

# EPPO Datasheet: *Closterovirus tristeza*

Last updated: 2020-06-19

## IDENTITY

**Preferred name:** *Closterovirus tristeza*

**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

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**EPPO Categorization:** A2 list

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**EU Categorization:** A1 Quarantine pest (Annex II A), PZ Quarantine pest (Annex III), RNQP (Annex IV)

**EPPO Code:** CTV000



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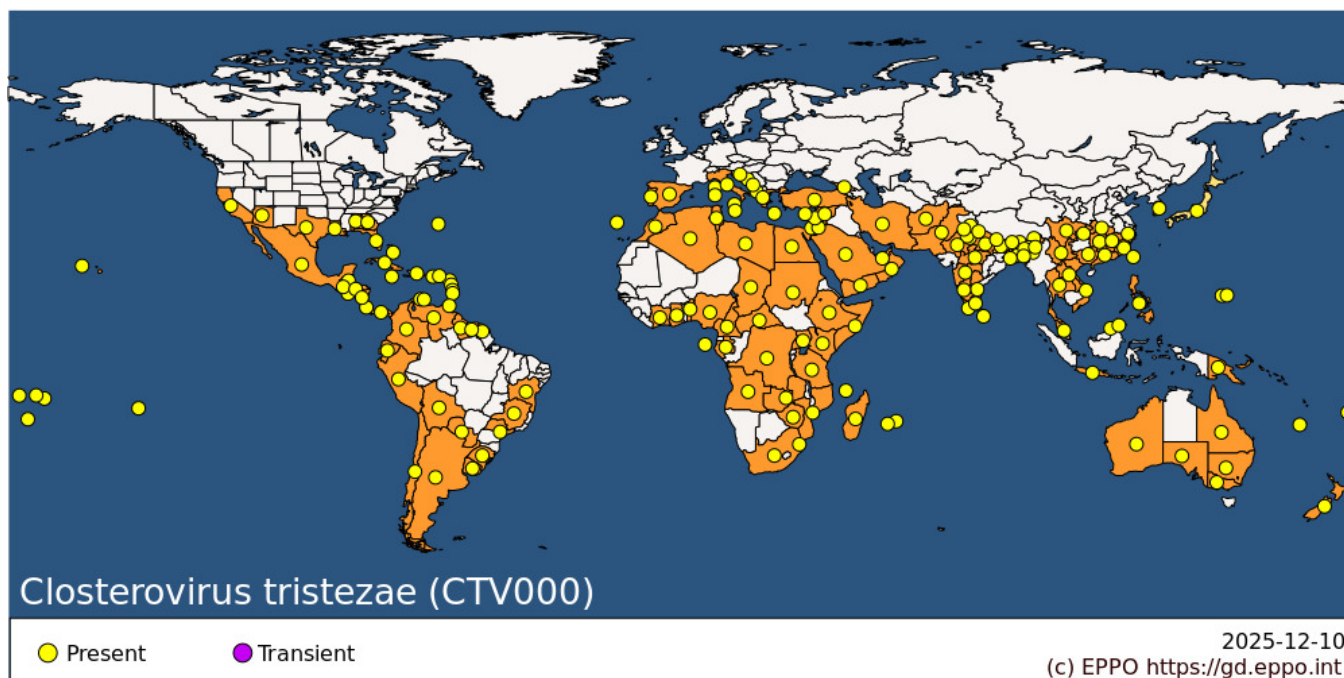
## HOSTS

*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. *limettoides*, *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), 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 (Domínguez *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).

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