



CCPP

Citrus Clonal Protection Program



Citrus Clonal Protection Program

The California Citrus Clonal Protection Program (CCPP) was established over 50 years ago (1956-Citrus Variety Improvement Program) and today stands as a cooperative program between the Department of Plant Pathology of the College of Natural and Agricultural Sciences in the University of California, Riverside (UCR) and the California citrus growers, represented by the California Citrus Research Board (CRB), the California Citrus Nursery Board (CCNB), and the California Department of Food and Agriculture (CDFA). The purpose of the CCPP is to provide a safe mechanism for the introduction into California of citrus varieties from any citrus-growing area of the world for research, variety improvement, or for use by the commercial industry of the state. This mechanism includes disease diagnosis and pathogen elimination followed by maintenance and distribution of true-to-type, primary citrus propagative material of the important fruit and rootstock varieties.

Introduction of Citrus Varieties

The citrus quarantine in California is a cooperative venture involving federal, state, and county departments of agriculture and the University of California (UC). The federal government represented by the USDA Animal and Plant Health Inspection Service (APHIS) is concerned with citrus pest exclusion from foreign sources entering the U.S. There are also instances where federal quarantines exist between states, which likewise govern the movement of citrus. The director of the University's CCPP has a USDA/APHIS-issued permit to import citrus budwood from foreign countries. There are specific stipulations spelled out on this permit regarding handling and treatment of materials in quarantine, which must be followed when citrus material enters the Riverside CCPP Quarantine Facilities at Rubidoux **1**. These stipulations are also required by the State of California and are enforced by the California Department of Food and Agriculture (CDFA). The agricultural code of the State of California states that ANY CITRUS MATERIAL ENTERING CALIFORNIA REGARDLESS OF ITS POINT OF ORIGIN, FOREIGN OR DOMESTIC, MUST ENTER THROUGH THE CCPP.

Disease Diagnosis

The CCPP program of importation, production, and distribution of disease-free propagative materials starts with a comprehensive indexing, or testing program, to detect graft-transmissible diseases, which may arrive in an import budline. Graft-transmissible diseases may be caused by viruses, viroids, or other pathogens (bacteria, phytoplasmas) and are vegetatively propagated with an infected budline. Graft-transmissible diseases can seriously affect fruit quality, production, tree health, and longevity.



Stubbourn: Yield Reduction



Tristeza: Quick Decline

Additionally, diseases from infected field propagations may be spread to neighboring orchards of healthy trees by insects or farming equipment. Detection of graft-transmissible diseases of citrus is based primarily upon biological indexing, which is accomplished by grafting tissue of the import onto specific citrus indicator seedlings. Specific indicator seedlings are used to detect specific diseases. Indicator varieties have been selected for sensitivity to disease and ability to express disease symptoms. In each index adequate positive control, or disease-infected seedlings along with healthy control seedlings of each indicator variety, are held under the same environmental conditions as test source seedlings. Controls are used as a comparison with the test source and also

as confirmation that environmental conditions in the greenhouse are optimal for plant growth and symptom

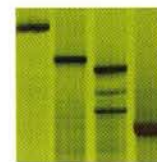


Citrus Indicator Graft Inoculation

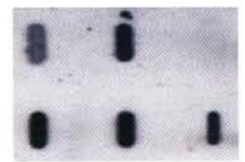


Sour Orange Infectious Variegation Positive & Healthy Control

expression. Examples of complementary laboratory techniques to the biological testing are: enzyme-linked immunosorbent assay (ELISA) used for the detection of citrus tristeza virus; sequential polyacrylamide gel electrophoresis (sPAGE); polymerase chain reaction (PCR) and hybridization used for the detection of citrus viroids; and culture in growth media for the detection of *Spiroplasma citri*, the causal agent of citrus stabborn disease **2**.



Sequential Polyacrylamide Gel Electrophoresis (sPAGE)



Hybridization

Disease diagnosis takes place in the Rubidoux Quarantine Facility in an insect-proof greenhouse with temperature and light controls that are required

for biological indexing. The Rubidoux Quarantine Facility is located within the city of Riverside adjacent to the original location of the Citrus Experiment Station (1907), isolated from the nearest commercial citrus and University experimental orchards by about 3 miles. It consists of approximately 5,000 square feet of greenhouse, 9,000 square feet of screenhouse, and a modular office and lab area.



**Rubidoux
Quarantine
Greenhouse**

Typically, when a new import is received by CCPP at the Rubidoux Quarantine Facility, propagations are made on rough lemon rootstocks to preserve the budline and to produce budwood for future index and/or therapy. These propagations are placed in a warm greenhouse and growth is pushed. In addition, some of the imported budwood is cultivated *in vitro* and the produced meristems are used for the shoot-tip-micrografting procedure. The remaining portion of the same import budwood is used to graft inoculate indicator seedlings in a screening index called a pre-index. The pre-index will indicate if the import budline is infected with citrus tristeza virus, vein enation virus, psorosis or psorosis-related pathogens, or citrus viroids. A very high percentage of new imports arrive infected with one or more of these bud-transmissible diseases.

Pathogen Elimination-Therapy

If the pre-index shows that the newly introduced variety is infected, it must be subjected to therapy procedures that can eliminate the disease or diseases from the budline. The CCPP employs two methods of therapy, thermaltherapy and shoot-tip-micrografting.

Thermaltherapy or heat treatment is performed by taking buds from the infected budline and grafting them onto citrange seedlings. The infected bud grafted onto each seedling is tightly and

completely wrapped with budding tape so that the bud will not flush during thermaltherapy. The citrange seedlings, each with an infected bud grafted on it, are placed into a hot greenhouse with temperatures maintained at 28-40 °C daytime and 25 °C nighttime for preconditioning to high temperatures for 30 days. Following preconditioning the seedlings are placed into a controlled temperature chamber which is set for 16 hour days at 40 °C and 8 hour nights set at 35 °C **3A**.



**Thermal
Therapy
Chamber**

Plants are maintained in the thermaltherapy chamber for a period of 3 months. Upon removal of the plants from the temperature chamber the buds are unwrapped. The rootstock seedling is lopped over and the top of the seedling is pushed into the potting soil such that the grafted bud will become the terminal bud. The plants are then placed in the greenhouse until sufficient budwood growth is produced from the grafted bud for further indexing. All imports received by CCPP originating outside the U.S. are routinely subjected to thermaltherapy as a precaution.

Shoot-tip-micrografting (STG) is the other form of disease clean-up therapy employed by CCPP. Some pathogens, particularly the citrus viroids (exocortis, cachexia), are difficult or impossible to eliminate by thermaltherapy, and are much more readily eliminated by STG. STG is a procedure in which several new growth tips slightly less than 1cm in length are taken from one of the original infected import propagations. Under a microscope, the apical meristem of about 0.15 mm, barely visible to the naked eye, is removed from the infected growth tip and grafted onto a seedling grown *in vitro*. If small enough when removed from the growth tip, the apical meristem is not yet developed enough to contain the pathogen and therefore the disease will not be present in the micro-



**Apical
Meristem
Isolation**

grafted propagation. STG propagations are returned to glass tubes and placed under light in a culture chamber. When the scion of the micrografted propagation reaches about 2cm, it is regrafted onto a clean rough lemon seedling and moved to the greenhouse. STG is used by CCPP to produce citrus clones free of bud-transmissible diseases **3B**.

Following any therapy procedures, all propagations produced during therapy must undergo thorough indexing again to determine disease status. If subsequent indexing shows that disease is still present then the plant material must be subjected again to therapy. This cycle of therapy and testing continues until the tests are negative. When a propagation of a budline tests negative in pre-indexing following therapy it may then enter the Variety Introduction (VI) index. Budwood from a selected therapy propagation of a budline, which has subsequently tested clean in pre-indexing, is grafted onto a host range of some 60 indicator seedlings and propagations in the complete VI Index. During the VI Index the budline is also tested for the presence of stubborn disease and is again double-checked by ELISA, sPAGE, and PCR. If a budline is shown to be free of known diseases in the VI Index it is then considered ready for release from quarantine.

Quarantine Release

When an introduced budline has tested negative for all known bud-transmissible diseases in the VI Index, the CCPP then applies for its release from both state and federal quarantine. The CCPP must first obtain release from CDEA by outlining the testing procedures and test results. Once released by the State of California, an application for federal quarantine release is sent to USDA/APHIS, containing the testing information and a copy of the letter of approval by the State of California for release from quarantine. The distribution of citrus material that has been released from quarantine is also a highly regulated

and carefully executed procedure that involves close interaction between CDFA, CCPP, and citrus nurserymen and growers. The time from introduction of a variety until quarantine release is approximately three years. Time varies based on the disease load of the introduced plant material.

Maintenance

The Lindcove Foundation and Evaluation Block

The newly introduced varieties that have tested “clean” are propagated under quarantine at Rubidoux on suitable rootstocks for field planting in the Foundation and Evaluation Block. The CCPP Foundation and Evaluation Block is located at the University of California Lindcove Research and Extension Center in the San Joaquin Valley of California. This is a field planting of about 20 acres and now contains over 1,100 trees and over 300 different scion and rootstock varieties. New



The Lindcove Foundation and Evaluation Block

budlines are being added to the Lindcove Foundation and Evaluation Block each year following quarantine release ⁴.

The Foundation and Evaluation Block is planted on fumigated soil and has a wide planting distance between rows and trees to allow for better visual evaluation of each tree. Each tree of the Foundation and Evaluation Block is examined several times each year by CCPP and interested University and industry people for trueness-to-type, fruit quality, budsports and chimeras, spontaneous genetic disorders, and symptoms of disease. Each tree is annually biologically re-indexed for tristeza for its entire lifetime and may be tested up to five more times annually by ELISA. Any tree showing abnormal growth characteristics or which test positive for disease are immediately removed from the block and the soil is fumigated prior to planting of another tree.

Lindcove Protected Foundation Block

Because of the increasing threat of natural spread of the tristeza virus around the

Foundation Block as well as the constant threat of exotic pathogens and pests, the CCPP also maintains a 40,000 square foot protected screenhouse at the Lindcove Research and Extension Center.



The Lindcove Protected Foundation Block (general view)



Ground and Potted Plantings (interior)

The CCPP maintains duplicate potted trees of all of the foundation stock varieties inside this insect-proof screenhouse. These potted screenhouse trees are maintained as backup budwood sources to the foundation field trees. Varieties that traditionally have high budwood demand may also be planted and maintained in the ground inside the protected screenhouse ⁴.

Registration of Trees and Budwood Availability

Prior to the distribution of any budwood from Foundation Blocks trees (protected and outdoors), they must be registered as budwood source trees with the CDFA. Registration by CDFA requires that after the trees have fruited satisfactorily for several years they be then re-indexed for tristeza, viroids, and psorosis. If these tests are all negative, the tree is then assigned a CDFA registration number, which must accompany any budwood distributed from that foundation tree. In order to remain as registered budwood sources CCPP re-indexes these Foundation Block trees annually for tristeza, every three years for viroids, and every six years for psorosis. Budwood from CCPP Lindcove Foundation Block trees is then released to California nurserymen and growers. Limited quantities of budwood are available and recipients normally use Foundation Block budwood to produce their own nursery or grower owned registered trees or nursery increase blocks, which are also regulated by CDFA.

There is also provision for distribution of limited amounts of “Early Release” budwood from container grown trees of some selected newer varieties. Following release from quarantine “Early Release” trees are maintained in a protected screen-

house at Lindcove. “Early Release” variety trees have not fruited but have otherwise undergone all the required indexing and are registered with CDFA. Recipients of small lots of “Early Release” budwood understand that the fruiting characteristics of that particular budline have not yet been evaluated by CCPP. A waiver of liability for budwood that may not be true-to-type or may contain budsports must be signed prior to receiving “Early Release” budwood. Budwood from the CCPP Foundation Block is cut three times per year, January, June and September. The CCPP primarily is serving the California citrus nurserymen and growers but clients outside the state can request budwood. Budwood cut dates, order forms, prices, and other information is available at www.ccpp.ucr.edu/budwood/budwood.html ⁵. Letters are sent out prior to each cut and announce the specific date of the cut as well as a list of available varieties. This letter and list may be obtained by writing: Citrus Clonal Protection Program, Department of Plant Pathology, University of California, Riverside, CA 92521.

Outreach

The CCPP makes all the information related to budwood distribution, variety evaluation, citrus disease, and management issues available to public via the web page www.ccpp.ucr.edu and publication in agricultural magazines, peer reviewed journals, and scientific conferences. Yearly Foundation-Evaluation Block inspections or “walk-throughs” are scheduled by the CCPP for the benefit of citrus nursery personnel and interested growers. The CCPP also is present at the Tulare International Farm Equipment Show and the Citrus Mutual Citrus Showcase with a booth displaying the citrus varieties of its collection and distributing information on the program and its objectives.



CCPP Fruit Display

1

Introduction of Citrus Varieties

U.C. Riverside Quarantine Facilities-Rubidoux



Greenhouse-
Disease Diagnosis



Office and Lab



Screenhouse-
Citrus Germplasm
Maintenance

2

Disease Diagnosis

A. Quarantine Greenhouse

Disease Expression:



Citrus indicators
Graft Inoculation



Mexican Lime-
Tristeza



Citrange-
Tatter Leaf



Sweet Orange-
Psorosis



Mandarin-
Cachexia



Citron-
Exocortis

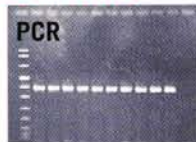


Mexican Lime-
Vein Enation

B. Laboratory



HYBRIDIZATION



PCR

Molecular



SPAGE



ELISA

Immunological



Spiroplasma citri

Culture & Microscopic Observation

3

Pathogen Elimination-Therapy

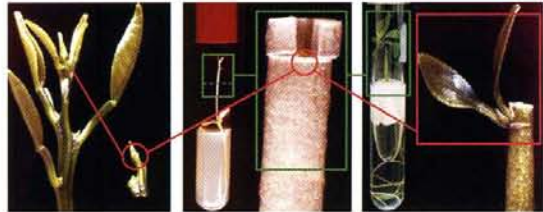
A. Thermaltherapy



Citrus Growing in
Chambers for 3 Months

40° C Days
35° C Nights

B. Shoot-Tip Micrografting



Apical Meristem
Isolation

Grafting in
Microscale

Growth *in vitro*

4

Maintenance

~300 Commercially Important Varieties - Yearly Additions
Disease Retesting & Variety Evaluation



Foundation & Evaluation Block



Protected Foundation Block

U.C. Lindcove Research & Extension Center

5

Budwood Availability



Citrus Budwood
Is Distributed
3 Times Per Year



More Information at

www.ccpp.ucr.edu/budwood/budwood.html

Sponsored by:



United States Department of Agriculture
Animal and Plant Health Inspection Service



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