

## CITRUS GENETIC RESOURCES IN CALIFORNIA

ONE OF THE LARGEST AND most diverse assemblages of citrus genetic resources in the world is maintained in California by a de facto conservation and utilization system that involves the federal government, the state government, the University of California, and the California citrus industry as represented by the Citrus Research Board (CRB). The three primary components of this system are the UC Citrus Variety Collection, the UC Citrus Clonal Protection Program, and the National Clonal Germplasm Repository for Citrus and Dates. The federal government is represented by the USDA ARS research units and individual scientists who work with citrus, the USDA NPGS which maintains repositories of citrus (see below for details on the NCGRCD), the USDA National Research Initiative which awards research funds to competitive research proposals that have included citrus research, and the USDA APHIS office which establishes and enforces US plant import and export regulations. The state government is represented by the California Dept. of Food and Agriculture which enforces for California the APHIS and California plant material import regulations (see below for details on the CAPP), administers the citrus marketing order under the California Marketing Act, and administers pathogen testing facilities. The University of California is represented by individual faculty scientists, the Division of Agriculture and Natural Resources and the Agricultural Experiment Station, the UC Riverside CNAS and several member departments, the CVC and CEBs (see below for details), and the UC Citrus Breeding Program. The CRB is the panel of growers and researchers who establish research priorities and direct funds raised by a California marketing order on citrus production toward those priorities. The goal of the CRB is to enhance the production and marketing of highest quality citrus fruits while being totally competitive in the domestic and international marketplace.

### CITRUS VARIETY COLLECTION (CVC)

The CVC, curated by T.L. Kahn, is the oldest component of the de facto California citrus conservation and utilization system. It was initiated in 1910 (SOOST et al. 1977) by staff of the Citrus Experiment Station and

USDA researchers soon after the establishment of the Citrus Experiment Station (CES) at the original site in Riverside on the slopes of Mount Rubidoux. In June of 1917, Webber, the first director of the CES, guided the installation of the Citrus Variety Collection on five acres of land adjacent to the new site of the CES in what is now the UC Riverside campus. The purposes of the CVC are threefold: 1) to conserve and evaluate true-ness-to-type of citrus and citrus relatives; 2) to provide a resource of citrus genetic diversity for research; and 3) to extend knowledge about citrus diversity.

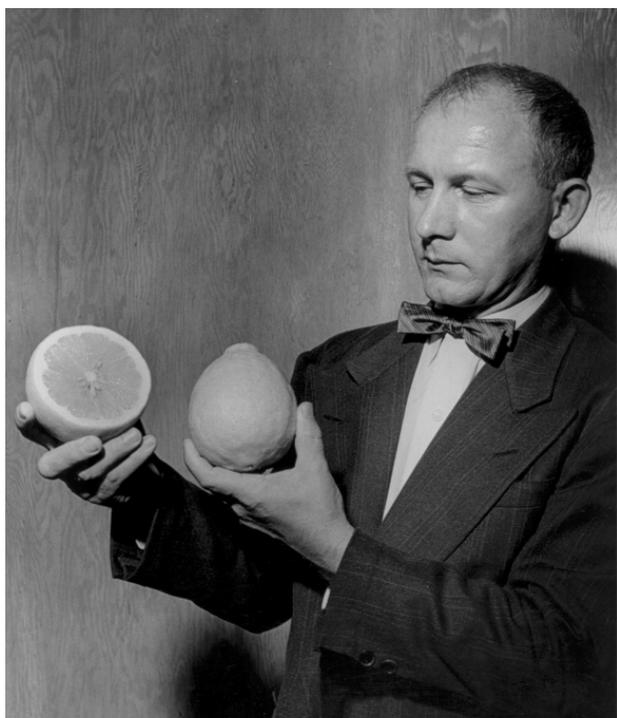
Over the 87 years since its founding, the collection has been supervised by 11 persons with some overlap in tenure between 1982 and 1995 (Table 2). From 1912 to 1936, under the direction of Webber, budwood was freely introduced into the collection from virtually all the citrus-growing regions of the world. Since 1910, when the collection began, it has included a total of approximately 4,000 citrus accessions. These field plantings were located adjacent to what is now UC Riverside. Initially most of the accessions were propagated on sour orange rootstock. In 1951, under the direction of W.P. Bitters, the collection was consolidated and repropagated onto sweet orange rootstock due to the failure of certain genotypes on sour orange rootstock (Figure 16). These new trees, as well as all new accessions on sweet orange rootstock added to the collection, were planted adjacent to

**Table 2.** Chronology of UCR Citrus Variety Collection supervision.

Curator	Date	Title
R. Smith	1909–1911	Superintendent of Whittier and Rubidoux Labs
E. Coit	1911–1912	Superintendent of Whittier and Rubidoux Labs
H.J. Webber	1912–1936	Director of Citrus Experiment Station
L.D. Batchelor	1936–1946	Director of Citrus Experiment Station
W.P. Bitters	1946–1982	Professor of Horticulture
R.K. Soost	1982–1986	Professor of Genetics
E.M. Nauer	1982–1989	Specialist
M.L. Roose	1986–1995	Professor of Genetics
R.W. Scora	1986–1995	Professor of Botany
K.D. Bowman	1990–1992	Senior Museum Scientist
T.L. Kahn	1995–present	Senior Museum Scientist

the older trees. In the mid-1950s, WALLACE (1956) indexed certain trees in the CVC and found CTV present in six trees (ROISTACHER 1981b). This presence of CTV and a later indexing of 98 trees from the collection by C.N. Roistacher and E.C. Calavan in 1963 precipitated the repropagation of the collection again in 1966 onto CTV-resistant, appropriate rootstocks including Troyer citrange and Carrizo citrange (ROISTACHER 1981b). During the mid- to late-1960s, seedling yellows, a severe isolate of CTV probably began to move within the CVC and into some of the field trees surrounding the CVC (ROISTACHER 1982). Annual inspections by Bitters of all the trees in the CVC determined that the number of trees declining each year was increasing at an exponential rate even though they were on CTV-tolerant rootstocks. This dramatic spread of seedling yellows between 1970 and 1980 was found to be due to a change in the transmissibility of CTV (ROISTACHER 1981a).

In 1981, with support of the UC Riverside CNAS, the CRB, and the State of California Employment Development Funds, a committee of UCR researchers appointed by the CNAS Dean initiated an intensive effort to index all citrus trees at the Citrus Research Center and remove those found positive for seedling yellows. The collection was again consolidated and repropagated, this time onto Carrizo citrange, C35, or another appropriate rootstock. These new trees were planted in 1983



**Figure 16.** W.P. Bitters, curator of the Citrus Variety Collection from 1946 to 1982, holding Ponderosa lemons (CRC #0294), ca. 1950. During his tenure, the CVC collection was greatly increased in number and diversity of accessions. Photo courtesy of Special Collections, Tomás Rivera Library, University of California, Riverside.

into their current locations in Fields 12A, 12B, 18A, and 18B. Additional land was allocated in these fields for expansion. Throughout the history of the CVC, new accessions have been added to the collection and others have been removed because they were very similar to other types present. A few were lost due to tree death. At the time of Bitters retirement in 1982, the CVC contained approximately 1,200 accessions. Since then some 400 accessions have been lost to attrition and selective removal of apparent duplication.

Currently, the CVC occupies 22.3 acres on the UCR campus, 2 acres at the UC South Coast Research and Extension Center (SCREC) in Irvine, California, and 2 acres at the UC Riverside Coachella Valley Agricultural Research Station (CVARS) in Thermal, California. The Citrus Variety Collection contains 865 accessions (identified in Appendix) within the genus *Citrus* and within 27 of the 33 related genera in the subfamily Aurantioideae of the Rutaceae. Approximately 670 of the 865 accessions are within the subgenus *Citrus* and encompass virtually all of the commercially important and historic citrus varieties of the world (Table 3 and Appendix). The majority (98%) of the accessions in the CVC have been assigned PI numbers by the USDA NPGS, either before or after inclusion in the CVC. About 580 of the those accessions (68%) exist only in the CVC. Thus, the CVC is a key resource for the NPGS.

## CITRUS EVALUATION BLOCKS (CEBS)

THERE ARE THREE COLLECTIONS of commercially important citrus varieties maintained in California, currently under the direction of the curator of the CVC. These Citrus Evaluation Blocks (CEBs, also known as Demonstration Blocks) are each collections of approximately 200 trees which include varieties that are also present in the CVC. One is located at the UC Lindcove Research and Extension Center (LREC), the second at SCREC, and the third at CVARS. The CEBs serve as demonstration material for periodic field days that allow industry representatives to evaluate new varieties. In addition, along with the CVC, they serve as sources of fruit for research conducted by the CVC curator to evaluate fruit quality traits for trueness-to-type and commercial potential and for fruit displays as part of CVC outreach activities. Funding for fruit evaluations comes from annual grants from the CRB. These grant funds cover the cost of a Staff Research Associate position whose duties include assistance with management of the CVC to the extent such activities are relevant to the objectives of both units.

The CEBs are maintained by the staffs of their respective centers or station. The CEBs at LREC and SCREC are projects subject to approval by the Research Advisory Committee for each center. Approved projects are allocated specific numbers of hours of care by staff at that center.

## CITRUS CLONAL PROTECTION PROGRAM (CCPP)

THE CCPP, DIRECTED BY D.J. Gumpf, has a two-fold mission: 1) it provides a safe mechanism for the introduction of citrus varieties from other citrus-growing areas of the world for research, variety improvement, or commercial production and 2) it provides the California citrus industry and researchers with a collection of important fruit and rootstock varieties which are tested and maintained free of bud-transmitted diseases. The history of the CCPP mirrors the development of our knowledge of citrus virus and viroid diseases (NAUER et al. 1967, REUTHER et al. 1972, CALAVAN et al. 1978, REUTHER 1981, GUMPF et al. 1997, BASH 1999, KRUEGER 1999b, DUNLAP 2000). Before 1930, no viral diseases of citrus had been identified. However, they existed and were common in citrus production areas in California and around the world. These viral diseases caused decreases in tree vigor, yields, and fruit quality. One of the most severe citrus virus diseases present in California was citrus psorosis virus, which greatly reduced the profitability of citriculture. In 1932, H.S. Fawcett of the Citrus Experiment Station, the father of citrus pathology, showed that psorosis was due to a virus and could be transmitted by the

use of infested budwood and mechanically on pruning tools. In 1937, a voluntary program to provide growers with a source of psorosis-free budwood produced under CDFA regulations in cooperation with UC was initiated.

In 1939, quick decline (CTV), the devastating bud-transmitted infection, was discovered in California. Spread by certain aphids as well as by humans, this disease wiped out millions of trees in the 1930s and 1940s. The danger of psorosis proved to be less than that of CTV (and stubborn disease), and use of the psorosis-free program declined.

By the early 1950s, it had become increasingly clear that many conclusions drawn from earlier citrus production experiments, especially rootstock trials and those involving orange scions, were not valid due to undetermined and random viral infections. Experimental plantings by J.W. Cameron and R.K. Soost of the Citrus Experiment Station comparing nucellar strains and old lines of commercial varieties generally showed that the nucellar strains which were virus-free were superior in vigor and yield.

It was becoming abundantly clear that a reliable source of virus-free, true-to-type budwood would be of great value to both researchers and growers. This led the Citrus Research Advisory Committee (a forerunner of today's CRB, composed largely of prominent growers and nurserymen) to request in 1957 that UC assume primary responsibility for developing and maintaining healthy citrus genetic resources, which were called 'primary foundation blocks' and to provide this material under regulations promulgated by CDFA.

This led to the establishment of the Citrus Variety Improvement Program (CVIP) in 1958. This was initially a cooperative project between the UC Riverside Depts. of Plant Pathology and Horticulture. The project leaders were initially Professors E.C. Calavan and W. Reuther. The CVIP was renamed the Citrus Clonal Protection Program in 1977 to indicate its functions more precisely and to make clear that the program is not concerned with variety testing and breeding, except to provide and maintain healthy plants. In 1979, Calavan retired and was replaced by D.J. Gumpf. In 1992, the Dept. of Plant Pathology took over sole responsibility for CCPP (see website at <http://www.ccpp.ucr.edu/index.html>).

The CCPP citrus collection, often referred to as the Lindcove Foundation Block, is a field planting of about 14.5 acres located at LREC in Tulare County in the San Joaquin Valley of California. This block contains over 1,000 trees of about 200 different scion and rootstock varieties of commercial importance. In 1996, a special CRB subcommittee charged with seeking a way to protect CCPP budwood sources recommended that the collection should be maintained in a greenhouse facility constructed to meet CDFA quarantine standards. The facility was completed during the summer of 1998 (Figure 17). It is anticipated that the trees propagated for

**Table 3.** Type and number of accessions in the CVC.

Type	Number
Mandarin	107
Lemon, lemon-type	91
Sweet orange and hybrid	75
Pummelo (shaddock)	62
Navel orange	48
Trifoliolate	48
Sour orange and hybrid	47
Trifoliolate hybrid	43
Citron and hybrid	39
Lime, lime-type	32
Grapefruit	30
<i>Citrus</i> subgenus <i>Papeda</i> and hybrid	27
Pummelo hybrid	25
Tangelo	20
Valencia orange	18
Blood orange	17
Kumquat and hybrid	17
Tangor	14
Rangpur type	11
Miscellaneous <i>Citrus</i> species	10
Calamondin and hybrid	6
Grapefruit hybrid	6
Miscellaneous species, not genus <i>Citrus</i>	72
<b>Total accessions</b>	<b>865</b>

the new greenhouse facility will be mature enough to start serving as a replacement for the Foundation Block as the primary source of budwood in 2001. Newly imported varieties are added to the collection each year after having been released from quarantine and fruited. The trees are registered by the CDFA and are retested for the presence of diseases at regular intervals. Any trees testing positive are pulled from the block. Evaluations of the trees and fruit produced on the trees are also done regularly.

The Lindcove Foundation Block is currently the primary source of budwood for the California citrus industry. Budwood from registered citrus trees in this collection is available for sale in limited quantities by UC in accordance with CDFA regulations for citrus registration and certification. Potential purchasers are supplied with a list of available cultivars, order forms, cutting date, and deadline for submitting orders. Budwood is cut three times each year. Individual nurseries or growers may use the trees produced from buds to propagate additional trees for 18 months (24 if the trees are re-tested during the first year). They may also register their own trees and then use those trees to propagate additional trees from them in a similar manner if CDFA requirements are met. Any citrus trees for commercial sale in California must be certified by CDFA as having met certain requirements before sale. These regulations and the CCPP have resulted in California having overall the lowest disease incidence and highest fruit quality of any citrus producing area in the world.

## **NATIONAL CLONAL GERMLASM REPOSITORY FOR CITRUS AND DATES (NCGRCD)**

THE MISSION OF THE NCGRCD, directed by R.R. Krueger, is to acquire, preserve, distribute, and evaluate



**Figure 17.** Budwood source trees for the Citrus Clonal Protection Program in a screenhouse-protected planting at the Lindcove Research and Extension Center. *Photo credit:* David J. Gumpf.

genetic diversity within *Citrus*, the 32 related Aurantioidae genera, and date palms and their relatives (*Phoenix* species) and to do research which supports these objectives (WILLIAMS 1990, 1992a,b, KRUEGER 1999a). The NCGRCD is a part of the USDA NPGS and is a cooperative effort between USDA-ARS and the UC Agricultural Experiment Station.

The NPGS emerged in 1974 as an umbrella system which incorporated genetic resource-related activities which had previously been parts of a wide range of agencies within and outside of ARS. Components incorporated into the NPGS included New Crops Research Branch, the Regional Plant Introduction Stations, the Plant Introduction Office, and other entities. The NPGS was established with the goal of collecting, evaluating, maintaining, and preserving plant genetic resources (SHANDS et al. 1988, WHITE et al. 1991, SHANDS 1995). Guidelines were developed for this system incorporating ideas suggested by RAVEN (1976), NATIONAL RESEARCH COUNCIL (1978), and others culminating in an operational program for the United States (SHANDS et al. 1988, NATIONAL RESEARCH COUNCIL 1991, SHANDS 1995).

The NCGRCD was established in 1987 on the UCR campus (see website at <http://www.ars-grin.gov/riv/>). This location was chosen to take advantage of the resources available at the Citrus Experiment Station, particularly the CCPP and the CVC. D.J. Gumpf of the CCPP was largely responsible for the conceptualization and specifications which resulted in the design of the facilities, and served as the University's chief contact and liaison with ARS. The first NCGRCD curator was T.E. Williams, who served from 1987 through 1993. He was succeeded by R.R. Krueger in 1994.

One of the NCGRCD's primary purposes is to exchange genetic resources with scientists all over the world. The stated mission of the NPGS is to facilitate and encourage the free exchange of genetic resources. Consequently, the NCGRCD distributes materials free of charge to qualified scientists, as do other units of the NPGS. Exchange of citrus genetic resources is highly regulated. Most citrus-producing countries have restrictions on the introduction of new citrus materials to prevent the concurrent introduction of new pathogens or strains of pathogens. Most countries will accept only pathogen-free citrus budwood. Therefore, the NCGRCD maintains a screenhouse collection of over 700 virus-free trees which represent nearly 350 accessions. These are the primary source of budwood for distribution. Approximately 55 accessions of citrus relatives are also maintained in greenhouse or screenhouse chambers (Figure 18).

There are several ways that the NCGRCD obtains new virus-free genetic resources. The CCPP was designated to be the primary vehicle for introduction and quarantine of new accessions which are received as clonal (vegetative) materials from sources outside California. The NCGRCD also receives material directly as seed after inspection in Beltsville, Maryland. Material

received as seed is generally undeveloped or semi-wild material, primarily citrus relatives, which usually come true-to-type from seed. Finally, to increase the genetic diversity of its virus-free collection, the NCGRCD is introducing accessions from the CVC into the screen-house collection. This is done by an internal quarantine process similar to the one outlined above for the introduction of foreign materials. This allows the acquisition of noncommercial types which are difficult to obtain and have a low priority for processing by CCPP. NCGRCD has submitted applications to APHIS to be permitted as a quarantine program. If approved, this would alleviate some of the pressure on the CCPP quarantine program and also increase the capacity to acquire new germplasm.

NCGRCD collaborates and interacts formally and informally with CCPP, CVC, and other researchers on a regular basis. Collaborative projects have included horticultural and molecular characterization of accessions in the CVC, development of improved anti-serum for increased sensitivity of ELISA testing, and varietal trials. This continues a productive association between the USDA and the Citrus Experiment Station that started with the activities of G.H. Powell and A.D. Shamel in the early 1900s (LAWTON and WEATHERS 1989).



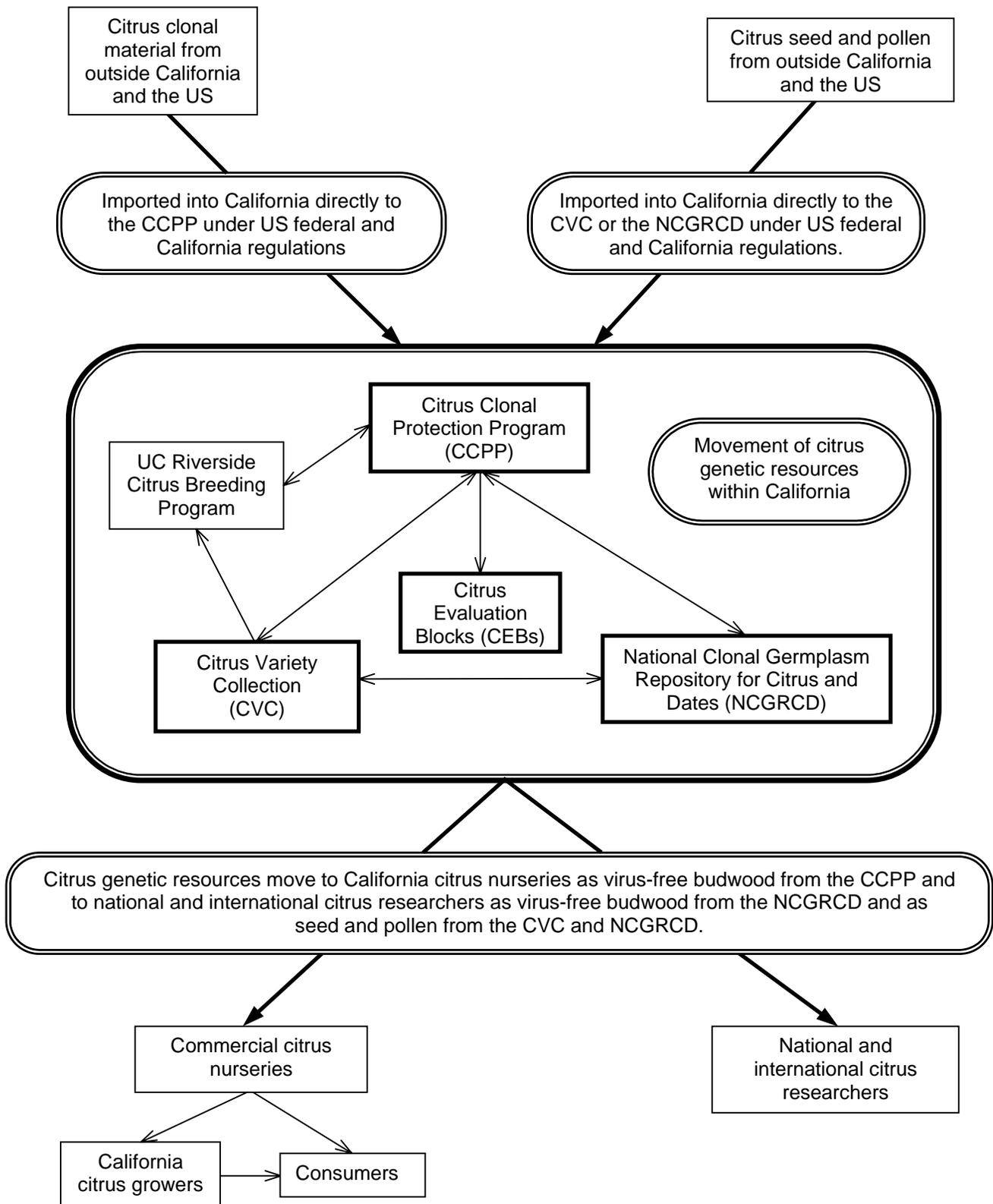
**Figure 18.** Seedlings of indicator plants for indexing, growing in cool-temperature chamber of NCGRCD greenhouse facilities. *Photo credit:* Polly M. Balance.

## INTERRELATIONSHIPS AMONG CVC, CEBS, CCPP, AND NCGRCD

THE UNIQUE WORKING RELATIONSHIPS among the CVC, CCPP, CEBS, and NCGRCD have evolved to produce a comprehensive citrus genetic resource conservation and utilization system for the State of California. Their individual goals are very closely interrelated but are not duplicative. Figure 19 illustrates the flow of genetic resources into and out of the system and among these four units and the UC Riverside Citrus Breeding Program which maintains some accessions used for breeding that are not in any of the other collections. New introductions that are to be incorporated into any one of the collections and which are imported as budwood must be introduced to the California system by way of the CCPP to prevent the inadvertent introduction of pathogens or pests not found in California. CCPP quarantines incoming clonal material and supplies budwood to the commercial industry in California. New cultivars entering California that appear to have direct commercial value are kept in the CCPP collection for evaluation and possible distribution. The same materials may be added to the CVC and NCGRCD collections depending on their specific needs and requests. Materials that are solely for germplasm enhancement are not kept by the CCPP, but are moved directly after quarantine to the CVC and NCGRCD facilities for maintenance. The NCGRCD distributes clonal materials to the national and international citrus research community. The CVC, which maintains the largest number of accessions and the largest amount of genetic diversity, is used as a resource for a myriad of research projects, extension activities, evaluation and characterization of accessions, as well as a source of materials for indexing/clean-up and nonclonal materials, and serves as a backup to the virus-free collections.

The CVC collection is a field planting exposed to natural infections of a number of diseases, notably CTV, which is pervasive in the area. Trees in the CVC are not registered by CDFA and so cannot serve as a budwood source for trees for commercial or experimental purposes or for national or international distribution. However, a permit was granted by the CDFA in 1996 to allow repropagation of citrus trees in the CVC that need to be retained in the collection from untested source trees in the CVC since many of the accessions present in the collection are found nowhere else in North America. Seed and pollen from CVC accessions can be distributed to researchers outside of California and the US, since seed and pollen generally do not transmit diseases critical to citrus production.

The CVC also has been a source for accessions maintained in the NCGRCD virus-free genetic resource collection. The CVC serves as a source of seeds, pollen, flowers, etc. for distribution by NCGRCD and as a field planting for NCGRCD accession evaluation and charac-



**Figure 19.** Movement of citrus genetic resources into, within, and out of the California system for conservation and utilization of citrus genetic resources.

terization activities. Materials obtained by the NCGRCD via exchange for incorporation into the virus-free collection need to be established in the field. Thus, both a virus-free collection maintained under screen and a field collection will continue to be necessary for NCGRCD in the future. Through the NCGRCD, the CVC has contributed to studies of cryopreservation of citrus seeds, pollen, and vegetative tissue; citrus taxonomy and genetics; and blight-associated proteins. Thus, the CVC plays an important role in fulfilling the NCGRCD mission and in contributing to citrus research world-wide.

The NCGRCD screenhouse collection is a protected and tested collection that may serve directly as a source for filling requests for budwood. NCGRCD distributes materials to scientists, scientific organizations, and governmental agencies throughout the world but

does not distribute directly to citrus growers except under unusual circumstances. The long-range goal of the NCGRCD is increasing the number of disease-free accessions of unimproved, wild-type genetic resources, breeding lines, and other nonelite material that it maintains, rather than concentrating on varieties of potential commercial importance, leaving that to the CCPP.

The small size of the NCGRCD trees and limited amount of screenhouse space also makes distribution of commercial quantities of budwood infeasible. The CCPP, on the other hand, is a field planting of mature trees which are capable of supplying commercial amounts of budwood. The number of trees planted in the Foundation Block of the CCPP can also be increased readily if demand increases.

