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# Virus-free Citrus Budwood Production and Tristeza Management Program in Texas Through Industry Partnership

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## ABSTRACT

**Citrus tristeza virus and its vector the brown citrus aphid, *Toxoptera citricida* Kirkaldy, are an imminent threat to the citrus industry of Texas. The current practice of growing grapefruit (*Citrus paradisi* Macf.) and oranges (*C. sinensis* [L.] Osb.) on sour orange (*C. aurantium* L.) rootstock would be a major limiting factor to citrus production when the brown citrus aphid becomes established in the Lower Rio Grande Valley. Proactive measures through the production of virus-free planting materials, research and development, appropriate laws and regulations and education are important to keep a viable citrus industry in Texas. The citrus industry, in partnership with the Texas A&M University System and state and federal regulatory agencies, has initiated a mandatory, virus-free budwood program and other measures to manage the threat of the tristeza virus-aphid complex.**

## RESUMEN

**El virus de la tristeza de los cítricos y su vector *Toxoptera citricida* Kirkaldy, constituyen una amenaza inminente para la industria citrícola de Texas. La práctica actual de cultivar toronja (*Citrus paradisi* Macf) y naranja (*C. sinensis* [L.] Osb.) sobre portainjerto de naranjo agrio (*C. aurantium* L.) podría ser un importante factor limitante para la producción de cítricos cuando *T. citricida* se establezca en la parte baja del Valle de Río Grande. Medidas de preparación ante esta amenaza, tales como la producción de materiales vegetativos libres de virus, el desarrollo de investigación, el establecimiento de leyes y regulaciones apropiadas y educación son importantes para mantener una industria citrícola viable en Texas. La industria citrícola, en sociedad con el sistema universitario de Texas A&M y las agencias reguladoras estatales y federales ha iniciado un programa obligatorio de producción de yemas libres de virus y otras medidas de preparación para manejar la amenaza del complejo áfido-virus de la tristeza.**

*Additional index words: citrus tristeza virus, CTV, brown citrus aphid, BrCA*

Commercial citrus production in Texas is located in the Lower Rio Grande Valley (LRGV) along the Mexican border. Citrus occupies over 33,000 acres with approximately 70 percent grapefruit (*Citrus paradisi* Macf.), 28 percent oranges (*C. sinensis* [L.] Osb.), and the rest with miscellaneous cultivars of tangerines (*C. reticulata* Blanco), Meyer lemon (*C. limon* [L] Burm.f.), etc. Among the grapefruit cultivars, the predominant one is 'Rio Red', while 'Marrs' and navel are the major orange varieties. The total annual economic impact of the Texas Citrus Industry is about $100-150 million, and it employs over 5000 people. Over 98 percent of the citrus in the LRGV is grown on sour orange (*C. aurantium* L.) rootstock, which is well-adapted to the soil types, climate, and other conditions of South Texas.

Tristeza, a disease caused by citrus tristeza virus (CTV), is very destructive to grapefruit and oranges, especially when budded on sour orange rootstock. Past and recent surveys for CTV have confirmed its presence in the LRGV and elsewhere in Texas (Olson, 1955; Davis et al., 1984; Skaria, 1993; Skaria et al., 1996); however, there are no obvious field symptoms of CTV in commercial orchards in the state. CTV can be transmitted very efficiently in the field by the brown citrus aphid (BrCA) (*Toxoptera citricida*, Kirkaldy). Severe tristeza epidemics have followed the introduction of this aphid in some areas where sour orange was the main rootstock (Rocha-Peña et al., 1995; Roistacher et al., 1991). The BrCA is not present in Texas but it may reach the state in the near future since it arrived in Florida in 1995 (Hardy, 1995) and in Belize in 1996 (P.S. Reddy, personal communication). This aphid can introduce severe CTV isolates from other areas and also transmit the virus from symptomless cultivars to susceptible ones. The possibility of the introduction of BrCA is a threat to the Texas Citrus Industry and the implementation of strategies for CTV and BrCA management is essential for a viable citrus industry in this area. In this paper we describe the efforts made toward the production of a virus and viroid-free budwood program and other management strategies that have been adopted in Texas.

***Citrus tristeza virus:*** Tristeza is ranked as the most important citrus virus disease in the world. The disease was

present in South Africa and Australia at the end of the nineteenth century where it was described originally as an incompatibility between sour orange and certain scions. The first major CTV disaster was reported in 1930 in the province of Corrientes, Argentina. The disease appeared in Brazil in 1937, where it was named tristeza, a Portuguese word meaning sadness. Approximately 30 million citrus trees on sour orange were lost to CTV infection in South America (Bar-Joseph, et al., 1981).

The term CTV covers different strains of the virus that produce at least five distinct biological reactions in citrus, based on the cultivar and the environment. The mild isolates do not produce noticeable symptoms on most commercial scion-rootstock combinations; however, they may cause slight stem pitting, vein clearing and flecking on Mexican lime plants kept in a cool greenhouse. The seedling yellows (SY) isolates may cause severe chlorosis and dwarfing of sour orange, lemon, and grapefruit. SY type reaction is normally found in greenhouse grown trees or top worked field trees. The sour orange decline or quick decline isolates produce decline symptoms of sweet orange on sour orange rootstock. Grapefruit and tangerine cultivars are also commonly affected. Infected trees show leaf yellowing, wilting, defoliation, and fruit hanging on dead trees. The bud union area may show needle-like pegs and pinholes in the wood and in the bark, respectively, and may also show a brown line at the bud union. The stem pitting on grapefruit isolates produce chlorosis, stunting, and stem pitting on the stems. In the field, grapefruit and pummelo may show large longitudinal ridges or ropes and the fruit on infected trees are rather small (Fig. 1). The stem pitting on sweet orange isolates produce chlorosis, stunting, and stem pitting. In the field, sweet orange trees also produce small fruit that are not marketable as fresh commodity and the twigs may become brittle and break easily. The presence of CTV isolates can be detected by biological indexing on 'Mexican' lime [*Citrus aurantifolia* (Christm.) Swing.] or by enzyme linked immunosorbent assay (ELISA) (Bar Joseph et al., 1979). Apart from BrCA, man is another agent capable of spreading CTV and other viruses and viroids by the inadvertent use of budwood from infected but symptomless trees for propagation.

***The brown citrus aphid:*** BrCA is the most efficient vector of CTV (Costa and Grant, 1951; Yokomi et al., 1994). It originated in Southeast Asia and has moved to several countries, including the United States. It probably reached South America in the 1920s when massive importations of citrus trees were made from South Africa and Australia (Roistacher et al., 1991). BrCA spread naturally northward to the Caribbean Basin and is currently present in Florida and Belize. It is possible that it will soon reach Texas either from Florida or from Belize through Mexico. The BrCA efficiently transmitted CTV in many parts of the world and contributed to the decline of several millions of citrus trees grown on sour orange rootstock ( Rocha-Peña et al., 1995 ). The rate of tree loss related to BrCA can be best illustrated by the case study reported from Venezuela where the aphid entered the country in 1976 through the southeast (Colombia) and southwest (Brazil) borders. The aphid was well established there by 1978 and resulted in the death of over four million trees on sour orange rootstock during the 1980s (Geraud, 1976, 1992; Mendt, 1992).

***CTV incidence in the LRGV and in East Texas:*** CTV was detected in the 1950's in the Lower Rio Grande Valley (Olson, 1955, 1956, 1958). Later surveys using ELISA detected CTV in commercial orchards, nurseries, and dooryard plantings (Davis et al. 1984; Skaria 1996; Skaria et al., 1993). CTV has been also found in East Texas (Malouf, 1959; Davis et al., 1984; Skaria et al, 1996). Symptomless cultivars planted in dooryards could be potential carriers of CTV. The incidence of CTV in samples, most of them collected from dooryard trees, in the LRGV and some areas northeast of the Valley since 1994 is shown in Table 1. CTV transmission studies using LRGV populations of aphids have shown transmission of CTV by *Aphis spiraecola* Patch (Smith and Farrald, 1988; Cutrer, 1998).

***Citrus Tatter Leaf Virus (CTLV) and Citrus Exocortis Viroid (CEV):*** The replacement of sour orange by alternative rootstocks which are tolerant to CTV could be a practical option for CTV management in the LRGV (Table 2). However, these rootstocks are sensitive to CTLV (Fig. 2) and CEV (Fig. 3). Commercial cultivars infected with these pathogens are symptomless when grown on sour orange rootstock. CTLV and CEV have been detected in several trees in various locations in the LRGV (Miao et al., 1996; da Graça and Skaria, 1996). The propagation of infected budwood from symptomless trees to susceptible rootstocks may drastically reduce the long term economic return from affected orchards.

**Table 1.** CTV incidence in the LRGV and in East Texas, 1994-1997.

| Year | Number of samples tested | CTV incidence % | |
|---|---|---|---|
| | | LRGV | East Texas |
| 1994-95 | 1,495 | 0.62% | 28.05% |
| 1995-96 | 5,108 | 0.39% | 19.35% |
| 1996-97 | 1,220 | 1.29% | 22.7% |

**Table 2.** Comparative reactions of sour orange and hybrids of trifoliate orange to different citrus pathogens.

| Rootstock | Phytophthora | Citrus Nematode | CTV | CEV | CTLV |
|---|---|---|---|---|---|
| Carrizo citrange | tolerant | tolerant | tolerant | susceptible | susceptible |
| Troyer citrange | tolerant | tolerant | tolerant | susceptible | susceptible |
| Swingle citrumelo | resistant | resistant | tolerant | tolerant? | susceptible |
| Sour orange | tolerant | susceptible | susceptible | tolerant | tolerant |

**Table 3.** Indicator plants, incubation period, and temperature regimes that are currently used for virus and viroid indexing in Texas.

| Disease | Indicator | Temp.(°C) | Incubation period |
|---|---|---|---|
| Tristeza (all strains) | Mexican lime | 24-27 | 3-5 weeks |
| Tristeza seedling yellows (SY) and grapefruit stem pitting (SP) | Grapefruit | 24-27 | 8-10 weeks |
| Tristeza SY | Sour orange | 24-27 | 8-10 weeks |
| Tristeza orange SP | Madame Vinous | 24-27 | 8-10 weeks |
| Greening | Madame Vinous | 20-25 | 10-12 weeks |
| Stubborn | Madame Vinous | 32-38 | 10-12 weeks |
| Psorosis | Madame Vinous | 24-27 | 3-4 weeks |
| Exocortis and related viroids | Etrog citron 861-S-1 | 32-40 | 4-6 months |
| | Tomato (exocortis only) | 32-40 | 2-3 weeks |
| Cachexia | Parson's special mandarin | 32-40 | 12-18 months |
| Tatter leaf | *Citrus excelsa* Wester | 24-27 | 5-7 weeks |
| | Rusk, Carrizo, or Troyer citranges, and Swingle citrumelo | 24-27 | 5-7 weeks |
| | Cowpea, kidney beans *Chenopodium quinoa* Willd. | 24-27 | 4-6 days |
| Concave gum | Dweet tangor | 24-27 | 5-8 weeks |
| | Sweet orange | 24-27 | 5-8 weeks |
| Infectious variegation | Dweet tangor | 20-22 | 4-6 weeks |
| | Lemon | 24-27 | 4-6 weeks |

## DEVELOPMENT OF THE VIRUS-FREE BUDWOOD PROGRAM

To obtain virus-free propagation materials, commercially important cultivars in Texas are being subjected to shoot tip grafting (STG) to eliminate viruses and viroids. Plants produced by STG are then subjected to various indexing procedures to assure freedom from pathogens. Budwood from cultivars that are important to the urban sector are being imported under quarantine from the California Citrus Clonal Protection Program (CCPP) and are tested by ELISA for CTV. Once clean materials are obtained, foundation trees will be established, from which nursery increase blocks will be developed to provide propagation materials.

***Shoot-tip grafting:*** STG is a technique that has been used successfully to produce virus-free citrus plants (Murashige et al., 1972; Navarro et al., 1975). It involves a precise excision of a minute (0.17 mm) shoot tip from the rest of the tissue below, which may be contaminated with a graft-transmissible pathogen, and its successful grafting on a decapitated rootstock, that was previously grown in sterile agar medium. Figure 4 shows the laboratory set up for STG. The grafted rootstock is then placed in a test tube containing an artificial liquid growing medium and kept under light for several weeks (Fig. 5). Our success rate of this operation was about 15%. Plants grown this way were transplanted into 4-inch pots (Fig. 6) and were kept in the greenhouse.

***Indexing:*** This step refers to a series of tests performed on plants developed by the STG technique to confirm the absence of graft-transmissible viruses and viroids in shoot tip grafted plants. The most reliable method is a comprehensive biological indexing using several citrus indicator plants (Roistacher, 1991). Inoculated plants are kept in a cool or warm section of a greenhouse for several weeks, depending upon the disease being indexed, and symptoms are recorded. The process is repeated over time to confirm the observations made. The indicator plants, the incubation period, and the temperature settings used for indexing are listed in Table 3. Herbaceous indicator plants can also be used for additional indexing for CTLV and CEV. The program uses part of a greenhouse facility which has separate compartments for cool and warm conditions to induce symptom expression of different viruses and viroids. The budwood coordinator is in charge of indexing and multiplication of the cultivars. The Texas Citrus Producers Board finances the positions of the budwood coordinator and a half-time greenhouse assistant.

Other methods that are used for indexing are ELISA and nucleic acid analysis by polyacrylamide gel electrophoresis (PAGE). ELISA is a technique based on an antigen-antibody reaction that is routinely used for the detection of plant and animal pathogens, including CTV (Bar-Joseph et al, 1979; Clark and Adams, 1977). Numerous variations of this technique are available, however, the common practice is to use a polystyrene plate as a solid phase to trap potential virus particles in plant sap, with the use of two antibodies, making a virus sandwich. A variation of this technique, using a nitrocellulose paper instead of the solid plate is called dot-blot ELISA. The above techniques are used in our indexing program. A monoclonal antibody, the CTV-MCA13 is useful for detecting certain severe type CTV isolates (Permar et al., 1990). Nucleic acid analysis by PAGE allows the detection of plant viroids (Semancik, 1991; Duran-Vila et al., 1993). This technique is based on the property of the viroid nucleic acid to move in an electric field in a polyacrylamide gel where it is visualized as a band when it is stained with ethidium bromide or silver. Since this system can detect citrus viroids even when the indexed plants are symptomless or show mild symptoms, it is an excellent tool for confirming the absence of these

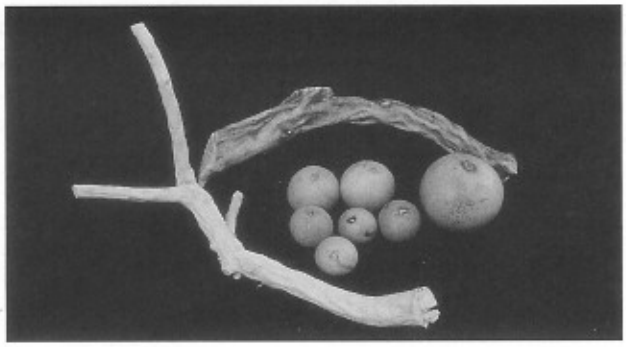

**Fig. 1.** Star Ruby grapefruit infected with stem pitting strain of CTV, showing ropy stem and small fruit.

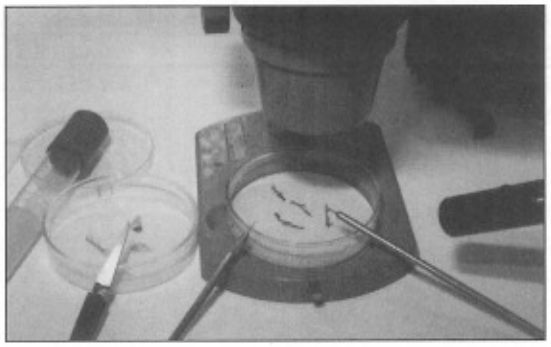

**Fig. 4.** Set-up for shoot-tip grafting under a laminar flow hood.

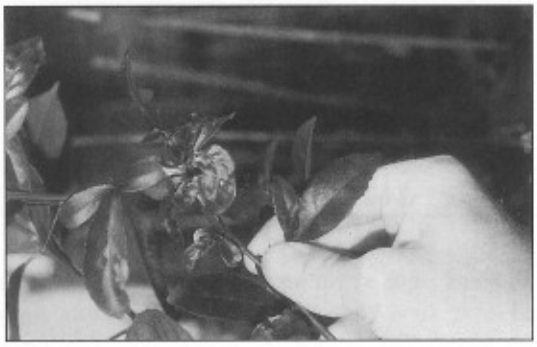

**Fig. 2.** Tatterleaf virus symptoms in Rusk citrange showing tattered and chlorotic leaves.

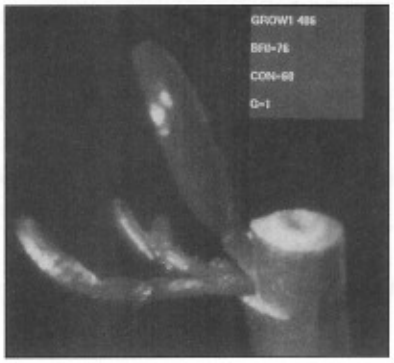


**Fig. 5.** Growth from a shoot-tip grafted meristem in a sterile culture tube.

**Fig. 3.** Exocortis symptoms in Etrog citron showing severe leaf epinasty.

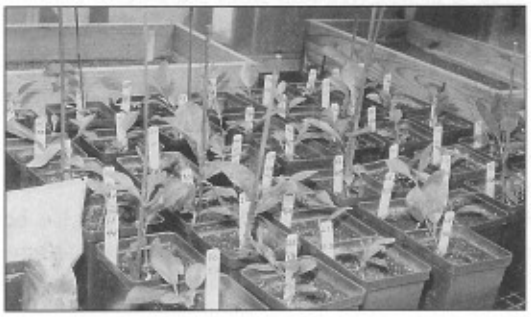

**Fig. 6.** Shoot-tip grafted plants transferred to pots and grown in a greenhouse.

**Fig. 7.** Shoot-tip grafted and virus-free plants grown in the greenhouse for bud multiplication.

**Table 4.** Citrus budwood selections that have been shoot-tip grafted, indexed, and or multiplied since 1994 and approximate number of virus-free buds available for multiplication at the end of 1997.

| Cultivars | Number of STG plants | Biological indexing status | Buds available |
|---|---|---|---|
| N-33 navel orange, source A | 2 | 2C | 500 |
| Marrs orange, source B | 3 | 1C, 2N | 300 |
| Rio Red grapefruit, source A | 2 | 1C, 1N | 250 |
| Star Ruby grapefruit, source A | 7 | 1C, 6N | 150 |
| Valencia orange, source A | 4 | 2NC, 2P* | 150 |
| Pineapple orange, source A | 3 | 1NC, 2P* | 100 |
| Hamlin orange, source A | 1 | 1C* | 0* |
| Everhard navel orange, source C | 2 | 1P, 1N | 0 |
| Pineapple orange, source B | 2 | 2P* | 0 |
| Henderson grapefruit, source B | 1 | 1P | 0 |
| N-33 navel orange, source B | 4 | 4N | 0 |
| Everhard navel orange, source B | 3 | 3N | 0 |
| Henderson grapefruit, source A | 3 | 3N | 0 |
| Marrs orange, source A | 3 | 3N | 0 |
| Orlando tangelo, source A | 3 | 3N | 0 |
| Pineapple orange, source D | 3 | 3N | 0 |
| Armstrong Early satsuma, source A | 2 | 2N | 0 |
| Eureka lemon, source A | 2 | 2N | 0 |
| Pineapple orange, source C | 2 | 2N | 0 |
| Rio Red grapefruit, source B | 2 | 2N | 0 |
| Eureka lemon, source B | 1 | 1N | 0 |
| Everhard navel orange, source A | 1 | 1N | 0 |
| Henderson grapefruit, source C | 1 | 1N | 0 |
| Marrs orange, source C | 1 | 1N | 0 |
| Marrs orange, source D | 1 | 1N | 0 |
| Marrs orange, source E | 1 | 1N | 0 |
| Star Ruby grapefruit, source B | 1 | 1N | 0 |
| Sunburst tangerine, source A | 1 | 1N | 0 |

C=indexing completed
NC=indexing nearly completed
P=indexing in progress
N=indexing not started yet
*=positive for exocortis viroid, not further indexed

pathogens in shoot tip grafted plants that show no symptoms after indexing on 'Etrog' citron.

***Foundation block:*** The virus-free plants will be planted in a foundation block starting in spring, 1998. These plants will be the source of clean budwood for future multiplication. The foundation block trees will be re-indexed periodically for viruses and viroids. Approximately eight acres at the Citrus Center in Weslaco will be leased from the Texas A&M University-Kingsville by the Citrus Foundation for use as the virus-free citrus foundation block. The first phase of planting will be 'Rio Red' and 'Star Ruby' grapefruit and 'Marrs', 'N-33' navel, 'Pineapple', and Valencia oranges on Troyer and Carrizo citrange and sour orange rootstocks. There will be four replicates of each scion-rootstock combination. A nursery increase block will be planted next to the foundation block to produce certified budwood for the industry. The revenue from budwood sales will be used for partial support of this program. Increase blocks will be established annually to produce budwood for a two year period, after which, the trees will be dug and sold as certified trees or the block will be destroyed.

## CURRENT STATUS OF STG AND INDEXING AS OF 1997

Over 62 plants from 16 different cultivars have been shoot-tip grafted from 1994 to 1997 (Table 4). Of these, five STG plants of 'Rio Red' and 'Star Ruby' grapefruit and 'Marrs' and 'N33'navel oranges have been completely indexed and are undergoing mass propagation (Fig. 7). The 'Pineapple' and 'Valencia' sweet oranges are nearing completion of indexing and are also being mass propagated. About 150 plants of the above scion cultivars have been grafted on different rootstocks in the greenhouse for multiplication of buds. All these cultivars will be further propagated for the increase block. The next two STG cultivars to be indexed are 'Henderson' grapefruit and 'Everhard' navel orange. Other virus-free citrus plants that are needed for non commercial or 'dooryard' planting will continue to be imported from the Citrus Clonal Protection Program in California.

## INDUSTRY SUPPORT

The Texas Citrus Industry created a CTV/BrCA task force to provide advice and assistance in managing tristeza disease and its vector. The task force developed a management plan that outlines the importance and the risk of CTV/BrCA and describes the role and responsibility of each agency involved with this problem. The most important aspect of this plan is the risk assessment procedure to be used when the BrCA and/or a severe strain of CTV is detected in the LRGV.

The CTV/BrCA Task Force has also formed an education committee which is charged with the responsibility of developing a plan to inform the public of the rules and regulations restricting importation of plant materials, and to enlist the public's help in identifying the BrCA and CTV. Such educational programs will help to prevent illegal movement of infected citrus material into Texas. Another part of the education program will be to update all segments of the industry and extension personnel about changes in the status of BrCA and CTV. TDA fruit fly trappers will assist in the distribution of educational materials on BrCA and CTV to property owners.

***Role of the Texas Department of Agriculture (TDA):*** TDA has the statutory authority to take action to limit the spread of the BrCA or CTV. The Risk Assessment Group of the Texas CTV/BrCA Task Force will make recommendations for needed actions. Regulatory tools which could be utilized by TDA include quarantines to regulate the movement of the BrCA or CTV and authority to remove citrus trees infected with severe types of CTV. The Commissioner of Agriculture has appointed a seven member Citrus Budwood Advisory Council to advise the Commissioner on issues related to BrCA and CTV.

***Mandatory Citrus Budwood Certification Program:*** In 1997, the Texas Legislature passed House Bill 2807, which amended the existing citrus budwood program. This law is aimed at protecting the Texas Citrus Industry from tristeza virus by requiring the use of budwood free of viruses and viroids. Prior to the 1997 legislation amendments, the use of virus-free budwood was a voluntary program. The amended legislation eliminated an exclusion for ornamental nurseries which propagate citrus trees for dooryard use. The legislation now applies to all citrus nurseries and growers in Texas. The law now gives the Commissioner of Agriculture the authority to adopt rules that will make the program mandatory as virus-free budwood becomes available. For example, when an adequate supply of a given cultivar like 'Rio Red' is available, the Commissioner of Agriculture can adopt a rule saying that citrus rootstocks cannot be budded with 'Rio Red' unless the budwood is certified virus-free.

***The National Citrus Research Council (NCRC):*** This is a body of growers, industry personnel, and scientists from each of the citrus producing states. NCRC has identified nine research priorities nationwide from a list of state priorities. In 1997, NCRC has been successful in obtaining $ 750,000 from federal sources for CTV management research programs. Texas is represented in NCRC by three scientists, one grower, and an industry member who is also the current chairman.

## CONCLUSION

BrCA infestation may be unavoidable in Texas and the industry will have to develop strategies to live with it. The use of alternative rootstocks and virus-free budwood for propagation, the establishment of programs to reduce aphid infestation through chemical and/or biological control and the education of the public about the dangers of inadvertent use of infected budwood for propagation are all important measures for maintaining a viable citrus industry in Texas. The Texas Citrus Industry has already taken an active role in developing a proactive CTV management program in partnership with the Texas A&M University System, state and federal agencies, and legislative support.

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