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Citrus Tristeza Virus: Events That Occur Before, During and After the Disease Epidemics

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ABSTRACT

Citrus tristeza virus (CTV) is a phloem-limited aphid-transmitted pathogen that causes one of the most economically important diseases of citrus worldwide. CTV occurs in practically all citrus areas of the world and its most dramatic effect is on scion trees grafted on sour orange (*Citrus aurantium*) rootstock. This article analyzes the current situation of the citrus industries of the Caribbean islands, Central America, Mexico, and United States because of the predominance of sour orange as a rootstock in these areas; the occurrence of CTV, the establishment of its most efficient vector, the brown citrus aphid, *Toxoptera citricida*, in the Caribbean Basin area, and the lack of adequate certification programs in all the countries of the region. Emphasis is given to the series of events that commonly happen in regard to the occurrence of epidemics of "tristeza", on the basis of the experiences in countries where "tristeza" epidemics have occurred. Knowledge of these experiences provides better anticipation of what to expect as the virus moves into new areas, and should help to avoid of delaying the implementation of timely actions because of the lack of adequate knowledge of the disease.

RESUMEN

El virus tristeza de los cítricos (CTV) es un patógeno restringido al floema y transmitido por áfidos, que causa una de las enfermedades económicamente más importantes de los cítricos en el mundo. El CTV está presente en prácticamente todas las áreas citrícolas del mundo y el efecto más dramático ocurre en árboles injertados en patrón de naranjo agrio (*Citrus aurantium*). El presente artículo analiza la situación actual de la citricultura de las Islas del Caribe, Centro América, México, y los Estados Unidos de América, debido al uso generalizado del naranjo agrio como principal portainjerto en estas áreas; la ocurrencia del CTV, el establecimiento de su insecto vector más eficiente, el áfido pardo, *Toxoptera citricida*, en la Cuenca del Caribe, y en la ausencia de programas adecuados de certificación en la mayoría de los paises de la región. Se hace énfasis en la serie de eventos que suceden relacionados con la ocurrencia de "tristeza" basados en las experiencias en paises donde han ocurrido epifitias de "tristeza". El conocimiento de estas experiencias son aleccionadoras y proporcionan una mejor anticipación de lo que puede pasar a medida que el virus ingresa en áreas nuevas, así como para evitar el retraso en la implementación de acciones oportunas debido a la falta de conocimiento de la enfermedad.

Additional index words: Toxoptera citricida, Aphis gossypii, ELISA, monoclonal antibodies, citrus virus diseases, citrus rootstocks, budwood certification programs

"Tristeza" disease is distributed worldwide and is the most economically important viral disease of citrus (Bar-Joseph et al. 1989; Lee and Rocha-Peña, 1992; Lee et al. 1994; Rocha-Peña et al. 1995). "Tristeza" killed more than 40 million trees grafted on sour orange (*Citrus aurantium* L.) between 1930 and 1980 in Argentina, Uruguay, and Brazil in South America; California and Florida in the United States; and Israel and Spain in the Mediterranean area (Lee et al. 1992; Rocha-Peña et al. 1995). Since 1980 "tristeza" has additionally killed over 6 million trees in Venezuela (Mendt, 1992; Ochoa et al. 1994), and caused severe unestimated losses in Colombia, Peru (Roistacher, 1988; Roistacher et al. 1991) and Florida (Brlansky et al. 1986).

Since 1990, there have been increasing reports of new incidences of "tristeza" in Central America and the Caribbean Basin area (Lastra et al. 1991a, 1991b, 1991c; Yokomi et al.

1994). At some specific locations, very severe CTV strains causing tree deterioration on plants grafted on CTV tolerant rootstocks have occurred (Ochoa and Trujillo, 1991; Roistacher, 1988). This situation, along with the well-documented northward movement of the brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy), in this Caribbean Basin area (Yokomi et al. 1994), creates great concern for the future of the citrus industries of this area in regard to "tristeza".

This article analyzes the current situation of the citrus industries of the Caribbean islands, Central America, Mexico, and United States because of: 1) the predominance of sour orange as a rootstock in these areas; 2) the prevalence of citrus tristeza virus in these areas; 3) the establishment of the BrCA in most Caribbean islands, southern and northern parts of Central America, and the state of Florida in the mainland of USA; and, 4) lack of adequate certification programs in the past in most of these citrus areas of the region resulting in untold losses due to viroids and viruses. Major emphasis is given to the chain of events happening with the occurrence of epidemics of "tristeza" on the basis of the experiences and events that took place in countries where "tristeza" epidemics have occurred. Knowledge of these experiences provides better anticipation of what to expect as the virus moves into new citrus areas, and should help in the implementation of timely actions for a proper management of the disease.

Citrus Tristeza Virus

"Tristeza" disease is caused by citrus tristeza closterovirus (CTV). The virus infects nearly all *Citrus* species, varieties, and intergeneric hybrids and some citrus relatives (Bar-Joseph and Lee, 1989; Garnsey and Müller, 1988). The different types of CTV strains, according to the syndromes they cause in the citrus hosts and scion/rootstock combinations, may be classified into four major groups (Bar-Joseph et al. 1989; Garnsey et al. 1987): 1) Decline inducing strains (CTV-D), cause decline and death of sweet orange {*Citrus sinensis* (L.) Osbeck}, grapefruit (*C. paradisi* Macf.), and mandarin (*C. reticulata* Blanco) plants on sour orange or alemow (*Citrus macrophylla* Webster) rootstocks. However, plants grafted on some other decline tolerant rootstocks, such as Cleopatra mandarin (*Citrus reshni* Hort. ex Tanaka), rough lemon (*C. jambhiri* Lush.), Volkamer lemon (*C. volkameriana* Pascuale), Rangpur lime (*C. limonia* Osbeck), trifoliate orange {*Poncirus trifoliata* (L.) Raf.}, citranges (*C. sinensis* x *P. trifoliata*), citrumelos (*C. paradisi* x *P. trifoliata*), etc., are normally symptomless when infected with CTV-D strains. The term "CTV tolerant", as most of the latter rootstocks are regarded, indicates they do not decline upon infection by CTV-D strains. 2) Stem pitting strains (CTV-SP) produce pitting or channels in the wood of some oranges, grapefruit or Tahiti lime trees, usually seen by removing the bark of branches or trunk of affected trees. The pitting on the wood may be produced either in the rootstock below the bud union, as occurs frequently with Rangpur lime, rough lemon or Volkamer lemon or above the bud union in the tree scion. Most CTV decline tolerant rootstocks such as citranges, citrumelos or even trifoliate orange also can be pitted by severe CTV-SP strains (Dawson and Mooney, 1998; C.N. Roistacher, unpublished). Affected trees usually do not die. A loss of plant vigor with resulting severe yield reduction results in economic losses upon infection by CTV-SP strains, even when trees are on CTV decline tolerant rootstocks. Some CTV-SP strains can debilitate an industry as occurred with navel oranges on CTV decline tolerant rootstocks in Peru (Roistacher, 1988) and Venezuela (Ochoa et al. 1994). 3) Seedling yellows strains (CTV-SY) cause a chlorotic or yellowing reaction in seedlings of sour orange, acid lemons {*Citrus limon* (L.) Burm. f}, and grapefruit. The seedling yellows reaction mostly occurs in the greenhouse in indexing, but can be a field problem when CTV-SY infected trees are topworked with sensitive cultivars. Most CTV-SY strains are usually but not always associated with CTV decline and stem pitting symptoms. Most CTV-D, CTV-SP and CTV-SY strains cause a severe decline of Mexican lime {*C. aurantifolia* (Christm.) Swingle} regardless of the rootstock. 4) There are some CTV strains, so called "mild" (CTV-M) that do not induce conspicuous symptoms in any citrus host, including Mexican lime plants, which is the most sensitive host for CTV (Bar-Joseph and Lee, 1989; Garnsey and Müller, 1988; Garnsey et al. 1987; Rocha-Peña et al. 1995). The above classification of all CTV isolates into four general categories describes the variability present in CTV (Bar-Joseph and Lee, 1989; Garnsey et al. 1987); however, as most of individual CTV strains may induce two or more of the above disease syndromes in different citrus hosts, a much detailed classification system is available (Lee et al. 1994; Rocha-Peña et al. 1995).

Virus properties. Citrus tristeza virus is a phloem-limited, flexuous closterovirus approximately 2,000 x 11 nm in size, transmitted by aphids in a semi-persistent manner. A single stranded positive sense RNA of 5.4-6.5 x 10^6 daltons has been isolated from purified virus preparations (Bar-Joseph and Lee, 1989). The virus is encapsidated by two structural proteins, a major coat protein of about 25,000 kDa (Sekiya et al. 1991) and a diverged coat protein of about 27 kDa (Febres et al. 1994). The latter is present at one end of the virus particle and covers about 4% of the entire virion (Febres et al. 1996). In infected tissue apparent proteolysis frequently produces coat proteins with apparent molecular weights of 23,000 (CP1) and 21,000 (CP2) (Lee et al. 1988a; Pappu et al. 1993). The entire genome of the CTV RNA has been sequenced (Karasev et al. 1995).

The virus is readily transmitted by budding and grafting (Bar-Joseph and Lee, 1989). Because CTV is restricted to phloem tissue, mechanical transmission has been accomplished with difficulty after slash inoculation of partially purified virus preparations into the stem of hosts such as citron (*Citrus medica* L.) and Mexican lime (Garnsey and Muller, 1988). Seed transmission has not been reported (Bar-Joseph and Lee, 1989).

Movement of CTV by man and vectors. The initial spread of CTV from field to field and to different geographical areas occurs via the use of infected propagative budwood sources. Subsequent dissemination in a given location is done by several aphid species, in a semi-persistent manner (Bar-Joseph and Lee, 1989; Lee and Rocha-Peña, 1992; Raccah et al. 1989; Roistacher and Bar-Joseph, 1987). The most efficient

vector for CTV is the BrCA, *T. citricida* (Yokomi et al. 1994). However, in several citrus-growing regions in the world, where *T. citricida* is still not present, *Aphis gossypii* Glover can also efficiently transmit some CTV strains (Bar-Joseph and Loebenstein, 1973; Hermoso de Mendoza et al. 1984; Roistacher et al. 1980; Yokomi and Garnsey, 1987). Other aphid species reported as CTV vectors, but with a low degree of efficiency include: *A. spiraecola* Patch (formerly *A. citricola* van der Goot) (Yokomi and Garnsey, 1987), *T. aurantii* Boyer de Fonscolombe, *A. craccivora* Koch and *Dactynotus jacae* L. (Roistacher and Bar-Joseph, 1987). *A. spiraecola* may be an important vector in some instances because of the high populations which occur (Yokomi and Oldfield, 1991).

Importance of sour orange as a rootstock in the citrus industries of the Caribbean Islands, Central America, Mexico and the United States

Sour orange has been an important rootstock for many diverse scions in most citrus areas due to its tolerance to *Phytophthora* foot root, good tree growth, excellent fruit quality, and good performance in different soil types (Castle et al. 1993; Forner-Valero, 1985; Rocha-Peña and Padrón-Chávez, 1992). Additionally, trees on sour orange rootstock are symptomless when infected by tatterleaf virus and viroid diseases such as exocortis and cachexia; have field tolerance to citrus blight, and are more cold tolerant than vigorous rootstocks, a valuable trait for Northern Mexico, Texas, Florida and California (Castle et al. 1993; Derrick et al. 1990a). If it were not for the hypersensitivity of sour orange to CTV-D strains, it would be a near perfect rootstock (Derrick et al. 1990a; Rocha-Peña and Padrón-Chávez, 1992).

Citrus is an increasingly important crop with expansion and demand for local consumption and production for export as fresh fruit and/or juice concentrate in most countries in the Caribbean islands and Central America. Citrus species and varieties in these areas are predominantly grown on sour orange rootstock, making these industries vulnerable to CTV and BrCA.

Much of the citrus in the Caribbean islands and Central America is less than 10 years old, and it is estimated that the hectareage will double or triple in the next decade. In Mexico, more than 400,000 hectares of citrus are distributed throughout the country with almost 90% on sour orange rootstock. Likewise, about one third of the 340,000 hectares of the Florida citrus industry still consisting of trees on sour orange rootstock. Most of the citrus industry of Texas of about 12,000 hectares and one third part of the 18,000 hectares in Arizona are on sour orange rootstock (Lastra et al. 1992; Yokomi et al. 1994). The citrus industries *en masse* of the Caribbean islands, Central America, Mexico, and United States, represent almost a million hectares of citrus with about 60 percent planted on sour orange rootstock.

Events that occur before, during, and after CTV epidemics

We describe here a chain of events experienced by citrus industries before, during, and after CTV epidemics. This information is based on experiences in Venezuela (Lee et al. 1994; Ochoa et al. 1994; Rocha-Peña et al. 1995), Spain (Moreno, 1992), Israel (Bar-Joseph et al. 1989), Brazil (Müller and Costa, 1992), and current events in Central America, Caribbean islands, and Mexico. These experiences are summarized to call attention to considerations needed in countries where CTV epidemics have not occurred yet, but are threatened by the invasion of the BrCA.

Conditions preceding CTV epidemics. Quite commonly the citrus industry is predominantly on sour orange if CTV has not caused major tree losses to date. This is because of the desirable properties of sour orange mentioned earlier. Use of sour orange as a rootstock hides the presence of potential problems which may seriously affect citrus production upon introduction of other rootstocks. In addition to common root pathogens, certain viroids and viruses are usually symptomless in sweet or grapefruit on sour orange. These are tatterleaf and vein enation viruses, and exocortis, cachexia and other citrus viroids. These diseases are commonly present and not recognized because of the lack of certification schemes where regular indexing would indicate the presence of viruses and viroids.

The continuous use of sour orange rootstock as a "monoculture" makes a citrus industry totally vulnerable to CTV. Commonly there is a lack of experience and information on the performance of alternative CTV tolerant rootstocks, their management requirements and susceptibility to common fungal and virus/viroid diseases (Rocha-Peña and Padrón-Chávez, 1992). Most citrus growers and nurserymen are skeptical about the threat of "tristeza" and are hesitant to alter current practices. Governmental officials are hesitant to take any direct action to prepare for CTV epidemics, mostly because of lack of funds or adequate public awareness of the problems and the resulting lower priorities.

It is well documented that CTV may be present for a long period of time in a latent or symptomless condition even on trees grafted on sour orange rootstock (Lastra et al. 1991b; Yokomi et al. 1994). In these situations, the detection of CTV infected trees may be made by using serological methods (Lastra et al. 1991b; Rocha-Peña and Lee, 1991) or by greenhouse indexing (Knorr et al. 1960; Roistacher, 1991, 1993). Nevertheless even with the positive detection of severe CTV strains in infected trees, the absence of visible disease field symptoms causes complacency among citrus growers and/or government officials who fail to acknowledge the presence and potential threat of CTV (Bar-Joseph et al. 1989). When CTV is present without dead trees to raise the level of concern, the credibility of CTV diagnosis by laboratory and/or greenhouse methods is often questioned. This situation is analogous to testing for the presence of human immunodeficiency virus (HIV), which may be present for several years before symptoms of AIDS appear.

When surveys for CTV detection indicate the presence of CTV infected trees in the field, eradication is one action which is usually considered. While in certain circumstances removal of infected trees is an appropriate decision, ultimately such actions are unpopular and rejected by citrus growers and citrus growers organizations (Bar-Joseph et al. 1989), especially if trees are still productive. This frequently causes antagonistic situations between the citrus growers, government, and

researchers, with the result of long costly delays or moratorium in implementing actions to protect the citrus industry against CTV epidemics (Bar-Joseph et al. 1989; Dodds et al., 1996; Ochoa et al. 1994).

Conscientious citrus growers and/or citrus grower organizations can prepare for CTV either by attending (FCM, 1993; Lastra et al. 1992; Lee et al. 1995) or organizing (Lee and Niblett 1994) scientific meetings, as well as visiting citrus industries of countries, such as Venezuela, Brazil, Argentina, Spain, etc. (Lastra et al. 1992; URCT, 1994; Yokomi et al. 1994), that have experienced CTV related problems. Citrus growers may establish trials to evaluate new rootstocks themselves, as well as to conduct surveys for CTV detection in their groves (Rocha-Peña, M.A., personal observations). Production and distribution of well illustrated and informative brochures and popular articles (Lee et al. 1992; Rocha-Peña et al. 1992; Rocha-Peña and Padrón-Chávez, 1992; Roistacher et al. 1991) has been another action commonly taken.

In some instances, actions such as the use of inarching may be considered and conducted by citrus growers as a measure to protect adult trees grafted on sour orange rootstock. These actions were observed during the "tristeza" epidemics in Brazil in the 1960s (Müller, G.W., personal communication), Venezuela in the 1980s (Ochoa et al. 1994), and have been observed in some parts of Mexico (Rocha-Peña, M.A., personal observations) and Belize (Lee, R.F., personal observations) in anticipation to the occurrence of the epidemics. While the inarching may be a useful measure to protect from decline some citrus trees of valuable germplasm collections, its use at a large scale seems to be expensive and impractical; likewise, the probable presence of latent infections of viroids and viruses on the receptor trees, may affect the performance and growth of the rootstocks to be inarched (Ochoa et al. 1994).

Events during CTV epidemics. Severe CTV-D and CTV-SP strains may be present in a latent condition for decades even when the majority of the industry is on sour orange rootstock (Lastra et al. 1991b; Lee et al. 1995; Yokomi et al. 1994). When *T. citricida* is not present, CTV strains usually start to be disseminated by the local aphid populations, mostly *A. gossypii*, after a prolonged lag period of 30-50 years (Bar-Joseph, 1978), then CTV epidemics may suddenly occur. This happened in California, Florida, Spain and Israel during the 1950s to 1970s (Bar-Joseph, 1978; Bar-Joseph and Loebenstain, 1973; Bar-Joseph et al. 1989; Lee et al. 1992; Rocha-Peña et al. 1995; Roistacher et al. 1991). By contrast, when *T. citricida* invades a country, the dissemination of latent severe CTV strains commonly occurs more rapidly, and CTV epidemics may destroy the citrus industry established on sour orange rootstock in a short period of 5 to 10 years. This happened in Argentina/Brazil in the 1940s (Costa, 1956) and Venezuela (Geraud, 1992; Mendt, 1992; Ochoa et al. 1994) and Peru in the 1980s (Roistacher, 1988).

When tree deaths first appear, there is a period of uncertainty for citrus growers, government officials, and some researchers, as the origin of the problem is researched and the possible solutions are discussed. Promptness in making proper decisions to deal with the problem, obtaining sufficient funds, and unified action by both government officials and citrus growers will lead to success to properly minimize the damage by CTV, whereas delays and procrastination will lead to failure. Survey campaigns for CTV detection, along with the removal of CTV-infected trees (especially those infected by severe strains) may help in the temporary management of the disease and may prolong use of sour orange as a rootstock (Bar-Joseph et al. 1989). Unfortunately, these measures are expensive and often citrus growers are not properly informed as to the problem and benefits of these actions. As a result the measures lack popular appeal, and ultimately CTV-D strains become endemic after several years. After sour orange rootstock can no longer be used due to risk of loss due to CTV-D strains, CTV tolerant rootstocks are used for re-planting, as has happened in Spain and Israel where *T. citricida* is not present (Bar-Joseph et al. 1989; Cambra et al. 1990; Moreno, 1992). When *T. citricida* is the primary aphid responsible of the CTV spread, the chances of stopping or timely management CTV epidemics are much lower, as was experienced in Argentina/Brazil in the 1940s (Costa, 1956), Venezuela (Geraud, 1992; Mendt, 1992; Ochoa et al. 1994) and Peru (Roistacher, 1988) in the 1980s, and currently underway CTV epidemics in Puerto Rico, Panama, Jamaica, Haiti, and Dominican Republic (Lee, R.F., personal observations).

When *T. citricida* is the primary vector of CTV spread, the most common management action is the removal of dead trees and the use of CTV tolerant rootstocks for re-planting. Information on the performance of CTV tolerant rootstocks with different scions in local soil types is usually lacking. Sufficient clean budwood for the desired scions and seed of CTV tolerant rootstocks are not available. Because of the urgency to recover the citrus industry, citrus growers use non-indexed and infected budwood sources and choose rootstocks on the basis of seed availability and performance generated from ecologically different areas. The result is a virus/viroid-infected citrus industry, which only becomes apparent 5 to 10 years later. All these events have been observed in Venezuela since 1980 (Ochoa et al. 1994). The dramatic effect of the use of viroid infected budwood sources on susceptible trifoliated rootstocks has been recently documented in Belize (Roistacher et al. 1996).

Events proceeding CTV epidemics. Historically, epidemics of CTV which result in the loss of sour orange as a rootstock, are followed by the obligatory use of CTV tolerant rootstocks to re-establish the citrus industry. Several problems then develop. Selection of CTV decline tolerant rootstocks without information as to their performance to local soil types and climatic conditions result in either poor grove performance and/or occurrence of *Phytophthora* root rot, especially in rootstocks such as Cleopatra mandarin, rough lemon, Volkamer lemon, and Rangpur lime. Exocortis and cachexia viroids, and probably other graft transmissible diseases such as citrange stunt, caused by citrus tatter leaf virus, causes dwarfing and decline of trees on *P. trifoliata* and its hybrid rootstocks; and woody gall, caused by citrus vein enation virus, cause low vigor and decline on trees on rough lemon, Volkamer lemon and related rootstocks. Concave gum, cristacortis, psorosis, and impietratura may be present in budwood illegally imported to

meet the demand for increased nursery propagations.

Inevitably, severe CTV-SP strains occur, probably from undetected infected sources or illegally imported budwood. The appearance of CTV-SP strains occurs relatively soon when *T. citricida* is the primary vector, whereas many years may pass before they appear when *T. citricida* is not present. The Capao Bonito CTV strain which occurred in Brazil after the CTV epidemics in the 1940s is the first example of a CTV strain causing stem pitting on a rootstock considered to be CTV tolerant, Rangpur lime rootstock (Müller and Costa, 1992; Müller et al. 1968). Severe CTV-SP strains on citrumelo rootstock occurred in Peru in the 1980s (Roistacher, 1988) and more recently, the Macapo and other severe CTV-SP strains have appeared in Venezuela (Ochoa et al. 1994; Ochoa and Trujillo, 1991). While the impact of CTV-D strains may be efficiently avoided by using CTV decline tolerant rootstocks, the only way to minimize the impact of CTV-SP strains is by mild strain cross protection (Bar-Joseph et al. 1989; Lee et al. 1994; Müller et al. 1988) or widespread planting of CTV-SP tolerant varieties, such as mandarins, which is a practical measure but not always an economically appropriate decision. The use of mild strain cross protection (Lee et al. 1994; Lee and Rocha-Peña, 1992), as a control strategy to reduce losses due to CTV-SP strains, has been applied successfully commercially to Pera sweet orange in Brazil (Müller et al. 1988), and to grapefruit in South Africa (Lee et al. 1995; Van Vureen, 1995) and Australia (Broadbent et al. 1991), and in Navel orange in Japan (Ieki, 1997).

Another consequence of the substitution of sour orange by CTV tolerant rootstocks after CTV-D epidemics is the appearance of citrus blight, a chronic decline of unknown etiology with an unknown means of spread which causes significant tree losses in many countries. Most CTV tolerant rootstocks are susceptible to citrus blight. More than 500,000 trees are removed from production annually because of citrus blight in Florida (Lee et al. 1988b). "Declinio dos citros", the Brazilian name for blight, appeared in Brazil in 1975 and by 1987 had removed more than 5 million trees from production (Tubelis et al. 1991). In Venezuela, citrus blight called "Decaimiento repentino" appeared after CTV tolerant rootstocks were used to re-establish the citrus industry after the CTV epidemics in 1980 (Ochoa et al. 1994; Ochoa et al. 1987). All the blight diseases, whether from Florida, Brazil or Venezuela (Ochoa et al. 1993), have the same diagnostic criteria including the consistent association of a 12 KDa protein present in blight affected plants (Derrick et al. 1990b), presence of amorphous plugs in the xylem (Brlansky et al. 1984), reduced water uptake in the trunk (Lee et al. 1984), and an accumulation of zinc in the trunk wood (Wutscher et al. 1977).

Following an epidemic of CTV-D losses, the industry often re-establishes on one or two types of rootstocks per plantation. As examples, Venezuela currently has 40 percent Volkamer lemon and 50 percent Cleopatra mandarin (Mendt, 1992); Brazil after the CTV-D epidemic in the 1940s used predominantly Rangpur lime and more recently sweet orange as rootstocks (Müller and Costa, 1992). Widespread use of Volkamer lemon led to "Decaimiento repentino" in Venezuela, with a high occurrence of declining and dead trees in recent years (Ochoa et al. 1994). In Brazil the establishment of nucellar sources in the 1950s and 1960s reduced the occurrence of diseases caused by viroids and other graft-transmissible agents, but these diseases and other graft transmissible diseases have been reintroduced. Rangpur lime and sweet orange rootstocks are susceptible to *Phytophthora*, and viroid-infected budlines on Rangpur lime rootstock result in less vigorous trees (Castle et al. 1993; Forner-Valero, 1985; Rocha-Peña and Padrón-Chávez, 1992).

When CTV-SP epidemics begin, citrus growers and/or farm advisers may attempt to recover affected trees by applying higher dosage of nitrogen fertilizations. This activity, far from solving the problem, will cause additional and unnecessary expenses to the citrus growers. This may happen because of the lack of knowledge about the viral nature of the disease and detrimental effects of CTV-SP strains in affected trees (Ochoa, F.M., personal observations).

Summary and Outlook

The citrus industries of the Caribbean islands, Central America, Mexico and the United States are at immediate risk because of the CTV/*T. citricida* complex. The immediate risk is due to the predominance of sour orange as a rootstock and the lack of budwood certification programs through most parts of this geographical area. Surveys for CTV in Central America and the Caribbean islands (Lastra et al. 1991b, 1991c; Lee et al. 1992; Yokomi et al. 1994) indicates long term use of non-indexed and infected budwood in many citrus areas.

Since 1995, considerable efforts have been made to implement individual citrus budwood certification programs in Florida (Rucks, 1995), Texas (Skaria et al. 1997), Mexico (SAGAR, 1998), Belize (Reddy et al. 1998), and Jamaica (Lee, R.F., unpublished, 1996); likewise, endeavours to locate a citrus clean stock germplasm distribution center, as well as a diagnostic laboratory center at regional level in the Caribbean area have long been pursued before FAO headquarters (Lee, R.F., unpublished, 1997).

The use of ELISA with strain specific monoclonal antibodies (Lastra et al. 1991b; Yokomi et al. 1994) has been a useful tool for the detection of severe CTV (mostly CTV-D and CTV-SP) strains in the Caribbean area (Lastra et al. 1991b, 1991c; 1992; Yokomi et al. 1994), but the lack of actual dead trees yet in many of these areas, makes it easy to ignore the urgent need to take action before the actual epidemic starts (Lee et al. 1995; Lee, R.F., personal observations).

In addition to the development of the MCA-13 monoclonal antibody (Permar et al. 1990), there have been other research achievements for the specific and rapid detection of severe CTV (CTV-D and CTV-SP) strains (Lee et al. 1996), such as specific nucleic acid amplification (Cevik et al. 1996a). Likewise, several methods for the specific discrimination of either CTV-M, CTV-D and CTV-SP strains have been developed by molecular hybridization with strain specific synthetic DNA probes (Cevik et al. 1996b), and by single stranded conformational polymorphism (Febres et al. 1995). Recently, a strain specific serological assay for CTV-SP on sweet orange has been reported (Nikolaeva et al. 1998). More research is still needed in this area to improve these methods

and eventually incorporate them into the integrated management disease programs.

Implementation of mandatory budwood certification programs takes time, action plans to deal with CTV-D and CTV-SP strains need to be considered and developed, the public needs to be educated, the presence of other graft-transmissible diseases needs to be determined, and trials with CTV decline tolerant rootstocks need to be established prior to the "tristeza"/*T. citricida* epidemics. The rapid movement and establishment of the BrCA in Panama, Costa Rica and Nicaragua in Central America, in most of the Caribbean islands, as well as the predicted movement into new areas (Lastra et al. 1992; Rocha-Peña et al. 1995; Yokomi et al. 1994) highlights the urgency of this situation. From the Venezuelan experience (Geraud, 1992; Mendt, 1992; Ochoa et al. 1994) the occurrence of CTV epidemics started within a period of 4-5 years after the BrCA invaded and colonized the citrus industry as no advance actions were taken in anticipation of the problem. Once sour orange was eliminated as a rootstock, the citrus industry replanted on CTV decline tolerant rootstocks only to have CTV-SP epidemics begin in another 5-10 years. More recently CTV-SP strains have occurred which severely stem pitted Volkamer lemon rootstocks further limiting the recovery of the citrus industry (Ochoa et al. 1994).

Problems with exocortis, cachexia, other citrus viroids, tatter leaf virus, woody gall and *Phytophthora* root rot are not apparent as long as sour orange is the predominant rootstock in a citrus industry (Derrick et al. 1990a; Rocha-Peña and Padrón-Chávez, 1992). These pathogens are symptomless on sweet orange and grapefruit on sour orange rootstock. Use of CTV decline tolerant rootstocks will quickly reveal the presence of many serious diseases: Troyer and Carrizo citranges (susceptible to exocortis, other citrus viroids and tatterleaf virus), Cleopatra mandarin (susceptible to cachexia), Rangpur lime (susceptible to exocortis), and rough lemon-type rootstocks (susceptible to woody gall). All rootstocks, except Cleopatra mandarin and sweet orange are susceptible to citrus blight. Cleopatra mandarin, Rangpur lime, Rough lemon, and Volkamer lemon are sensitive to *Phytophthora* in heavy soils with poor drainage systems (Castle et al. 1993; Forner-Valero, 1985; Rocha-Peña and Padrón-Chávez, 1992). The lack of certification programs and lack of virus-free bud sources encourages the illegally importation of budwood which may harbor most of these viruses and viroids (Roistacher, 1993).

The prosperous future of the citrus industries of Central America, the Caribbean islands, Mexico, and the United States in regard to CTV depends upon the understanding of the problem by citrus growers, nurserymen, farm advisers, and government officials, and their willingness to implement direct actions, specifically certification programs to minimize the economic impact of CTV epidemics. The current scenarios of the citrus industries of the American Continent with regard to the occurrence of CTV and prevalence of aphid vectors are summarized in Table 1.

The key points to prepare a citrus industry with regard to the possible occurrence of CTV and arrival of the BrCA are summarized. Each citrus area needs to be aware of its particular situations and use actions as appropriate for its

Table 1. Scenarios of citrus tristeza virus (CTV) occurrence in the major citrus areas of the Western Hemisphere according to the prevalence of aphid vectors[1].

Scenario	Cirtus Area	Predominant Roostocks	CTV Occurrence	Primary Vector
A	South America	*Poncirus trifoliata*, Citranges, Cleopatra mandarin Volkamer lemon, Rangpur lime, etc.	CTV endemic, stem pitting strains commonly found	*Toxoptera citricida*
B	Haiti, Puerto Rico, Dominican Republic, Jamaica, Panama	Sour orange[2]	CTV present as symptomless infections, outbreaks occurring (1995-1998) 3-10 years following introduction of *T. citricida*	*Toxoptera citricida*
C	Most Carribean Islands; El Salvador, Cuba, Costa Rica Belize, Nicaragua[3]	Sour orange[2]	CTV present as symptomless infections	*Toxoptera citricida* and probably *Aphis gossypi* and *A. spiraeecola*
D	Florida	Rough lemon, citranges, citrumelos, sour orange, etc.	CTV endemic, recurrent outbreaks in plants on sour ornage rootstock since 1975	*Aphis gossypii* and probably *A. spiraeecola* and *Toxoptera citricida*
E	Mexico, Texas	Sour orange[2]	CTV present as symptomless infections	*Aphis gossypii, Aphis spiraecola*
F	California, Southern	Citranges	CTV endemic, decline strains prevalent	*Aphis gossypii*
G	California, Central Valley	Citranges and sour orange	CTV incidence low due to ongoing suppression program	*Aphis gossypii Aphis spiraecola*

[1]Sources: Lastra et al., 1991a, 1991b, 1991c; 1992; Hardy 1995; Lee et al., 1994, 1995; Reddy et al. 1997; Lee, R.F. personal observations, 1995-1998.

[2]Additional CTV tolerant rootstocks (e.g. citranges, citrumelos, Cleopatra mandarin, Volkamer lemon, etc.), are being commonly used for new plantations.

[3]Some disease symptoms have been observed before the appearance of *T. citricida* in 1996 (Lee et al. 1994).

special needs and circumstances.

1) Establishment of citrus budwood certification programs in each country of this geographical area. These programs must be mandatory and strictly enforced by a regulatory agency either at state or country level. Experiences from Venezuela, Brazil and Florida show that voluntary certification programs become ineffective after a few years (Lee and Rocha-Peña, 1992; Roistacher, 1993). The models of the Citrus Improvement and Certification Program of Spain (Navarro et al. 1988) and the South African Citrus Improvement Programme (Lee et al. 1992) which are mandatory are suggested as schemes to observe the components to be included in well managed effective budwood citrus certification programs.

The continued propagation on sour orange rootstock in nurseries and their use in citrus groves despite the absence of visible disease symptoms in the field should de discouraged. In Spain, this was prohibited by law (Moreno, 1992).

2) Campaigns for CTV detection and removal of inoculum sources. Survey campaigns should be conducted in each citrus area to estimate the extent of the CTV occurrence and the presence and prevalence of CTV-D and CTV-SP strains. This information provides the basis to determine the feasibility to reduce CTV inoculum sources. The thresholds of CTV occurrence to impose tree removal campaigns as well as the areas subjected to the program must be evaluated and clearly stipulated in advance of the beginning of the work. All the decisions in this regard (*i.e.* surveys for CTV detection and removal of infected trees) must be carefully analyzed and taken jointly by government, growers, and scientific personnel, and must have adequate and permanent technical and financial support (Bar-Joseph et al. 1989). The comprehension and conscientious assimilation of the CTV situation by citrus growers and nurserymen determines their willingness to actively participate. The establishment of tree removal campaigns without strict regulations, sufficient funds, and grower participation will inevitably lead to failure.

3) Regular surveys for the detection of the brown citrus aphid should be implemented in those countries of Central America where there are still no records of the presence of the BrCA (e.g. Guatemala, El Salvador), Mexico and United States (mostly Texas) in order to determine its first appearance in a citrus area and to accelerate the campaigns against it and the occurrence of CTV epidemics.

4) The substitution of sour orange by "CTV decline tolerant" rootstocks and mandatory use of virus/viroid free budwood to minimize the impact of future CTV-D epidemics should be evaluated. The following considerations are listed: A) Careful selection of the most appropriate rootstocks according to specific soil properties, local climatic conditions, presence of soil-borne pathogens, as well as the scions/rootstock combinations. There are guides for the selection of the rootstocks (Castle et al. 1993; Forner-Valero, 1985; Rocha-Peña and Padrón-Chávez, 1992). It must be emphasized, however, that each grove must be carefully and individually analyzed before a rootstock selection is made. B) Use diversified rootstocks in citrus groves. Most rootstocks other than sour orange have specific properties and/or limitations in management, performance, susceptibility to different pathogens (virus, viroids, *Phytophthora*, nematodes, etc.), soil conditions and climatic situations (Castle et al. 1993; Forner-Valero, 1985; Rocha-Peña and Padrón-Chávez, 1992). These differences in rootstock performance may be compensated for by using at least two or three different rootstocks for each plantation. C) Virus/viroid free budwood must be available for propagation on the alternative rootstocks. If sufficient clean budwood is not available, consideration could be given to importation from programs that have virus/viroid tested budwood sources, such as Spain (Navarro et al. 1988) and Cuba (IICF, 1992). Clean budwood must be used to establish increase or multiplication blocks which are then used for large scale propagation of nursery plants. D) Learn the management and expected performance of alternative rootstocks under specific soil and climatic conditions in both nurseries and groves. The widespread use of sour orange rootstocks in most citrus industries that have not yet experienced CTV epidemics has not allowed familiarity with the specific management and performance of other rootstocks under local soil and climatic conditions (Rocha-Peña and Padrón-Chávez, 1992).

5) Education campaigns are needed to advise citrus growers, nurserymen, farm advisers, and government officials about the seriousness of the threat that the CTV/BrCA complex poses. There have been some scientific meetings (FCM, 1993; Lastra et al. 1991a, 1992; Lee and Niblett, 1994; Lee et al. 1995) held in Costa Rica, Venezuela, Florida, and Mexico, which have been specifically devoted to this topic and the threat it represents to the citrus industries of Central America, the Caribbean islands, Mexico and United States. These meetings have been attended by nearly a thousand persons from 30 different countries. Some of the meetings have been organized and sponsored entirely by citrus grower organizations (Lee and Niblett, 1994). Numerous publications, many fully illustrated, have been published in both Spanish (Ochoa et al. 1994; Rocha-Peña et al. 1992; Rocha-Peña and Padrón-Chávez, 1992) and English (Lastra et al. 1991a, 1991b, 1991c, 1992; Lee et al. 1992, 1994,1995; Rocha-Peña et al. 1995; Roistacher, 1993; Roistacher et al. 1991; Yokomi et al. 1994) to explain the threat that the CTV/BrCA complex represents to the citrus industries of the Central America, the Caribbean islands, Mexico and United States.

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