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QUALITY TREE PROGRAM FOR FLORIDA CITRUS

P. RUCKS*
President, Florida Citrus Nurserymen's Association
**Jack Berry Groves*
P.O. Box 459
LaBelle, FL 33935

Abstract. **In order to provide the best possible nursery material to the Florida citrus industry, the Florida Citrus Nurserymen's Association (FCNA) and the Florida Citrus Production Managers' Association (FCPMA) have worked together to develop a Quality Tree Program (QTP). This QTP would provide for changes in the current Florida Citrus Budwood Registration Program (FCBRP) by making it mandatory that all citrus nursery tree stock grown in Florida be propagated from registered sources, with required recurring testing to be made of registered sources for severe strains of citrus tristeza virus (CTV) yearly and for psorosis and viroids every six years. Seed source trees for *Poncirus trifoliata* and *P. trifoliata* hybrids would be indexed only for psorosis once every six years unless they were propagated from foundation material. Use of nursery increase blocks would be encouraged with virus-tested budwood for these blocks being provided by the FCBRP, and the increase blocks would be tested for presence of severe CTV between the 12th and 18th month of their life span (24 months in the field or 30 months if screened). The need to have a mandatory FCBRP has been recognized: Florida faces the imminent threat of invasion of the brown citrus aphid, *Toxoptera citricida*, the most efficient vector of CTV. This would greatly increase the risk that stem pitting strains of CTV would be transmitted from possible "sleeping source trees" in the state into cultivars enabling more efficient aphid transmission and introduction in budwood source trees. Also, recent indexing has indicated the presence of psorosis-like agents in some registered sources. The Florida Division of Plant Industry (DPI) would have the responsibility for enforcement of the mandatory QTP, but the QTP would be governed by an Advisory Committee composed of members from FCNA, FCPMA, and research scientists. Once the law is passed making the QTP mandatory, enforcement of the law would begin after a three-year grace period. This grace period would allow time for the existing non-qualified citrus nursery stock to be sold or consumed, and to allow sufficient time for an ample amount of registered screenhouse scion/seed start material to be available to nurserymen. The disease testing of registered sources belonging to nurserymen and private owners would be done by commercial and private diagnostic labs which would be certified by DPI. It should be encouraged that all scion and seed source material currently being used be tested now with the approved testing techniques. This testing would be considered to fulfill the recurring indexing requirements once the QTP becomes law and is enforced.**

Historical Perspective

The Florida Citrus Budwood Registration Program (FCBRP) was initiated in January 1953, following a discussion the previous year as to the need and benefit of a citrus registration program at the Florida State Horticultural Society (FSHS) (19). Florida growers were concerned about tree losses caused by citrus tristeza virus (CTV) in Brazil and Argentina, the graft-transmissible nature of psorosis (scaly bark) had just been elucidated in California, the graft transmission of cachexia (xyloporosis) and "scaly butt" disease (exocortis) on trifoliate orange had been demonstrated. A committee composed of production managers, scientists and plant regulatory personnel formulated the policy and procedures which formed the basis of operation of the FCBRP. The voluntary program was established to select vigorous, productive, true-to-type citrus trees which were then biologically tested for CTV, psorosis, cachexia, and exocortis. Budwood from these virus-tested trees were made available to nurserymen and growers for propagation of registered nursery stock.

Changes have been made in the FCBRP in the past 40 years of operation (26). Beginning in 1961, it was unlawful to propagate psorosis in 9 major commercial varieties. In 1967 an additional 7 varieties were included as psorosis-free propagations. In 1967, it was realized that exocortis was spread by contaminated pruning tools. Recommendations were made to nurserymen for better sanitation practices and additional trees were tested for presence of exocortis. In the beginning of the FCBRP, scion trees were removed from registered status if CTV, identified by the Mexican lime index, was present. However, this practice was discontinued in 1964 because of the concern that there would not be enough budwood source trees because of the increasing rate that CTV was moving into the trees (14). CTV had been recognized to be present in Florida since 1952 (9), but tree losses were not occurring in Florida as they were in Brazil, Argentina and California. For many decades, CTV was not a problem in Florida but the complexity and severity of CTV changed over time. In the 1950s, CTV decline in trees on sour orange rootstock was noted at scattered, isolated locations in Orange, Lake and Seminole counties and in the Ft. Pierce area (10,11; M. Cohen, personal communication). In the 1960s the spread of CTV decline on sour orange was observed in the Ft. Pierce area (3), but no problems were noted elsewhere. In 1975 a destructive outbreak of tree loss due to CTV occurred in western Orange and southern Lake counties (7). A survey conducted in 1979 of budwood trees being used for propagation on sour orange rootstocks indicated most sweet orange scion and about half of the grapefruit scions were CTV infected, but biological indexing of random samples indicated few severe CTV and seedling yellows strains of CTV (8). The CTV situation changed during the late 1970s and early 1980s. In the early 1980s, numerous instances were found where budlings on sour orange rootstock were stunted and not growing well. CTV induced losses to producing trees on sour orange rootstock reached epidemic proportions in the Indian River and Southwest Florida Flatwoods citrus areas, with some isolated groves losing 50% of their trees on sour orange rootstock in a year (4). Sour orange rootstock had remained popular for citrus in Florida until just recently (3,26). In 1963, about one third of the nursery trees were on sour orange. After the 1962 freeze, the popularity of sour orange continued. Citrus blight losses reached epidemic proportions in the early 1970s, beginning in the Flatwoods citrus areas and moving northward though the citrus area, thus maintaining the demand for sour orange as a blight-tolerant rootstock (24). By 1987 due to frequent occurrence of stunting of budlings on sour orange rootstock and the epidemic of CTV induced decline of producing trees on sour orange rootstock, only 6% of the trees propagated were on sour orange and only 2-3% since then (26). Surveys of budwood source trees in the 1980s indicated many severe strains of CTV were present, and seedling yellows strains were common. A biological index using sour orange liners as indicator plants in 1984 indicated over one third of the registered budwood source trees were infected with decline inducing CTV strains; with budlings from these sources having less than 50% of the stem diameter of budlings propagated with the same scion varieties propagated virus-free or with mild CTV strains (25,14).

With the recognition of the prevalence of decline inducing CTV strains present in registered mother trees and the extent of their distribution throughout the industry, fundamental changes in the policy and procedures of FCBRP were proposed in 1987 (23). These changes, devised by a group of regulators, scientists, and nurserymen after studying other budwood programs, were aimed at preventing viruses vectored by insects from getting into propagation sources. The changes were implemented into the policy of the FCBRP in 1991 (26). Small quantities of virus-tested propagating material were established in protected greenhouses and screenhouses. Selected virus-tested trees were inoculated with mild CTV strains shown to have some cross protecting ability and also are maintained under protected conditions. Budwood from these clean (or mild CTV strain inoculated for cross protection) foundation material is made available to nurserymen for use in nursery increase blocks which serve as budwood sources for propagation of larger commercial quantities of registered citrus nursery stock. The increase blocks can be used for up to 24 months as a source of registered budwood. From experience in Florida, at least 50 buds may be collected from each nursery increase plant per year. ELISA testing for CTV indicates that with the present situation in Florida, it is rare that CTV becomes established in the nursery increase blocks in the 24 month life span (C. Youtsey, personal communication). Foundation material must still bear true-to-type fruit before buds may be cut. Nurserymen and scion grove owners may established their registered scion trees under protected conditions.

The Need For Improvement

It is being generally realized that the present voluntary FCBRP could be improved. In the past season, over 16 million plants were propagated, less that 4 million of these were registered. At the present, there are 44,636 registered scion trees in 70 scion blocks (C. Youtsey, personal communication). While the FCBRP tries to index trees being used heavily for propagation, they only have resources to index 2,000 scion trees per year. Many trees have never been re-indexed for CTV, psorosis or viroids since they were registered. There is confusion in the industry as to what registered trees are; some nurseries claim to sell registered trees while they are only certified to be nematode-free and pay a "registration fee" for this certification of the nursery site. While there are claims that some budwood is a good as registered or first generation, incidences of unthrifty trees on Carrizo, Swingle and trifoliate rootstocks are common. Cachexia has occurred on *Citrus macrophylla* rootstock, and some strains of cachexia cause symptoms on Cleopatra mandarin, a commonly used rootstock in Florida. Recent indexing for causal agents associated with Rio Grande Gummosis (RGG) in the Indian River indicated that 79% of the grapefruit trees samples in 5 different groves were infected with psorosis-like agents, and a follow-up survey of registered Ruby Red grapefruit scion trees indicated about a 15% infection rate with psorosis-like agents (13). This could be eliminated as a problem by proper indexing for psorosis under controlled greenhouse conditions.

Implementation of a mandatory citrus certification will help ensure Florida growers can remain competitive in the world market while having to compete with areas have cheaper labor and access to pesticides which are illegal to use in the USA. When considering diseases which are graft-transmissible which could become widespread quickly throughout the Florida Citrus Industry, the situation will only become worse as time goes on. Florida is facing an imminent invasion by *Toxoptera citricida*, commonly called the brown citrus aphid (BrCA), the most efficient vector of CTV (15). If events occur in Florida as has occurred in other countries upon the arrival of the BrCA (17,18), severe strains of CTV causing stem pitting in grapefruit and sweet orange scion appear in the years following the introduction of BrCA. This is usually followed by the occurrence of severe strains which also cause stem pitting on rootstocks considered to be CTV tolerant. The severe stem pitting strains are rapidly widespread through the industry by movement through nursery material, limiting production of citrus in areas where citrus has previously been a major cash crop. Recent indexing of Meyer lemon sources in Florida has indicated the presence of CTV strains capable of causing stem pitting in grapefruit (S. Garnsey, personal communication). These strains have not been transmitted by aphid species presently in Florida, but have been by the BrCA in the high-level quarantine facilities at Fredrick, MD (L. Marais & R. Yokomi, personal communication).

In addition to CTV, other exotic diseases having insect vectors could cause economic damage to citrus in Florida if they have been introduced via illegal budwood entry into the state and if they were present in propagating material being distributed throughout the state. Examples are:

Naturally spread psorosis in parts of Argentina and northern Brazil where unidentified aerial vectors cause spread into virus-free nucellar material (22). In these areas, psorosis is considered the most important limiting factor of citrus production, even with the presence of severe CTV strains and the BrCA.

Citrus variegated chlorosis (**CVC**), caused by a xylem-inhabiting, systemic bacteria, *Xylella fastidiosa* (12). This disease was first found in one location in 1987, now it is widespread in Sao Paulo State, Brazil and has been reported to be present in Argentina and Uruguay. The disease is spread by leafhopper insects. By 1992, 2 million trees had been rendered nonproductive by CVC and in 1993 an estimated 12 per cent of the citrus in Sao Paulo state has been affected (O. Possos, personal communication).

Citrus greening disease (5) caused by a phloem-limited bacterium which has not been well characterized, is a limiting factor of citrus production in parts of China, India, Southern Africa. It is spread by psyllid insects. One vector, *Diaphorina citri*, is present in Brazil, Argentina, Paraguay, Uruguay, and Bolivia, but the greening disease has not been reported in the Western Hemisphere.

Stubborn disease is caused by *Spiroplasma citri*, a mycoplasmalike organism, and vectored by several species of leafhoppers (6). Stubborn is present in California, but the significance of the disease has been minimized by propagating nursery trees from stubborn-free budwood sources and use of vector-proof enclosures if the nursery is in a stubborn infested area.

Witches' broom disease is caused by a mycoplasmalike organism and vectored by leafhopper species (1). It originated in Oman, and has almost entirely eliminated production of limes in that country.

The Proposed Quality Tree Program

The FCNA, in cooperation with the FCPMA, have recognized the importance of maintaining virus-free foundation material by the FCBRP, the need to protect their registered scion trees from potential insect vectors, the need to conduct recurring indexing for CTV, psorosis, and viroids on all registered scion trees and to monitor for the presence of severe CTV in nursery increase blocks and to not propagate severe strains of CTV in nursery material, and most important of all: the FCBRP must be mandatory for the protection of all the Florida Citrus Industry and governed by a standing advisory committee composed of nurserymen, production managers and scientists to implement the establishment of the QTP and who will advise the FCBRP.

Under the QTP graft transmissible diseases of citrus, including severe strains of citrus tristeza virus as determined by reactivity to the severe strain differentiating monoclonal antibody MCA-13 (20), will no longer be propagated. This program, coupled with the current procedures of the FCBRP which allows for protected budwood source trees and use of nursery increase blocks, provides an important safeguard against propagation of insect vectored and graft transmissible diseases including severe strains of citrus tristeza virus, psorosis, citrus viroids, and exotic diseases which could be introduced into Florida in the future.

The QTP would be guided and directed by the standing advisory committee. Recommendations duly passed by members and adopted by this committee would be made mandatory. These recommendations would apply to all citrus propagated in the state of Florida. All citrus trees propagated would be registered.

A standing technical subcommittee would meet on an "as needed" basis to set standards for citrus disease testing, make recommendations on potential disease problems, evaluate new testing procedures, standardize techniques for sampling and testing, and establish quality control and certification of testing laboratories.

The FCBRP would be responsible for testing all foundation trees for psorosis, cachexia, tristeza, exocortis and other citrus viroids. Division of Plant Industry (DPI) would be responsible for certifying nurseries to be free of citrus damaging nematodes, which currently includes *Radopholus citrophilus, Tylenchulus semipenitranes,* and *Pratylenchus coffaea.*

Diagnostic laboratories certified by the FCBRP (DPI) would test budwood field trees in scion blocks and nursery increase blocks belonging to nurserymen and scion grove owners as follows:[1]

1. Scion Blocks

(Costs are estimated based on information from a private laboratory. Costs may vary depending on number of trees tested and sampling requirements.)

[1]This information is subject to change as the Quality Tree Program is Further developed.

2. Nursery Increase Blocks

Tristeza - Budwood for nursery increase blocks will come from trees which have borne fruit at the DPI Foundation Farm under screen. The blocks will be tested once between the 12th and 18th month of a 24-month nursery increase block's life span. Random samples would be taken to give a reasonable probability of detecting a 1% infection level. Cost is estimated at $300.00 per 1000 trees (tested with 10% sampling to give a 90% probability of detection of a one percent CTV infection rate). If over 1% infection rate of severe strains of tristeza is found, then all trees being used for budwood need to be retested (at an estimated cost of $2.00 per tree).

3. Screen Houses

Registered budwood source trees and nursery increase blocks would be tested using the same protocol as other similar source material as described above. For nursery increase blocks maintained under screen or in greenhouses, the life span will be increased to 30 months or a period of time as defined by DPI. If the life span for nursery increase blocks maintained under screen or in greenhouses is longer than 30 months, testing would be required as per scion blocks.

4. Seed Source Trees

Seed source trees of *Poncirus trifoliata* and *P. trifoliata* hybrids would be evaluated for psorosis only using the protocol described above unless the rootstock budwood originated from DPI foundation material and is not budded on *P. trifoliata* or *P. trifoliata* hybrid rootstock. Seed imported into the state must meet or exceed the state requirements.

Virus	Estimated Cost/Tree	Period in years	Type of assay[2]	Length of assay
Tristeza[3]	$ 9.00	1	Serological	days
Psorosis	$50.00	6	Bioassay on Madam Vinous[4]	3-6 months
Exocortis & other viroids	$70.00	6	Bioassay on Citron	8-12 months
Cachexia & Group II Viroids			PCR pending	days

Expected Benefits of the Quality Tree Program

What are the benefits of having virus-free nursery material for the Florida Citrus Industry and having recurring indexing of propagating material to ensure a graft transmissible disor-

[2]The guidelines currently accepted for indexing for graft-transmissible agents of citrus are as defined in "Graft-transmissible diseases of citrus. Handbook for detection and diagnosis" (C. N. Roistacher, Ed.), FAO, Rome, 1991. 286 pp (20). These are subject to change pending approval of additional methods by the Technical Subcommittee and the Standard Advisory Committee.

[3]Tristeza testing will be done only between the 1 Oct. through 30 May when lower temperatures result in higher concentration of the virus in the plant, enabling better detection.

[4]Biological indexing in this manner will also detect the presence of concave gum, blind pocket, crinkly leaf and infectious variegation diseases which are reported to be present in Florida as well as impietratura and cristacortis diseases which have not been reported in Florida.

der does not accidently slip into the system and get spread to multiple sites in the state before it is noticed? Each of the diseases to be indexed cause debilitating effects to citrus production. Psorosis in parts of South America is considered to be the most limiting factor of citrus production. Viroids can cause extreme dwarfing of trees on Carrizo, Swingle or trifoliata rootstocks, cachexia can cause dwarfing and unthrifty trees on *C. macrophylla* or Cleopatra mandarin rootstock. While there are already strains of CTV in the state which cause decline in trees on sour orange rootstock and Florida has lost the use of sour orange rootstock, desirable because of high fruit quality, blight tolerance, and cold tolerance, there are no stem pitting strains of CTV known to be present in commercial citrus in Florida. However, recent indexing of Meyer lemon from several areas in the state indicate strains of CTV are present capable of causing stem pitting in grapefruit. When the BrCA enters Florida, that efficient vector will be able to transmit these severe strains while the aphids present in Florida now cannot. Once these stem pitting strains of CTV gets into sweet orange and/or grapefruit varieties instead of just lemon, it will be transmitted even more efficiently by aphids. If these strains infect scion trees and be propagated through nursery propagations, they could easily be distributed to multiple sites throughout the industry; and this could happen on a grand scale if this nursery material was distributed through retail discount stores. A review of what has happened in Venezuela after the invasion of the BrCA would be a good indication of what would happen in Florida if a programs such as the QTP is not instituted and made mandatory.

In Venezuela, the BrCA entered the southeast part of the country in 1979. Venezuela citrus was almost totally on sour orange rootstock at that time. The BrCA moved about 300 km per year, and invaded the citrus area in the northeast and in the north central areas about the same time. By 1980, the first trees died of quick decline, by the mid-1980s the quick decline reached epidemic proportions. About 8 million trees on sour orange rootstock have now been killed due to CTV. The Venezuelan industry replanted on CTV tolerant rootstocks; most of the rootstocks used were affected severely by citrus blight. Blight was not even recognized to be present in the country while the trees were on sour orange. In addition to blight, stem pitting and vein corking due to severe strains of CTV began to appear on sweet orange trees, especially Valencia and Hamlin cultivars, and grapefruit. The stem pitting CTV strains became a production problem about 10 years after the BrCA became established. More recently, strains of CTV causing stem pitting on rough lemon and Volkamer lemon rootstocks have occurred and are becoming widespread. Also, severe stem pitting is occurring on Tahiti lime, a plant grown in Brazil because of its CTV tolerance in the area where very severe stem pitting on Pera sweet orange occurs.

Yield loss due to a virus is difficult to obtain because often appropriate virus-free controls are not present, and because of logistic problems. However in South Africa, data has been collected of yields between mild strain cross protected and non-cross protected Marsh grapefruit on rough lemon rootstock (16). The mean production over the past 10 years was 45 tons/ha and 25 tons/ha for cross protected and non-cross protected trees, respectively, a yield reduction of about 45 per cent. In addition to total yield loss, there is a significant reduction in fruit size which results in a much poorer pack out for fresh fruit. Because of the prevalence of severe CTV strains in South Africa, there is no comparison with virus-free trees. The Nartia mild strain used for cross protection causes some mild stem pitting itself, thus the yield loss is conservative. If this loss was projected to the Florida grapefruit crop for this year (1993-94), the yield would be 27 million boxes instead of 49 million. With the BrCA destined to enter Florida within the next year or two and with knowledge that stem pitting strains are already present in Florida (in Meyer lemon), we may be obtaining similar yield loss data in Florida grapefruit in another decade if a mandatory budwood program with recurring indexing for the most important virus diseases is not instituted.

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THE MOLECULAR BASIS FOR THE ANTIGENIC DIVERSITY OF CITRUS TRISTEZA VIRUS: IMPLICATIONS FOR VIRUS DETECTION[1]

H. R. PAPPU, S. S. PAPPU, R. F. LEE[1], M. CAMBRA[2], P. MORENO[2], S. M. GARNSEY[3] AND C. L. NIBLETT.
Plant Pathology Department, P.O. Box 110680
University of Florida, Gainesville, FL 32611, USA

[1]Citrus Research and Education Center,
Lake Alfred, FL 33850, USA

[2]IVIA, Valencia, Spain, [3]USDA-ARS
[3]Horticultural Research Laboratory,
2120 Camden Road, Orlando, FL 32803, USA.

Abstract. **Citrus tristeza virus (CTV) displays a wide range of serological diversity. Strains have been reported from various countries that do not react with some of the CTV-specific monoclonal antibodies (MAb). We investigated the molecular basis for the emergence of these serotypes of CTV. While CTV-specific MAb MCA13 reacts predominantly with severe strains that cause quick decline and/or stem pitting, 3DF1 is a broad spectrum MAb that reacts with a majority of the CTV strains. To map the antigenic determinants recognized by these MAbs, capsid protein (CP) genes of several serologically and biologically diverse strains from different parts of the world were cloned and sequenced. Sequence comparisons showed that phenylalanine (Phe) at position 124 of the CP was conserved among all the MCA13-reactive strains. This was replaced by a tyrosine (Tyr) in MCA13-nonreactive strains. Sequence comparisons between 3DF1-reactive and nonreactive strains showed that amino acids Aspartic acid (Asp), Lysine, and Phe were conserved at positions 2, 13 and 28 in the CPs of all the 3DF1-reactive strains, respectively. These were replaced by Glycine, Threonine/Asp, and Tyr in 3DF1-nonreactive strains. The amino acid differences in both cases were due to single nucleotide changes in their respective codons. Mutations were introduced that altered the codons in the cloned CP genes of selected strains and their effect on the reactivity of the two MAbs was evaluated by Western blot analysis. For MCA13, its reactivity dependent on the presence of phenylalanine residue at position 124, for 3DF1, the second amino acid, asp was found to be critical for its reactivity. Our data indicate that point mutations in the CP gene resulting in single amino acid substitutions can result in the loss of a viral epitope. This can drastically alter or eliminate the reactivity of a CTV-specific MAb. This provides the molecular basis for the existence/emergence of new serotypes of CTV and indicates the necessity of using a combination of MAbs of differing specificities or a polyclonal antibody for the effective detection of diverse strains of CTV.**

Citrus tristeza virus (CTV) causes the most economically important viral disease of citrus (Bar-Joseph et al. 1989). The virus, a member of the closterovirus group, contains a single-stranded, positive-sense RNA genome of approximately 20,000 nucleotides. Virus particles are flexuous, thread-like and are 2000 nm long (Bar-Joseph and Lee, 1990). CTV is transmitted to other citrus hosts by grafting during citrus propagation and also by several species of aphids. CTV infection causes a diverse range of symptoms in citrus. Strains have been described that vary in severity in a single host, and that induce different biological effects, depending on the host or scion/rootstock combination of the citrus host involved. The most common, economically important symptoms include decline or death of trees grafted on sour orange rootstock, and/or stem pitting of the scion irrespective of the rootstock. Some strains of CTV produce mild symptoms only in Mexican lime and do not cause decline or stem pitting (Garnsey et al., 1987).

One of the most effective control strategies for CTV is the early detection and identification of infection in the field and use of certified virus-free propagating material. The CTV capsid protein (CP) is antigenic and when purified virus is injected into animals such as mice or rabbits, antibodies specific to the viral protein are formed. Multiple antigenic sites (epitopes) are present in the viral CP and polyclonal antisera contain antibodies to a diverse array of epitopes. In contrast, monoclonal antibodies produced by hybridoma technology are specific to a single epitope. While polyclonal antisera react to nearly all strains of a virus, MAbs may not display such broad specificity.

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