Citrus tristeza virus

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in

D'Onghia A.M. (ed.), Djelouah K. (ed.), Roistacher C.N. (ed.).
Citrus tristeza virus and Toxoptera citricidus: a serious threat to the Mediterranean citrus industry

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 65

2009
pages 19-33

Citrus tristeza virus

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I – Identity

1. Preferred scientific name

Citrus tristeza virus (CTV)

2. Taxonomic position

CTV is a member of the closterovirus group which have thread-like, flexuous virions, insect vectors, cause characteristic cytopathological structures (inclusion bodies) in infected phloem tissues, and have a positive sense, single-stranded RNA genome of up to 20 kb. Based on molecular characterization of CTV and other members of the closterovirus group, enough genetic diversity occurs among members to propose that the closteroviruses belong to the family Closteroviridae which contains homologues of cellular heat-shock proteins (Dolja *et al*. 1994). The Closteroviridae are composed of three genera: Citrivirus (CTV = type member) which has one 19.3 kb genome component and 12 open reading frames (ORFs); Closterovirus (beet yellows virus = type member) which has one 15.4 kb genome and 9 ORFs; and Biclovirus (lettuce infectious yellows = type member) which has two genome components of 8.1 and 7.1 kb and 9 ORFs.

3. Internationally used common names

Tristeza
Stem pitting (SP)
Hassaku dwarf
Podredumbre de las raicillas

4. Other common names

Citrus quick decline (QD)
Grapefruit stem pitting
Sweet orange stem pitting
Seedling yellows (SY)
Lime dieback disease
Pummelo yellow dwarf (China)

5. Notes on taxonomy and nomenclature

Failure of some citrus selections to graft propagate successfully on sour orange rootstocks was observed in different areas prior to 1900, but an association of this condition with a disease was only gradually established through observations in South Africa and Java (Roistacher 1995, Wallace 1978). The disease appeared in Argentina in 1931 and in southern Brazil in 1937 after importation of infected plants from South Africa and Moreira (1942) referred to it as tristeza, which in Portuguese and Spanish means melancholy or sadness. The probable viral etiology of CTV

was demonstrated when Meneghini (1946) showed that the causal agent was transmissible by the oriental citrus aphid (= brown citrus aphid), *Aphis citricida* (= *Toxoptera citricida* (Kirkaldy)). This was quickly followed by reports in California and South Africa that a quick decline of sour orange rooted citrus was bud transmissible. A lime dieback disease in Africa was eventually associated with CTV infection (Wallace 1978, Bar-Joseph *et al*. 1989). Some isolates of CTV induce a seedling yellows (SY) reaction in inoculated sour orange and grapefruit plants. SY was once thought to be associated with a separate virus (Fraser 1959, McClean 1974). Generally, the most severe strains of CTV cause stem pitting in various cultivars regardless of rootstock and also develops on seedling trees. Severe stem pitting in susceptible cultivars leads to a loss in tree vigor and a decrease in fruit quality and quantity. Extensive diversity among CTV isolates has been well established and molecular characterization studies now in progress suggest that CTV may well be complex of related viruses.

II – Hosts

Natural hosts for CTV include nearly all citrus species, interspecific hybrids, some citrus relatives and some intergeneric hybrids. The only natural noncitrus host that has been reported is *Passiflora* (Kitajima *et al*. 1974, Müller *et al*. 1974)). Some of the more important economic hosts are: sweet orange [*Citrus sinensis* (L.) Osb.], grapefruit [*C. paradisi* Macf.], mandarins [*C. reticulata* Blanco], limes [*C. aurantifolia* (Christm.) Swing.) and *C. latifolia* Tan.], lemons [*C. limon* (L.) Burm. f.], pummelo [*C. grandis* (L.) Osb.], tangelos [*C. reticulata* x *C. paradisi*], tangors [*C. reticulata* x *C. sinensis*], calamondin [*C. madurensis* Lour.], and kumquat [*Fortunella margarita* (Lour.) Swing.].

Sweet lime [*C. limettiodes* Tan), citron, [*C. medica* L.], and combava (*C. hystrix* (DC,], Swing.] are also infected, as are commonly used rootstock varieties such as Rangpur lime [*C. limonia* Osb.], rough lemon [*C. jambhiri* Lush.], sour orange [*C. aurantium* L.], volkamer lemon [*C. volkameriana* Ten. and Pasq.], and alemow [*C. macrophylla* Wester].

1. Affected plant stages

The virus is phloem limited and can be detected in leaves, stems, fruits, and roots. Greatest concentrations of virions are found in young growth under relatively mild temperature conditions.

2. Notes on host range

Many natural hosts of CTV remain essentially symptomless when infected by most CTV isolates. Mandarins, sweet oranges and rough lemon are among common tolerant hosts. Some citrus species show a selective susceptibility and are readily infected by some CTV isolates and not by others (Garnsey *et al*. 1996c). *Poncirus trifoliata* (L.) Raf., a citrus relative commonly used as a rootstock, is highly resistant to nearly all isolates of CTV and this resistance is also found in some trifoliate orange hybrids.

Other hosts which have been experimentally infected either by aphid, graft, or mechanical inoculation include: *Aegle marmelos* (L.) Corr. Serr., *Aeglopsis chevalieri* Swingle, *Afraegle paniculata* (Schum.) Engl., *Citropsis gilletiana* Swingle and M. Kellerman, *Microcitrus australis (*Planch.) Swingle, *Pamburus missionis* (Wight) Swingle (Müller and Garnsey 1984); *Passiflora gracilis* Jacq., *P. caerulea L.*, *P. incense*, and *P. incarnata* (Roistacher and Bar-Joseph 1987). CTV can replicate in protoplasts of *Nicotiana benthamiana* (Navas-Castillo *et al*. 1995).

III – Identification

1. Virus morphology and characteristics

CTV particles are flexuous rods 10-11 nm in diameter and 2,000 nm long. The particles are easily sheared and extracts from partially purified preparations typically contain many broken particles of various lengths. The capsid protein subunits are helically arranged along the particle with a basic pitch of 3.7 nm and ten subunits in each turn of the helix (Bar-Joseph *et al*. 1972). The nucleic acid is single-stranded positive sense RNA with a molecular weight of about 6.5 x 10^6 which is composed of 19,296 nucleotides (nt) in the isolate T36 from Florida (Karasev *et al*. 1995) or 19226 nt in the isolate VT from Israel (Mawassi *et al* 1996). These encode 12 open reading frames (ORF) potentially coding for at least 17 protein products. Nine 3' co-terminal subgenomic RNAs have been found in infected citrus tissue (Hilf *et al*. 1995). One ORF encodes the capsid protein of approximately 25,000 daltons, another (p27) encodes a divergent coat protein which has been shown to coat one end of the virion forming a "rattlesnake" structure, and a third encodes a homologue of HSP70 heat shock protein which is also found in other closteroviruses. Functions of other ORFs have been inferred from sequence comparisons with other viruses, but not confirmed (Karasev *et al*. 1995). Sequence comparisons between strains are in progress and indicate a relatively high level of conservation toward the 3' end and divergence up to 40 % toward the 5' terminal (Mawassi *et al*. 1996).

Many CTV-infected plants contain defective RNAs which are composed of portions of the 5' and 3' terminal sequences of the CTV genome (Mawassi *et al*. 1995). Some of the defective RNAs have been found to contain small portions of non-virus encoded RNA linking the 5' and 3' terminal portions. Defective RNAs are often detected as prominent bands in purified extracts of double-stranded RNA from infected tissues. The significance of defective RNAs on symptom expression has not been determined (Mawassi *et al*. 1995).

2. Symptoms

Virulence is affected by the CTV isolate and environmental conditions. Since there are hundreds of citrus species, hybrids, and citrus relatives, an isolate's virulence should be defined in terms of specific hosts. Being phloem-limited, most CTV symptoms are associated with viral disruption of phloem and its function. Some isolates cause few symptoms, even in plants that are normally reactive such as Mexican lime (Bové *et al*. 1988). Most CTV strains cause vein flecking, leaf cupping, a transient leaf epinasty in young leaves, and some stem pitting on CTV-sensitive plants such as Mexican lime, *C. macrophylla,* or *C. hystrix.* These vary from mild to severe and impact fruit quality when severe.

Some isolates of CTV cause a decline when field trees of sweet orange, mandarins, or grapefruit grafted on sour orange rootstocks become infected. This decline is associated with a virus-induced phloem necrosis at the budunion which blocks normal translocation of carbohydrates to the root system. As the root system deteriorates, trees begin to decline. Symptoms appear a year or more after infection and may occur gradually over several years or very suddenly (QD). Canopy symptoms are wilting, chlorosis and an abnormal crop of small fruit which may persist after tree death in trees affected by QD. Clinical symptoms can often be seen by removing a patch of bark across the budunion. Trees which decline slowly usually will have thicker bark immediately below the union and the face of the bark surface below the union will have many small conical pits (honeycombing) corresponding to bristle-like protuberances from the wood (Schneider 1954). Trees affected by QD lack honeycombing, but will frequently show a yellow brown stain at the budunion. If budwood from scions infected with decline strains of CTV are propagated on sour orange seedlings, the budlings may be stunted and chlorotic, but rarely collapse and die (Brlansky *et al*. 1986).

Other CTV strains cause stem pitting in commercial cultivars of grapefruit, lime, and sweet orange. Stem pitting does not kill trees, but affected trees may have thin canopies and produce fewer fruit of reduced size and quality (Marais *et al*. 1996). Dieback occurs in severely affected limes. Chronically infected trees sometimes show a bumpy or ropy appearance of trunks and limbs on larger trees. The twigs and limbs are brittle and easily broken. Early stages of stem pitting are detectable only upon removal of bark. These pits consist of depressions in the outer wood with corresponding pegs or projections on the inner bark face. Stem pitting severity can range from very few small pits to many pits to a more general disruption of the cambium which results in many fine, sandpaper-like pits and abnormally thickened bark. CTV strains which cause stem pitting in grapefruit do not necessarily cause stem pitting in sweet orange, and vice versa. CTV strains have also been reported which cause stem pitting in mandarin, rough lemon, and Gou Tou rootstocks.

Some strains of CTV also induce a seedling yellows (SY) reaction (a dwarfing and general chlorosis) in inoculated grapefruit, lemon, and sour orange seedlings. The SY reaction usually, but not always, accompanies the presence of decline on sour orange and/or stem pitting strains of CTV.

3. Similarities to other diseases

Tristeza-induced decline can be confused with other decline diseases affecting citrus including foot rot and blight. Association of decline with trees only on sour orange rootstocks in the absence of external lesions in the bark of the trunk or major roots is often an indication of CTV (Whiteside *et al*. 1988). Extensive root damage caused by root weevils, nematodes, gophers, or waterlogging may, however, cause tree decline in trees grafted on sour orange rootstock. Citrus blight, an infectious decline disease of unknown etiology, can also affect trees on sour orange rootstock. This decline can usually be differentiated in the field from CTV-induced decline on sour orange rootstock because blight is associated with a xylem dysfunction. There are no budunion symptoms of blight, blighted trees have a delayed flush rather than a precocious flush and deterioration of the root system follows development of decline but does not cause it (Garnsey and Young 1975).

CTV is the most frequent cause of stem pitting in citrus, but other virus and viruslike agents can also cause stem pitting. Cristacortis can cause large pits in limbs and trunks of some cultivars. Cachexia, a citrus viroid, can also produce stem pits with gumming on susceptible rootstocks, such as *C. macrophylla*, and on some mandarins.

Other phloem pathogens of citrus, such as greening, can induce visual canopy effects similar to those caused by severe stem pitting isolates of CTV, especially in grapefruit. Co-infection of greening and CTV is common in Asian citrus areas. Greening alone does not cause stem pitting and CTV alone does not induce leaf mottling and rarely causes stunting or chlorosis in mandarins (Whiteside *et al*. 1988).

4. Diagnostic methods

The traditional method to detect CTV is to graft-inoculate *Citrus aurantifolia* (Christm.) Swing. (Key lime or Mexican lime) plants (Roistacher 1991). This host reacts to most isolates by developing clearing or flecks in the veins of new leaves formed after inoculation. In some cases the severity of reaction in limes is indicative of the isolate's virulence in other hosts, but the correlation is not exact. To determine CTV-decline effects, sweet orange plants grafted on sour orange rootstock are inoculated and monitored for symptoms of dwarfing and chlorosis over 6-12 months. However, some isolates that cause decline of field trees grafted on sour orange are symptomless in the same condition under glasshouse or screenhouse conditions (Ballester-Olmos *et al*. 1993). Stem pitting is determined by inoculating seedlings of an appropriate or susceptible the cultivar, usually sweet orange or grapefruit, and monitoring growth over 12-15 months. The main stem

of the indicator plants is stripped of bark and examined for stem pitting. To test an isolate for SY reaction, the isolate is grafted to a seedling of sour orange, lemon, or grapefruit and observed for 6-12 months in the greenhouse for stunting and chlorosis symptoms. This is often considered a presumptive indication for a severe isolate of CTV, but some virulent stem pitting isolates do not cause SY and vice versa. Some isolates induce SY, but do not cause stem pitting in grapefruit or sweet orange.

Serological methods, such as ELISA in microplates (Garnsey and Cambra 1991) or in tissue prints (Garnsey *et al*. 1993), are routinely used for CTV detection (Garnsey and Cambra 1991). Polyclonal antisera have been produced to a number of different isolates and these detect nearly all isolates. Monoclonal antibodies (Mabs) have also been produced (Permar *et al*. 1990, Tsai and Su 1991, Vela *et al*. 1986). Some react to epitopes which are widely conserved among diverse CTV isolates and these also provide nearly universal detection, especially if two are used in combination (Cambra *et al*. 1993). Other Mabs are more isolate specific, and one (MCA13) has been used to differentiate mild isolates from those that cause decline and stem pitting in Florida (Permar *et al*. 1990). No monoclonal antibody has been developed which reacts specifically to only decline or stem pitting CTV isolates. A variety of other serological tests have also been utilized to detect CTV (Cambra *et al*. 1991, Rocha-Peña and Lee 1991).

Electron microscopy (EM) also has been used for CTV detection. Immunospecific EM, is very sensitive and specific for diagnosis (Garnsey *et al*. 1980). Light microscopy has been used for the detection of characteristic CTV-induced inclusion bodies in the phloem of infected plants (Edwardson and Christie 1978, Garnsey *et al*. 1980, Brlansky *et al*. 1988).

Analysis of double-stranded RNAs in infected tissues can result in characteristic patterns that are diagnostic for CTV (Dodds and Bar-Joseph 1983) and may discriminate between some isolates (Dodds *et al*. 1987, Moreno *et al*. 1990, Guerri *et al*. 1991).

With the recent completion of the sequencing of the T36 CTV isolate, assays have been developed utilizing reverse transcriptase polymerase chain reaction (RT-PCR) and/or hybridization with specific probes. Use of these assays allow testing of homology at discrete areas of the viral genome. Development of these assay provide highly sensitive detection methods which can differentiate CTV strains having different biological activities (Albiach *et al*. 1995, Gillings *et al*. 1993).

IV – Geographic distribution

Tristeza apparently originated in Asia and existed there for many years in tolerant cultivars which were either propagated vegetatively as cuttings or by seed. New areas of citriculture in other continents were first established from seed and were free of CTV infection. Subsequently, CTV has been introduced into nearly all citrus-growing areas via virus-infected budwood or plants. In many areas infections have become wide spread due to propagation and secondary spread by aphids. In some areas little or no secondary spread has occurred from the few existing infected trees.

CTV is very common in commercial citrus in southeast Asia, Australia, southern Africa, India, Japan, South America, and most Pacific Islands, and where the brown citrus aphid is present nearly all field-grown trees are infected. CTV is widespread in parts of Spain, Florida, parts of California, portions of Central America and most of the Caribbean islands. CTV is present, but not widespread in parts of the Mediterranean region and Mexico (Tab. 1).

Table 1. Geographic distribution of CTV

COUNTRY	STATUS	REFERENCES
ASIA		
-Brunei Darussalam	P	Büchen-Osmond *et al*. 1996
-China	W	Wallace 1978
-India	W	Wallace 1978
-Indonesia	W	Wallace 1978
-Iran	P	Büchen-Osmond *et al*. 1996
-Israel	L,W	Wallace 1978
-Japan	W	Wallace 1978
-Korea, Rep.	P	Büchen-Osmond *et al*. 1996
-Malaysia	W	Wallace 1978
-Nepal	W	Büchen-Osmond *et al*. 1996
-Pakistan	W	Büchen-Osmond *et al*. 1996
-Philippines	W	Wallace 1978
-Saudia Arabia	P	Büchen-Osmond *et al*. 1996
-Sri Lanka	W	Büchen-Osmond *et al*. 1996
-Taiwan	W	Wallace 1978
-Thailand	W	Büchen-Osmond *et al*. 1996
-Turkey	P	Büchen-Osmond *et al*. 1996
-Vietnam	W	Büchen-Osmond *et al*. 1996
AFRICA		
-Algeria	P	Büchen-Osmond *et al*. 1996
-Benin	P	Bové and Vogel 1981
-Cameroon	P	Büchen-Osmond *et al*. 1996
-Comores	P	Bové and Vogel 1981
-Central African Republic	P	Büchen-Osmond *et al*. 1996
-Chad	P	Büchen-Osmond *et al*. 1996
-Egypt	P	Wallace 1978
-Ethiopia	P	Büchen-Osmond *et al*. 1996
-Gabon	P	Büchen-Osmond *et al*. 1996
-Ghana	P	Wallace 1978
-Ivory Coast	P	Bové and Vogel 1981
-Kenya	P	Büchen-Osmond *et al*. 1996
-Madagascar	P	Bové and Vogel 1981
-Mauritius	W	Bové and Vogel 1981
-Morocco	P	Wallace 1978
-Mozambique	P	Büchen-Osmond *et al*. 1996
-Nigeria	P	Büchen-Osmond *et al*. 1996
-Reunion	W	Bové and Vogel 1981
-South Africa	W	Wallace 1978
-Tanzania	P	Büchen-Osmond *et al*. 1996
-Uganda	P	Büchen-Osmond *et al*. 1996
-Zaire	P	Büchen-Osmond *et al*. 1996
-Zambia	P	Büchen-Osmond *et al*. 1996
-Zimbabwe	W	Wallace 1978
NORTH and CENTRAL AMERICA		
-Antigua and Barbuda	P	Büchen-Osmond *et al*. 1996
-Belize	L	Yokomi *et al*. 1994
-Bermuda	P	Büchen-Osmond *et al*. 1996
-Costa Rica	W	Yokomi *et al*. 1994
-Cuba	L	Lee *et al*. 1995
-Dominican Republic	W	Yokomi *et al*. 1994
-El Salvador	P	Yokomi *et al*. 1994
-Honduras	P	Yokomi *et al*. 1994
-Guatemala	P	Yokomi *et al*. 1994

COUNTRY	STATUS	REFERENCES
-Jamaica	W	Yokomi *et al*. 1994
-Mexico	P	Yokomi *et al*. 1994
-Netherlands Antilles	P	Büchen-Osmond *et al*. 1996
-Nicaragua	P	Yokomi *et al*. 1994
-Panama	W	Yokomi *et al*. 1994
-Puerto Rico	W	Yokomi *et al*. 1994
-Trinidad and Tobago	W	Yokomi *et al*. 1994
-USA		
Arizona	P	Wallace 1978
California	L	Wallace 1978
Florida	W	Wallace 1978
Hawaii	W	Garnsey *et al*. 1991
Texas	P	Wallace 1978
SOUTH AMERICA		
-Argentina	W	Wallace 1978
-Bolivia	W	Büchen-Osmond *et al*. 1996
-Brazil	W	Wallace 1978
-Chile	W	Wallace 1978
-Colombia	W	Büchen-Osmond *et al*. 1996
-Ecuador	W	Büchen-Osmond *et al*. 1996
-Guyana	P	Wallace 1978
-Paraguay	W	Büchen-Osmond *et al*. 1996
-Peru	W	Büchen-Osmond *et al*. 1996
-Surinam	W	Wallace 1978
-Uruguay	W	Wallace 1978
-Venezuela	W	Wallace 1978
EUROPE		
-Italy	P	Wallace 1978
-Madeira	W	Lee *et al*. 1995
-Portugal	P	Büchen-Osmond *et al*. 1996
-Spain	W	Wallace 1978
-Former Yugoslavia	P	Bové and Vogel 1981
OCEANIA		
-American Samoa	P	Büchen-Osmond *et al*. 1996
-Australia	W	Wallace 1978
New South Wales	W	Lee *et al*. 1995
Queensland	W	Lee *et al*. 1995
-Fiji	W	Wallace 1978
-French Polynesia	W	Büchen-Osmond *et al*. 1996
-New Caledonia	W	Büchen-Osmond *et al*. 1996
-New Zealand	W	Büchen-Osmond *et al*. 1996
-Tonga	W	Bové and Vogel 1981
-Western Samoa	W	Büchen-Osmond *et al*. 1996

L: Low spread; P: Present; W: Widespread.

V – Biology and ecology

1. Transmission

Natural spread of CTV is primarily through propagation of infected budwood and by aphids. CTV is not seed-borne. Propagation of new plants from buds from infected plants is responsible for long-distance spread of CTV and extensive bud propagation from a single plant can rapidly increase foci of inoculum. Topworking old citrus plantings to new scion varieties using infected buds has been a common way of CTV spread in some countries. Tree to tree spread is by aphids.

CTV is semipersistently transmitted by several aphid species (Bar-Joseph and Lee 1989). Aphid vectors acquire the virus from an infected tree with feeding times ranging from 5 min. to hours, however, not by brief probes. The transmission efficiency of the vector increases as the acquisition and feeding times are increased up to 24 hours. There is no latent period, and the virus does not multiply or circulate in the aphid. The time required to inoculate a plant is the same as for acquisition. Aphids remain viruliferous for 24-48 hours after feeding on infected plants. Many aphids species that feed on an infected citrus tree can acquire CTV, as detected by ELISA (Cambra *et al*. 1981), but only a few species can transmit it to new plants. *T. citricida* is the most efficient vector, and where it exists, is often the most abundant aphid on citrus (Yokomi *et al*. 1994). Most isolates of CTV, including severe stem pitting isolates, are effectively vectored by *T. citricida*, but a few isolates are vectored less efficiently. *Aphis gossypii* Glover (melon or cotton aphid) can transmit some isolates efficiently and is the most important CTV vector in regions where *T. citricida* is not present (Hermoso de Mendoza *et al*. 1984, Roistacher *et al*. 1984, Yokomi *et al*. 1989, Ballester-Olmos *et al*. 1993). In contrast to *T. citricida*, it has a wide host range and only occasionally colonizes citrus. *Toxoptera aurantii* (Boyer de Fonscolombe) can transmit some CTV isolates but is less efficient than brown citrus aphid or melon aphid (Hermoso de Mendoza *et al*. 1984). It also has a wide host range and occasionally colonizes citrus. *A. spiraecola* Patch (spirea aphid) is an inefficient vector of CTV under experimental conditions, but is very common on citrus worldwide and also is extremely polyphagous (Hermoso de Mendoza *et al*. 1984, Yokomi and Garnsey 1987). These aphids, except *T. citricida*, are distributed worldwide. Several other aphids have been shown to transmit CTV experimentally, but are not likely to be significant.

2. Notes on transmission

CTV is readily graft transmissible if a union is formed between the phloem of the donor and receptor host. A variety of graft-inoculation methods are used to experimentally transmit the virus (Roistacher 1991). Buds, sections of leaves that include veins, and stem pieces can all be used as inoculum. Mechanical transmission of CTV is difficult and has only been done experimentally by slash-inoculation of the stems of receptor plants with concentrated extracts from CTV-infected plants. CTV can also be transmitted experimentally by dodder.

3. Epidemiology

Primary infections of CTV are usually established via propagation of infected plants. Epidemics of CTV decline observed in many countries began with importation and propagation of infected plants in areas heavily planted with CTV-free trees on sour orange. When efficient vectors were present epidemics of decline often followed. Although CTV epidemiology is significantly affected by the citrus cultivar and horticultural practices, the most important factors are the CTV isolate and the aphid vector. When *T. citricida* is present, temporal and spatial spread of CTV spread is increased (Garnsey *et al*. 1996b). This aphid has a narrow host range and migrants move from citrus to citrus to start new aphid colonies and, in this process, can transmit CTV if they are viruliferous. High aphid populations also coincide with new flush which is favorable for virus acquisition and inoculation. The other vectors are much less efficient than *T. citricida*, and also have a wider host range. Migrants may originate in other crops prior to feeding on citrus and may feed on a different plant species after leaving citrus. Therefore, aphid host range and feeding behavior likely affect pattern and rate of spread (Gottwald *et al*. 1996b). It is assumed that aphid population levels may be correlated with rates of spread, but threshold levels for minimum and maximum levels of transmission have not been established. Natural spread is generally slow in desert regions where natural thermotherapy may keep inoculum at lower levels in plants and may vary seasonally in temperate areas as well. CTV spread rate in sweet oranges is generally higher than that observed in grapefruit (Moreno *et al*. 1988, Gottwald *et al*. 1996a). CTV isolates in Meyer lemon and some mandarins have not spread appreciably from these hosts unless *T. citricida* is present. The latent periods between inoculation and systemic infection and between

infection and symptom expression also affect evaluation of disease development. Presence of other strains may also influence rate of virus movement (Hermoso de Mendoza *et al*. 1984).

VI – Pest significance

1. Economic impact

CTV is the most economically important virus pathogen of citrus worldwide. Millions of citrus trees on sour orange have been killed by CTV decline epidemics in Argentina, Brazil, Venezuela, Peru, Florida, California, Israel, Spain, and other locations. It is estimated that world wide there are over 200 million trees on sour orange rootstock which are at risk to this disease. Sour orange is popular because it produces a vigorous tree with high quality fruit, is adaptable to many soil conditions including high lime and salt content and has tolerance to many other viruses, viroids, and virus-like pathogens and Phytophthora. Use of tristeza-tolerant rootstocks often risks losses from other factors. In addition to decline, many severe CTV isolates cause stem pitting diseases of susceptible scions cultivars and these occur even when tolerant rootstocks are used. Stem pitting weakens trees and eventually reduces fruit size, quality, and quantity (Marais *et al*. 1996). Grapefruit and lime are very sensitive to stem pitting. Sweet orange is more tolerant but can be severely affected by some isolates.

2. Phytosanitary Risk

The phytosanitary risk for CTV is associated with importation of infected plants or budwood for propagation in a new citrus-growing area. The risk associated with dry tissue or fresh fruit is negligible.

VII – Control

Control strategies for CTV differ according to the incidence and severity of the CTV isolates in an area and with the cultivars and rootstocks used. No single control strategy is applicable in all situations (Garnsey *et al*. 1996a, Lee *et al*. 1994).

1. Exclusion and Quarantine

When CTV is absent or rare, preventive efforts should be made to avoid introduction of CTV into the growing area by having quarantines on importation of live citrus tissue. A practical and safe method to legally introduce cultivars from other regions and to free these of infection is necessary and reduces industry pressure to illegally introduce new cultivars or germplasm resources. Procedures for safe international movement of citrus germplasm have been devised (Frison and Taher 1991).

2. Certification Programs

Careful control of propagating material remains the single most effective means to avoid rapid and extensive CTV epidemics. Most commercial citrus are clonally propagated by using buds from a selected scion cultivar to a nucellar seedling as a rootstock. Budwood is usually taken from a mature, vigorous tree and used directly or increased in a nursery block to produce thousands of buds from a single source. Thus, propagation of CTV-infected trees can be prevented by using virus-free scion trees protected from natural infection by isolation or use of insect-free screenhouses or by shoot tip grafting (Navarro 1993). Rapid indexing tests are available to verify freedom from CTV infection.

3. Eradication and suppression

If a few trees become infected in a CTV-free area and indigenous aphids are poor vectors, natural spread can be slowed appreciably by a vigilant eradication and suppression program. However, an effective survey program is essential and when CTV is detected, infected trees must be removed immediately and surveillance maintained (Garnsey *et al*. 1996a). Eradication is rarely effective once infections are well established, especially in the presence of favorable vector conditions.

4. Resistant/tolerant rootstocks

Numerous rootstocks are tolerant or resistant to CTV decline and use of these is essential for economic production of citrus in many areas. Some examples are Cleopatra and Sunki mandarins, rough lemon, Rangpur lime, trifoliate orange, and trifoliate orange hybrids such as Troyer and Carrizo citranges and Swingle citrumelo. CTV resistant/tolerant rootstocks are often susceptible to other problems such as citrus blight, viroids, nematodes, or poor soil conditions.

5. Tolerant Ssions

Most mandarins are generally tolerant to CTV, although some hybrids, such as some tangelos, are seriously affected by stem pitting. In most areas in Asia where CTV isolates are severe, mandarins are the principal varieties produced due, in part, to their tolerance to stem pitting. There are no CTV-tolerant limes although Persian limes are more tolerant than small acid limes. All grapefruits are susceptible to grapefruit stem pitting isolates of CTV. Sweet oranges vary in susceptibility to sweet orange stem pitting, but none are truly tolerant. Pera orange, a major variety in Brazil, is very susceptible while Valencia is one of the more tolerant cultivars.

6. Cross protection

Infection with a mild isolate of CTV may protect a tree from becoming infected with or showing symptoms of a more virulent strain CTV (Gonsalves and Garnsey 1989). This is a strategy for control of stem pitting in areas where severe isolates of CTV and the brown citrus aphid are endemic. Cross protection is for production of grapefruit in South Africa (Van Vuuren *et al*. 1993) and Australia (Broadbent *et al*. 1991), and Pera sweet orange and Galego lime in Brazil (Müller and Costa 1972, Costa and Müller 1980). In these countries, protective isolates have been selected from vigorous trees that remained in areas destroyed by the disease and their protective capacity confirmed in controlled experiments. Protection often is effective only between certain isolates and many mild isolates show little protective effect (Roistacher *et al*. 1993). Furthermore, mild protective isolates are often effective only in the specific cultivar in which they were selected. Effective long-term cross protection against decline of trees grafted on sour orange rootstock has not been demonstrated, though significant delay of symptom onset has been observed with some mild isolates (Yokomi *et al*. 1991, Moreno *et al*. 1993c).

Cross protection is an empiric practice and the basis for the strain interaction involved is not understood. One of the difficulties to implement an effective cross protection is that many CTV isolates contain a mixture of strains that differ at molecular level and in symptom expression (Moreno *et al*. 1993a). The balance of strains in a CTV isolate may change depending on the host and other factors (Moreno *et al*. 1993b) and, hence, its specific interactions with other isolates. Therefore, until additional understanding on the molecular mechanism involved in this interaction will be available, cross protection has to be considered as a practical procedure to delay or reduce damage caused by severe isolates in some citrus cultivars grown in specific areas.

New technologies for citrus transformation (Moore *et al*. 1992, Peña *et al*. 1995) and the increasing knowledge on CTV genome (Karasev *et al*. 1995) may allow production of transgenic citrus plants with resistance mediated by viral sequences.

7. Vector control

Vector suppression is an unproven strategy for CTV control. In the case of semipersistently transmitted viruses, viruliferous winged aphids may inoculate citrus trees several kilometers from the donor tree. It is not clear what level of vector control is necessary to reduce spread of CTV. However, vector control may have potential to reduce secondary spread (Gourmet *et al*. 1994). Biological controls to restrict build up of citrus aphids, especially *T. citricida*, may be feasible (Tang and Yokomi 1996). Although insecticides may not act quickly enough to prevent primary infection by viruliferous aphids, they could reduce local aphid populations and decrease rate of secondary spread. Insecticidal control of vector populations may have use in specific situations such as in a citrus nursery or to protect budwood sources. A long residual systemic insecticide with minimum impact on biological control agents is preferred. CTV titer is highest when trees are forming new shoots in spring and fall. Aphid flights also peak at this time and, hence, these periods should be targeted for control actions.

8. Integrated disease msanagement

Incidence and spread of CTV is a complex process that involves interaction of the plant, pathogen and vector. Conventional approaches have been directed at one or several components for CTV control. An integrated disease management (IDM) strategy should incorporate as many elements as possible based on our fundamental knowledge of the disease (Garnsey *et al*. 1996a).

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