# Control of Virus Diseases of Citrus

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## Contents



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## Abstract

Citrus is thought to have originated in Southeast Asia and horticulturally desirable clonal selections have been clonally cultivated for hundreds of years. While some citrus species have nucellar embryony, most cultivation of citrus has been by clonal propagation to ensure that propagated plants have the same traits as the parent selection. Clonal propagation also avoids juvenility, and the propagated plants produce fruit sooner. Because of the clonal propagation of citrus, citrus has accumulated a large number of viruses; many of these viruses are asymptomatic until a susceptible rootstock and/or scion is encountered. The viruses reported to occur in citrus will be summarized in this review. Methods of therapy to clean selected clones from viruses will be reviewed; the use of quarantine, clean stock, and certification programs for control of citrus viruses and other strategies to control insect spread citrus viruses, such as mild strain cross-protection and the use of pest management areas will be discussed.

## 1. INTRODUCTION

Citrus and its relatives are native to Southeast Asia, New Caledonia, and Australia (Webber, Reuther, & Lawton, 1967). Citrus has been cultivated for hundreds of years and moved around the world, as seed and propagation materials, without regard to what viruses may be present until relatively recently. As a result, citrus probably has many more virus and virus-like diseases as compared to other commodities.

Citrus is clonally propagated (Swingle & Reese, 1967). There are several reasons for this. While some citrus species have nucellar embryony, and seedlings are genetically the same as the parent tree, seedling citrus plants express juvenility traits that are undesirable: excessive thorniness, upright growth, and lack of flower/fruit production for several years. Clonal propagation avoids juvenility and ensures that propagated plants will grow and produce fruit genetically like the original tree used as the source of buds. Citrus is normally grown on a rootstock. Research and experience has shown that rootstocks can influence the quality of fruit, tree size, and impart a level of tolerance to root rots, high water tables, and poor soil type (Castle, 1995).

Many citrus viruses occur as latent infections in some scions, or cultivars, but express symptoms when the cultivar is propagated on a susceptible rootstock or scion/rootstock combination. For example tristeza decline is not expressed when trees are grown on rough lemon rootstock, but when the trees are propagated on sour orange rootstock, decline and eventually death of the tree occurs. Many "old-line" bud sources are infected with multiple

viruses and graft transmissible diseases, many which will be expressed only if the trees is propagated on a susceptible rootstock or reaches an advance age when the symptoms are then expressed. An example is psorosis disease which may not produce symptoms in the scion until the tree is from 5 to 12 years old (Roistacher, 1991).

In this review, the frequently encountered virus diseases of citrus will be briefly summarized with emphasis on their characteristics and properties which influence detection and their control. This is followed by a review of the control methods/strategies for citrus viruses.

## 2. COMMONLY ENCOUNTERED CITRUS VIRUSES AND GRAFT-TRANSMISSIBLE DISEASES

### 2.1. *Citrus tristeza virus*

*Citrus tristeza virus* (CTV) is probably the most economically important virus disease of citrus in the world, having killed more than 50 million trees on sour orange rootstock, beginning with the epidemic in Argentina and Brazil in the 1930s (Lee, Baker, & Rocha-Pena, 1994). CTV is a member of the closterovirus group, having a flexuous filament about 2000 by 12 nm in size, with a positive-sense RNA genome of about 20 kb, containing 12 open reading frames and two untranslated regions (Dawson, 2010). The virus is transmitted in a semi-persistent manner by a number of aphid species, with *Aphis gossypii* and *Toxoptera citricida* being the most important. *A. gossypii* is the most common vector in area where *T. citricida* is not yet present, such as Israel, the Mediterranean basin and the near East, and Texas, Arizona, and California (Rocha-Peña et al., 1995). In citrus producing areas where *T. citricida* has been introduced, the severity of CTV has increased (Halbert et al., 2004; Rocha-Peña et al., 1995).

CTV expresses a number of biological activities: decline on sour orange rootstock, seedling yellows in lemon and grapefruit, stem pitting on grapefruit and/or sweet orange. While the decline on sour orange symptom of CTV can be overcome by planting on CTV-tolerant rootstocks, the stem pitting activity is much more difficult to control. The stem pitting symptom of CTV is readily apparent in susceptible hosts, but the decline on sour strains and seedling yellows strains are latent in most varieties and cultivars, and become apparent only when propagated on susceptible rootstocks or in grapefruit or lemon varieties, respectively. Seedling yellows strains are almost always accompanied by decline on sour orange and/or stem pitting activities. Biological indexing is required to determine the biological activity

of a specific CTV isolate. The biological indexing procedure has been standardized to include a battery of five indicator plants: Mexican lime as a sensitive universal indicator; sweet orange on sour orange rootstock to monitor for decline on sour orange; Duncan grapefruit seedlings to monitor for stem pitting on grapefruit; Madame vinous sweet orange seedlings to monitor for stem pitting on sweet orange; and sour orange as an indicator for seedling yellows (Garnsey, Barrett, & Hutchison, 1987; Garnsey, Gumpf, et al., 1987; Garnsey et al., 2005). Seedling yellows may also be detected on the Duncan grapefruit seedling indicators.

Quarantine, clean stock, and citrus certification programs are effective in preventing the introduction of new isolates of CTV into a citrus area, and in minimizing the spread of severe CTV isolates within a citrus area. In areas where stem pitting strains of CTV are not present, tristeza decline on sour orange may be controlled by the use of CTV tolerant rootstocks, such as *Poncirus trifoliata* and hybrids with *P. trifoliata* as one of the parents. When stem pitting strains of CTV are present, mild strain cross-protection (MSCP) has been shown to be a useful management strategy in Brazil, Australia, and Japan (Lee & Keremane, 2013; Roistacher, da Graça, & Müller, 2010). In California, large pest management areas for eradiation or suppression of tristeza have been established where citrus is surveyed on a regular basis, and when CTV is found, the infected trees are eradicated (Gottwald, Polek, & Riley, 2002). This has maintained CTV at very low levels in the Central Valley of California for the past five decades. *P. trifoliata* is immune to most strains of CTV: this immunity gene has been identified and may be useful in the future in developing horticulturally desirable citrus varieties which are immune to CTV (Garnsey, Barrett, et al., 1987; Garnsey, Gumpf, et al., 1987). However, initially in New Zealand and later in other citrus areas, isolates of CTV have been found which break the immunity shown by most isolates of CTV in *P. trifoliata* (Dawson & Mooney, 2000).

## 2.2. Citrus psorosis virus (including citrus ringspot)

Citrus psorosis was one of the first diseases realized to be graft transmissible and is characterized as a disease of citrus which induces bark scaling lesions on the trunk and limbs of sweet orange, mandarins, and grapefruit (Fawcett, 1934). The discovery that the disease could be graft transmitted to seedlings enabled the use of biological indexing to detect citrus psorosis virus (CPsV) in trees used for budwood. The ability to detect CPsV in trees led to the development of the first certification program in 1937 where budwood source trees could be declared to be psorosis-free (Hiltabrand, 1957).

The citrus ringspot disease was first reported in the late 1960s and was characterized by the development of localized ringspots when extracts from bark lesions from citrus were rubbed on the leaves of *Chenopodium quinoa*. Citrus ringspot disease was once considered a different disease from psorosis, but molecular characterization revealed that it is CPsV (Barthe, Ceccardi, Manjunath, & Derrick, 1998; Roistacher, 1993). Using citrus ringspot isolates because of their ability to cause local lesions when inoculated onto *C. quinoa*, it was realized that there were two centrifugal components of the associated virus when separated on sucrose density gradients, and both components were required for infectivity. Upon further characterization of the virus, three single stranded, negative-sense RNAs were found, with the positive strand of RNA1 encoding a p24 (unknown function) and the RNA-dependent RNA polymerase (280 kDa protein); RNA2 positive strand encoding a p54 of unknown function, and RNA3 positive strand encoding the coat protein (CP) (48 kDa) (Barthe et al., 1998; Derrick et al., 1988). The molecular characterization revealed that what formerly was referred to as psorosis A (no bark scaling) and psorosis B (with bark scaling) and citrus ringspot (bark scaling and causing localized ringspots on *C. quinoa*) are all isolates of CPsV, now the type member of the *Ophiovirus* genus (Milne, Garcia, & Grau, 2000).

CPsV has a means of natural spread, but the means of natural spread has not been identified (Roistacher, 1993). In Argentina, the natural spread of CPsV has made the virus hard to control, even when clean stock programs have been implemented. CPsV is present in most old-line bud sources and commonly found when testing imported germplasm for viruses. Symptoms of CPsV occur on sweet orange, mandarin, and grapefruit, although the bark scaling symptom is not displayed on trees in the field until the trees are 3–7 years of age, or even older. Sour orange and lemon will show symptoms on young leaves but usually do not show bark scaling symptoms.

Control of CPsV is by use of quarantine, clean stock and certification programs to prevent the introduction of new isolates or strains and to limit distribution of the disease within a growing region. Because natural spread can occur, although the means of spread is not known, foundation trees and mother trees should be re-indexed on a recurring basis (every 3–4 years) to ensure they are free of CPsV.

## 2.3. Concave gum disease

Concave gum disease (CGD) is a disease of unknown etiology, although it is graft transmitted. CGD was first reported in 1936, and is widespread in old-line budwood (Fawcett, 1936). The disease is characterized by

concavities on the trunk and limbs, with gum-filled deposits in the wood beneath the concavities. The bark cracks occasionally and gum exudes from the cracks. Upon biological indexing, oak leaf patterns and vein clearing occur in sweet orange and Sweettangor indicator plants. As with CPsV, the control of the disease is by the use of quarantine, clean stock, and certification programs. In Florida, there appears to be a natural spread, especially in grapefruit (Powell, Pelosi, Sonoda, & Lee, 1998).

## 2.4. Impietratura disease

Impietratura disease is a graft transmissible disease presumably caused by an uncharacterized citrus virus (Caruso, Davino, & Terranova, 1993; Roistacher, 1991). The disease occurs in most countries in the Mediterranean basin. The leaves of affected trees show vein clearing and oak leaf patterns in the spring and some, but not all, of the fruit are small, malformed, and hard. The symptomatic fruit will display gum pockets in the albedo which appear as small bumps of the surface of the fruit. Most varieties will show symptoms, whereas citrons appear to be more tolerant. The disease results in fruit drop and many of the fruit are not marketable because they are malformed and hard. Upon biological indexing, the symptoms on sweet orange indicator plants are similar to CPsV (Roistacher, 1991).

## 2.5. Cristacortis disease

Cristacortis disease is a graft transmissible disease presumed to be caused by an uncharacterized citrus virus (Roistacher, 1991). It was first reported from Corsica (Vogel & Bové, 1968) and has been reported also in Italy, Spain, Northern Africa, Turkey, Mauritius, and San Paulo, Brazil. The disease is characterized by vertical depressions or pockets in the wood, with gumming occurring at the bottom of the depression when the bark is cut away. The symptoms appear similar to stem pitting caused by some strains of CTV, but the pits can occur on sour orange rootstocks, also on mandarins which tend to be more tolerant of CTV induced stem pitting. Leaf symptoms are vein flecking and oak leaf patterns, similar to those produced by CPsV. Natural spread is not reported. Quarantine, clean stock, and certification programs effectively control this disease.

## 2.6. Citrus vein enation

Citrus vein enation (CVEV), also known as woody gall, was first described in California as a graft-transmissible disease which caused enations in the leaf

veins of sour orange (Wallace & Drake, 1953). CVEV also caused woody galls on the trunks or rootstocks of acid lime, rough lemon, Rangpur lime, and *Citrus volkameriana*. The disease is transmitted in a persistent manner by several aphid species including *T. citricida*, *Myzus persicae*, and *A. gossypii* (Roistacher, 1991). The disease has been reported from Australia, California, India, Japan, Peru, and Spain. Using electron microscopy, spherical virus particles about 28 nm in diameter have been visualized, leading to the suggestion that the virus associated with citrus vein enation was a luteovirus (Maharaj & da Graça, 1988). Recently, an Enamovirus has been associated with CVEV and sequenced by deep sequencing of the small RNAs, confirming that the virus associated with CVE is a luteovirus (Vives et al., 2013). Control of the disease involves using propagation material that is free of CVEV and to protect budwood source trees from aphids to keep the budwood source trees from becoming infected via aphids.

## 2.7. Citrus blight disease

Citrus blight is a disease of unknown etiology, but the disease is reproducibly graft transmitted using inoculum that comes from the rootstock (Tucker, Lee, Timmer, Albrigo, & Brlansky, 1984). Different rootstocks vary in susceptibility to citrus blight as indicated by the age where blight symptoms become evident (Derrick & Timmer, 2000). Rough lemon, Rangpur lime, *P. trifoliata*, and Carrizo citrange are especially susceptible, whereas Cleopatra mandarin, sour orange, and sweet orange are more tolerant. Once trees develop the disease, the decline is irreversible, and the trees become unproductive. Early symptoms of citrus blight are zinc deficiency symptoms in the leaves, zinc accumulation in the phloem, and eventually high zinc levels occur in the xylem. Blockage of xylem tissues with amorphous plugs occurs, resulting in reduced water uptake (Lee, Marais, Timmer, & Graham, 1984). The canopy of affected trees thins and upper shoots develop small leaves, pointing upright, the foliage has a dull green color. Overall tree decline continues with twig dieback and root loss. Other physiological changes follow such as off season flowering and production of water sprouts inside the canopy (Tucker et al., 1984). The first incidence of blight occurs at random in a citrus grove, but the subsequent incidences of blight tend to be within one to two trees of trees which are already symptomatic, suggesting a natural means of spread that is yet unknown (Castle & Gottwald, 2005). A serological assay has been developed to test for citrus blight (Derrick et al., 1990). Management of citrus blight includes use of blight tolerant rootstocks and testing of budwood source trees

for lack of reactivity with the blight-associated protein (Derrick et al., 1990). Blight losses currently are estimated at about 4–7% of the tree population in Florida (Wang & Brlansky, 2013).

## 2.8. Citrus viroids

Citrus viroids are a group of small, low molecular weight (371–330 nt), circular, infectious RNA molecules which cause various symptoms in citrus and citrus relatives (Table 1) (Brlansky & Timmer, 2014; Duran-Vila, Roistacher, Rivera-Bustamante, & Semancik, 1988). Viroids are not encapsidated in a protein. The discovery that Etrog citron was a good indicator plant for viroids (Calavan, Frolich, Carpenter, Roistacher, & Christiansen, 1964) and the development of the sequential polyacrylamide gel electrophoresis (sPAGE) for diagnosis of the different viroids (Flores, Duran-Vila, Pallas, & Semancik, 1985) has led to the discovery of additional viroid pathogens present in citrus (Ito et al., 2001, 2007). Viroids are easily transmitted mechanically, and care must be made to sterilize clippers, saws, and other tools used to cut or prune trees to prevent spread (Roistacher, 1991). There are no reported vectors. Quarantine, clean stock, and certification programs are effective in control of viroids.

*Citrus exocortis viroid* (CEVd) was the first citrus viroid described (Roistacher, 1991). CEVd is associated with bark shelling on trees grown on *P. trifoliata* or *P. trifoliata* hybrids (citranges and citrumelos), and can cause dwarfing of trees grafted on these rootstocks. CEVd is the largest citrus viroid at 371 nt, and produces the most pronounced symptoms of leaf epinasty, stunting, and necrosis of the leaf midvein. CEVd is a species of the genus *Pospiviroid*.

Cachexia, previously also called xyloporosis and synonymous with *Hop stunt viroid*, (Childs, 1950), was first demonstrated to be viroid-like in 1980 when Roistacher, Nauer, and Wagner (1980) demonstrated the ability to mechanically transmit the disease and found that thermotherapy would not eliminate the pathogen. The viroid was first characterized by Semancik, Roistacher, Rivera-Bustamante, and Duran-Vila (1988). Cachexia causes dwarfing and poor growth of tangelo, mandarin and *Citrus macrophylla* as scions or as rootstocks. On susceptible trees, cachexia causes depressions in the wood of the tree with corresponding pegs in the bark. Cachexia (299 nt) is a species in the *Hostuviroid* genus.

*Citrus bark cracking viroid*, synonymous with Citrus viroid IV, causes leaf drooping on Etrog citron indicator plants, and general necrosis of the leaf midribs. *Citrus bark cracking viroid* (284 nt) is a species in the *Cocadviroid* genus.

**Table 1** Properties of viroids reported from citrus

| Viroid | Size (bases)[a] | Symptoms in citron | Parson's special mandarin reactivity | Symptoms on *Poncirus trifoliata* |
|---|---|---|---|---|
| Citrus exocortis viroid (CEV) | 394–369 | Stunting, epinasty, midvein necrosis, petiole necrosis, leaf tip browning | None | Bark scaling, stunting |
| Citrus viroid 1 | CV-1a: 340 CV-1b: 330 | Stunting, leaf bending, point necrosis of midvein | None | Unknown |
| Citrus viroid II-a, b | 305–295 | Slight petiole necrosis and leaf tip browning | None (CVd IIa) | Bark cracking |
| Citrus viroid IIb | | Asymptomatic in citron | Strong symptoms | Unknown |
| Citrus viroid IIc | | Asymptomatic in citron | Strong symptoms | Unknown |
| Citrus viroid III-a to d | 291–296 | Stunting, leaf dropping, necrosis of midvein, petiole necrosis | None | Grooving possible |
| Citrus viroid IV | 275 | Stunting, leaf dropping, necrosis of midvein, petiole necrosis | None | Unknown |
| Citrus viroid V[b] | 293–294 | Mild stunting, mild bark cracking and gum exudates | None | Unknown |
| Citrus viroid VI[c] | 328–331 | Stunting, mild leaf bending, petiole necrosis | None | Unknown |

[a]From https://ebi.ac.uk/genomes/viroid.html; Genome Pages—Viroid.
[b]Serra, Barbosa, Daròs, Flores, and Duran-Vila (2007).
[c]Ito, Icki, Ozaki, and Ito (2001).

*Citrus bent leaf viroid*, synonymous with Citrus viroid I, causes leaf bending on Etrog citron and point necrosis on the leaf midribs. *Citrus bent leaf viroid* (318 nt) is a species in the *Apscaviroid* genus.

Citrus dwarfing viroids, synonymous with Citrus viroid III, cause dwarfing on *P. trifoliata*, citrange, and citrumelo rootstocks. Citrus viroid IIIa (297 nt) and Citrus viroid IIIb (294 nt) are species in the *Apscaviroid* genus.

Citrus viroid V produces mild symptoms in Etrog citron. Citrus viroid V (294 nt) is a species in the *Apscaviroid* genus (Serra et al., 2007).

Citrus viroid VI produces mild symptoms in Etrog citron. Citrus viroid VI (330 nt) is a species in the *Apscaviroid* genus.

## 2.9. Citrus tatterleaf virus

Citrus tatterleaf virus (CiTLV), synonyms *Citrange stunt virus*, *Apple stem grooving virus*, was first reported in 1962 from Meyer lemon which had been imported from China in 1908 (Wallace & Drake, 1962). CiTLV causes stunting or dwarfing, necrosis at the bud union, and virus-induced bud union incompatibility on scions grafted onto *P. trifoliata*, citrange, or citrumelo rootstocks (Iwanami, Kano, & Koizumi, 1991). The virus is carried asymptomatically in most citrus varieties, and symptoms often are not apparent until the tree is 3–7 years of age (Roistacher, 1991). The major method of transmission is by use of infected propagation materials. CiTLV is widespread in China, Japan, and Korea and has been reported in South Africa, Australia, and in the United States via the importation of Meyer lemon from China. Control of CiTLV is by use of quarantine, clean stock, and certification programs.

## 2.10. Citrus leaf blotch

Citrus leaf blotch (CLBV) was first reported from Nagami kumquat on Troyer citrange rootstock plants that had a bud union crease (Vives, Galipienso, Navarro, Moreno, & Guerri, 2002). The CLBV is a member of the family *Flexiviridae*. The virus has filamentous virions about 960 × 14 nm in size with a single-stranded, positive-sense genomic RNA of 8747 nt. CLBV is seed transmitted and there are no known vectors. A similar if not identical virus was intercepted in California while indexing a Cleopatra mandarin variety introduced from Florida in 1968 and called *Dweet mottle virus* (DMV) (Krueger, Bash, & Lee, 2005). Sequencing of DMV has confirmed a 96% homology with CLBV, but the reported host range differs slightly (Hajeri, Ramadugu, Keremane, Vidalakis, & Lee, 2010). CLBV has been associated with bud union crease with *P. trifoliata* or trifoliate hybrid rootstocks with Pera sweet orange in Brazil, Marsh grapefruit and Roble sweet orange in Florida, and Nules Clementine plants in Italy, and Nules, Clementine, Navelina, and Navelate sweet orange in Spain (Vives et al., 2002).

## 2.11. Measles disease

Measles is a disease which causes pale yellow spots on leaves as they mature. The affected trees generally appear smaller and branches have a droopy appearance. Usually only a few trees in a grove will show symptoms and will

continue to show symptoms year after year. There appears to be a natural spread but it is very slow with only a few additional trees developing symptoms once the disorder appears in a grove. While initially thought to be a genetic disorder, the measles symptoms has been graft transmitted in Florida (Lee, Derrick, Futch, & Tucker, 1993) and in California (Lee, Yokomi, & Vidalakis, 2008), but the causal agent has not been identified. Sour orange seedlings in a cool (20–27 °C) greenhouse are good indicator plants for measles disease.

## 2.12. Yellow vein

Yellow vein is a graft transmissible disease reported from California in the 1950s by Weathers (1960). The disease is characterized by yellow veins in the leaves. The fruit also may develop irregular yellow blotches in the rind and are smaller and flatter than normal fruit. Many varieties and types of citrus show symptoms of yellow vein when graft inoculated, but many of these plants recovery and do not show yellow veins on the leaves of new flushes. Limes and lemons appear to be the most symptomatic. There is no evidence of natural spread. A similar disease has been reported in Pakistan (Bové, 1995). The molecular characterization of *Citrus yellow vein clearing virus* has been recently reported (Loconsole et al., 2012).

## 2.13. Citrus leprosis

Citrus leprosis is one of the most important citrus viral diseases and has spread northward from South America to Central America and Mexico in recent years. The leprosis disease is vectored by the *Brevipalpus* species mites (commonly called flat mites) in a persistent manner (Rodrigues, Kitajima, Chilers, & Chagas, 2003). Once thought to be cause by a Rhabdovirus, research has revealed that while there are no difference in symptomology, there are two types of citrus leprosis viruses: cytoplasmic and nuclear (Guerra-Moreno, Manjunath, Brlanksy, & Lee, 2005). The cytoplasmic-type leprosis (CiLV-C) is limited to the cytoplasm. CiLV-C is the type member of a new genus *Celivirus*, has membrane-bound bacilliform virions 50–60 × 110–120 nm. The genome is a bipartitie, single-stranded, positive-sense RNA with RNA1 having 8729 nts and RNA2 having 4969 nts (Locali-Fabris et al., 2006). Recently, a variant isolate of CiLV-C was reported from Colombia which is different enough from the previous characterized isolates of CiLV-C from Panama and Brazil that serological and RT-PCR tests designed for the detection of these previous characterized

isolates did not detect the Colombian variant, referred to as CiLV-C2 (Roy et al., 2013). CiLV-C2 has a similar genome to CiLV-C with the RNA1 having 8717 nts and RNA2 with 4989 nts. Both RNA1 and RNA2 had only 58% and 50% homology with RNA1 and RNA2 of CiLV-C. Symptoms produced by CiLV-C and CiLV-C2 are similar.

The genome sequence of the nuclear-type leprosis virus (CiLV-N) obtained from Mexico was recently reported (Roy et al., 2013). RNA1 had 6268 nts and RNA2 had 5847 nts. The genome organization closely resembles that reported for *Orchid fleck virus* with 90–91% homology with OFV at the nucleotide level and 93–98% homology with amino acid sequence identities.

Quarantine, clean stock, and certification programs are a key to prevent leprosis from becoming established in new areas and slowing spread within a citrus area. Mite control slows the spread of the disease once it is established in an area. For isolated trees in a grove which become infected with leprosis, probably because the tree did not receive the miticide application, severe pruning to eliminate all symptoms of leprosis, followed by miticide applications are successful in recovering the trees.

## 2.14. *Citrus yellow mosaic*

*Citrus yellow mosaic* (CYMV) (synonym *Citrus mosaic badnavirus*) is an important disease in India where infection rates are as high as 70% (Ahlawat et al., 1996). The disease infects most citrus cultivars and relatives, oranges, grapefruit, and mandarins usually show strong symptoms, while Mexican lime is symptomless. The mature leaves show bright yellow mottling for vein flecking, fruit production is reduced and on chronically infected trees, symptoms are present on the fruit. The disease is spread by the citrus mealy bug, *Planococcus citri* (Huang & Hartung, 2001). Spread by the mealy bug vector appears to be limited, widespread spread is by the use of infected propagating source materials.

CYMV is a badnavirus with nonenveloped bacilliform particles $150 \times 30$ nm in size having a DNA genome of 7559 bp in length and six putative open reading frames, all on the plus-strand of the genome (Huang & Hartung, 2001). Control is by the use of quarantine, clean stock, and certification programs, and control of the mealy bug vector.

## 2.15. Satsuma dwarf

Satsuma dwarf is a virus disease originally described in Japan where it causes serious problems (Changyong, Xueyuan, Yuanhui, & Xinhua, 1993; Cui,

Gu, & Roistacher, 1991). Trees infected with *Satsuma dwarf virus* (SDV) have spoon shaped leaves, enations, multiple flushing, stunting or dwarfing, fewer leaves, and small fruit having a thick peel (Tanaka, 1972). There are variants of SDV, with one variant being called *Citrus mosaic virus* (not to be confused with CYMV virus which is a badnavirus) (Ahlawat, Chenulu, Viswanath, Pandey, & Bhagabati, 1985; Dakshinamurti & Reddy, 1975). The SDV virion is an isometric virus particle about 26 nm in diameter. Two RNAs are present, RNA1 with about 7000 nt and RNA2 with about 5400 nt (Karasev, Han, & Iwanami, 2001). SDV has been reported from Japan, China, Iran, Korea, and Turkey, while the citrus mosaic variant of SDV has been reported only from Japan.

SDV is readily transmitted by grafting, and soil transmission has been reported but not confirmed (Koizumi, Kano, Ieki, & Mae, 1988). No vector has been reported.

## 3. OTHER INSECT-SPREAD DISEASES CAUSED BY PROKARYOTES WHICH NEED TO BE CONSIDERED IN CONTROL/MANAGEMENT OF CITRUS VIRUSES AND VIRUS-LIKE DISEASES

### 3.1. Huanglongbing

Huanglongbing (HLB), also known as citrus greening disease, is perhaps the most important citrus disease in areas where the disease and its vector are both present (Bové, 2006). The symptoms of include an asymmetrical mottling of the leaves; frequently the midribs of the leaves are yellowed. Sectors of the canopy decline and dieback, followed by the decline and dieback of the entire canopy. Once the tree is nearly totally infected, the tree will produce yellow shoots. The yellow shoots are characteristic of HLB, and HLB in Chinese translates to "yellow shoot." Symptomatic fruit are lobsided, usually contain aborted seed, and have an off flavor. Fruit production is reduced, symptomatic fruit is small, and fruit often drops prematurely. Over 2–3 years the tree declines and dies.

Three forms of HLB have been identified: the Asiatic form which is associated with *Candidatus* Liberibacter asiaticus (Las); the African form which is associated with *Candidatus* L. africanus (Laf); and the American form which is associated with *Candidatus* L. americanus (Lam) (Jagoueix, Bové, & Garnier, 1994; Teixeira et al., 2005). The Asiatic form of HLB expresses symptoms at a warmer temperature whereas the African from which expresses strongest symptoms under cooler temperatures. The American

form expresses symptoms under cooler temperatures and extended periods of warmer temperature eliminate the disease from infected plants (Lopes et al., 2009). In Brazil where Lam was first reported, the Las has out competed Lam and Lam is rarely encountered now under field conditions (Lopes et al., 2009).The host range of HLB is limited to citrus and citrus relatives.

There are two psyllid vectors of HLB: *Diaphorina citri* and *Trioza erytreae* (Bové, 2006). Both species of the vector can transmit either the Asiatic form or the African form of HLB, and *D. citri* has been shown to transmit the American form in Brazil (Bové, 2006; Lopes et al., 2010). *T. erytreae* occurs mostly in Africa, while the *D. citri* occurs in northern Africa, Asia, and the Americas. *D. citri* is the more efficient vector (Bové, 2006).

Control of HLB requires quarantine, clean stock, and certification programs in order to produce healthy plants and prevent movement of infected nursery stock. The psyllid vectors must be controlled. Florida and Texas have begun using area-wide sprays to reduce the psyllid population during the winter months when the psyllids are not as active and only the overwintering adults are present (Bassanezi et al., 2013). In areas where HLB is not already established, a three pronged approach to control is effective: regular surveys to identify early symptoms on trees which are then removed; control of the psyllid vector by survey and pesticide application; and use of clean plant material for replanting (Bové, 2006). Detection of the bacterium associated with HLB is by PCR or real-time PCR. Testing psyllids for the presence of the bacterium associated with HLB by real-time PCR has proven to provide an earlier warning of the presence of the disease in an area where HLB is not already established (Manjunath, Halbert, Ramadugu, Webb, & Lee, 2008).

## 3.2. Stubborn disease

Stubborn was described as a disease in 1944 in California. It is now known to be caused by a helical mollicute *Spiroplasma citri* and Koch's postulates have been fulfilled (Bové et al., 2002; Saglio et al., 1973). The *S. citri* genome has been sequenced (Ye et al., 1992). Stubborn causes stunting, hence the name stubborn as the infected plants do not grow. This is especially apparent in when young trees become infected (Roistacher, 1991). Fruit on affected trees are often lopsided, small in size, and have aborted seed. Color inversion is often seen with the stylar end remaining green and the peduncular end showing color. The symptoms on the tree canopy are often localized into sectors, especially when larger trees become infected. Leaves are small,

cupped, and often have an upright appearance. Mottling can occur on the leaves. Diagnosis of stubborn is by *in vitro* culture, or by use of PCR and/or real-time PCR (Yokomi, Mello, Fletcher, & Saponari, 2010). For biological indexing, Madame Vinous sweet orange seedling maintained in warm conditions (37 °C) day temperature and 27 °C night temperature are inoculated with bud chips or side shoot grafts (Roistacher, 1991). Symptoms expressed under these conditions are slow growth, small cupped leaves, short internodes between leaves, and leaf mottling. Most citrus varieties and cultivars are susceptible to stubborn.

Stubborn has several planthoppers as vectors: *Circulifer tenellus* and *Scaphytopius nitidus* common in California and Arizona, and *Circulifer haematocepts* (syn. *Neoaliturus haematocepts*) in the Mediterranean region (Calavan & Bové, 1989). The *Spiroplasma* multiplies in the vector but there is no evidence of transovarial passage. Most spread of stubborn in citrus is primary spread: there is little evidence of citrus to citrus transmission (Yokomi et al., 2010). The leafhopper vectors have a wide host range and citrus is a temporary host if more preferred hosts are not available. Stubborn has not been reported in tropical or subtropical regions.

Control is by the use of quarantine, clean stock, and certification programs to ensure that healthy plants are planted in the field. The movement of the planthopper vectors is seasonal, and planting of new citrus when the vectors are likely to move to the young actively growing citrus should be avoided. Young plants which become infected with stubborn should be removed and replaced with a healthy plant. The use of trap plants which are more attractive to the vectors than citrus has been reported (Schwarz, 1965).

## 3.3. Citrus variegated chlorosis

Citrus variegated chlorosis (CVC) first appeared in Brazil in 1987 and rapidly became established throughout the Brazil citrus industry (Lee, Derrick, Beretta, Chagas, & Rosetti, 1991). CVC has spread to countries surrounding Brazil and most recently was reported in Costa Rica (Aguilar et al., 2005). The disease was named because of the chlorotic appearance of the tree, resembling a zinc deficiency, once the tree becomes infected. Newly affected trees show sectoring of symptoms, while chronically infected trees have a yellow appearance. As the leave mature, small, light brown gummy lesions form on the underside of the leaves, corresponding to the yellow chlorotic areas on the upper side of the leaf. As the leaf ages, the lesions on the underneath of the leaf may become dark brown and even become

necrotic. Fruit size on CVC affected trees is greatly reduced and the fruit has a hard rind. Normal fruit thinning after blossom set does not occur on a CVC affected tree, resulting in the tree carrying more fruit that is smaller. CVC affected fruit ripens earlier than fruit on a healthy tree and the sugar content is higher. Fruit tends to occur in clusters, rather than one or two fruit as normal, especially on Pera sweet orange trees. CVC affected trees become stunted, the branches dieback, and the canopy thins, but the tree rarely dies. The disease has been shown to be caused by a pathovar of *Xylella fastidiosa* (Hartung, Beretta, Brlansky, Spisso, & Lee, 1994).

CVC is vectored by numerous sharpshooters; several species have been identified as vectors (Hopkins & Purcell, 2002). Control of CVC is by quarantine, clean stock, and certification programs so that clean propagation materials are used and plants going to the field are healthy. Vector control is important, and sharpshooter populations are controlled by insecticide applications.

## 4. METHODS OF CONTROL OF GRAFT-TRANSMISSIBLE PATHOGENS OF CITRUS

Control of citrus viruses begins though three programs: (1) quarantine (safe introduction of germplasm into an area or region), (2) clean stock program (to test, therapy if necessary, and produce/provide sources of pathogen-tested propagating stock for an industry), and (3) certification to ensure the maintenance and use of pathogen-tested propagating material for commercial use (Lee, 2000; Lee, Lehman, & Navarro, 1999; Navarro, 1993). These three programs will be discussed in greater detail, Figs. 1 and 2 illustrate the relationship among these three programs.

### 4.1. Quarantine programs

Quarantine programs allow for the safe introduction of germplasm into an area or region. In a viable citrus industry, there is a constant demand for new varieties for possible commercial production or for use in citrus breeding programs. A quarantine program provides a mechanism for introduction of new varieties or cultivars into a citrus area without introducing citrus viruses or other graft-transmissible pathogens. Quarantine programs require regulatory authority and are operated under the guidance of the Ministry of Agriculture or the state Department of Agriculture.

Desired germplasm is requested using an import permit so that everyone concerned knows the material is being imported, and once the material

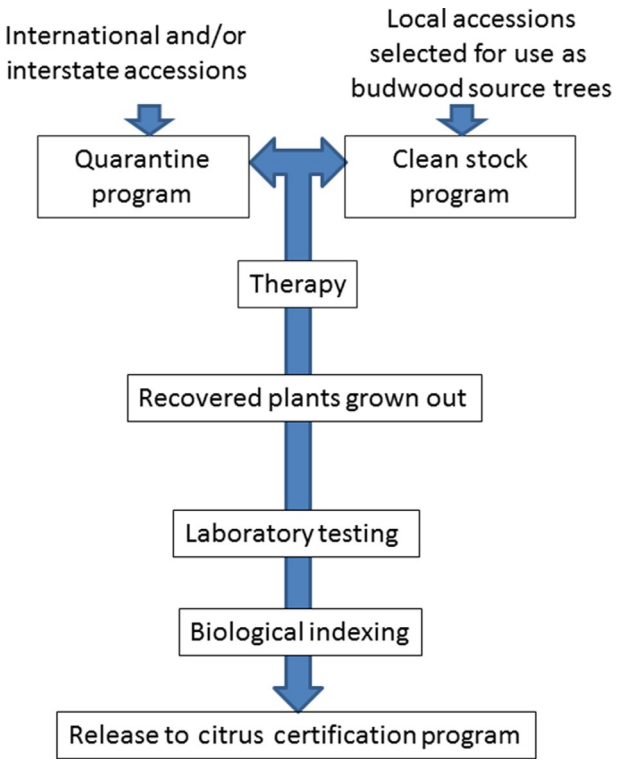


**Figure 1** Diagram showing the relationship between a quarantine program and a clean stock program and the general scheme of therapy and testing before incoming accessions may be released into the citrus certification program or made available to the citrus industry.

arrives, it is usually inspected to make sure insects, fungi, and other pests are not present. The imported germplasm is then established in a quarantine greenhouse or in a region removed from the main citrus production area, or by the use of *in vitro* quarantine by placing the surface sterilized budstick in media so that emerging buds may be used for shoot tip grafting (Lee, 2000; Navarro, 1993). The imported germplasm must be therapied to eliminate graft-transmissible pathogens.

The traditional methods of therapy have been thermotherapy (Grant, 1957) or shoot tip grafting (Navarro, Roistacher, & Mirashige, 1975). More recently, cryotherapy has been applied to eliminate graft-transmissible pathogens (Wang, Panis, Engelmann, Lambardi, & Valkonen, 2009).

Thermotherapy involves subjecting the buds, grafted onto a rootstock, to an extended heat cycle (Roistacher, 1991). Thermotherapy of citrus

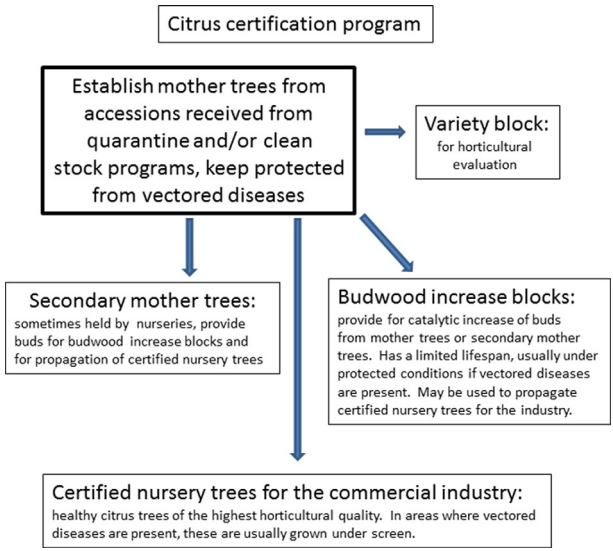


**Figure 2** Diagram of the general structure of a citrus certification program. The clean material received from the quarantine or clean stock program is used to establish the mother trees and maintained under conditions to protect them from exposure to vector-borne pathogens. Buds cut from the mother trees may be used to establish secondary mother trees, usually maintained under protected conditions in a commercial nursery, for budwood increase blocks to rapidly increase the number of buds available for propagation, or for direct propagation of certified nursery trees. A variety block is established from buds obtained from the mother trees to evaluate the horticultural trueness to type of the budline and to obtain production data.

requires grafting a bud onto a rootstock, usually a citrange, keeping the bud wrapped with grafting tape so that it does not grow or push, and placing the grafted rootstock in a heat treatment chamber having a 16-h light cycle at 40 °C and 8-h dark cycle at 30 °C for 12–16 weeks. Once the heat treated plants are removed, the grafting tape is removed, and the buds are allowed to push. Once the buds have grown for 12–15 weeks, testing is performed to determine if the pathogens have been eliminated. This method is effective at removing most citrus pathogens which are detected by biological indexing under cool conditions (Table 2), but it is not effective at eliminating citrus viroids or CiTLV. The disadvantage of this approach is the time required for thermotherapy, 12–16 weeks.

Therapy by use of shoot tip grafting has been the approach of choice for removal of citrus viruses (Navarro et al., 1975). Using this procedure, the meristem tips, about 0.1 mm thick, are cut from emerging buds and grafted onto etiolated rootstocks grown *in vitro*. The tips are usually free of graft-

**Table 2** A summary of the required tests for citrus viruses and virus-like diseases for release from quarantine status in California[a]

| Virus/disease | Indicator plant | Symptoms | Index condition[b] | Laboratory diagnostic[c] |
|---|---|---|---|---|
| *Citrus tristeza virus*/tristeza | Mexican lime | Leaf vein clearing, leaf cupping, stunting, stem pitting | Cool | ELISA |
| *Citrus psorosis virus*/psorosis including citrus ringspot and pathogens which test similar (impietratura, concave gum, cristicortis) | Sweet orange, Dweet tangor | Leaf vein flecking, oak-leaf patterns on young leaves, bark scaling may occur with some isolates; psorosis and ringspot will cause shock symptoms | Cool | |
| *Infectious variegation virus*, *Citrus leaf rugose virus*, *Citrus crinkly leaf virus* | Sour orange, may be supplemented with Etrog citron, Eureka lemon | Leaf variegation and/or crinkle on sour orange; chlorotic spotting on lemon | Cool | |
| *Apple stem grooving* virus syn. *Citrus tatter leaf virus*/citrange stunt | *Citrus excelsa*, rusk citrange on rough lemon | *C. excelsa*: deformed leaves with chlorotic zones; rusk citrange: deformed leaves with chlorotic leaf blotch, stem with zigzag growth | Cool | |
| *Citrus leaf blotch virus*/*Dweet mottle virus* | Dweet tangor | Mottle pattern on leaves | Cool | RT-PCR |
| Citrus viroids including exocortis, | Arizona 861-SI Etrog citron | Leaf epinasty and/or stunting, midvein necrosis, | Warm | sPAGE from inoculated citrons |

*Continued*

**Table 2** A summary of the required tests for citrus viruses and virus-like diseases for release from quarantine status in California—cont'd

| Virus/disease | Indicator plant | Symptoms | Index condition | Laboratory diagnostic |
|---|---|---|---|---|
| cachexia, and five other viroid species | on rough lemon | petiole necrosis, leaf tip browning | | grown under warm conditions |
| *Spiroplasma citri*/stubborn | Sweet orange | Stunting, small leaves, leaf cupping | Warm | Culture from source tree held under warm conditions |
| *Citrus vein enation virus*/vein enation | Sour orange/ Mexican lime | Vein enations; galls may form on Mexican lime where wounds occur | Cool | RT-PCR |
| Huanglongbing associated with *Candidatus* Liberibacter species/citrus greening disease | Source plant observation | Asymmetrical mottle on leaves, yellowing (zinc deficiency symptoms) | Warm (Asiatic form); cool (African and American forms) | qPCR, observation of source plant |
| *Xylella fastidiosa*/citrus variegated chlorosis | Source plant observation | Zinc deficiency symptoms, gummy lesion on underside of leaves | Warm | PCR |
| Miscellaneous and unknown | Source plant | | | dsRNA |

ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcriptase polymerase chain reaction; sPAGE, sequential polyacrylamide gel electrophoresis; qPCR, quantitative real-time polymerase chain reaction; PCR, polymerase chain reaction; dsRNA, double-stranded RNA.
[a]When performing biological indexing, positive controls *in planta* must be available in order to perform complete and definite testing. Exotic pathogens (such as Huanglongbing and *Xylella fastidiosa* may be used as DNA extracts for positive control for the laboratory testing).
[b]Cool indexing conditions is 24–27 °C, while warm indexing conditions is 28–40 °C.
[c]All laboratory testing is performed on the source plant unless otherwise described.

transmissible pathogens, and the resultant recovered plant is usually free of pathogens and does not undergo juvenility. This approach has been shown to be effective at elimination of all citrus viruses (Navarro, Civerolo, Juarez, & Garnsey, 1989). A modification of the procedure is referred to as *in vitro* quarantine (Lee et al., 1999). Here the budstick, once received

under permit, is surface sterilized, placed in media in a glass tube and placed under lights at 25–26 °C. When the buds emerge, shoot tips are cut and grafted as in the normal shoot tip grafting protocol. Depending on the skill of the technician performing the shoot tip grafting procedure, it is most likely the resultant recovered plant will be free of pathogens.

An alternative method of therapy, referred to a cryotherapy, is where vegetative buds are excised, treated in a buffer to remove excess water within the cells, frozen in liquid nitrogen, then recovered under controlled conditions and grafted onto an etiolated rootstock as done with shoot tip grafting (Wang et al., 2009). The protocol we are using for citrus is very similar to that used for cryopreservation of citrus (Volk, Bonnart, Krueger, & Lee, 2012), except that the freezing step is limited to 1–2 h. The advantage of this procedure is that vegetative buds are used, not the more tender emerging buds as in shoot tip grafting. The buds are 1–1.5 mm when cut. After the freezing step, about 0.5 of the base of the bud is excised and the resultant tip is grafted to the etiolated rootstock.

Regardless of the method used to therapy the germplasm received in quarantine, it is important that the therapied germplasm be indexed for all known graft-transmissible pathogens before being released from quarantine. This usually requires both biological and laboratory testing. Table 2 summarizes the pathogens tested and the test used for each pathogen as required in California. If the germplasm has been obtained from a region where a virus or virus-like pathogen is present, that pathogen should be tested for. For example, germplasm originating from Florida should be tested for citrus blight.

## 4.2. Clean Stock Programs

Clean stock programs provide for recovery of healthy sources of local cultivars and provide a mechanism whereby pathogen-tested propagating material is available to the local citrus industry. A clean stock program is often carried on by a research institution utilizing the expertise of specialists in plant pathology, horticulture, and possibly other departments, but the clean stock program may be operated by the regulatory agency having jurisdiction over the citrus production area.

There are several steps in a clean stock program (see generalized scheme in Fig. 1). The clean stock program selects mother trees from local cultivars, the selection of potential mother trees should be made with regard to horticultural characteristics without pathogen content being considered. However, it is usually recommended that a measurable trait or phenotype be used to make the best use of limited resources. Examples of measurable traits or

phenotypes are earlier maturation, consistent production of more fruit, higher color score on fruit, etc. Following the selection of potential mother trees, the process for cleanup is similar to the quarantine program. The trees are indexed to determine what citrus viruses are present. Therapy follows, either shoot tip grafting or thermotherapy, depending on viruses present and expertise and facilities available. Following therapy, the resultant recovered potential mother trees must be completely indexed for all graft-transmissible pathogens present in the area.

An important component of a clean stock program is the horticultural evaluation. The availability of the data from the horticultural evaluation is valuable to the local industry in helping growers decide if they want to grow a particular cultivar or variety. In Florida, the productivity on a per tree basis over a 15–20 year period was increased by 10–15% because the growers could evaluate the publically available data obtained from the horticultural evaluation trials from the accessions being therapied in the Florida clean stock program (Lee, 2000). The clean stock program maintains a source of mother trees, usually under protected conditions to prevent possible insect vectors access, and makes budwood available upon request.

### 4.3. Certification Programs

Certification programs are important to guarantee the sanitary status and horticultural quality of propagating material for the commercial production of nursery plants (Lee, 2000; Lee et al., 1999; Navarro, 1993). Certification programs must have regulatory authority and are usually operated by a state or provincial agency having the legal authority to impose restrictions. All the propagation of citrus is done by commercial nurseries, but the primary foundation trees are usually maintained by the agency in charge of the certification program. In a certification program, testing of primary foundation trees on a recurring basis is done for commonly occurring citrus viruses or viroids and nursery increase blocks may be tested for pathogens known to be insect vectored in the region. A typical scheme for a certification program is shown in Fig. 2.

## 5. OTHER METHODS OF CONTROL

### 5.1. Pest management areas

Pest management areas for eradication or suppression of citrus viruses and other pathogens have been used effectively in California. As an example,

in California pest management areas for the suppression of CTV were established in the Central Valley of California in 1963 (Gottwald et al., 2002). An epidemic of CTV decline on sour orange had occurred in the southern California production area resulting a wide spread incidence of tristeza, but CTV was rarely found in the Central Valley production area (Fresno, Kern, Madera, and Tulare counties). To control losses due to CTV, the Central California Citrus Pest Control Agency was charged with locating and removing CTV-infected trees. This agency was later renamed the Central California Tristeza Eradication Agency (CCTEA) with responsibility through an agreement of the Joint Operations of the Tristeza Eradication Program (Joint Powers Agreement or JPA) of what was then five citrus pest control districts in Fresno, Tulare, and Kern counties. Two of the districts withdrew in 1995 and 1996, leaving three pest management districts. The program is funded by a special assessment on growers depending on the acreage of citrus in the district.

The purpose of the CCTEA is to identify and eradicate CTV in a timely, orderly, and cost effective manner and to encourage and support appropriate research programs that pursue method to eliminate the threat of CTV (Gottwald et al., 2002). The program has been charged with ensuring that all trees planted in the suppressive areas remain virus-free by identifying reservoirs of CTV so the trees can be eliminated. While the approach for surveying and eliminating CTV has changed over the years and technology has been developed and applied, the area still has a very low incidence of CTV after five decades.

## 5.2. Mild strain cross-protection

MSCP is the phenomenon which occurs when a mild isolate of a virus is inoculated into a plant, and when that plant is later inoculated (or challenged) with a severe isolate of the same virus, the symptoms of the severe isolate are suppressed or delayed in expression (Lee, Brlansky, Garnsey, & Yokomi, 1987). MSCP is not the same as virus resistance. MSCP as a management strategy is useful only when (1) the disease is endemic with no other possibility of control; (2) there is confidence that the mild isolate being used will not spread and cause damage in other crops or act in combination with other viruses to cause more severe damage; (3) there is some level of confidence that the mild isolate being used is stable (especially if it has been derived by mutation) and will continue to be mild when purposefully inoculated into many plants.

MSCP of citrus viruses has been a useful method to control, or manage, disease losses due to severe viruses, especially with tristeza (Bederski, Roistacher, Silvestre, & Müller, 2010; da Graça & van Vuuren, 2010; Lee & Keremane, 2013; Roistacher et al., 2010). Empirically selected mild isolates of CTV have been used in several citrus production areas to maintain the ability to produce fruit despite the presence of severe stem pitting strains of CTV; Pera sweet orange in Brazil (Müller & Costa, 1987), grapefruit in Australia and South Africa, limes in India, Hassuku dwarf on pummelo in Japan. More recently, in Florida isolates of CTV were empirically selected which protected against CTV decline on sour orange rootstock (Lee & Keremane, 2013).

The mechanism of MSCP in citrus is not understood, and it is probable that several mechanisms are involved in the phenomena perhaps depending on the virus and/or host. The mechanisms of protein mediated protection, such as CP-mediated resistance, RNA-mediated resistance, and RNA silencing have been suggested as mechanisms of cross protection. The practical cross protection employed at the field level so far has involved selection of cross protection strains by empirical methods, but as a better understanding of the mechanisms(s) determine the efficiency and effectiveness of MSCP, better and quicker methods of selection of isolates for MSCP should develop in the foreseeable future. The ability to transform plants, including citrus, should enable the development of a better understanding of MSCP at the molecular level with specific viruses.

## 5.3. CP-mediated resistance

CP-mediated resistance to *Tobacco mosaic virus* (TMV) was demonstrated by Sherwood and Fulton (1982) and in *Cucumber mosaic virus* by Dodds, Lee, and Tiffany (1985). In transgenic plants, the level of expression of the CP seems to determine if resistance is expressed for not, with the higher expression of the CP having better protection against challenge (Powell, Sanders, Tumer, Fraley, & Beachy, 1990). CP-mediated resistance has been reported to be less strain specific than forms of resistance involving RNA (Lomonossoff, 1995). Dominguez et al. (2002) reported pathogen-derived resistance to CTV in 10–33% of the Mexican lime plants transformed with the CP gene of CTV.

## 5.4. RNA-mediated resistance

Using TMV as the model system, RNA-mediated resistance to explain cross protection was suggested by Palukaitis and Zaitlin (1984). In this model, the

protecting virus produces excessive positive-sense RNA which hybridizes to the newly formed minus-strand RNA and this stops the further replication and translation of the challenge virus. This protection by RNA hybridization theory has been demonstrated at the cell level but not at the whole plant level.

Virus resistance due to an expressed transgene with sequence homology to the virus is a form of RNA-mediated resistance (Febres, Lee, & Moore, 2008; Lindbo, Silvarosales, Proebsting, & Dougherty, 1993; Ratcliff, Harrison, & Baulcombe, 1997; Waterhouse, Graham, & Wang, 1998). This RNA-mediated resistance, also called gene silencing, and has been termed RNA interference or RNAi. Silencing is initiated in plants by dsRNA that is processed into small interfering RNA (siRNA) of about 21–26 nt. The siRNAs then mediate RNA degradation of complementary sequences; a process known as posttransciptional gene silencing (PTGS). SiRNAs can also direct methylation of complementary DNA sequences (Mette, Aufsatz, van der Winden, Matzke, & Matzke, 2000; Wassenegger, Heimes, Riedel, & Sanger, 1994). Thus, methylation of the transcribed sequence is associated with PTGS. Methylation of promoter sequences inhibits transcription. RNAi has been proposed to be a natural plant defense mechanism against viruses and transposons and is also involved in the regulation of the expression levels of certain gene (Voinnet, 2002; Waterhouse, Wang, & Lough, 2001).

RNA-mediated protection against CTV has been reported in Duncan grapefruit transformed with the 3′ end of the CTV genome (Febres et al., 2008). The most resistant line showed no transgene mRNA accumulation and promoter methylation of cytosines; both characteristic of PTGS.

Most likely MSCP uses one of these two methods as suggested by experiments with transformed plants, either CP-mediated resistance or RNA-mediated resistance, or a combination. The virus and even strain of the virus probably determines the mechanism used. Regardless, at least with CTV, mild strain cross-protection, using empirically selected strains, has been demonstrated to be a viable control mechanism. Further research will provide more information as to the nature of this protection.

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