Pathogen Testing in The Florida Mandatory Citrus Budwood Protection Program

P. J. Sieburth

ABSTRACT. The Florida Citrus Budwood Protection Program became a mandatory certified clean stock program on January 1, 1997. Budwood source trees that are used to propagate new trees for commercial use or the grower's own use are required to be registered in the program. These source trees must be tested annually for citrus tristeza virus (CTV) and every 6 yr for exocortis, cachexia and group III viroids, psorosis and tatterleaf viruses. The CTV testing is done by commercial and research laboratories certified by the Florida Bureau of Citrus Budwood Registration. Certification of laboratories requires correctly identifying a blind panel of 20 CTV isolates using ELISA. All other testing for other pathogens is done by the Florida Bureau of Citrus Budwood Registration. Citrus viroids have been indexed using citron indicator plants in the greenhouse and Orlando tangelo indicators in the field. In the summer of 1998, PCR testing for citrus viroid groups II, III and CEV was initiated. PCR testing will be used in conjunction with citron indexing for viroid indexing. Psorosis testing is now being done by biological indexing on sweet orange seedlings in an air-conditioned greenhouse. Tatterleaf virus indexing has been done in the field using *Poncirus trifoliata*. In the future, tatterleaf virus testing using PCR will be implemented.

Key words. Certification, virus indexing, viroids, citrus.

Florida has had a voluntary Citrus Budwood Protection Program since 1953 (9). Not all growers participated in the voluntary program, and a significant number of propagations were made using non-registered material. There were several large plantings propagated from non-registered budwood that had visible viroid symptoms. It was because of the poorly performing plantings that the production managers helped push for the mandatory program (7). The Florida program became mandatory for registering and testing trees on January 1, 1997. The new mandatory program was developed with input from growers and research scientists over a 3-yr period (1). Under the mandatory program, all propagations of citrus are regulated. Budwood source trees that are used to propagate new trees for commercial use or the grower's own use are required to be registered in the Citrus Budwood Protection Program. All budwood cutting is witnessed, and those data are entered into the bureau's records and computer database. Nurseries may have increase blocks as well as traditional field-grown scion trees as budwood sources. Increase blocks are budded from foundation trees or parent trees and are valid for 24 mo. Registration for increase blocks is valid for 33 mo if they are tested for freedom from citrus tristeza virus (CTV) between the 22nd and 24th mo, and for 48 mo if they are kept under screen and tested for freedom from CTV again between the 34th and 36th mo. Scion trees must be tested annually for CTV, and every 6 yr for viroids.

The bureau receives oversight from the Citrus Budwood Technical Advisory Committee, which is composed of growers, scientists and Florida Department of Agriculture representatives. The committee's scientific working group, which is composed of scientists and Florida Department of Agriculture representatives, gives guidance to the testing program. The bureau has personnel assigned to the following areas: three in the laboratory, three and one half in the greenhouse, four inspectors, two grove crew, four office staff and one bureau chief.

Budwood sources must be tested annually between Sept. 1 and May 1 for CTV. All laboratories must be certified to test for CTV through a certification process administered by the bureau. Budwood sources

must be free of isolates of CTV that react positively with the monoclonal antibody, MCA-13 (4). The CTV testing is done by the bureau, commercial laboratories and research laboratories which have been certified to test for CTV by the Bureau of Citrus Budwood Registration. Testing for all pathogens other than CTV is done solely by the Bureau of Citrus Budwood Registration.

Laboratory certification for CTV testing is an important part of the program. Commercial laboratory certification consists of an on-site visit, answering a series of questions to document all aspects of the testing process, and successfully identifying the CTV status of a test panel of 20 samples. The samples are composed of healthy controls with no tristeza, mild isolates and isolates that react positively with MCA-13. The numbering of each of these types in the blind panel is changed every year. The samples that are used contain a diversity of isolates recovered from surveys of citrus in the state by biological assays. Laboratories must correctly identify all 20 samples. Two commercial laboratories and five research laboratories were certified in 1998.

One commercial laboratory uses direct tissue blots, all other laboratories use ELISA. Test results from the private laboratories are reviewed by the bureau for accuracy before the data are entered into our databases. The Bureau of Citrus Budwood Registration tests its own budwood sources which include: 2,000 foundation grove trees in the southwestern part of the state not under screen, 65 trees under screen at the same location; 500 field trees and 100 trees under screen at the Florida Citrus Arboretum in Winter Haven; and over 500 trees under screen at the Dundee Foundation Grove location. All trees are tested annually during spring flush, and heavily used budwood sources are tested again during fall flush. In the 1997-1998 fiscal yr, 4,500 CTV tests were conducted by the bureau. All other budwood sources are tested for CTV by the certified commercial or research laboratories.

The infection rate of CTV in scion groves was impacted by the brown citrus aphid (BrCA), *Toxoptera citricida*. The BrCA was first detected in southern Florida in November of 1995 (2). The rate of spread of severe CTV in scion groves in Florida that are not under screen has increased since the introduction of the BrCA; 52% of the scion trees have CTV, many of these have been cross-protected with mild strains of CTV. Currently, the CTV incidence of severe CTV infections in scion groves ranges from 0 to 40%. The average incidence of severe CTV was 4.7% in scion groves. There was a 5.5% incidence of severe CTV in the Immokalee Foundation Grove. Trees that test severe by reacting to MCA-13 are suspended from the budwood program; however, trees that test severe in the Immokalee foundation grove are removed. The incidence of infection had remained around 1.3% over the last seven years until this current year. Of trees in scion groves of commercial nurseries, 18.7% are under screen with six commercial nurseries having screenhouses, and 59% of the budwood bureau's foundation trees are protected from insects in three screenhouses and two greenhouses.

Budwood sources are tested for viroids a minimum of once every 6 yr. Testing is initiated as a biological index using Etrog citron Arizona 861 S-1 indicator plants budded onto vigorous lemon and lime rootstocks. This allows the detection of CEVd and CVd III. Samples from the citron are taken for use in PCR to detect CVd II. In May 1998, the budwood bureau's PCR (5, 8) passed certification by correctly identifying a total of 38 samples from two different researchers for citrus viroids II, III and CEVd. PCR for CEVd, CVd II

and CVd III is also used on parent trees and as an aid to growers with trees displaying viroid symptoms. Between 1,100 and 2,400 trees a year are tested for viroids. The incidence of viroids in scion grove trees tested during this fiscal year was 0.4%. When a tree tests positive for viroids, the four surrounding trees are suspended from the program until the viroid-positive tree is removed and the four surrounding trees are tested for freedom of viroids. This encourages the growers to remove the viroid-infected tree. After the four surrounding trees are retested and a negative test result is obtained, they are reinstated in the program.

Psorosis testing is done by biological indexing on Madam Vinous sweet orange indicator plants. The greenhouse has recently been air-conditioned which has extended the testing season by two months as well as to increase the sensitivity of our test. We have the capacity to conduct 136 tests every two years. The majority of the positives detected are apparently concave gum (6) rather than psorosis.

Tatterleaf virus indexing is done in the field using *Poncirus trifoliata* or *P. trifoliata* hybrids, but in the future, PCR testing will be implemented.

Shoot-tip grafting (STG) is used to free germplasm from pathogens present in parent trees. These pathogens are detected during the initial screening process. There are currently 39 candidates for STG, most of the plants are being freed of severe CTV. Budsticks are sterilized, put into media and the shoots forced in a heat therapy chamber according to the method of Navarro (3). In 1997 we transferred 12 shoot-tip grafts representing five shoot-tip graft candidates from the laboratory to the greenhouse.

For CTV testing, we hope to move away from an ELISA test based on the monoclonal antibody MCA-13, and replace it with a laboratory test that selectively identifies isolates of CTV that cause stem-pitting in sweet orange and grapefruit when these tests are developed. The testing capacity for psorosis and concave gum will be increased by enlarging the air-conditioned greenhouse, and for tatterleaf virus both biological indexing and PCR testing will be used.

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