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Citrus Tristeza Virus in Hawai‘i

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Citrus tristeza virus (CTV) is the most economically important pathogen of citrus worldwide (Figure 1). CTV is the causal agent of "tristeza" (also known as "quick decline"), which has destroyed over 50 million citrus trees growing on sour orange rootstocks globally (Figure 2). CTV is also responsible for another viral disease known as stem pitting (Figures 3, 4, 5) which reduces fruit quality and negatively impacts the production of limes, grapefruits, and sweet oranges (Figures 6, 7) (72). Numerous strains of CTV exist. Mild strains that infect sweet orange, mandarins, and many other cultivars sometimes do not cause symptoms and result in less severe crop losses. CTV is vectored by multiple aphid species in Hawai‘i (Figures 8, 9, 10), where it poses a continuing threat to citrus cultivation throughout the state. Here we discuss CTV, the diseases it causes, and some integrated practices for its management.

Figure 1. Tristeza or quick decline caused by *Citrus tristeza virus* in citrus grown on a sour orange rootstock. Photograph courtesy of S. M. Garnsey, University of Florida.

Hawai‘i imported nearly 20 million pounds of fresh citrus fruits in 2008, while producing only a few hundred thousand pounds of fresh citrus for local markets (71) (Table 1). Although Hawai‘i possesses a favorable climate for citrus cultivation, citrus farming ranks relatively low in crop value and total acreage among agricultural commodities. Among the factors contributing to the paucity of productive citrus farms in Hawai‘i are plant diseases, in particular tristeza and stem pitting caused by CTV.

Farmers in Brazil coined the disease name "tristeza," which means "sadness" in Portuguese, because of the severity of symptoms and the large crop losses caused there by the virus in the 1930s.

The pathogen, CTV

When, how, or from where CTV first entered Hawai‘i is unknown. However, the first report of tristeza and stem pitting in the Islands was at the University of Hawai‘i at Mānoa's Poamoho Agricultural Experiment Station on the island of O‘ahu in 1952 (33) (Table 2). A subsequent survey of citrus trees elsewhere on O‘ahu indicated that disease symptoms caused by CTV were widespread in citrus throughout Hawai‘i.

CTV is a member of the virus family *Closteroviridae*, genus *Closterovirus*, of which *Beet yellows virus* (BYV) is the type member. Several review articles and book chapters have focused on this virus family (1, 2, 16, 44) or specifically on CTV (29, 49, 58). The closteroviruses are positive-strand RNA viruses with long, flexuous particles. A typical CTV particle is approximately 2000 nm in length and 12 nm in diameter (Figure 11). CTV is limited to the phloem tissues of infected hosts. The natural host range for CTV is confined to members of the plant family *Rutaceae*, of which citrus species are members (Tables 3, 4). Some *Passiflora* species are the

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Table 1. Market supply of fresh citrus in Hawai‘i (2008) (from 71).

Citrus	Inshipments (Pounds)	Produced in Hawai‘i (Pounds)
Grapefruit	1,307,000	46,000
Lemon	3,501,000	55,000
Lime	1,957,000	76,000
Orange	12,381,000	(data not available)
Tangerine	2,000,000	98,000

Table 2. Quick decline and stem-pitting symptoms observed at the Poamoho Agricultural Experiment Station on the island of O‘ahu in 1952, the first report of tristeza disease in Hawai‘i (from 33).

Disease Severity	Citrus Varieties and Types Exposed to CTV at Poamoho[1]
Severe[2]	Lime: ‘Mexican’
	Limequat (cross between Key lime and kumquat)
Moderate	Lime: ‘Kusaie,’ ‘Rangpur’
	Mediterranean sweet orange
	Navel orange: ‘Golden Buckeye,’ ‘Washington,’ ‘Carter’
Mild	Grapefruit: ‘Marsh,’ ‘Davis,’ ‘Bowden,’ ‘Sunshine’
Unaffected (asymptomatic)[3]	Kumquat: ‘Nagami,’ ‘Nippon’
	Lemon: ‘Eureka,’ ‘Villafranca,’ ‘Meyer,’ rough lemon
	Lime: Tahitian lime
	Mandarin orange: ‘Wase,’ ‘Kara’
	Orange: Hawaiian (Kona), ‘Valencia’

[1]All trees were grown on rough lemon rootstocks. The year of these data was 1952. [2]Severe symptoms included stunted growth, distorted limbs, and stem pitting. [3]Lack of symptoms (unaffected) does not necessarily indicate host resistance to CTV.

Figure 2. Aerial view of a Florida citrus grove affected by tristeza or quick decline. Photograph courtesy of S. M. Garnsey, University of Florida.

only known non-rutaceous hosts (66).

Numerous strains of CTV have been described that vary in virulence and the severity of the diseases they cause. Some strains of CTV cause tristeza, but not stem pitting. Similarly, some strains cause stem pitting in oranges, but not in grapefruit. The most reliable and effective method for differentiating the CTV strains is through the use of indicator plants (30, 74). Garnsey et al. (30) have developed a standardized bioassay for strain detection using the following indicator plants: ‘Mexican’ lime, sour orange, ‘Duncan’ grapefruit, ‘Madam Vinous’ sweet orange, and navel sweet orange on a sour orange rootstock. Symptom development in these indicator plants following inoculation with an unknown CTV strain helps to determine whether the strain can cause quick decline or stem pitting, as well as indicating the severity of symptoms. Severe strains of CTV will cause tristeza and/or stem-pitting symptoms, whereas milder strains cause less severe disease or may infect citrus plants asymptomatically. Some strains of CTV may induce symptoms differentially in one host but not in another (49).

Based on the wide range of genetic marker patterns, a diverse population of CTV strains exists in Hawai‘i. Mixed infections consisting of various CTV strains also commonly occur in Hawaiian citrus; observed symptoms are usually caused by the more severe strain in the mixture.

CTV is limited worldwide to tropical and subtropical regions. Currently, CTV is found in North America (35, 73), Central America and the Caribbean (35, 37, 79), South America (35), Australia (6), Oceania (33), Asia (56), Africa (49, 76), and some Mediterranean countries (7, 36, 49). CTV and the diseases it causes exist throughout the Hawaiian Islands, where the incidence

Figure 3. Compare the smooth stem of a healthy citrus tree (above) versus the stem pitting caused by *Citrus tristeza virus* (CTV) (below). Peel back the epidermis of young stems to observe this symptom, which is diagnostic for this disease.

Figure 4. Stem pitting caused by *Citrus tristeza virus* (CTV) in Mexican lime (*C. aurantifolia*): symptoms caused by a very mild strain of CTV (above) versus a more severe CTV strain (below).

of CTV-infected citrus plants is high. Garnsey et al. (28) reported virus incidence of 91% (41 out of 45 plants) on O‘ahu, Maui, and the island of Hawai‘i, although most of the samples tested appear to have spread from the Big Island. A recent and more comprehensive survey (52) found CTV incidence of 74% (298 of 405 plants).

Diseases caused by CTV and symptoms

There are three principal diseases caused by CTV in citrus trees: tristeza, stem pitting, and seedling yellows (Figure 12) (Table 5). The severity of these diseases is dependent on the strain of CTV present and on the susceptibility of the host. Tristeza is a decline of different scion cultivars grafted onto sour orange rootstocks. This decline can occur over a period of several years, or in only a few months (this rapid form of the disease is also known as "quick decline"). Trees with tristeza initially appear water stressed; this stage is followed by defoliation and death. In Hawai‘i, tristeza is rarely encountered because sour orange rootstocks are no longer used.

Stem pitting is a disease most commonly seen in grapefruits, sweet oranges, and some lime cultivars. Trees with severe stem pitting appear stunted, with chlorotic leaves that often display "vein-clearing" symptoms (Figure 13). Twigs and small branches on these trees are brittle and can be snapped with little effort. When the bark is removed from twigs or branches, the wood will have small pinhole-like pits, or long grooves that give it a rope-like texture. In Hawai‘i, stem pitting is the most prevalent and important disease of citrus caused by CTV.

Seedling yellows is a disease of sour orange, lemon, and grapefruit seedlings. Susceptible seedlings infected with these CTV strains become stunted and have small, chlorotic leaves. Seedling yellows is most devastating in nursery operations, and therefore not an important

Table 3. Genera of the family *Rutaceae*, subfamily *Aurantioidae*, tribe *Citrae*, subtribe *Citrinae* (from 69, 70).

Genus	Number of Species	Geographic Origin
Severinia	6	S China, SE Asia
Pleiospermium	5	S Asia, Oceania
Burkillanthus	1	SE Asia, Oceania
Limnocitrus	1	SE Asia
Hesperathusa	1	S and SE Asia
Citropsis	11	Central Africa
Atalantia	11	S and SE Asia
Fortunella	4	S China
Eremocitrus	1	Australia
Poncirus	2	Central and N China
Clymenia	1	Oceania
Microcitrus	6	Australia
Citrus	16	S and SE Asia, S China

Figure 5. Stem pitting of a Minneola tangelo (*C. paradisi* x *C. reticulata*), a diagnostic symptom for citrus tristeza.

disease for growers in Hawai‘i who have established trees and who import all their planting stocks.

Insect vectors and transmission of CTV

CTV is transmitted by several aphid species in a semi-persistent manner (Table 6). The brown citrus aphid (*Toxoptera citricida*) (Figures 8, 9) is by far the most efficient vector of CTV, followed by the melon aphid (*Aphis gossypii*) (Figure 10). The black citrus aphid (*T. aurantii*) and spirea (or citrus) aphid (*A. spiraecola=A. citricola*) are inefficient vectors of CTV, or are only able to transmit certain strains of the virus. These aphids, however, can build up large populations in citrus groves and therefore may contribute to the spread of CTV in some circumstances (Table 6). All of these aphid species are present in Hawai‘i.

Although CTV is not seed-transmissible, it is readily transmitted through grafting. Since most citrus species are vegetatively propagated, dissemination of infected

Table 4. Notable species in the genus *Citrus* (from 69).

Species	Year Named	Conventional Name	Geographic Origin	Suspected Parentage
Subgenus *Citrus*				
C. medica L.	1753	Citron	India	True species
C. aurantium L.	1753	Sour orange	China	*C. reticulata* x *C. grandis*
C. sinensis Osbeck	1757	Sweet orange	China	*C. reticulata* x *C. grandis*
C. grandis Osbeck	1765	Pummelo	China	True species
C. limon (L.) Burm.f.	1766	Lemon	India	*C. medica* x *C. grandis* x *Microcitrus*
C. reticulata Blanco	1837	Mandarin	China	True species
C. aurantifolia Christm.	1913	Common lime	Malaya	*C. medica* x *C. grandis* x *Microcitrus*
C. paradisi Macf.	1930	Grapefruit		*C. grandis* x *C. sinensis*
C. tachibana Tan.	1924	Tachibana	Japan	Unknown
C. indica Tan.	1931	Indian wild orange	India	Unknown
Subgenus *Papeda*				
C. hystrix D.C.	1813	Mauritius papeda	S.E. Asia	Unknown
C. macroptera Mont.	1860	Melanesian papeda	S.E. Asia	Unknown
C. celebica Koord.	1898	Celebes papeda	Celebes	Unknown
C. ichangensis Swing.	1913	Ichang papeda	China	Unknown
C. micrantha Webster	1915	Papeda	Philippines	Unknown
C. latipes	1928	Khasi papeda	Assam	Unknown

budwood has greatly increased the distribution of CTV worldwide. CTV can also be transmitted by parasitic plants called dodder (*Cuscuta* sp.), but this means of transmission is not thought to be important in the spread of the virus.

Disease diagnosis and CTV detection

Accurate diagnosis of citrus diseases caused by CTV and characterization of the virus in infected plants is critical for any successful implementation of management strategies. For example, tristeza occurs only when sour orange rootstocks are used. In Hawai‘i, this roostock is rarely used, and therefore this disease is rare. Yet many citrus trees displaying wilt and decline symptoms on rootstocks other than sour orange are often misdiagnosed as having tristeza when in fact they are in decline due to other causes such as citrus blight or foot rot. The cause of citrus blight is currently not known, but it can be distinguished from tristeza in the field and laboratory. Foot rot, caused by *Phytophthora* spp., can also be easily distinguished from tristeza (Table 8). In addition, foliar symptoms of the milder forms of CTV-induced diseases may resemble nutrient deficiencies in citrus plants. Stem pitting can be diagnosed positively in the field by the grooves or pinholes in the wood of young branches when the bark is removed (Figures 3, 4).

There are many methods used for laboratory detection of CTV strains within plants and even within insect vectors when disease symptoms are absent. These methods vary in terms of sensitivity, cost, reliability, and equipment needed or other technical aspects (Table 7). For example, CTV can be indexed on Mexican lime, where symptoms of vein-clearing develop 3–6 weeks after inoculation (Figure 13).

CTV disease management

Effective management of CTV is essential for successful commercial cultivation of citrus in Hawai‘i. For the industry to expand in the Islands, additional management strategies must be adopted for CTV. Yet, with high disease incidence, geographically widespread pathogen distribution, and high diversity of CTV populations in Hawai‘i, the development of such management strategies is a daunting task. Currently there is no single management strategy used to control CTV-caused diseases; rather, various strategies are integrated. The choice of which strategies to implement depends upon the incidence and strains of CTV in any particular region.

In regions where CTV incidence is low, management efforts should focus on quarantine of incoming materials, budwood certification programs, and suppression or eradication programs (29). In these low-incidence regions, the biggest threat to citrus growers is the introduction of infected materials as planting stocks.

Figure 6. Yellowing and dieback of a Mexican lime (*C. aurantifolia*) tree in Hawai‘i infected with a severe stem-pitting strain of *Citrus tristeza virus*.

Figure 7. Reduced fruit size for Mexican lime (*C. aurantifolia*) plants with stem pitting caused by *Citrus tristeza virus* (CTV): healthy fruits (left) versus fruits infected with CTV (right).

Table 5. Citrus diseases caused by *Citrus tristeza virus* (CTV).[1]

Disease	Symptoms and information
Tristeza or quick decline[2]	Occurs when a sweet orange, grapefruit, or tangerine scion is budded onto a sour orange rootstock. In the field, symptoms can begin within a month of infection and are typified by yellowing and loss of foliage but retention of fruit, followed by death of the tree. This is a disease of the bud union, and pinholes and a brownish margin are often present at the union.
Stem pitting[3]	Symptoms of stem pitting on grapefruit and stem pitting on sweet orange vary, as some strains of CTV are able to induce symptoms in one host but not the other. Unlike quick decline, this disease is not limited to the bud union. Rather, it is expressed as lengthy grooves in the trunks and limbs of trees caused by disruption of the vasculature. Diseased trees often produce fewer fruits having lesser quality.
Seedling yellows	Sour orange, lemon, and grapefruit seedlings inoculated with certain strains of CTV become severely chlorotic and stunted. These symptoms are most commonly encountered when seedlings are grown in greenhouses.

[1]Strains of CTV are often classified by which diseases they cause, as these diseases are often strain specific. For example, a strain that causes quick decline may not cause stem pitting. Many CTV strains are mild and do not produce any of these main diseases, although they may cause a symptom called “vein-clearing” in Mexican lime. Trees infected with mild strains will still produce, although the yield and quality of fruit may be somewhat lower than uninfected trees of modern citrus growers. [2]Rare disease in Hawai‘i, as sour orange rootstock is not commonly used. [3]Stem pitting is the most commonly occurring, contemporary CTV-caused disease in Hawai‘i, as sour orange rootstocks (subject to quick decline) are no longer used by growers.

However, there appear to be no CTV-free areas among the Hawaiian Islands where quarantine procedures for new plantings might be implemented. Removing infected plants to establish CTV-free areas and replacing them with virus-free planting material would also be ineffective, as viruliferous brown citrus aphids would soon re-infest the virus-free plantings.

Mild CTV strains that confer cross protection against severe CTV strains are well established in some citrus-production areas in Hawai‘i. Many productive trees in Hawai‘i are infected with these cross-protecting strains. Resistant or tolerant citrus varieties such as pummelo (*C. maxima* Merr.) and some mandarins (*C. reticulata* L.), popular in local markets, may be grown productively.

Figure 8. A colony of brown citrus aphids (*Toxoptera citricida*) herded by long-legged ants on Volkamer lemon (*C. volkameriana*) foliage. These insects can vector *Citrus tristeza virus*.

Quarantine, certification, and suppression/eradication programs

Quarantine programs test incoming citrus planting stocks for CTV and other citrus pathogens that may harm a region’s citrus industry. Citrus trees are usually clonally propagated by grafting, and CTV is graft-transmissible. Thus, an important aspect of CTV management is maintaining CTV-free bud stocks. Buds from a single tree can be used to produce hundreds of new plants that may be distributed over great distances. Many CTV epidemics were started or enhanced by the distribution of infected budwood (3). Budwood certification programs are designed to keep such budwood free of CTV and other graft-transmissible pathogens. In such programs, primary budwood source trees are regularly monitored for infections and may be further protected by insect-proof screening, or moved to locations far removed from CTV-infected trees (29). In order to reduce the number of CTV-infected trees in a region, surveys are routinely conducted to detect infected trees and have them removed. Outside of Hawai‘i, such suppression programs are generally undertaken only if the CTV incidence is

Figure 9. A colony of brown citrus aphids (*Toxoptera citricida*), CTV vectors, herded by ants on Ladu tangerine (*C. reticulata* Ladu) foliage.

Figure 10. A colony of melon aphids (*Aphis gossypii*) on Fairchild tangerine (*C. reticulata* Fairchild) foliage in Hawai‘i. These insects can vector CTV.

<5%. However, determining the incidence of CTV in a region is difficult where disease incidence is low, as a large number of samples must be processed to accurately estimate disease incidence. Several survey techniques have been designed to maximize the area and number of trees sampled, while minimizing the number of samples processed, often by combining samples from adjacent trees (41). If any of these combined samples are positive, a more intensive survey is conducted within the affected area to determine the extent of the infection. Although these are often called “eradication” programs, usually not all infected trees are removed, given the time lag between initial infection and when the virus is detectable by laboratory assays. If successfully implemented, however, a CTV-suppression program can be cost effective and may keep CTV at acceptable levels (29).

The control methods described above can also be applied to regions where so-called mild strains of CTV are common and where the goal is to control severe strains. Strain-selective control approaches, however, require a method to discriminate between the more desirable strains and those severe strains to be eradicated. In Florida, which has populations of both mild and disease-inducing strains, control is focused mainly on managing the more severe, stem-pitting strains of CTV. An important component of this management strategy is the use of the antibody MCA-13, which can discriminate between mild and severe Florida strains (62) to screen trees. Trees testing positive for MCA-13 are identified for removal by the testing process. This reduces the effort and expense of removing a large number of trees, and growers do not lose productive trees whose health is strengthened by the protective effects of infection by milder strains of CTV. Additionally, budwood harboring CTV infections that are not reactive to MCA-13 can be propagated and distributed on a limited basis (29). A similar strain-selective control strategy was employed in California when a stem-pitting strain was introduced at a single location. Although no antibodies were available that could discriminate between the introduced strain and endemic mild strains, indicator plants were used to identify infected trees. These trees were removed and the introduced strain of CTV was eradicated (29).

Citrus varieties having natural resistance to or tolerance for CTV

A first-management strategy to employ is to plant CTV-tolerant or -resistant citrus varieties (27, 54). For example, tristeza, which is a disease of the graft union when sour orange rootstocks are used, can be avoided by using non-susceptible rootstocks. Sour orange was once a popular citrus rootstock due to its tolerance to other plant diseases such as foot rot, caused by *Phytophthora* spp. Thus, these diseases often once again become a problem when non-sour orange, tristeza-resistant rootstocks are used.

Rigorous testing of various tristeza-resistant rootstocks under local conditions is the best way to determine which rootstocks to use. In Hawai‘i, such testing has revealed that ‘Cleopatra’ and ‘Sunki’ mandarins, ‘Heen Naran’ tangerine, and ‘Rangpur’ lime rootstocks grow best under our local conditions (39). However, the use of resistant rootstocks will not effectively manage the more

Table 6. Vectors of *Citrus tristeza virus* (CTV).

Vector of CTV[1]	Information
Humans (grafting, dispersal of infected plants)	The most effective vector of CTV.
	Virus has spread to nearly all citrus-producing regions of the world via transport of infected budwood and by grafting.
Toxoptera citricida (formerly *Aphis citricidus*): Brown citrus aphid	Virus acquisition time: less than 10 minutes.
	Single aphid transmission efficiency: 20% (the most efficient insect vector of CTV). Transmits most strains of CTV (72).
	The geographic range of the brown citrus aphid has been constantly expanding over the last few decades through South America, Central America, and parts of the continental United States (35, 65).
	The brown citrus aphid has a narrow host range and has been present in Hawai‘i since at least 1906, when it was first described (45).
Aphis gossypii: Melon aphid	Virus acquisition time: less than 30 minutes
	Single aphid transmission efficiency: 0.5-1.1%, much less efficient than the brown citrus aphid.
	Transmits most strains of CTV.
	The melon aphid has a much wider host range than the brown citrus aphid, including hundreds of plant species.
Aphis citricola/*Aphis spiraecola*: Citrus aphid/Spirea aphid	Virus acquisition time: not available.
	Single aphid transmission efficiency: not available.
	Transmits only a few strains of CTV.
	The citrus or spirea aphid is a poor vector of CTV but builds large populations on citrus trees, increasing the chance of transmission.
Toxoptera aurantii: Black citrus aphid	Virus acquisition time: not available.
	Single aphid transmission efficiency: not available.
	Transmits very few strains of CTV
	The black citrus aphid is a very poor vector of CTV but builds large populations on citrus trees, increasing the chance of transmission.

[1]Vectors are listed in order of their relative importance, from most to least important.

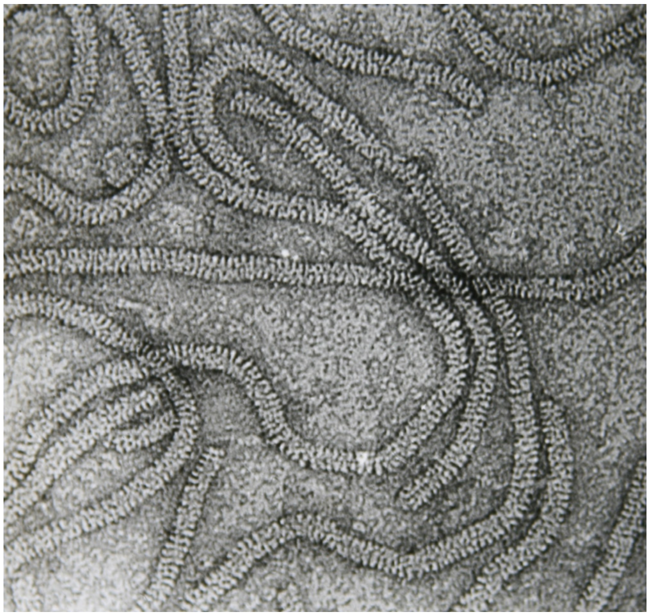

Figure 11. Transmission electron micrograph of negatively stained, purified *Citrus tristeza virus* particles. These particles measure about 12nm in width and 2000nm in length. Photograph courtesy of M. Bar-Joseph, Volcani Institute of Agricultural Research.

severe, stem-pitting strains of CTV, as the symptoms manifest in the scion. Most economically important citrus varieties, however, do not show resistance to stem pitting. Yet, some varieties of pummelo appear to be immune or highly resistant to CTV, including the stem-pitting strains (20, 31). A large pummelo growing in Waiākea, Hawai‘i, surrounded by citrus trees harboring tristeza and stem-pitting strains, has remained CTV-free for many years, despite numerous natural and artificial attempts at inoculation (28, 31). ‘Persian’ limes, white grapefruits, ‘Valencia’ oranges, and most mandarin varieties are the varieties most resistant to stem pitting (29).

Various citrus relatives such as *Poncirus trifoliata* were also thought to be immune to CTV (27), although it has been recently demonstrated that some strains of CTV can overcome this resistance (9).

Mild-strain cross protection

Mild-strain cross protection (MSCP) is another strategy for controlling diseases caused by CTV where virus incidence is high. In this approach, healthy plants are inoculated with a mild strain of CTV, which thereafter

Table 7. Description of methods used for diagnostic detection of *Citrus tristeza virus* (CTV).

CTV Detection Method	Use and Comments
Biological indicator plants: Mexican lime (C. aurantifolia) seedlings	Requires several months for symptoms to develop, and milder strains often cannot be detected.
Electron microscopy	Expensive, time consuming, and therefore not effective for assaying a large number of samples, and may not be useful for low-titer infections.
Light microscopy to detect CTV inclusion bodies stained with Azure A in phloem tissues	Inexpensive, simple.
Isolation of dsRNA from citrus	Complicated by variables such as virus strain, host species/variety, and time of year, which often alter the results (15).
In situ immunofluorescence	Simple and easy way to detect CTV inclusion bodies (5).
Serology-based methods (monoclonal and polyclonal antibodies), including tissue blot immunoassays (TBIAs) and enzyme-linked immunosorbent assay (ELISA). The double-antibody sandwich (DAS-ELISA) is the most popular detection method.	The most commonly used methods for CTV detection. These methods are quick, inexpensive, reliable, and easy to perform, allowing large numbers of samples to be processed in a short period of time. ELISA is perhaps the most commonly used detection technique for CTV (64).
Reverse transcriptase-polymerase chain reaction (RT-PCR).	For CTV detection in plant (42, 50) and aphid (51) tissues; although more technically challenging than ELISA, it has unparalleled sensitivity and can detect CTV when ELISA cannot (50).
Quantitative (real-time) RT-PCR	Technically challenging but has high sensitivity and reliability.

Figure 12. Right: seedling yellows disease caused by *Citrus tristeza virus*. Left: healthy controls. Photograph courtesy of W.O. Dawson, University of Florida.

Figure 13. Vein-clearing in the leaf of a Mexican lime (*C. aurantifolia*) infected with *Citrus tristeza virus*.

confers resistance against subsequent infections by more severe strains of CTV. The more widespread such cross-protection programs are in a geographic region, the more effective they are (81). Several different strategies have been employed to find and test potential mild strains for use in cross protection (8, 47, 59, 67, 80), often with positive results (8, 43, 55, 63). With the recent development of infectious clones of both CTV (68) and CTV defective RNAs (dRNAs) (77), however, future MSCP programs may utilize strains of CTV or dRNAs that are engineered for optimal protection (29). Some plant nurseries in Hawai'i sell cross-protected citrus plants to growers.

However, there are some risks associated with mild-strain cross protection. First, mild strains are plant pathogens and may reduce the productivity of infected plants. This strategy is only used in those regions such as Australia, Brazil, Florida, New Zealand, and South Africa where the potential losses from CTV are so high that growers are willing to accept some losses in fruit yields to remain in production. Second, the resistance provided by cross protection is not complete and generally does not last for the life of a tree. Depending on the effectiveness of the mild strain, the effects of MSCP may last for two to ten growing seasons (43). There is also a concern that mild strains may spread to other hosts where their pathogenic effects might be more severe (26). In addition, a synergistic reaction may occur between the mild strain and an invading unrelated virus (26), causing severe disease. Finally, a mild strain may mutate to a more virulent form, resulting in severe disease.

Developing citrus varieties resistant to CTV

The most promising and useful strategy for controlling CTV where epidemics are caused by severe strains is the development of resistant citrus varieties. There are three approaches used to develop such varieties: 1) conventional breeding, 2) somatic hybridization, and 3) genetic engineering.

Conventional plant breeding usually involves crossing a susceptible, desired variety with a closely related, resistant variety (54). Unfortunately, conventional breeding in citrus is difficult due to many factors, including

Table 8. Distinguishing three common decline diseases of citrus.

	Blight	Foot Rot	Tristeza
Pathogen	Unknown (14)	*Phytophthora* spp.	*Citrus tristeza virus*
Prevalence in Hawai‘i	Common	Common	Rare
Canopy dieback	Sectoral	Sectoral to even	Even
Temporary regrowth	Yes	Yes	No
Gummosis/lesions at graft union (bark on)	No	Yes	No
Dark line or pitting at graft union (bark off)	No	No	Yes
High zinc content in xylem	Yes	No	No
Starch depletion in roots by iodine test (21)	No	No	Yes
Low water uptake by xylem by water injection test (48)	Yes	No	No
Control	Blight-tolerant rootstocks	Pesticide application, use of resistant rootstock, grafting union at least 18” from soil line	Switching from sour orange rootstock

complex reproductive biology, compatibility factors, weak zygotic embryos, partial or complete pollen/ovule sterility in important cultivars, and long juvenility periods (58).

Somatic hybridization of protoplasts derived from citrus and related species can overcome the compatibility barriers encountered in conventional plant breeding. This method is being used to develop plants that are potentially tolerant to CTV diseases as well as other important diseases such as citrus blight (53).

Genetic engineering is arguably the most promising method for incorporating resistance to CTV into host plants. The first transgenic citrus were developed by inserting DNA sequences directly into citrus protoplasts (40, 46, 75). This approach, however, was largely abandoned when more efficient *Agrobacterium*-based transformation protocols were developed (57, 60). Transgenic plants have since been reported for species of the genera *Citrus* (*C. aurantifolia*, *C. aurantium*, *C. grandis*, *C. limon*, *C. paradisi*, *C. reticulata*, *C. sinensis*); *Poncirus* (*P. trifoliata* and its hybrids); and *Fortunella* (*F. crassifolia*) (61, 78).

There are two different approaches for developing resistance via *Agrobacterium*-mediated transformation. The first is to identify citrus or sexually compatible relatives that are resistant to the virus. Although CTV can replicate in protoplasts of resistant varieties, the virus lacks effective cell-to-cell and long-distance movement within resistant plants (25). The genes responsible for this type of CTV resistance could then be identified and used to transform commercially important varieties (27). Although the putative resistance genes from *P. trifoliata* have been identified (10) and mapped (11, 12, 13), the recent discovery of CTV strains that can overcome this resistance (9) has made this approach less attractive. The second approach for developing resistance via *Agrobacterium*-mediated transformation is to induce a natural resistance mechanism known as post-transcriptional gene silencing (PTGS) in the plants. PTGS can be induced by the introduction of specific transgenes into a plant genome. The plant recognizes the introduced sequences as foreign and subsequently degrades the transcripts of the gene and any other sequence that shares significant nucleotide homology, including pathogenic viral sequences. This technique has been successfully employed in several crops, most successfully in papaya, to produce plants that are resistant to some strains of papaya ringspot virus (22, 34).

A great deal of effort has been devoted to the development of transgenic CTV-resistant citrus (58). The first attempts used functional coat protein genes from mild and severe strains of CTV to produce transgenic plants (18). Most transgenic lines that resulted were as susceptible to CTV as the non-transgenic controls. However, some transgenic lines exhibited resistance in the form of immunity or as a significant delay in the development of symptoms. These resistant plants had variable levels of coat protein expression, ranging from undetectable to relatively high, suggesting that PTGS and/or other resistance mechanisms may be involved. Although it is unclear how genetically identical plants of the same transgenic line may display variable resistance in their phenotypes, it was proposed that epigenetic effects were involved (18, 19). Additional transgenic citrus plants have since been created using functional CTV coat protein genes (32, 38), but their resistance to CTV has yet to be reported. Other studies have shown that citrus plants encoding untranslatable coat protein transgenes were found not to be resistant to CTV (17, 24).

Other genes from CTV have been used to generate transgenic citrus plants in the hopes of producing virus resistant plants. Febres et al. (24) transformed grapefruit with RdRp gene sequences, but found that all were susceptible to CTV. When truncated untranslatable p23 genes were introduced into citrus plants, different transgenic lines displayed variable resistance phenotypes similar to those previously described for coat protein gene sequences (19). For any given line, some plants were immune, some had delayed development of disease symptoms, and others were susceptible to the virus. Similar results were also obtained when p23 and 3'-UTR nucleotide sequences were linked and introduced to citrus plants in an inverted-repeat configuration (4) or using only the 3'-UTR (23). These studies suggest it may be possible to generate CTV-resistant citrus plants using the PTGS mechanism.

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