Citrus Tristeza: Biology, Potential Impact, and Control Strategies

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Abstract

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Tristeza disease, caused by citrus tristeza closterovirus (CTV) is the most economically important disease of citrus in the world. CTV occurs worldwide, often unknowingly imported in propagating or breeding material. Several aphid species transmit CTV in a semi-persistent manner with the brown citrus aphid (BrCA), *Toxoptera citricida*, being the most efficient. CTV causes a multitude of symptoms including death of trees on sour orange rootstock and stem pitting of scions regardless of rootstock. Millions of trees on sour orange have been killed by CTV in South America and the Caribbean Basin following the introduction of the BrCA and subsequent spread of the aphid and CTV. Epidemics of CTV decline are occurring in several Caribbean countries. Sour orange is a favored rootstock when CTV is not a problem because of its tolerance to calcareous soils, root rot pathogens, production of high quality fruit, and tolerance to a variety of other viruses/viroids which limit productivity of citrus using CTV-tolerant rootstocks. The impact of the recent spread of the BrCA in the Caribbean Basin and the management strategies for control of CTV are discussed.

Movement and Spread of CTV

Citrus tristeza closterovirus (CTV) probably originated in Southeast Asia where *Citrus* originated. CTV has been distributed around the world in the movement of *Citrus* germplasm, especially before the viral nature of tristeza disease was understood (1).

While the long distance spread of CTV is usually by movement of infected nursery or breeding material, the local spread is by aphid vectors. The virus is transmitted semi-persistently (12). There is no latent period needed before transmission can occur once the aphid acquires the virus, but the aphid remains viruliferous for only 24-48 hours. While the aphid can acquire CTV with short acquisition time, the longer the acquisition feed (up to 24 hours), the more likely it is that CTV can be transmitted. The brown citrus aphid (BrCA), *Toxoptera citricida*, is the most efficient vector of CTV. *Aphis gossypii*, common called the melon or cotton aphid, is the most common aphid vector in areas where the BrCA is not present. In side by side comparisons, the BrCA is about 25 times more efficient at transmitting CTV as compared to *A. gossypii* (31). *A. spiraecola*, the green aphid, and *T. aurantii*, the black citrus aphid, are commonly present as vectors of CTV, but are less efficient vectors than the melon aphid. Studies in Cuba (2), Florida (8), and Belize/Mexico (M. Rocha-Pena, personal comm.) indicate that the BrCA migrates about 200 kilometers a year. This rate of spread may be faster if nursery materials are not disinfested to kill aphids before movement to other locations.

Symptomatology

CTV isolates express a multitude of symptoms (18, 26). Some isolates are mild and do not produce noticeable

symptoms on any commercially desirable citrus cultivars and, only under optimum temperature, will produce symptoms on CTV-sensitive indicator plants such as Mexican lime. Some isolates express a symptom referred to as seedling yellows (SY), a chlorosis and dwarfing of lemon, sour orange, and grapefruit hosts. Some isolates of CTV, commonly called decline isolates of CTV (CTV-D) cause decline and death of orange, grapefruit and/or mandarin scions on sour orange rootstock. The decline may occur suddenly with little forewarning of the impending death of the tree, or gradually over a period of several years where the trunk immediately above the budunion begins to bulge, and honeycombing occurs beneath the sour orange rootstock. Other isolates cause stem pitting on scions (CTV-SP), regardless of the rootstock the tree is grown on. The stem pitting can affect only grapefruit, or only sweet orange, or both sweet orange and grapefruit. Stem pitting can also occur on rootstocks that are normally considered to be CTV tolerant, such as Cleopatra mandarin, Rangpur lime, rough lemon, and Volkamer lemon. A standard host range of five indicator plants has been established (5): Mexican lime as a universal sensitive indicator; sour orange seedlings as an indicator for SY; sweet orange on sour orange rootstock as an indicator of CTV-D; Madam Vinous sweet orange seedlings as an indicator for stem pitting on sweet orange; and Duncan grapefruit seedlings as an indicator for stem pitting on grapefruit. Biological indexing requires 12-15 months under optimal growth conditions. Symptoms expressed due to CTV strains present in a field isolate may be expressed in any combination. Thus, 10 categories have been defined for CTV biological reactivity, plus an 11^{th} category (category 0) for isolates which are very mild and may be detected only by serological assay and not by symptom development (12) (Table 1).

Rapid Methods of CTV Strain Differentiation

The molecular characterization of CTV has progress rapidly in the past few years. This increase in knowledge has resulted in the development of methods which can rapidly predict the biological activity of a given CTV isolate. A monoclonal antibody, MCA-13, has been selected which reacts selectively to most CTV-D and CTV-SP strains and not to mild strains in Florida (25). An association between the amino acid sequence of the CTV coat protein gene and biological activity has been reported (23, 24). This has allowed the development of a procedure, referred to strain specific probes (21), to differentiate mild, CTV- D and CTV-SP isolates from each other. Single strand conformational polymorphism (SSCP) analyses of the PCR products from the coat protein gene has been used for strain differentiation also (30). Perhaps a more sensitive approach is the colony hybridization approach (6) which permits the detection of minor CTV strains which could cause CTV-SP or CTV-D but which would be overlooked because these strains represent only a small percentage of the total strains present in the isolate.

Impact of the BrCA and CTV in the Caribbean Basin

The BrCA was introduced into the Americas in the 1930s in Argentina and Brazil (1). Following this introduction, this efficient vector moved northward, and was first reported in Venezuela in 1976. The following sequence of events occurred in Venezuela beginning following the introduction of the BrCA (22).

When the BrCA first entered Venezuela, CTV was present but not causing problems on citrus. Almost all the 6 million trees comprising the citrus industry were on sour orange rootstock. By 1979, the aphid had spread throughout all citrus producing areas of Venezuela. The first outbreak of CTV decline on sour orange occurred in the north-central region in 1980. By 1987, 6 million trees had been killed by CTV induced decline on sour orange. The farmers initially responded to the loss of their trees by replanting new trees on sour orange rootstock. It soon became apparent that no trees could be grown on sour orange in the presence of CTV, so then CTV-tolerant rootstocks were used.

Table 1. Categories of Biological Reactivity to Citrus Tristeza Virus Isolates

Category	Mexican lime Rx (Universal detection)	Sweet orange/ sour orange Rx	Seedling Yellows Rx	Grapefruit stem pitting	Sweet orange stem pitting
0 (Mild)	-	-	-	-	-
I	+	-	-	-	-
II	+	+	-	-	-
III	+	+	+	-	-
IV	+	+	+	+	-
V	+	+	+	-	+
VI	+	-	+	+	-
VII	+	-	-	+	-
VIII	+	-	-	+	-
IX	+	-	-	-	+
X	+	+	+	+	+

Volkamer lemon was a commonly used rootstock, it produced vigorous, productive trees. When the trees on Volkamer lemon reached 4-6 years of age, they were affected by a condition referred to as sudden decline (22). Closer study revealed that sudden decline was the same as citrus blight in Florida, except in the tropical climate of Venezuela, it is much more severe resulting in the affected trees dying which is not common in Florida. Citrus blight also severely affects *Poncirus trifoliata*, and citrange rootstocks, limiting their usefulness as CTV-tolerant rootstocks. The presence of other viruses also became apparent, depending on what CTV-tolerant rootstock was used. Cachexia causes dwarfing and stem pitting on mandarin-type rootstocks. Viroids cause dwarfing and low productivity in trees on citrange, citrumelo, or *P. trifoliata* rootstocks. Citrus tatter leaf, also called citrange stunt, affects citrange, citrumelo, or *P. trifoliata* rootstocks. Woody gall, when present in budwood, produces galls on rough lemon and lemon-type rootstocks. Inevitably, farmers propagated their new trees on the CTV-tolerant rootstocks using budwood sources which had performed well on sour orange rootstock, then discovered the budwood source was contaminated with one or more of these viruses which, collectively, render all CTV-tolerant rootstocks non-productive.

In Venezuela, as well as other countries where the BrCA has become established, the complexity and severity of CTV changed probably as a result of transmission by the more efficient vector. Isolates of CTV which cause stem pitting appeared. The stem pitting may be on grapefruit, or on sweet orange, or both hosts. These stem pitting isolates spread through the country rapidly because of the unregulated movement of infected nursery material. Once planted in the field, the aphid vectors further spread the severe isolates within the grove. These stem pitting strains of CTV cause a loss of tree vigor, reduced yield, and reduced quality of fruit. From yield tests in South Africa, stem pitting on grapefruit can reduce yields by 45 percent and sweet orange yields by up to 26 percent (16). Thus 15 years after the first report of the BrCA in Venezuela, all trees had been lost on sour orange rootstock, and stem pitting strains which cannot be controlled by use of CTV tolerant rootstocks were widespread in all citrus areas.

The last phase of the increasing complexity of CTV after introduction of the BrCA is the appearance of very severe strains of CTV which will cause stem pitting on rootstocks which are considered to be CTV-tolerant. In Venezuela, CTV isolates have appeared and spread which cause very severe stem pitting on rough lemon and Volkamer lemon rootstocks, and even stem pitting on Cleopatra mandarin rootstocks. The first report of a CTV isolate which could cause stem pitting on a CTV-tolerant rootstock was the Capao Bonito isolate reported in 1963 from Brazil, which causes severe stem pitting on Rangpur lime rootstock (19). In Brazil, the effects of the Capao Bonito strain have been minimal because of a strict quarantine placed on the region where this severe strain was found. In Venezuela, the CTV isolates causing stem pitting on CTV-tolerant rootstocks have been spread through the industry by the movement of infected nursery material. Thus twenty years after the BrCA was first reported in Venezuela, there are still about 6 million citrus trees, but the overall productivity is less than 1 box/tree.

Following the spread of the BrCA through the Caribbean Basin, this scenario of events, which has occurred in Venezuela, is occurring country by country. This scenario of events is destined to continue unless timely measures are taken to implement mandatory citrus certification programs. The clock is already counting off the time. Table 2 summarizes the first report for presence of the BrCA and the date of the first outbreak of CTV decline on sour orange rootstock for countries in the Caribbean Basin.

Table 2. Dates of First Report of *Toxoptera citricidus* (BrCA) and Destructive Citrus Tristeza Virus Outbreak for Caribbean Basin Area

Country	BrCA first reported	CTV outbreak recorded
Bahamas	1996	None
Belize	1996	None
Costa Rica	1989	None
Cuba	1993	None
Dominican Republic	1992	1998 (7)
Florida/USA	1995	CTV decline already endemic (3)
Haiti	1992	1997[a]
Jamaica	1993	1997 (10)
Nicaragua	1991	None
Panama	1985	1995[b]
Puerto Rico	1992	1996 (32)

[a] D. D=Adeski and R. F. Lee, unpublished.
[b] J. Bernal, personal communication

Threats to citrus in the Caribbean Basin from other exotic pests

Other important vector-borne graft-transmissible diseases of citrus pose a grave threat to continued citrus production in the Caribbean Basin. Citrus variegated chlorosis, caused by *Xylella fastidiosa*, was first reported in 1987 at one location in northern Sao Paulo State, Brazil (15). Presently this devastating disease is present in all citrus growing regions of Brazil, in Argentina and Paraguay (11, 15). This disease is spread by xylem feeding plant hoppers, commonly called sharpshooters. These insects are widespread in Brazil, present in Florida, and throughout the Caribbean. Once a tree is infected with CVC, it is rendered totally non-productive within three years. Coupled with a mite transmitted virus disease, citrus leprosis, a deadly disease complex is formed which has killed thousands of trees in Sao Paulo State, Brazil. The survey conducted by FundeCitrus in 1998 estimated that 29 percent of all citrus trees in Sao Paulo State were infected with CVC (11). The rapidity of spread of CVC, the reduction of yield, and ability to form a disease complex with leprosis which kills trees surely mark CVC as one of the most deadliest citrus diseases known.

The Asian vector of huanglongbing (citrus greening), *Diaphorina citri*, has been present in Brazil for many years. In June 1998, this psyllid was found in four counties in southern Florida (8). To our knowledge, citrus greening does not occur in the Western Hemisphere. However, *D. citri* has been expanding its geographical area, and may be present in several countries of the Caribbean Basin. With the vector already present, the risk is much greater of establishment and spread of citrus greening if the phloem-inhabiting bacterial pathogen should be introduced. In Asia and parts of Africa, citrus greening is considered to be the limiting factor of citrus production (28).

There are additional exotic pests which could have an impact on citrus production in the Caribbean: citrus chlorotic dwarf, caused by a graft-transmissible virus-like pathogen in Turkey which adversely affects many citrus varieties and is vectored by the citrus whitefly (9); witches' broom disease of lime, caused by a phytoplasma, which causes death of acid lime, sweet lime, and some other varieties and is vectored by a leaf hopper (4); and citrus leprosis with a mite vector. This virus causes lesions which enlarge to girdle the trunk resulting in dieback, and fruit with lesions drop prematurely (11).

Similarity of the Caribbean Basin to the Mediterranean

The BrCA has already invaded and become established in most countries of the Caribbean Basin. The scenario of events following (losing trees on sour orange rootstock, finding budwood sources are latently infected with virus-like agents which will affect their productivity on CTV-tolerant rootstocks, increasing severity of CTV isolates) is already occurring. A similar scenario could be repeated in the Mediterranean area. The BrCA was introduced to Maderia Island, west of Portugal and Morocco in the Atlantic, in 1994 (20). Research has indicated the increasing complexity of the CTV strains present on the islands (20). When the BrCA reaches the citrus areas surrounding the Mediterranean, the same scenario could be replayed, country by country. Of the citrus producing countries in the region, only Spain has a mandatory citrus certification program.

Economic losses

What are the economic losses when a farmer plants trees on a CTV-tolerant rootstock, then finds the budwood was contaminated with a virus which affects the productivity of trees on the rootstock he has chosen? Roistacher *et al* (29) calculated this based on Valencia sweet orange affected with a citrus viroid on Rangpur lime rootstock groves in Belize. From an analysis of all costs associated with establishing and production, including initial capital, maintenance, pick and haul, and grove maintenance, the return on the grove planted with virus-free certified budwood was US$ 10,898 per ha/ 220 trees, compared to US$ -5,147 for the viroid infected grove under the same conditions and management.

The costs of living with a chronic decline disease of citrus, such as citrus greening, in Thailand has been reviewed by Roistacher (28). From the interpolation of data, a one hectare citrus grove in Thailand would return a cumulative loss of US$/ha of $8,292 and $3,660 for a grove life expectancy of 6 and 8 years, respectively. A cumulative profit of $3,383 and $125 would be realized if the life expectance of the grove were 10 years and 20 years, respectively. While the exact numbers will vary depending on local costs, this trend would be valid anywhere. The longer the grove remains as a healthy block of trees, the greater the return on the investment. If a chronic disease, such as citrus greening, shortens the life span of the grove, the profitability will be marginal or maybe not realized at all.

Management of CTV

The most important strategy for management of CTV is by the efficient operation of three separate but integrated programs: quarantine, clean stock, and certification (16).

Quarantine programs are for the safe introduction of select horticultural germplasm. Often it is desirable to import citrus species and varieties for commercial or scientific purposes. Uncontrolled importation of germplasm has the risk of introducing new pests and diseases which could cause economic losses. The risk can be minimized by allowing for importation through quarantine stations under controlled conditions. The most common approach is to establish the newly imported material under isolated quarantine conditions. The potential graft-transmissible pathogens are eliminated by shoot tip grafting procedures, followed by a through indexing of the pathogens that are suspect, based on previous indexing of the original imported material. Once the cleaning of the imported germplasm is completed, the germplasm is released to the clean stock program.

Clean stock programs provide for the testing and therapy of local germplasm and the maintenance of pathogen-free propagating stock, while quarantine programs are aimed at preventing the introduction of additional pathogen on imported germplasm. The clean stock program provides for selection of mother trees of local cultivars based on performance records. The selected mother trees are indexed for presence of graft-transmissible pathogens. If such pathogens are found, they are eliminated by shoot tip grafting and/or thermal therapy. The pathogen-free status of the recovered plants are verified by indexing at regular intervals. Horticultural evaluations are made of the healthy plants, propagated from the therapied mother trees. Trueness of type of the fruit and freedom from mutations are verified by the horticultural evaluations. Healthy plants are maintained under protected conditions to provide germplasm to the certification program.

Certification programs provide for the distribution of sanitary, true-to-type propagating materials for use in commercial nurseries. They also control the horticultural quality of nursery plants. Certification programs impose legal regulations governing nursery operations and require periodic indexing and inspection of trees used for nursery propagation. Usually they are operated by a governmental agency having legal authority to impose restrictions and to inspect nurseries, while commercial propagation occurs in private nurseries.

Properly operated quarantine/clean stock/certification programs provide a means for farmers to start with healthy plants of high genetic potential, even in areas where graft-transmissible pathogens having insect vectors are endemic. These programs provide the starting point of an integrated pest management system to enable productivity of citrus. New cultivars and varieties can be efficiently introduced, mild strain cross protection (if used in the area) can be effectively implemented, and widespread dissemination of all graft-transmissible pathogens is avoided.

Mild strain cross protection (MSCP) is the phenomenon which occurs when a plant previously infected with a mild strain of a virus does not display the symptoms of a second, more severe strain of the same virus introduced later into the same plant (14). In areas where severe CTV isolates are endemic, MSCP enable continued production of some cultivars (18). For example, in South Africa all citrus are planted in the field with a mild isolate for MSCP. Initially they used the Nartia mild strain for everything, recently they have selected better mild isolates for MSCP in limes and sweet orange. In Australia, all grapefruit are planted with a cross protecting isolate. Growth of Pera sweet orange in Brazil is possible due to MSCP. While most MSCP mild isolates are chosen for their ability to protect against CTV-SP, in Florida selections have been made to extend tree life on sour orange rootstock (13). Five mild isolates have been demonstrated to be beneficial in extending the tree life on sour orange rootstock in the presence of CTV-D (17). Mature producing trees on sour orange rootstock in Florida have been inoculated with selected mild strains, and the productive life of the trees have been extended. MSCP should be considered for use only as a last attempt to maintain citrus production because severe strains of CTV are causing such great losses.

Selection of mild isolates for use in MSCP can be a long, empirical process. The best starting point is to collect from the horticulturally superior surviving trees in an area where CTV has caused severe losses (21). Because success of MSCP depends on the ability to identify an isolate which will protect against the severe isolates present in an area, it is unlikely that cross protecting mild strains could be identified until severe losses have been incurred due to severe strains of CTV. Recently, we have tried to speed the selection process by utilizing single aphid transmission with the BrCA, coupled by quick challenge treatments using several severe isolates of CTV found in the area, and complemented by the use of molecular probes which provide an estimate of whether or not a given isolate contains mild, CTV-D or CTV-SP strains (21).

Caution should be used with the term mild strain (12). In Florida, the term mild strain suggests a isolate of CTV which does not cause any detectable symptoms on citrus except perhaps on Mexican lime or other CTV-sensitive hosts. However in Brazil, as an example, a mild strain could well be an isolate which contains SY, CTV-D, and even mild to moderate CTV-SP, but compared to severe strains present in Brazil which contain strong SY, CTV-D, and severe CTV-SP often on CTV-tolerant rootstocks such as Rangpur lime, it is mild. If the same isolate was in Florida, it would be considered severe.

Virus Resistance. If research has been done to select mild strains of CTV which protect against the symptom expression of severe strains present in an area, MSCP can be immediately utilized as a part of an integrated pest management strategy to extend tree life and productivity. CTV resistance and tolerance exists in rootstocks which are presently used commercially. However, CTV-SP strains affect the scion and are not controlled by using immune or tolerant rootstocks. In the future, genetically engineered CTV-resistant plants should be ready. Research efforts are underway utilizing parts of the CTV genome to obtain virus resistance, also to identify, isolate, and transform the immunity gene present in *Poncirus trifoliata* into commercially desirable citrus.

Suppression or eradication of CTV can be considered only if the incidence of CTV is very low. Continuous monitoring for presence of CTV and prompt removal of infected trees can keep CTV incidence at very low levels, as witnessed in the Central Valley of California (27). Quarantine, clean stock/ certification programs must be in operation in order suppression/eradication programs to have a chance for success. Suppression/eradication programs are expensive to operate and must be maintained over a long time.

الملخص

لي، ريتشارد. 2000. فيروس التدهور السريع: حياتيته، تأثيراته المحتملة، واستراتيجيات المكافحة. مجلة وقاية النبات العربية. 18: 143-148.

يعدّ مرض التريستيزا، الذي يحدثه فيروس التدهور السريع المرض الأكثر أهمية من الناحية الإقتصادية على أشجار الحمضيات/ الموالح في العالم. وينتشر الفيروس على نحو عالمي، ويتم إدخاله بشكل غير مباشر مع مواد الإكثار. وتسهم عدة أنواع من المنّ في نقل المرض بالطريقة الشبه باقية، ويعدّ المنّ البني للحمضيات *Toxoptera citricida* أكثر الأنواع كفاءة في نقل المرض. ويحدث المرض أعراضاً متعددة بما في ذلك موت الأشجار المطعمة على النارنج وتنقر الساق في الطعوم بغض النظر عن الأصول. وقد تسبب المرض في موت ملايين الأشجار المطعمة على النارنج في أمريكا الجنوبية وحوض الكاريبي إثر دخول المن البني الناقل وانتشاره مع الفيروس فيما بعد. وتحدث إصابات وبائية بالمرض في عدة دول كاريبية. ومن المعلوم أن النارنج هو الأصل المفضل في المناطق التي لا ينتشر فيها الفيروس، لتحمله العالي للأتربة الكلسية، وممرضات التربة، وإنتاج ثمار عالية النوعية، ولتحمله أيضاً لمجموعة من فيروسات/ فايروئيدات أخرى تحدّ من إنتاجية الأشجار. وسيناقش الباحث تأثير الإنتشار الحديث للمنّ البني في حوض الكاريبي واستراتيجيات مكافحة مرض التدهور السريع.

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