was very commonly used for rootstocks and because of the high *A. gossypii* populations that provided a very efficient means for the spread of CTV. The replacement of sour orange for CTV-tolerant rootstocks in the whole industry (citrange hybrids, *C. macrophylla*, *C. volkameriana*, *C. limetoides*), combined with a stringent mandatory certification programme, were the only means of recovering the citrus industry in Spain. Because of these measures, and because of the absence of SP-causing isolates, the current impact of CTV in Spain is now considered low, and is limited to the very few remaining instances of plots grafted on susceptible rootstocks. Thus, despite a widespread occurrence and very high prevalence of CTV in orchards in the commercial citrus production areas of Spain and the replanting of certified tolerant rootstock/scion combinations has virtually eliminated the impact of CTV.

In contrast, outbreaks of CTV with severe impact due to tristeza decline have been reported from Sicily (Davino et al., 2005) and other parts of Italy, from Crete (Owen et al., 2014) and from some other EU MSs. In Sicily, outbreaks of CTV, recorded in two locations, were from mild virus isolates that did not cause decline. The CTV outbreak at a third location most likely resulted from the introduction of a decline-inducing CTV isolate causing severe symptoms in sweet orange, consisting of dwarfing and dieback of the branches, size reduction and interveinal chlorosis of leaves, size reduction and elongation of fruits, and root death. These are the typical symptoms associated with tristeza decline, which were expressed because the local Tarocco sweet orange trees were grafted on sour orange.

Given that the ability to cause tristeza decline in susceptible rootstock/scion combinations is a property shared by the majority of European (and non-European) CTV isolates, and because the citrus industry still heavily relies on sour orange rootstocks in Mediterranean countries (both European and non-European) producing citrus (Cambra and Gorris, 2003), decline symptoms, culminating in the death of trees, are observed in most relevant EU regions outside of Spain, with only a few mitigating circumstances:

- local use of tolerant rootstocks preventing, as in Spain, the development of tristeza decline;
- infection by mild isolates unable to cause decline;
- local conditions affecting either the CTV isolate or the local aphid populations and limiting the spread of CTV.

In addition to these local parameters, the existence of efficient voluntary certification systems contributes to limiting the spread of CTV and can contribute to limiting its impact.

So far, there is no evidence of the severe CTV SP syndrome in EU citrus orchards (Mariano Cambra, Valencian Institute for Agricultural Research, personal communication, 2014), despite the fact that: (1) irrespective of the rootstocks used, a range of the planted species and varieties grown in the EU are susceptible; and (2) sequence variants closely related to those of the non-European SP-inducing CTV isolates have been reported from several EU MSs. There are uncertainties as to the reasons underlying this situation and as to its potential long-term evolution.

There is no identified CTV environmental impact, as this virus affects only cultivated species.

3.6. Currently applied control methods in the EU

Given the current context of CTV in the EU, characterised by the apparent absence of SP-inducing European isolates, currently applied control methods target either CTV itself, or the tristeza decline syndrome. The most efficient way to tackle tristeza decline is to replace susceptible trees with trees grafted on tolerant rootstocks, as was done in Spain. This effectively eliminates the decline disease but does not affect CTV spread, resulting in a high prevalence of symptomless, but infected, trees. It should be stressed that, although very efficient, this strategy has two limitations: (1) the industry-wide replacement of trees is a costly process that can only be envisaged to occur over many years; and (2) this approach is viable only in the absence of SP-inducing isolates, because the tolerant trees are not protected against the negative consequence of SP.

The most efficient control method for CTV involves a rigorous application of certification of planting materials, which includes the use of virus-free budwood for grafting and rootstocks, in combination with outbreak eradication efforts. Certification schemes are prescribed by EPPO (1998). It should be stressed that certification is particularly important when deploying CTV-tolerant rootstocks because, contrary to sour orange, these rootstocks tend to be very susceptible to other citrus viruses or viroids that may be present latently in uncertified budwood material (Navarro, 1986; Navarro et al., 2002; Cambra et al., 2000a). In Spain, where (1) mandatory certification programmes for nursery materials are strictly followed, (2) vigilant quarantine measures are followed to prevent introduction of SP-inducing non-European CTV isolates, (3) tolerant rootstocks are used in commercial plantations, and (4) old citrus trees are successively replaced with improved, CTV-tolerant planting materials, the impact of CTV is today considered low. This is despite a high infection rate and widespread occurrence of CTV in Spanish citrus orchards. Thus, as shown in Spain, widespread use of certified tolerant rootstocks and planting materials has the potential to achieve comprehensive CTV control after a costly and lengthy transition phase. The success of such a strategy, which is dependent on the absence of SP-inducing isolates, becomes evident only after conversion of existing orchards, and requires the concerted action of all stakeholders, industry and regulatory bodies alike.

The most efficient CTV vector, *T. citricida*, is a regulated pest with distribution limited to parts of Portugal and Spain that are not important for commercial citrus production. Vector control with insecticides could be used as an additional strategy for the containment of outbreaks. However, long-term control of CTV in orchards through an action against its most widespread European vector, *A. gossypii*, is unlikely to be effective given the wide host range of this species and its short acquisition and transmission feeding periods.

3.7. Uncertainty

Generally speaking, CTV is a very important and very well-studied virus; as a consequence, there are very few uncertainties when it comes to the parameters analysed in the present pest categorisation, such as its taxonomy, its general biology, its detection, its vectors and spread mechanisms, and the various syndromes it causes in its hosts. It should be stressed, however, that because of the loose and inconsistent use of terms such as "strain" and "mild" or "severe" isolate, the literature is frequently confusing.

When it comes to European isolates, several aspects of the current pest categorisation have met with significant uncertainties. The first of these concerns the criteria used to define a European isolate. While the geographical criteria are clear, the boundary between a freshly introduced non-European isolate, and one that has established and spread to the point that it is now recognised as a European one, is somewhat unclear since there is no obvious threshold point in this gradual process that could be used to clearly separate the two.

There are also some uncertainties when it comes to the precise geographical distribution of CTV isolates, in particular when it comes to areas where CTV is still deemed to be absent, or to the situation for ornamental citrus plants. Other uncertainties concern the potential contribution of ornamental *Rutaceae* species outside of the *Citrus*, *Fortunella* and *Poncirus* genera to the epidemiology and spread of CTV.

The most critical area of uncertainty concerns the pathological properties of European CTV isolates and, in particular, whether or not some European isolates possess the ability to cause SP symptoms in sweet orange. Although isolates closely related to non-European isolates able to cause SP have been reported in several EU MSs, SP symptoms have never been observed in EU citrus groves. While such European isolates may conceivably be unable to cause SP, it is also possible that they have not so far been able to express their ability to induce SP for a range of reasons, including local environmental conditions, the local varieties in which they have been observed, or co-infection with other isolates preventing the expression of SP symptoms. This highlights that the connection between virus genome sequence and disease is not well established. As control and management of SP- and non-SP-inducing isolates of CTV require different strategies (because the use of tolerant rootstocks does not protect against SP) the uncertainties concerning the existence of European SP isolates are of particular importance. Given that the limited data available in the literature have been analysed in the present pest categorisation, this issue is unlikely to be clarified by a detailed pest risk assessement and will require further specific research efforts.

CONCLUSIONS

The Panel summarises in Table 8 its conclusions on the key elements addressed in this scientific opinion in consideration of the pest categorisation criteria defined in ISPM 11 and ISPM 21 and of the additional questions formulated in the terms of reference.

Table 8: The Panel's conclusions on the pest categorisation criteria defined in the International Standards for Phytosanitary Measures (ISPM) No 11 and No 21 and on the additional questions formulated in the terms of reference

Criterion of pest categorisation	Panel's conclusions on ISPM 11 criterion *Provide answers to the questions in the column below*	Panel's conclusions on ISPM 21 criterion *Provide answers to the questions in the column below*	List of main uncertainties *List key uncertainties*
Identity of the pest	*Is the identity of the pest clearly defined? Do clearly discriminative detection methods exist for the pest?* CTV is a well-characterised virus and its taxonomy is clear. Reliable detection and identification tests are available. However, European isolates cannot be discriminated from non-European ones on the basis of their molecular properties		While the geographical criteria defining a European isolate are clear, the boundary between a freshly introduced non-European isolate and one that has established and spread to the point that it is now recognized as a European one is somewhat unclear. Literature is complex on intraspecific diversity
Absence/presence of the pest in the risk assessment area	*Is the pest absent from all or a defined part of the risk assessment area?* European isolates of CTV are present with variable prevalence in seven of the eight EU MSs with a significant citrus industry. CTV is reported as eradicated in Malta	*Is the pest present in the risk assessment area?* European isolates of CTV are present in seven of the eight EU MSs with a significant citrus industry	Uncertainties mostly concern the biological properties and prevalence of the isolates present in various countries or hosts

Regulatory status	*Mention in which annexes of 2000/29/EC and the marketing directives the associated hosts are listed without further analysis Indicate also whether the hosts and/or commodities for which the pest is regulated in AIIAI or II are comprehensive of the host range* European isolates of CTV are listed on Annexes IIAII and IIB of Council Directive 2000/29/EC. CTV's most efficient vector, *Toxoptera citricida*, is listed in Annex IIAI		Blurred boundary between a freshly introduced non-European isolate and one that has established and spread to the point that it is now recognised as a European one is somewhat unclear
Potential establishment and spread	*Does the risk assessment area have ecological conditions (including climate and those in protected conditions) suitable for the establishment and spread of the pest?* *Indicate whether the host plants are also grown in areas of the EU where the pest is absent* *And, where relevant, are host species (or near relatives), alternate hosts and vectors present in the risk assessment area?* European isolates of CTV are already widely present in citrus-producing EU MSs. Ecoclimatic conditions are not expected to affect further establishment. CTV has the potential to spread both by the activity of aphid vectors and through the movement of plants for planting	*Are plants for planting a pathway for introduction and spread of the pest?* CTV citrus hosts are vegetatively propagated and CTV can therefore be associated with the plants for planting pathway	
Potential for consequences in the risk assessment area	*What are the potential for consequences in the risk assessment area?* *Provide a summary of impact in terms of yield and quality losses and environmental consequences* Given the widespread use of sour orange as a rootstock in the citrus industry of most European countries, the potential impact through tristeza decline is high No CTV environmental impact is clearly identified	*If applicable is there indication of impact(s) of the pest as a result of the intended use of the plants for planting?* Given the widespread use of sour orange as a rootstock in the citrus industry of most European countries, the potential impact through tristeza decline is high No CTV environmental impact is clearly identified	Significant uncertainty about whether European CTV isolates have the ability to cause SP symptoms in sweet orange

Conclusion on pest categorisation	*Provide an overall summary of the above points* CTV is a well-defined virus with well-established diagnostics, and European isolates of CTV are regulated in its citrus hosts. They are present in seven of the eight EU MSs with a significant citrus industry. Ecoclimatic conditions are not expected to affect further establishment and spread in these countries. CTV has the potential to spread both by the activity of aphid vectors and through the movement of plants for planting. European isolates of CTV have the potential to cause very significant impact in the European citrus industries relying on sour orange rootstocks	*Provide an overall summary of the above points* CTV is a well-defined virus with well-established diagnostics, and European isolates of CTV are regulated in its citrus hosts. They are present in seven of the eight EU MSs with a significant citrus industry. Ecoclimatic conditions are not expected to affect further establishment and spread in these countries. CTV has the potential to spread both by the activity of aphid vectors and through the movement of plants for planting. European isolates of CTV have the potential to cause very significant impact in the European citrus industries relying on sour orange rootstocks	The most critical area of uncertainty concerns the pathological properties of European CTV isolates and, in particular, whether some European isolates possess the ability to cause SP symptoms in sweet orange
Conclusion on specific ToR questions	*If the pest is already present in the EU, provide a brief summary of:* – *the analysis of the present distribution of the organism in comparison with the distribution of the main hosts, and the distribution of hardiness/climate zones, indicating in particular if in the risk assessment area, the pest is absent from areas where host plants are present and where the ecological conditions (including climate and those in protected conditions) are suitable for its establishment,* European isolates of CTV are present in seven of the eight EU MSs with a significant citrus industry. They are reported as eradicated from Malta and as present with few occurrences or restricted distribution and/or part of the country still not affected for Cyprus, Greece, Italy and Portugal. Ecoclimatic conditions are not expected to affect further establishment in countries where citrus are grown outside *and* – *the analysis of the observed impacts of the organism in the risk assessment area* So far, there is no evidence of expression in the EU of the severe CTV stem pitting syndrome. Observed impact is therefore the consequence of CTV spread in citrus industries still heavily reliant on susceptible rootstocks such as sour orange As such, impact is currently very limited in Spain for which the whole industry has replaced sour orange with CTV-tolerant rootstocks		The most critical area of uncertainty concerns the pathological properties of European CTV isolates and, in particular, whether some European isolates possess the ability to cause SP symptoms in sweet orange

REFERENCES

Attard D, Gatt M, Agius MA, Muscat A and Leone Ganado C, 2009. Citrus tristeza virus (CTV) survey in the Maltese Islands 1999–2005. In: Citrus tristeza virus and *Toxoptera citricidus*: a serious threat to the Mediterranean citrus industry. Eds D'Onghia AM, Djelouah K and Roistacher CN. Bari: Options Méditerranéennes: Série B. Etudes et Recherches. No 65, 76–78.

Ayllón MA, López C, Navas-Castillo J, Garnsey SM, Guerri J, Flores R and Moreno P, 2001. Polymorphism of the 5′ terminal region of Citrus tristeza virus (CTV) RNA: incidence of three sequence types in isolates of different origin and pathogenicity. Archives of Virology, 146, 27–40.

Bar-Joseph M and Dawson WO, 2008. Citrus tristeza virus. In: Encyclopedia of Virology. Eds Mahy BWJ and Van Regenmortel MHV. Elsevier Ltd., Amsterdam, 520–525.

Bar-Joseph M, Marcus R and Lee RF, 1989. The continuous challenge of citrus tristeza virus control. Annual Review of Phytopathology, 27, 291–316.

Bar-Joseph M, Batuman O and Roistacher C, 2010. The history of Citrus tristeza virus—Revisited. In: Citrus tristeza virus complex and tristeza diseases. Eds Karasev AV and Hilf ME. APS Press, St Paul, MN, USA, 3–26.

Bertolini E, Moreno A, Capote N, Olmos A, De Luis A, Vidal E, Pérez-Panadés J and Cambra M, 2008. Quantitative detection of Citrus tristeza virus in plant tissues and single aphids by real-time RT-PCR. European Journal of Plant Pathology, 120, 177–188.

Cambra M and Gorris MT, 2003. La tristeza de los cítricos en la cuenca del Mediterráneo. Phytoma-España, 153, 42–48.

Cambra M, Gorris MT, Marroquín C, Román MP, Olmos A, Martínez MC, Hermoso de Mendoza A, López A and Navarro L, 2000a. Incidence and epidemiology of Citrus tristeza virus in the Valencian Community of Spain. Virus Research, 71, 85–95.

Cambra M, Gorris MT, Román MP, Terrada E, Garnsey SM, Camarasa E, Olmos A and Colomer M, 2000b. Routine detection of citrus tristeza virus by direct Immunoprinting-ELISA method using specific monoclonal and recombinant antibodies. Proceedings of the 14th Conference of the International Organization of Citrus Virologists. IOCV, Riverside, CA, USA, pp. 34–41.

Campolo O, Chiera E, Malacrinò A, Laudani F, Fontana A, Albanese GR and Palmeri V, 2014. Acquisition and transmission of selected CTV isolates by Aphis gossypii. Journal of Asia-Pacific Entomology, 17, 493–498.

Cerni S, Skoric D, Ruscic J, Krajacic M, Papic T, Djelouah K and Nolasco G, 2009. East Adriatic—a reservoir region of severe Citrus tristeza virus strains. European Journal of Plant Pathology, 124, 701–706.

Chatzivassiliou EK and Nolasco G, 2014. Detection of a new variant of Citrus tristeza virus in Greek citrus crops. Phytopathologia Mediterranea, 53, 140–147.

Davino S, Rubio L and Davino M, 2005. Molecular analysis suggests that recent Citrus tristeza virus outbreaks in Italy were originated by at least two independent introductions. European Journal of Plant Pathology, 111, 289–293.

Davino S, Willemsen A, Panno S, Davino M, Catara A, Elena SF and Rubio L, 2013. Emergence and Phylodynamics of Citrus tristeza virus in Sicily, Italy. PLoS ONE, 8, e66700.

Dawson WO, Garsey SM, Tatineni S, Folimonova SY, Harper SJ and Gowda S, 2013. Citrus tristeza virus–host interactions. Frontiers in Microbiology, 4, 11–20. doi: 10.3389/fmicb.2013.00088. eCollection 2013.

EFSA PLH Panel (EFSA Panel on Plant Health), 2010. Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options by EFSA. EFSA Journal 2010;8(2):1495, 66 pp. doi:10.2093/j.efsa.2010.1495

EFSA PLH Panel (EFSA Panel on Plant Health), 2014. Scientific opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with the identification and evaluation of risk reduction options. EFSA Journal 2014; 12(2):3557, 243 pp. doi:10.2903/j.efsa.2014.3557

EPPO (European and Mediterranean Plant Protection Organization), 1998. Certification schemes. Pathogen-tested citrus trees and rootstocks. Paris: EPPO/OEPP, PM 4/12, 1–12.

EPPO (European and Mediterranean Plant Protection Organization), 2004. Diagnostic protocols for regulated pests, PM 7/31. Citrus tristeza closterovirus. Bulletin OEPP/EPPO Bulletin, 34, 155-157.

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

Ferretti L, Fontana A, Sciarroni R, Schimio R, Loconsole G, Albanese G and Saponari M, 2014. Molecular and biological evidence for a severe seedling yellows strain of Citrus tristeza virus spreading in southern Italy. Phytopathologia Mediterranea, 53, 3–13.

Garnsey SM and Cambra M, 1991. Enzyme-linked immunosorbent assay (ELISA) for citrus pathogens. In: Graft-transmissible diseases of citrus. Handbook for detection and diagnosis. Ed. Roistacher CN. FAO, Rome, Italy, 193–216.

Garnsey SM, Gonsalves D and Purcifull DE, 1977. Mechanical Transmission of Citrus Tristeza Virus. Phytopathology 67, 965–968.

Garnsey SM and Lee RF, 1988. Tristeza. 48–50. In: Compendium of citrus diseases. Eds Whiteside JO, Garnsey SM and Timmer LW. APS Press, St Paul, MN, USA, 80 pp.

Garnsey SM and Müller GW, 1988. Efficiency of Mechanical Transmission of Citrus Tristeza Virus. In: Proceedings of the 10th Conference of the International Organisation of Citrus Virologists. Riverside, pp. 46-54.

Garnsey SM, Permar TA, Cambra M and Henderson CT, 1993. Direct tissue blot immunoassay (DTBIA) for detection of citrus tristeza virus (CTV). In: Proceedings of the 12th Conference of the International Organisation of Citrus Virologists. Eds Moreno P, Da Graça J and Timmer LW. IOCV, Riverside, CA, USA, 39–50.

Garnsey SM, Civerolo EL, Gumpf DJ, Paul C, Hilf ME, Lee RF, Brlansky R, Yokomi RK and Hartung JS, 2005. Biological characterization of an international collection of Citrus tristeza virus (CTV) Isolates. Proceedings of the 16th Conference of the International Organisation of Citrus Virologists, 2004. IOVC, Riverside, CA, USA, 491, pp. 75–93.

Gottwald TR, 2010. Concepts in the epidemiology of Citrus tristeza virus. In: Citrus tristeza virus complex and Tristeza diseases. Eds Karasev AV and Hilf ME. APS Press, St Paul, MN, USA, 119–131.

Gottwald TR, Cambra M, Moreno P, Camarasa E and Piquer J, 1995. Spatial and temporal analyses of Citrus tristeza virus in Eastern Spain. Phytopathology, 86, 45–55.

Gottwald TR, Garnsey SM, Cambra M, Moreno P, Irey M and Borbón J, 1997. Comparative effects of aphid vector species on increase and spread of citrus tristeza virus. Fruits, 52, 397–404.

Gottwald TR, Polek ML and Riley K, 2002. History, present incidence, and spatial distribution of Citrus tristeza virus in the California Central Valley. In: Proceedings of the 15th Conference of the International Organisation of Citrus Virologists. Eds Duran-Vila N, Milne RG and da Graça JV. IOCV, Riverside, CA, USA, 83–94. Available online: www.ivia.es

Harper SJ, 2013. Citrus tristeza virus: evolution of complex and varied genotypic groups. Frontiers in Microbiology, 4. doi: 10.3389/fmicb.2013.00093. eCollection 2013.

Harper SJ, Dawson TE and Pearson MN, 2010. Isolates of Citrus tristeza virus that overcome *Poncirus trifoliata* resistance comprise a novel strain. Archives of Virology, 155, 471–480.

Hermoso de Mendoza A, Ballester-Olmos JF and Pina-Lorca JA, 1984. Transmission of citrus tristeza virus by aphids (Homoptera, Aphididae) in Spain. In: Proceedings of the 9th Conference of the International Organization of Citrus Virologists. Eds Garnsey SM, Timmer LW and Dodds JA. IOCV, Riverside, CA, USA, 23–27.

Hilf ME and Garnsey SM, 2002. Citrus tristeza virus in Florida: A synthesis of historical and contemporary biological, serological and genetic data. Proceedings of the 15th Conference of the International Organization of Citrus Virologists. IOCV, Riverside, CA, USA, 13–16.

Karasev VA and Bar-Joseph M, 2010. Citrus tristeza virus and the taxonomy of Closteroviridae. In: Citrus tristeza virus complex and tristeza diseases. Eds Karasev AV and Hilf ME. APS Press, St Paul, MN, USA, 119–131.

Kitajima EW, Müller GW and Costa AS, 1974. Electron microscopy of tristeza-infected *Passiflora gracilis* Jacq. In: Proceedings of the 6th Conference of the International Organization of Citrus Virologists. Eds Weathers LG and Cohen M. University of California, Division of Agricultural Sciences, Berkeley, USA, 79–82.

McClean APD, 1957. Tristeza virus of citrus: evidence for absence of seed transmission. The Plant Disease Reporter, 41, 821.

Martelli GP, Agranovsky AA, Bar-Joseph M, Boscia D, Candresse T, Coutts RHA, Dolja VV, Hu JS, Jelkmann W, Karasev AV, Martin RR, Minafra A, Namba S and Vetten HJ, 2012. Closteroviridae. In: Virus Taxonomy, 9th Report of the International Committee on Taxonomy of Viruses. Eds King AMQ, Adams MJ, Carstens EB and Lefkowitz EJ. Elsevier Academic Press, London, UK, 987–1001.

Melzer MJ, Borth WB, Sether DM, Ferreira S, Gonsalves D and Hu JS (2010). Genetic diversity and evidence for recent modular recombination in Hawaiian Citrus tristeza virus. Virus Genes, 40, 111–118. doi:10.1007/s11262- 009–0409–3

Michaud JP, 1998. A review of the literature on *Toxoptera citricida* (Kirkaldi) (Homoptera: Aphididae). Florida Entomologist, 81(1), 37–61.

Moreno P and Garnsey SM, 2010. Citrus Tristeza diseases—A worldwide perspective. In: Citrus tristeza virus complex and tristeza diseases. Eds Karasev AV and Hilf ME. APS Press, St Paul, MN, USA, pp. 27–49.

Moreno P, Ambrós S, Albiach-Martí MR, Guerri J and Peña L, 2008. Citrus tristeza virus: a pathogen that changed the course of the citrus industry. Molecular Plant Pathology, 9(2), 251–268. doi: 10.1111/J.1364–3703.2007.00455.X

Müller GW and Garnsey SM, 1984. Susceptibility of citrus varieties, species, citrus relatives, and non-rutaceous plants to slash-cut mechanical inoculation with citrus tristeza virus (CTV). In: Proceedings of the Ninth Conference of the International Organization of Citrus Virologists. Eds Garnsey SM, Timmer LW and Dodds JA. IOVC, Riverside, CA, USA, 33–40.

Müller GW, Costa AS, Kitajima EW and Camargo IJB, 1974. Additional evidence that tristeza virus multiplies in *Passiflora* spp. In: Proceedings of the 6th Conference of the International Organization of Citrus Virologists. Eds Weathers LG and Choen M. University of California, Division of Agricultural Sciences, Berkeley, CA, USA, 75–78.

Navarro L, 1986. Citrus certification in Mediterranean countries. Bulletin OEPP/EPPO Bulletin, 16, 227–238.

Navarro L, Pina JA, Juárez J, Ballester-Olmos JF, Arregui JM, Ortega C, Navarro A, Duran-Vila N, Guerri J, Moreno P, Cambra M and Zaragoza S, 2002. The citrus variety improvement program in Spain in the period 1975–2001. In: Proceedings of the 15th Conference of the International Organization of Citrus Virologists. Eds Duran-Vila N, Milne RG and Da Graça JV. IOCV, Riverside, CA, USA, 306–316.

Nolasco G, de Blas C, Torres V and Ponz F, 1993. A method combining immunocapture and PCR amplification in a microtiter plate for the routine diagnosis of plant viruses and subviral pathogens. Journal of Virological Methods, 45, 201–218.

Nolasco G, Santos C, Silva G and Fonseca F, 2009. Development of an asymmetric PCR-ELISA typing method for Citrus tristeza virus based on the coat protein gene. Journal of Virological Methods, 155, 97–108.

Olmos A, Dasí MA, Candresse T and Cambra M, 1996. Print capture PCR: a simple and highly sensitive method for the detection of plum pox virus (PPV) in plant tissues. Nucleic Acids Research, 24, 2192–2193.

Owen C, Mathioudakis MAG, Gal P, Nol N, Kalliampakou K, Figas A, Bellan A, Iparaguirre A, Rubio L and Livieratos J, 2014. Evolution and molecular epidemiology of Citrus tristeza virus on Crete: recent introduction of a severe strain. Journal of Phytopathology, 162, 1–5.

Permar TA, Garnsey SM, Gumpf DJ and Lee RF, 1989. A monoclonal antibody that discriminates strains of citrus tristeza virus. Phytopathology, 80, 224–228.

Pina JA, Moreno P, Juarez J, Guerri J, Cambra M, Gorris MT and Navarro L, 2005. A new procedure to index Citrus tristeza virus-induced decline on sour orange rootstock. Proceedings of the 16th Conference of the International Organisation of Citrus Virologists, 2004. IOCV, Riverside, CA, USA, 491.

Raccah B, Loebenstein G and Bar-Joseph M, 1976. Transmission of citrus tristeza virus by melon aphid. Phytopathology, 66, 1102–1104.

Roistacher CN and Bar-Joseph M, 1987. Transmission of Citrus tristeza virus by *Aphis gossypii* and by graft inoculationto and from *Passiflora* spp. Phytophylactica, 19, 179–182.

Roy A and Brlansky RH, 2009. Population dynamics of a Florida Citrus tristeza virus isolate and aphid-transmitted subisolates: identification of three genotypic groups and recombinants after aphid transmission. Phytopathology, 99, 1297–1306.

Roy A, Choudhary N, Hartung JS and Brlansky RH, 2013. The prevalence of the Citrus tristeza virus trifoliate resistance breaking genotype among Puerto Rican isolates. Plant Diseases, 97, 1227–1234.

Rubio L, Ayllón MA, Guerri J, Pappu H, Niblett C and Moreno P, 1996. Differentiation of citrus tristeza closterovirus (CTV) isolates by single-strand conformation polymorphism analysis of the coat protein gene. Annals of Applied Biology, 129, 479–489.

Rubio L, Ayllón MA, Kong P, Fernández A, Polek ML, Guerri J, Moreno P and Falk BW, 2001. Genetic variation of Citrus tristeza virus isolates from California and Spain: evidence for mixed infections and recombination. Journal of Virology, 75, 8054–8062.

Ruiz-Ruiz S, Moreno P, Guerri J, Ambros S, 2006. The complete nucleotide sequence of a severe stem pitting isolate of Citrus tristeza virus from Spain: comparison with isolates from different origins. Archives of Virology, 151, 387–398.

Sambade A, López C, Rubio L, Flores R, Guerri J and Moreno P, 2003. Polymorphism of a specific region in the gene p23 of Citrus tristeza. Archives of Virology, 148, 2325-2340.

Satyanarayana T, Gowda S, Ayllón MA and Dawson WO, 2004. Closterovirus bipolar virion: Evidence for initiation of assembly by minor coat protein and its restriction to the genomic RNA 5′ region. Proceedings of the National Academy of Sciences of the USA. 101, 799–804.

Varveri C, Olmos A, Pina JA and Marroquin CMC, 2014. Biological and molecular characterisation of a distinct Citrus tristeza virus isolate originated from a lemon tree in Greece. Plant Pathology. doi: 10.1111/ppa.12308

Vidal E, Yokomi RK, Moreno A, Bertolini E and Cambra M, 2012. Calculation of diagnostic parameters of advanced serological and molecular tissue-print methods for detection of Citrus tristeza virus. A model for other plant pathogens. Phytopathology, 102, 114–121. doi: org/10.1094/PHYTO-05–11–0139

Vives MC, Rubio L, López C, Navas-Castillo J, Albiach-Martí MR, Dawson WO, Guerri J, Flores R and Moreno P, 1999. The complete genome sequence of the major component of a mild Citrus tristeza virus isolate. Journal of General Virology, 80, 811–816.

Vives MC, Rubio L, Sambade A, Mirkov TE, Moreno P and Guerri J, 2005. Evidence of multiple recombination events between two RNA sequence variants within a Citrus tristeza virus isolate. Virology, 331, 232–237. doi: 10.1016/j.virol.2004.10.037

Wallace JM and Drake RJ, 1951. Recent developments in studies of quick decline and related diseases. Phytopathology, 41, 785–793.

Wang JB, Bozan O, Kwon SJ, Dang T, Rucker T, Yokomi RK, Lee RF, Folimonova SY, Krueger RR, Bash J, Greer G, Diaz J, Serna R and Vidalakis G, 2013. Past and future of a century old Citrus tristeza virus collection: a California citrus germplasm tale. Frontiers in Microbiology, 4. doi: 10.3389/fmicb.2013.00366

Weathers LG and Hartung MK, 1964. Transmission of citrus viruses by dodder, *Cuscuta subinclusa*. Plant Disease Reporter, 48, 102–102.

Yokomi RK and Garnsey SM, 1987. Transmission of citrus tristeza virus by *Aphis gossypii* and *Aphis citricola* in Florida. Phytophylactica, 19, 169–172.

Yokomi RK, Lastra R, Stoetzel MB, Damsteegt VD, Lee RF, Garnsey SM, Gottwald TR, Rocha-Peña MA and Niblett CL, 1994. Establishment of the brown citrus ahid (Homoptera: Aphidide) in Central America and the Caribbean Basin. Journal of Economic Entomology, 88, 1078–1085.

Yokomi RK, Garnsey SM, Civerolo EL and Gumpf D, 1989. Transmission of exotic citrus tristeza isolates by a Florida colony of *Aphis gossypii*. Plant Disease, 73, 552–556.

ABBREVIATIONS

CTV	*Citrus tristeza virus*
EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organization
EPPO-PQR	European and Mediterranean Plant Protection Organization Plant Quarantine Retrieval system
EU	European Union
INSDC	International Nucleotide Sequence Database Collaboration
ISPM	International Standards for Phytosanitary Measures
MS	Member State
NPPO	National Plant Protection Organisation
ORF	open reading frame
PLH Panel	Plant Health Panel
PRA	pest risk analysis
RB	resistance breaking
SP	stem pitting disease
SY	seedling yellows