# Phytosanitary Status of California Citrus

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ABSTRACT. The certification/registration program for citrus nursery stock in California is one of the oldest in the world and has served as a model for other programs. Although this program functions efficiently in its present form, new challenges sometimes arise that force modifications or improvements to the program. Some recent proposed changes to regulations are inclusion of mandatory stubborn testing to complement the current tristeza, psorosis, and exocortis tests. To better protect nurseries, revision of the existing interior tristeza quarantine regulations for areas not participating in a mandatory tree removal program is being considered. However, as of this writing, these proposed changes have not been implemented. Recent reports from Spain of seed-transmission of Citrus leaf blotch virus (CLBV) have prompted a re-examination of existing bud sources in California. The apparently close identity of CLBV and Dweet mottle virus, which was first reported in the 1960s, has probably protected the industry from wide-spread contamination with CLBV since current biological indexing would have detected this virus. The Citrus Clonal Protection Program (CCPP) and National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) bud sources are currently being tested for CLBV and infected sources will be re-sanitized. We are also attempting to establish laboratory-based back up tests for all currently utilized biological indexes. These and other challenges underscore the need for eternal vigilance at the NCGRCD and the CCPP, the two programs primarily concerned with the phytosanitary health of the California citrus industry.

The phytosanitary status of California citrus is largely determined by the registration program for nursery trees and the interactions between various units supporting this program. The main program supporting registration is the Citrus Clonal Protection Program (CCPP), which is a cooperative venture between the University of California, Riverside (UCR), the California Department of Food and Agriculture (CDFA), and the United States Department of Agriculture (USDA). The CCPP provides a mechanism for the safe introduction of new varieties originating from outside the state, the sanitation of varieties already in the state, and maintenance of foundation materials that provide the basis for all propagative buds utilized by the California citrus nursery industry. The USDA-ARS National Clonal Germplasm Repository for Citrus & Dates (NCGRCD) also provides a mechanism for the introduction of new citrus materials into California. The mission of the Repository is such that its support of the nursery industry is less direct than that of the CCPP. However, the ability of the Repository to safely introduce research materials also contributes to the phytosanitary status of California citrus nurseries. The CCPP and NCGRCD work closely together on various issues and the result is a synergism that benefits the California citrus industry.

The CCPP and the citrus nursery tree registration program evolved from the original voluntary psorosis-free program established in 1937. Presently, the citrus nursery tree registration program provides the California citrus nursery industry with a source of pathogen-tested budwood that must be utilized for propagation of citrus trees for sale within the state of California. Citrus introduced from outside the USA goes through quarantine at the CCPP or NCGCRD facilities in Riverside after being inspected in Beltsville, Maryland for the pres-

ence of insects, fungi, and soil. After initial testing and (in some cases) therapy, the introductions are placed in the Virus Index (VI), which tests for a wide range of viral diseases using greenhouse and lab procedures as prescribed by State and Federal regulations. If any pathogens are detected in the VI, the material is subjected to shoot-tip grafting and/or thermal therapy for elimination of viruses and viroids. Trees are regenerated after therapy and re-indexed. This cycle is repeated until the material is 'clean' (free of all known citrus viruses).

The CCPP also maintains the primary sources of citrus budwood in California. The Foundation Block at the UC Lindcove Research and Extension Center (LREC) in Tulare County is a pathogen-tested field planting of about 1,000 trees, representing about 200 varieties, which serves as the primary source of budwood for California's commercial industry. The trees are registered with CDFA at about 5 yr of age and are re-tested by CCPP several times yearly for *Citrus tristeza virus* (CTV), every 3 yr for viroids, and every 5 yr for *Citrus psorosis virus* (CPsV). Trees are removed if infected, off-type, or are otherwise abnormal. In order to protect the Foundation Block (FB), all trees at LREC (not just those in the FB) are tested for CTV annually and are removed if positive. LREC is surrounded by the Tulare County Pest Control District, which currently has withdrawn from the program for removal of CTV infected trees. The phytosanitary status of the FB thus has become threatened, and therefore in 1998 a screenhouse was erected at LREC and a Protected Foundation Block (PFB) established therein. Currently, buds are still cut from the field-planted Foundation Block, but in the future the PFB will become the main source of primary buds in California. The screenhouse will prevent aphid-vectored virus infection of these invaluable buds in the event that CTV becomes endemic at LREC. In addition, it will prevent infection of the foundation trees by the stubborn disease agent, which like CTV is vector-transmitted and is endemic in the area near LREC.

Graft-transmissible pathogens of citrus reported from California include CTV, CPsV, *Citrus variegation virus* (CVV), *Citrus tatterleaf virus* (CTLV), *Citrus vein enation virus* (CVEV), *Citrus concave gum*, *Citrus yellow vein*, *Citrus fatal yellows*, various viroids, such as those that cause exocortis and cachexia, as well as *Spiroplasma citri* and the genetic disorder wood pocket (2). There have not been any reports of additional graft-transmissible pathogens of citrus becoming established in California since the publication by French (2) in 1989. Introduction of new varieties is tightly regulated and is done legally only by either the CCPP or NCGRCD, thus eliminating the chance of inadvertent introduction of new pathogens. Regulation of introductions is supported by the industry, since it is in their best interests to prevent the introduction of potentially devastating diseases. A few attempted unauthorized introductions have been detected during the routine surveys conducted by the Central California Tristeza Eradication Agency (CCTEA) or by other means and have been interdicted by CDFA and/or APHIS officials.

Regarding the recently identified *Citrus leaf blotch virus* (CLBV) (4), the overall status of this virus in California is unknown at this time. The recent report of seed transmission of CLBV (6) makes it a concern for the citrus nursery industry. Consequently, we have recently assayed all rootstock varieties, kumquats, and Fukumoto navels in the FB at LREC utilizing RT-PCR (5) with *Dweet mottle virus* (DMV) and a CLBV sample from R. F. Lee used as positive controls. Whereas the DMV

positives consistently produced a positive result from the RT-PCR, none of the FB trees did so. In addition, all trees maintained in the Repository Protected Collection were tested in the same manner and no CLBV was detected. If any positives were to be detected, the trees would be re-sanitized.

The apparent close identity of CLBV and DMV (10) has probably prevented CLBV from becoming established in California. All introductions of new citrus germplasm are indexed into Dweet tangor and other indicators to detect DMV. This would detect CLBV, which also gives a reaction in Dweet tangor (3), even if the actual identity of the virus was not known at the time of the index. Any apparent positives, even if misidentified, would have been removed from the variety by thermal therapy or shoot-tip grafting before release. DMV was first detected in this manner in the 1960s in a Cleopatra mandarin that had been introduced to California from Florida. Given the apparent close identity of CLBV and DMV, we are sequencing the DMV genome to determine whether these two viruses are identical or closely related.

The CCPP provides budwood to commercial nurseries, which may in turn either propagate certified trees for planting in commercial groves or for producing an increase block. Budwood may be taken from the increase block for 18 mo and used for the production of certified trees. After 18 mo, the increase block is destroyed. Increase stock may also be produced from registered mother trees that are tested annually for CTV, every 3 yr for exocortis and every 5 yr for psorosis. Registered mother trees are removed from registration if found infected with any of these pathogens. Seed source trees are also registered and tested for psorosis every 6 yr. All citrus trees for sale are produced from budwood from registered trees and are certified by CDFA after visual inspection and a mandatory nematode certification.

California has a state mandated internal quarantine intended to prevent spread of CTV. Citrus-producing areas of the state are designated as quarantine (where CTV is endemic but no control or suppressive program is conducted), regulated (CTV may occur but trees are not generally infected and there may be a control program in place), and suppressive (where CTV has been detected and a control or suppression program is conducted). Since the 1960s, most of the citrus production area in central California has been 'suppressive'. Nursery trees may be moved within the suppressive area, but may not enter from the quarantine or regulated areas except under special permit issued by the CDFA at the Departmental level. A tree removal program based upon detection of CTV by wide-scale surveys conducted by CCTEA has kept CTV incidence at very low levels in the suppressive area. However, recently the Tulare County Pest Control District (TCPCD), one of the districts in the suppressive area, withdrew its participation in the tree removal program. In response, a Nursery Protection Task Force was assembled to address the problem. The recommendation of the task force in August 2002 was that the TCPCD be re-designated as a 'limited regulated' area, where citrus would be moved only within the limited regulated or quarantine areas, unless a special permit is issued from the CDFA. This would help prevent infection of trees in areas still participating in the program from inoculum from the limited regulated area. Due to various factors, these recommendations were not implemented. CDFA has recently rejected this proposal and instead proposed that the non-participatory area be re-designated as a quarantine area. This would mean that nurseries could only ship trees within the

TCPCD except under a permit issued by CDFA at the Departmental level. This proposal has great potential consequences for the California citrus nursery industry, particularly for nurseries within the proposed new quarantine area. However, there will be a period of public comment and negotiation, so the final disposition of this issue remains unclear.

Another proposal briefly considered was to add testing for stubborn disease to the required nursery testing. Currently, stubborn detection in nursery trees is only done visually. However, the lack of an adequate testing method prevented this proposal from being acceptable to the industry and lead to the work discussed in Rangel et al. (9). It has become apparent that stubborn disease is more widely distributed within California than previously recognized, and that its economic effects might be greater than previously realized. Some growers have expressed the belief that stubborn results in greater economic losses than CTV, depending upon the area of the state. Stubborn effects are greater in warmer areas, where higher temperatures might mitigate to some extent effects of CTV. We have detected stubborn as far north as Madera County, and suspect that it might also be present in the smaller more northerly production areas. It also has been detected in the few remaining groves in the South Coast and Intermediate Valley production areas (confirming reports from the last century) and may be present in Ventura County. However, extension personnel in Ventura have not reported apparent symptoms in groves.

Another recent concern in California has been the incidence of *Xylella fastidiosa* (Xf). Although *Xf* strains which cause citrus variegated chlorosis (CVC) do not occur in California, various others do (2), including strains which induce diseases such as Pierce's Disease in grapes and almond leaf scorch which may sometimes enter citrus. These other strains apparently do not survive or multiply in citrus but may still result in a false PCR result using a PCR-based test for *Xf* (Purcell, pers. comm.). The detection of any strain of *Xf* has prevented shipments of citrus nursery stock in some instances. The glass-winged sharpshooter, *Homalodisca coagulata*, was introduced into California in the 1990s and has become established and invasive. This insect is an efficient vector of *Xf*, including CVC strains (1). It has apparently increased rates of infection of grapes near citrus groves, since citrus is an efficient host plant for *H. coagulata* (8). However, the PD strain has never been reported infecting citrus in California (Purcell, pers. comm.) although it has been in Florida (7). Thus, the potential threat to citrus from *Xf* continues to require vigilance by the regulatory agencies, and the role of citrus in disseminating non-CVC strains to other crops is apparently more due to its support of glassy-winged sharpshooter populations than as a reservoir for the bacterium.

As mentioned previously, biological indexing is invaluable in detecting known and in some cases unknown pathogens, but ideally, should be supplemented with laboratory tests. In California, the success of the biological indexing program has to some extent impeded the development or implementation of laboratory-based tests. We are currently initiating comparisons of laboratory tests and biological indexing for detection of such pathogens as CTLV, CPsV, CVV and citrus viroids. This will allow us to estimate the efficacy of these tests with California strains of the pathogens, and suggest whether implementation would be beneficial. We also are currently developing laboratory assays for CVEV and the concave gum pathogen. We hope to have supplemental or backup laboratory tests in place

for all pathogens for which we currently utilize biological indexing.

Overall, the phytosanitary status of California citrus remains high. However, new and ongoing challenges, both scientific and political, continue the need for eternal vigilance at the NCGRCD and the CCPP, the two programs primarily concerned with the phytosanitary health of the California citrus industry.

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