EPPO Datasheet: *Closterovirus tristezae*

Last updated: 2020-06-19

IDENTITY

Preferred name: *Closterovirus tristezae*
Taxonomic position: Viruses and viroids: Riboviria: Orthornavirae: Kitrinoviricota: Alsuviricetes: Martellivirales: Closteroviridae
Other scientific names: *CTV*, *Citrus tristeza closterovirus*, *Citrus tristeza virus*
Common names: bud-union decline of citrus, die-back of lime, quick decline of citrus, seedling yellows of citrus, stem pitting of grapefruit, tristeza of citrus
view more common names online...
EPPO Categorization: A2 list
view more categorizations online...
EU Categorization: Quarantine pest ((EU) 2019/2072 Annex II A), PZ Quarantine pest ((EU) 2019/2072 Annex III), RNQP ((EU) 2019/2072 Annex IV)
EPPO Code: CTV000



more photos...

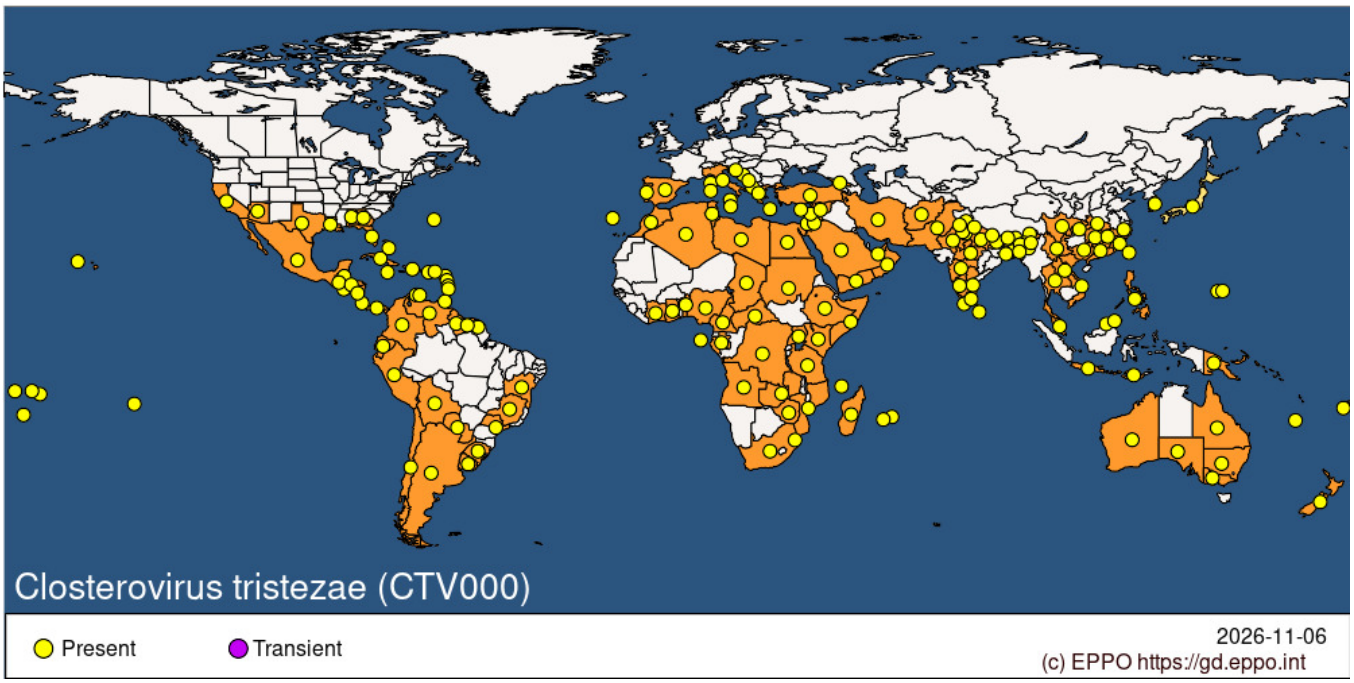
HOSTS

Citrus tristeza virus (CTV) infects most species and hybrids of the *Citrus* genus, as well as a large number of other Rutaceae. Within the Euro-Mediterranean area, sweet orange (*Citrus sinensis*) and mandarin (*C. reticulata*) are the most widely cultivated hosts, followed by lemon (*C. limon*) and grapefruit (*C. paradisi*), and the tristeza sensitive sour orange (*C. aurantium*) is still considered to be the main citrus rootstock used. *Passiflora* sp. has been experimentally infected and is the only known non-rutaceous host species (Garnsey *et al*., 1998; Roberts *et al*., 2001).

Host list: *Citroncirus webberi*, *Citrus hybrids*, *Citrus hystrix*, *Citrus maxima*, *Citrus medica*, *Citrus myrtifolia*, *Citrus reticulata*, *Citrus trifoliata*, *Citrus x aurantiifolia var. macrophylla*, *Citrus x aurantiifolia*, *Citrus x aurantium var. clementina*, *Citrus x aurantium var. deliciosa*, *Citrus x aurantium var. paradisi*, *Citrus x aurantium var. sinensis* , *Citrus x aurantium var. unshiu*, *Citrus x aurantium*, *Citrus x junos*, *Citrus x latifolia*, *Citrus x limon var. limetta*, *Citrus x limon var. limettioides*, *Citrus x limon var. meyerii*, *Citrus x limon*, *Citrus x limonia*, *Citrus*, *Clausena*, *Fortunella japonica*, *Fortunella margarita*, *Fortunella*

GEOGRAPHICAL DISTRIBUTION

Within the Euro-Mediterranean region, severe CTV outbreaks were reported, firstly in Spain (1950s) and Israel (1970s) (Moreno and Garnsey, 2010) then later on in Italy (2002) (Davino *et al.*, 2003 and 2005). Other CTV foci were reported from Algeria, Cyprus, Egypt, Greece, Lebanon, Morocco, Malta, Syria and Turkey (Djelouah and D'Onghia, 2001) and it is likely that CTV has the potential to establish wherever citrus are grown. Most isolates from the Mediterranean area have been characterized as mild CTV strains, although isolates associated with severe pathogenic behavior have been detected in some cases (Cerni *et al*., 2009; Yahiaoui *et al*., 2015).



EPPO Region: Albania, Algeria, Bosnia and Herzegovina, Croatia, Cyprus, France (Corse), Georgia, Greece (mainland, Kriti), Israel, Italy (mainland, Sardegna, Sicilia), Jordan, Malta, Montenegro, Morocco, Portugal (mainland, Madeira), Spain (mainland), Tunisia, Türkiye
Africa: Algeria, Angola, Benin, Cameroon, Central African Republic, Chad, Comoros, Congo, The Democratic Republic of the, Cote d'Ivoire, Egypt, Eswatini, Ethiopia, Gabon, Ghana, Kenya, Libya, Madagascar, Mauritius, Morocco, Mozambique, Nigeria, Reunion, Sao Tome and Principe, Somalia, South Africa, Sudan, Tanzania, United Republic of, Tunisia, Uganda, Zambia, Zimbabwe
Asia: Afghanistan, Bangladesh, Bhutan, Brunei Darussalam, China (Chongqing, Fujian, Guangdong, Guangxi, Hubei, Hunan, Jiangxi, Sichuan, Yunnan, Zhejiang), India (Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Delhi, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal), Indonesia (Java, Nusa Tenggara), Iran, Islamic Republic of, Israel, Japan, Jordan, Korea, Republic of, Lao People's Democratic Republic, Lebanon, Malaysia (Sabah, West), Nepal, Oman, Pakistan, Philippines, Saudi Arabia, Sri Lanka, Syrian Arab Republic, Taiwan, Thailand, United Arab Emirates, Vietnam, Yemen
North America: Mexico, United States of America (Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Texas)
Central America and Caribbean: Antigua and Barbuda, Aruba, Bahamas, Belize, Bermuda, Costa Rica, Cuba, Dominican Republic, El Salvador, Guadeloupe, Guatemala, Honduras, Jamaica, Martinique, Netherlands Antilles, Nicaragua, Panama, Puerto Rico, Saint Lucia, Trinidad and Tobago, Virgin Islands (British)
South America: Argentina, Bolivia, Brazil (Bahia, Minas Gerais, Rio Grande do Sul, Sao Paulo), Chile, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Uruguay, Venezuela
Oceania: American Samoa, Australia (New South Wales, Queensland, South Australia, Victoria, Western Australia), Fiji, French Polynesia, Guam, New Caledonia, New Zealand, Northern Mariana Islands, Papua New Guinea, Samoa, Tonga, Wallis and Futuna Islands

BIOLOGY

In the field, CTV is spread by several aphid species (Homoptera: Aphididae) when feeding on citrus trees. The virus is transmitted in a semi-persistent manner (Bar-Joseph *et al.*, 1983), and is reported to have no latent period. The acquisition and inoculation periods range from 30 min to 24 h (Bar-Joseph *et al.*, 1989). CTV can be transmitted by grafting during propagation of citrus planting material. It has also been transmitted from plant to plant throughout dodder (*Cuscuta americana* and *C. subglutinosa*) (Timmer *et al.*, 2000). CTV is not a seed-borne virus (Roberts *et al.*, 2001).

The most efficient aphid vector is *Aphis* (*Toxoptera) citricidus* (Yokomi *et al.*, 1994), followed by *Aphis gossypii*. *A. citricidus* is still absent from most citrus-growing areas of the EPPO region, but it was discovered in the early 2000s

in Portugal and parts of Spain. It has been shown that *A. citricidus* is up to 25 times more efficient at transmitting CTV isolates than *A. gossypii* (Gotwald, 2010). Although *A. gossypii* is less efficient than *A. citricidus* in transmitting the particularly severe CTV strains, it remains the main vector of CTV, in particular in most Mediterranean areas where *A. citricidus* still does not occur (Gottwald *et al.*, 1997; Roberts *et al.*, 2001, Roistacher *et al*., 1980; Roistacher and Bar-Joseph, 1984, Cambra *et al.*, 2000; Niblett *et al.*, 2000). In addition to these two main vector species, other aphid species can play a significant role in spreading the virus, such as *A. spiraecola*, *A. aurantii, A. craccivora* and *Myzus persicae* (Rocha-Peña *et al*., 1995; Yokomi *et al*., 1994; Marroquin *et al*., 2004).

DETECTION AND IDENTIFICATION

Symptoms

CTV isolates differ in their pathogenicity to citrus plants depending on the virus strain, the variety of citrus, and the scion-rootstock combination (Yoshida, 1996). Mild CTV infections can occur latently for a long period and generally incite barely noticeable symptoms of both vein clearing and flecking, only on leaves of Mexican lime, through biological indexing (Roistacher, 1991). Economically damaging CTV infections are associated to the type of CTV strains and can conversely induce quick decline (QD), seedling yellows (SY) and stem pitting (SP) symptoms (Roistacher, 2006).

Quick decline is a scion-rootstock incompatibility reaction caused by CTV on trees which have been graft-propagated on sour orange (*C. aurantium*) rootstock (Moreno and Garnsey, 2010). The canopy of infected trees suddenly becomes stunted, wilted, defoliated and dies within a few months. In some cases of latent infections, symptoms of inverse pitting or 'honeycombing' could be observed in the inner scion-rootstock interface. Stem pitting (SP) is usually associated with severe infections which affect the main trunk, the small branches and the twigs of grapefruit and sweet orange (*C. x paradisi* and *C. sinensis*) regardless to the rootstock, by inducing deep pits in the wood under enlarged cheesy bark, accompanied by a general growth cessation of the trees and easy breakage of the twigs (Roistacher, 1991; Rocha-Peña *et al*., 1995). Seedling yellows is mostly observed in sour orange, lemon, and grapefruit (*C. aurantium, C. limon* and *C. x paradisi*) seedlings; it is characterized by general stunting, production of small and pale leaves, reduced root system, and sometimes complete cessation of plant growth (Roistacher, 2006).

Morphology

CTV is a *Closterovirus* with flexuous filamentous rod-shaped virions, a positive sense ssRNA genome of 19296 nucleotides, packaged in 11 nm × 2000 nm threadlike particles, organized in 12 open reading frames (ORFs) and two 5' and 3' untranslated regions (UTRs) (Bar-Joseph *et al*., 1979; Karasev *et al*., 1995). The 5' half of the CTV genome encompasses the 'replication gene block' encoding proteins associated with viral replication function (Satyanarayana *et al*., 1999). While the remaining 3' half of the genome showed 90% of nucleotide homology (Gowda *et al*., 2003), it contains at least 10 ORFs encoding for the two capsid proteins (CP and CPm) of 25 and 27 kDa, respectively, in addition to p6, p13, p18, p20, p23, p33, p65 and p61 proteins (Hilf *et al*., 1995; Ayllon *et al* ., 2003).

Detection and inspection methods

Mexican lime (*C. aurantiifolia*) is still the appropriate universal plant indicator for detecting and discriminating most of the CTV isolates; whereby, graft inoculation of the virus commonly elicits typical vein clearing and leaf cupping on the new flushes under relatively cool conditions (24-27°C day/18-21°C night). In the presence of severe CTV infections, this woody indicator can also display vein corking.

Seedlings of grapefruit, lemon, and sour orange are highly sensitive to CTV-SY isolates; while, Duncan grapefruit and Madame vinous sweet orange seedlings provide a satisfactory response to CTV-SP inducing strains (Roistacher, 1991; Lee *et al*., 1996).

The development of serological tools, made the mass detection of CTV isolates feasible, first through the Enzyme Linked Immunosorbent Assay (ELISA) technique, and later on with the Direct Tissue Blot Immunoassay (DTBIA), which was more convenient for large-scale surveys, owing to its sensitivity and ease of use under field conditions (Cambra *et al.*, 2000; Djelouah and D'Onghia, 2001). The MCA 13 monoclonal antibody (Mab) raised against T36 strains (Florida) commonly cross-reacts against severe CTV isolates (Permar *et al*., 1990). Subsequently, knowledge

of the full-length CTV nucleotide sequences, transformed CTV diagnosis and constituted a starting point for the development of many genome-based procedures, including molecular hybridization (Hilf *et al*., 1999; Barbarossa and Savino, 2006), RT-PCR based techniques (Nolasco *et al.*, 1993; Hung *et al.*, 2000; Roy *et al.*, 2005; Bertolini *et al*., 2008; Saponari *et al.*, 2008), direct detection by tissue-print, and squash capture real-time RT-PCR (Cambra *et al* ., 2015). To date, several molecular tests for CTV have also been described based on Restriction Fragment Length Polymorphism (RFLP) (Gilling *et al*., 1993), Single-strand conformation polymorphism (SSCP) (Rubio *et al.* , 1996), and Multiple molecular markers (MMM) genotyping (Hilf *et al.*, 2005).

Interestingly, remote sensing using hyperspectral imaging techniques are becoming reliable methods for the early detection of CTV infected trees in citrus orchards. This enabled a detection strategy based on pre-visual symptoms (Gualano *et al.*, 2009; D'Onghia *et al.*, 2015).

PATHWAYS FOR MOVEMENT

At field level, the CTV pathosystem can be characterized mostly by the predominant vector species since aphids are responsible for the inter-plant dissemination of tristeza infections (Gottwald *et al*., 1998). Among the reasons for which *A. citricidus* is considered to be the most threatening vector may be the fact that it breeds exclusively on citrus species (Yokomi *et al*., 2010), but also its ability to transmit preferentially severe stem pitting strains in contrast to the other aphid vectors (Roberts *et al.*, 2001). In this context, the occurrence of *A. citricidus* in Portugal and Spain represents a serious threat to the citrus industry across the Euro-Mediterranean area (Ilharco *et al.*, 2005).

Disease spread within and between countries is mainly ensured by trade or movements of infected plants for planting (budwood). In the EPPO region, the cultivation of ornamental citrus in pots is becoming popular, and the import of these ornamental plants from CTV-infected areas may also present a risk.

PEST SIGNIFICANCE

Economic impact

Tristeza is one of the most devastating diseases of citrus worldwide (Roistacher, 1991). Massive epidemics occurred in Argentina in 1930, and Brazil in 1937, and resulted in the loss of 18 and 10 million trees, respectively. Within the Euro-Mediterranean region, repeated and severe CTV outbreaks occurred in the 1950s in Spain, where more than 40 million trees were killed (Moreno and Garnsey, 2010). In the 2000s, outbreaks also took place in Italy and caused the dieback of over 400 000 trees in Apulia and Sicily (Davino *et al*., 2003 and 2005). Finally, in the Mediterranean area, as the CTV sensitive sour orange is continuing to be the main rootstock used, this represents a great potential for future epidemics.

Control

Large-scale surveys are of utmost importance to detect newly established foci of CTV in an area, as early as possible. Once detected, a rapid elimination of the virus reservoirs either from individual trees or entire planted areas is the most effective practice to avoid the inoculum dispersal and to delay an epidemic (Gottwald, 2010). However, where the disease becomes endemic, and the probabilities of natural dissemination are high, the use of CTV tolerant rootstocks and mild strain cross-protection is a possible solution to extend the economic life of citrus trees without tristeza decline and stem pitting diseases (Garnsey *et al*., 1998). The most widely used tristeza-decline tolerant rootstocks are *P. trifoliata* and its hybrids Carrizo and Troyer citrange (*C. sinensis* x *P. trifoliata*), as well as Swingle citrumelo (*C. paradisi* x *P. trifoliata*) and Rangpur lime (*C. limonia*) (Moreno *et al*., 2008). Moreover, the use of rough lemon and mandarins as rootstocks instead of sour orange enabled a good production of oranges in South Africa despite the co-existence of severe CTV strains and the vector *A. citricidus* (Bar-Joseph *et al*., 2010). More recently, some North African and Near Eastern countries started an extensive replacement of sour orange rootstock, using Volkamer lemon (*Citrus volkameriana*) for its adaptability to the arid areas. Nevertheless, some resistance breaking isolates of CTV were found on *P. trifoliata* in California (Yokomi *et al*., 2017) and Morocco (Afechtal *et al.*, 2018).

Tristeza-decline mild strain cross-protection programs in Southern America allowed the survival of more than 90

million protected trees during the three decades between 1950 and 1980 (Costa and Müller, 1980). Similar results were obtained in the 1990s in Australia (Broadbent *et al*., 1991), South Africa (van Vuuren *et al*., 1991), Florida and Venezuela (Lee and Rocha-Peña, 1992; Ochoa *et al*., 1993). However, failure of cross-protection has also been reported from Australia, California, Florida and South Africa (Roistacher *et al*., 2010). The most plausible explanation for this failure is that the uneven distribution of CTV in host tissue makes certain parts of the plant susceptible to further infections. Breakdown of CTV pre-immunization could also be due to an increased exposure of the hosts to severe SP or SY-CTV variants, recorded to be easily transmissible by *A. citricidus*, and the arrival of *A . citricidus* into new areas has probably hastened this phenomenon. Another interpretation is that mixed CTV infections used for the pre-immunization might include potentially severe variants that may induce severe syndromes on a particular host and under specific environmental conditions. Nevertheless, studies conducted by Roistacher and co-workers (2010) on a new approach for finding protective isolates highlighted that severe tristeza stem pitting isolates could be attenuated after aphid passage throughout *Passiflora* sp. and generated isolates which showed potential for cross-protection.

To date, breeding strategies to incorporate resistance genes in commercial cultivars are considered to be the best approach to obtain more durable CTV resistant plants (Moreno *et al*., 2008). The fact that hybrids between *P. trifoliata*, the only resistant species sexually compatible with citrus, and *Citrus* spp., can be produced makes the resistance to CTV a suitable trait to be introduced into the cultivated varieties, either by sexual hybridization or by genetic transformation after cloning of the resistance gene (Mestre *et al*., 1997). Research is being carried out to develop transgenic citrus varieties that are resistant to CTV. For example, transgenic Mexican lime plants inoculated with the CP gene from some severe and mild CTV strain yielded varying degrees of resistance (Domìnguez *et al*., 2002).

Phytosanitary risk

Tristeza is a serious threat for the citrus-growing countries in the EPPO region, where the highly susceptible sour orange rootstock is still extensively used, and where aphid vectors are widely present. Spain, Israel, and recently Italy are the most affected countries in the region. In these countries, CTV isolates belonging to both T30-like genotype associated with relatively mild infections and VT-like genotype associated with severe infections inducing stem pitting (SP) and seedling yellows (SY) have been identified. Recent molecular investigations underlined the prevalence of both genotypes in the Euro-Mediterranean area as pure and mixed infections; they also disclosed the occurrence of T36-like genotypes (Florida) inducing quick decline on sour orange rootstock in the Balkans but reported the presence of infections with unusual biological behavior in this area (Cerni *et al*., 2009; Djelouah *et al.*, 2009; Yahiaoui *et al.*, 2015).

From the epidemiological point of view, representative isolates from Italy, inducing both mild and severe seedling yellows (SY) symptoms were shown to be highly transmissible by *A. gossypii* (Yahiaoui *et al*., 2015). Thus, co-abundance of severe strains of CTV and potential aphid vectors may favour the development of new epidemics in the EPPO region. Finally, the establishment of *A. citricidus* in Spain and Portugal further adds to this risk (Ilharco *et al.*, 2005).

PHYTOSANITARY MEASURES

In the absence of curative treatments against CTV, prophylactic measures are still the only effective way to avoid infection of hosts or dissemination into CTV-free areas. Therefore, the application of strict quarantine rules, such as prohibiting imports of plant material from high risk countries, can be recommended to prevent the introduction of non-indigenous and severe strains into the area (Frison and Taher, 1991). It can also be recommended that planting material imported from countries where CTV occurs should be treated against vectors; and that fruits should be free from peduncles and leaves, washed and waxed, or subjected to appropriate treatments. In addition, newly introduced cultivars should be grown in isolated areas, propagated, and regularly monitored for the presence of the virus (Garnsey *et al.*, 1998). Large-scale specific surveys for CTV using appropriate diagnostic methods, as well as rapid eradication of eventual new foci of CTV, are of utmost importance to maintain the disease incidence at manageable levels (Gottwald, 2010).

Regulatory actions gave encouraging results against CTV in many countries when strengthened by the consistent use of *Citrus* sanitation and certification programs (D'Onghia, 2009). Even in countries where CTV is already established, certification programs helped to stop the spread of severe CTV strains into newly planted areas, and to

avoid damage from other graft-transmissible pathogens when tolerant rootstocks are replacing the highly susceptible sour orange rootstock (Garnsey *et al.*, 1998).

REFERENCES

Abukraa H, Djelouah K & Kafu A (2009) Historical review of *Citrus tristeza virus* (CTV) in Libya. In: D'Onghia AM, Djelouah K, Roistacher CN (eds.). *Citrus tristeza virus and Toxoptera citricidus: a serious threat to the Mediterranean citrus industry*. *Options Méditerranéennes:* Series B. Studies and Research, CIHEAM, Bari no. 65, 103-105.

Afechtal M, D'Onghia AM, Cocuzzaa GEM & Djelouah K (2018) First report of the Citrus tristeza virus resistance-breaking strain in Morocco. *Journal of Plant Pathology* **100**, 351. https://doi.org/10.1007/s42161-018-0059-1

Ayllón MA, Gowda S, Satyanayanana T, Karasev AV, Adkins, S, Mawassi M, Guerri J, Moreno P & Dawson WO (2003) Effects of modification of the transcription initiation site context on *Citrus tristeza virus* subgenomic RNA synthesis. *Journal of Virology* **77**, 9232-9243.

Bar-Joseph M, Marcus R & Lee RF (1989) The continuous challenge of citrus tristeza virus control. *Annual Review of Phytopathology* **27**, 292-316.

Bar-Joseph M, Roistacher CN & Garnsey SM (1983) The epidemiology and control of citrus tristeza disease. In: Plumb, RT, Thresh JM (eds) *Plant virus epidemiology*. Blackwell Scientific Publications, Oxford, UK, pp. 61-72.

Bar-Joseph M, Garnsey SM, Gonsalves D, Moscovitz M, Purcifull DE, Clark MF & Loebenstein G (1979) The use of enzyme-linked immunosorbent assay for detection of citrus tristeza virus. *Phytopathology* **69**, 190-194.

Barbarossa L & Savino V (2006) Sensitive and specific digoxigenin labelled RNA probes for routine detection of *Citrus tristeza virus* by dot-blot hybridization. *Journal of Phytopathology* **154**, 329-335.

Bertolini E, Moreno A, Capote N, Olmos A, De Lius A, Vidal E, Perez-Panades J & 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. https://doi.org/10.1007/s10658-007-9206-9

Bové JM & Vogel R (1981) *Description and illustration of virus and virus-like diseases of citrus*. Setco-IRFA, Paris, France.

Broadbent P, Bevington KB & Coote BG (1991) Control of stem pitting of grapefruit in Australia by mild strain protection. In Brlansky RH, Lee RF & Timmer LW (eds) *Proceedings of the 11th Conference of the International Organization of Citrus Virologists*, IOCV, Riverside, California,. pp. 64-70.

Cambra M, Vidal E, Martinez C & Bertolini E (2015) Tissue print and squash capture real-time RT-PCR method for direct detection of Citrus tristeza virus (CTV) in plant or vector tissues. In: Antonino F. Catara et al. (eds.), Citrus tristeza virus: Methods and protocols, *Methods in Molecular Biology*, vol. 2015, Chapter 5. https://doi.org/10.1007/978-1-4939-9558-5_5

Cambra M, Olmos A, Gorris MT, Marroquìn C, Esteban O, Garnsey SM, Llauger R, Batista L, Penà I & Hermozo de Mendoza A (2000) Detection of citrus tristeza virus by print capture and squash capture-PCR in plant tissues and single aphids. In: Da Graça, J.K., Lee, R.F. & Yokomi, R. (eds). *Proceeding of the 14th Conference of the international Organization of Citrus Virologists*, IOCV, Riverside, 42-49.

Cerni S, Skoric D, Ruscic J, Krajacic M, Papic T, Djelouah K & Nolasco G (2009) East Adriatic-a reservoir region of severe *Citrus tristeza virus* strains. *European Journal of Plant Pathology* **124**, p 701. https://doi.org/10.1007/s10658-009-9444-0

Costa AS & Müller GW (1980) Tristeza control by cross-protection: A US-Brazil cooperation success. *Plant Disease* **73**, 692-597.

D'Onghia AM (2009) The CIHEAM-Mediterranean Research Network on Certification of Citrus (MNCC): the regional programme for the control of Citrus tristeza virus and its major vector *Toxoptera citricidus* in the

Mediterranean. In: *Citrus tristeza virus and Toxoptera citricidus: A serious threat to the Mediterranean citrus industry. Options Méditerranéennes,* Series B: Studies and Research, CIHEAM, Bari no. 65, pp 9-12.

D'Onghia AM and Lacirignola C (2009) The Mediterranean citriculture: productions and perspectives. In : D'Onghia AM (ed.), Djelouah K (ed.), Roistacher CN (ed.). Citrus tristeza virus and *Toxoptera citricidus*: a serious threat to the Mediterranean citrus industry. Bari : CIHEAM, 2009. p. 13-17 (Options Méditerranéennes : Série B. Etudes et Recherches; n. 65)

D'Onghia AM, Santoro F, Alnaasan Y, Gualano S, Valentini F, Djelouah K & Figorito B (2015) Improved method for assessing incidence of *Citrus tristeza virus* in large scale monitoring. *Phytopathologia Mediterranea* **54**, 55-63. https://doi.org/10.14601/Phytopathol_Mediterr-14503

Davino S, Rubio M & 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, Davino M, Sambade A, Guardo M & Caruso A (2003) The first *Citrus tristeza virus* outbreak found in a relevant citrus producing area of Sicily, Italy. *Plant Disease* **87**, p 314.

Djelouah K., Cerni S., Fonseca F., Santos C., Silva G., D'Onghia AM & Nolasco G (2009) Diversity of the coat protein gene of *Citrus tristeza virus* (CTV) in the Mediterranean region. In : D'Onghia AM, Djelouah K, Roistacher CN (eds). *Citrus tristeza virus and Toxoptera citricidus: a serious threat to the Mediterranean citrus industry. Options Méditerranéennes,* Series B. Studies and Research, CIHEAM, Bari no. 65, 159-163.

Djelouah K & D'Onghia AM (2001) Occurrence and spread of citrus tristeza in the Mediterranean area. Proceedings on Production and exchange of virus-free plant propagating material in the Mediterranean region. *Options Méditerranéennes,* Series B. Studies and Research, CIHEAM, Bari no. 35, 43-50.

Dodds JA, Morris TJ & Jordan RL (1994) Plant viral double-stranded RNA. *Annual Review of Phytopathology* **22**, 151-168.

Domínguez A, Hermoso de Mendoza A, Guerri J, Cambra M, Navarro L, Moreno P & Peña L (2002) Pathogen-derived resistance to *Citrus tristeza virus* (CTV) in transgenic Mexican lime (*Citrus aurantifolia* (Christm.) Swing.) plants expressing its *p*25 coat protein gene. *Molecular Breeding*. 10, 1-10.

Frison EA & Taher MM (1991) *FAO/IBPGR technical guidelines for the safe movement of citrus germoplasm*. *FAO Rome Eds*, Italy.

Garnsey SM, Gottwald TR & Yokomi RK (1998) Control strategies for Citrus tristeza virus. In: Hadidi A, Khetarpal RK and Koganezawa H (eds) *Plant Disease Control,* APS Press (US), 639-658.

Gillings M, Broadbent P, Indsto J & Lee R (1993) Characterization of isolates and strains of citrus tristeza closterovirus using restriction analysis of the coat protein gene amplified by the polymerase chain reaction. *Journal of Virological Methods* **44**, 305-317.

Gottwald TR (2010) Aphid transmission and epidemiology of Citrus tristeza virus. In: Karasev AV and Hilf ME (eds) *Citrus tristeza virus: complex and tristeza disease.* APS Press (US), 133-150.

Gottwald TR, Garnsey SM, & Barbòn JC (1998) Increase and patterns of spread of *Citrus tristeza virus* infections in Costa Rica and the Dominican Republic in the presence of the brown citrus aphid, *Toxoptera citricida*. *Phytopathology* **88**, 603-608.

Gottwald TR, Garnsey SM, Cambra M, Moreno P, Irey M & Borbòn J (1997) Comparative effects of aphid vector species on increase and spread of *citrus tristeza virus*. *Fruits* **52** (6), 397-404.

Gualano S, Santoro F, Djelouah K & D'Onghia AM (2012) Proximal and remote sensing in the monitoring of citrus tristeza virus (CTV) infected trees, preliminary results. *Acta Horticulturae* **940**, 641-646.

Gowda S, Satyanarayana T, Ayllon MA, Moreno P, Flores R & Dawson WO (2003) The conserved structures of the

5’ nontranslated region of Citrus tristeza virus are involved in replication and virion assembly. *Virology* **317**, 50-64.

Hilf ME, Mavrodieva VA & Garnsey SM (2005) Genetic marker analysis of a global collection of isolates of citrus tristeza virus: characterization and distribution of CTV genotypes and association with symptoms. *Phytopathology* **95** , 909-917.

Hilf M E, Karasev A, Maria R, Albiach M, Dawson WO & Garnsey SM (1999) Two paths of sequence divergence in the Citrus tristeza virus complex. *Phytopathology* **89**, 336-342.

Hilf ME, Karasev AV, Pappu HR, Gumpf DJ, Niblett CL & Garnsey SM (1995) Characterization of *Citrus tristeza virus* subgenomic RNAs in infected tissue. *Virology* **208**, 576-582.

Hung TH, Wu ML & Su HJ (2000) A rapid method based on the one step reverse transcriptase-polymerase chain reaction (RT-PCR) technique for detection of different strains of *citrus tristeza virus. Journal of Phytopathology* **148** , 469-475.

Ilharco FA, Sousa-Silva CR & Alvarez A (2005) First report of *Toxoptera citricidus* (Kirkaldy), (Homoptera, Aphidoidea) in Spain and continental Portugal. *Agronomia Lusitana* **51**, 19-21.

Jarrar S, Djelouah K, D'Onghia AM & Savino V (2000). First record of citrus tristeza virus in Palestine. *Journal of Plant Pathology* **82**, p 243.

Karasev AV, Boyko VP, Gowda S, Nikolaeva OV, Hilf ME, Koonin EV, Niblett CL, Cline K, Gumpf DJ, Lee RF, Garnsey SM, Lewandowski DJ & Dawson WO (1995) Complete sequence of the citrus tristeza virus RNA genome. *Virology* **208**, 511-520.

Lee RF, Pappu HR, Pappu SS, Rocha-Peña MA, Febres VJ, Manjunath KL, Nikolaeva OV, Karasev A, Cevik B, Akbulut M, Bencher D, Anderson EJ, Price M, Ochoa-Corona FM & Niblett CL (1996) Progress on strain differentiation of *Citrus tristeza virus*. *Phytopathology* **14**(2), 79-87.

Lee RF & Rocha-Peña MA (1992) *Citrus tristeza virus.* In: Kumar J, Chaube HS, Singh US and Mukhopdhyay AN (eds) *Diseases of fruit crops, Plant Diseases of International Importance,* volume III, Prentice Hall, Englewood Cliffs, New Jersey, 226-249.

Marroquin C, Olmos A, Gorris MT, Bertoloni E, Martìnez MC, Carbonell EA, De Mendoza AH & Cambra (2004) Estimation of the number of aphids carrying Citrus tristeza virus that visit adult citrus trees. *Virus Research* **100**, 101-108.

Mestre PF, Asins MJ, Carbonell EA & Navarro L (1997) New gene(s) involved in the resistance of *Poncirus trifoliata* (L.) Raf. to *Citrus tristeza virus*. *Theoretical and Applied Genetics* **95**, 691-695.

Moreno P & Garnsey SM (2010) Citrus tristeza diseases: A worldwide perspective. In Karasev AV and Hilf ME (eds) *Citrus tristeza virus: complex and tristeza disease.* APS Press (US), 27-49.

Moreno P, Ambrós S, Albiach-Marti MR, Guerri J & Peña L (2008) Citrus tristeza virus: a pathogen that changed the course of the citrus industry. *Molecular Plant Pathology* **9**, 251-268.

Niblett CL, Genc H, Cevik B, Halbert S, Brown L, Nolasco G, Bonacalza B, Manjunath KL, Febres VJ, Pappu HR & Lee RF (2000) Progress in strain differentiation of Citrus tristeza virus and its application to the epidemiology of citrus tristeza disease. *Virus Research* **71**, 97-106.

Nolasco G, Deblas C, Torres V & Ponz F (1993) A method combining immunocapture and PCR amplification in a microtiter plate for the detection of plant-viruses and subviral pathogens. *Journal of Virological Methods* **45**, 201-218.

Ochoa F, Carballo O, Trujillo G, Mayoral de Izaquirre ML & Lee RF (1993) Biological characterization and evaluation of cross-protection potential of citrus tristeza isolates in Venezuela. In: Moreno P, da Graça JV and Timmer LW (eds) *Proceedings of the 12th Conference of the International Organization of Citrus Virologists.*

IOCV, Riverside, California, 1-7.

Permar TA, Garnsey SM, Gumpf DJ & Lee RF (1990) A monoclonal antibody that discriminate strains of citrus tristeza virus. *Phytopathology* **80**, 224-228.

Roberts PD, McGovern RJ, Lee RF & Niblett CL (2001) Tristeza. *Florida Cooperative Extension Service*, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, 10 pp. http://plantpath.ifas.ufl.edu

Rocha-Peña MA, Lee RF, Lastra R, Niblett CL, Ochoa-Corona FM, Garnsey SM & Yokomi RK (1995) *Citrus tristeza virus* and its aphid vector *Toxoptera citricida*: Threats to citrus production in the Caribbean and central and North America. *Plant Disease* **79** (5), 437-444.

Roistacher CN, da Graca JV & Muller GW (2010) Cross protection against Citrus tristeza virus – A review. In: *Proceedings of the 9th Conference of the International Organization of Citrus Virologists17(17)*, IOCV, Riverside, CA. 28 pp

Roistacher CN (2006) EcoPort slide shows on the internet related to citrus and citrus diseases. http://ecoport.org/ep?SearchType=domainContents&id=9&type=group .

Roistacher CN (1991) Graft-transmissible diseases of citrus. Handbook for detection and diagnosis. FAO Eds, Rome, 286 pp.

Roistacher CN & Bar-Joseph M (1984) Transmission of tristeza and seedling yellows tristeza virus by *Aphis gossypii* from sweet orange, grapefruit and lemon to Mexican lime, grapefruit and lemon. In: *Proceedings of the 9th Conference of the International Organization of Citrus Virologists*, IOCV, Riverside, CA, 9-18.

Roistacher CN, Nauer EM, Kishaba A & Calavan EC (1980) Transmission of citrus tristeza virus by *Aphis gossypii* reflecting changes in virus transmissibility in California. In: Calavan EC, Garnsey SM and Timmer LW (eds) *Proceedings of the 8th Conference of the International Organizaiton of Citrus Virologists.* IOCV, Riverside, CA, 76-82.

Roy A, Fayad A, Barthe G & Brlansky RH (2005) A multiplex polymerase chain reaction method for reliable, sensitive and simultaneous detection of multiple viruses in citrus trees. *Journal of Virological Methods* **129**, 47-55.

Rubio L. Ayllòn M.A. Guerri J. Pappu HR, Niblett CL & Moreno P (1996) Differentiation of Citrus tristeza virus (CTV) isolates by single-stranded conformation polymorphism analysis of the coat protein gene. *Annals of Applied Biology* **129**, 479-489.

Saponari M, Keremane ML & Yokomi RK (2008) Quantitative detection of Citrus tristeza virus (CTV) in citrus and aphids by real-time reverse transcription-PCR (TaqMan). *Journal of Virological Methods* **147**, 43-53.

Satyanarayana T, Gowda S, Boyko VP, Albiach-Martõ ÂMR, Mawassi M, Navas-Castillo J, Karasev AV, Dolja V, Hilf ME, Lewandowski DJ, Moreno P, Bar-Joseph M, Garnsey SM & Dawson WO (1999) An engineered closterovirus RNA replicon and analysis of heterologous terminal sequences for replication. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 7433-7438.

Van Vuuren SP, Collins RP & da Graça JV (1991) The performance of exotic Citrus tristeza virus isolates as preimmunizing agents for sweet orange on sour orange rootstock under natural disease pressure in South Africa. In: Brlansky RH, Lee RF and Timmer LW (eds) *Proceedings of the 11th Conference of the International Organization of Citrus Virologists,* IOCV, Riverside, California, 60-63.

Yahiaoui D, Djelouah K, D'Onghia AM & Catara A (2015) Genetic evidence of potential virulent Citrus tristeza virus isolates in Mediterranean areas. *Journal of Plant Pathology* **97**(2), 243-248.

Yoshida T (1996) Graft compatibility of Citrus with plants in the Aurantioideae and their susceptibility to citrus tristeza virus. *Plant Disease* **80**, 414-417.

Yokomi RK, Selvaraj V, Maheshwari Y, Saponari M, Giampetruzzi A, Chiumenti M & Hajeri S (2017)

Identification and characterization of *Citrus tristeza virus* isolates breaking resistance in trifoliate orange in California. *Phytopathology* **107**, 901-908.

Yokomi RK, Polek M & Gumpf DJ (2010) Transmission and spread of Citrus tristeza virus in Central California. In: Karasev AV and Hilf ME (eds) *Citrus tristeza virus: complex and tristeza disease.* APS Press (US), 151-165.

Yokomi RK, Lastra R, Stoetzel MB, Damgstreet VD, Lee RF, Garnsey SM, Rocha-Peña MA & Niblett CL (1994) Establishment of the brown citrus aphid *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae) in Central America and the Caribbean Basin, and its transmission of *Citrus tristeza virus. Journal of Economic Entomology* **87**, 1078-1085.

ACKNOWLEDGEMENTS

This datasheet was extensively revised in 2020 by Dorsaf Yahiaoui, PhD (The Technical Center of Citriculture - Tunisia). Her valuable contribution is gratefully acknowledged.

How to cite this datasheet?

EPPO (2026) *Closterovirus tristezae*. EPPO datasheets on pests recommended for regulation. Available online. https://gd.eppo.int

Datasheet history

This datasheet was first published in the EPPO Bulletin in 1978 and revised in the two editions of 'Quarantine Pests for Europe' in 1992 and 1997, as well as in 2020. It is now maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', 'Hosts', and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

CABI/EPPO (1992/1997) Quarantine Pests for Europe (1st and 2nd edition). CABI, Wallingford (GB).

EPPO (1978) Data sheets on quarantine organisms No. 93, Citrus tristeza virus. *EPPO Bulletin* **8**(2), 91-99.

Co-funded by the European Union