

**USDA-ARS National Clonal Germplasm Repository for Citrus & Dates
Riverside, California**

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The National Clonal Germplasm Repository for Citrus & Dates (NCGRCD) in Riverside, California, is a cooperative venture between the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) and the Agricultural Experiment Station of the University of California, Riverside (UCR). The mission of the Repository is to acquire, preserve, distribute, and evaluate germplasm of *Citrus*, 32 related Aurantioideae genera, and date palms (*Phoenix dactylifera*) and related species.

The Repository was established in 1987 on the campus of UCR, which provides a number of services and support to NCGRCD through a Research Support Agreement (RSA), through the Dept of Agricultural Operations. Specific Cooperative Agreements are in place with Drs. Mikeal L Roose, Tracy L Kahn, and C Thomas Chao of the Dept of Botany & Plant Science. More information on these SCA*s is presented below. Two direct funded SCA*s are administered by the Repository, with Dr Michael Stanghellini, Dept of Plant Pathology (Biology and Control of Soil-borne Pathogens in Greenhouse Production) and Dr Thomas Bellows, Dept of Entomology (Biological Control of the Giant Whitefly). These pass-through agreements do not directly benefit the Repository program and will not be discussed in this report. Additional information on cooperation between the NCGRCD and the University is detailed in the appropriate sections. Additional UC facilities utilized include the Coachella Valley Agricultural Research Station (CVARS), located in Thermal, and the South Coast Research and Extension Center (SCREC), located in Irvine.

The Repository is served administratively by the ARS Riverside Location staff and by the ARS Pacific West Area (PWA) staff, located in Albany. The Repository is a part of the USDA National Plant Germplasm System (NPGS), under National Program 301: Plant, Microbial, and Insect Genetic Resources, Genomics, and Genetic improvement.

Citrus and Date Genetic Vulnerability

**Prepared by the Citrus & Date Crop Germplasm Committee
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Introduction

Citrus is one of the world's major fruit crops. It is widely grown in most areas with suitable climates – tropical, subtropical, and borderline subtropical/temperate. In the United States, Citrus is an important crop in Florida and California, and is locally important in Arizona and Texas. Current statistics for acreage, production, and farm-gate value may be accessed at <http://usda.mannlib.cornell.edu/reports/nassr/fruit/zcf-bb/>. The referenced page also has links to individual state statistics if these are needed. Citrus is such an important commodity that USDA divides fruit crop production into 'Citrus Fruits' and 'Noncitrus Fruits'.

During the period 2002 – 2004, there were approximately 1.0 million acres planted with citrus. During the 2003 – 2004 season, the total was approximately 984,000 bearing acres (Florida, 680,000 Acres; California, 250,000 acres; Arizona, 27,000 acres; Texas, 27,000 acres). Production of oranges, grapefruit, lemons, and other citrus during this period were approximately 7 million, 2 million, 800,000, and 500,000 tons, respectively, and farm gate receipts averaged \$ 2.4 billion (Florida, \$ 1.4 billion; California, \$ 893 million; Arizona, \$ 46 million; Texas, \$29 million). The actual value of production is higher when added value, such as export and juice production, is considered.

Florida grows approximately 70 % of the oranges and grapefruit produced in the US, while California and Arizona produce almost all of the lemons. Nearly 90 % of the citrus produced in Florida is for processing. In contrast, fresh market fruit accounts for approximately 70 % of the citrus production of Arizona, California, and Texas. Approximately 70 % of the US orange crop and 50 % of the US lemon and grapefruit crops are processed. The US enjoys a favorable balance of trade with citrus, exporting nearly 10 times the tonnage that is imported.

Citrus is produced throughout central and southern Florida, with the newer plantings in the south to avoid freezes. The bulk of the acreage is now south of Lake County. The warm, humid semi-tropical climate of Florida enables the production of large quantities of fruit suitable for processing. Citrus is grown in several different climatic areas in California. The cool, coastal valleys (eg, Ventura County) are suited for the production of lemons. High quality sweet oranges are grown in the intermediate valleys (eg, Tulare County), which have semi-arid, sub-

tropical climates. The desert valleys (eg, Coachella valley) have hot, arid climates suitable for the production of grapefruit and certain types of lemons and mandarins. Citrus in Arizona is grown in areas similar to the desert areas of California (eg, the Yuma area), and production is similar. Citrus in Texas is grown in a warm, humid area (the Rio Grande valley) suitable for grapefruit production. There is of course some overlap in the types of fruits produced in the different growing areas. There are also some small acreages of satsumas along the Gulf Coast, but these are generally not included in agricultural statistics.

Citrus is an extremely important crop on a world-wide basis, and is grown wherever the climate is suitable. Total world-wide production of citrus is estimated at over 73 million metric tons. The 5 largest citrus-producing countries during 2002 – 2004 were Brazil, USA, China, México, and Spain (http://www.fao.org/es/ESC/common/ecg/28189_en_FinalBull2003.pdf; see also http://www.fas.usda.gov/http/circular/2004/08-04/8-31-04_Citrus_Feature.pdf). Brazil and Florida produce primarily citrus fruit destined for the juice or concentrate market, while China, México, Spain, and California produce primarily fresh-market fruit. Spain is the world's largest exporter of citrus fruit. Sweet oranges and mandarins are the most import types of fruit in the export/import markets. Citrus is also widely produced in dooryard plantings for personal and local consumption.

Dates are a minor crop compared to citrus, since their climatic requirements are more stringent. In the US, the consumer demand is also much lower than for citrus. There are only about 5,000 acres grown in the hot, arid low desert areas of California and Arizona (Coachella, Imperial, and Bard valleys). Dates produce farm gate receipts of about \$ 20 million annually, making them locally important in the desert valleys. Dates are included in the 'Noncitrus Fruits' category, and their statistics may be found at <http://usda.mannlib.cornell.edu/reports/nassr/fruit/pnf-bb/>.

Dates are widely grown and consumed in the arid regions of the Middle East, North Africa, and the Indian subcontinent, and have great cultural significance people living in these areas and for Muslims worldwide. The US is not a significant producer of dates on a global scale. The most important date-producing countries are Saudi Arabia, Egypt, Iran, and Pakistan. As with citrus, dates are grown extensively in dooryard and local market situations.

Status of Crop Vulnerability

Plant germplasm is living tissue from which new plants can be grown. It contains the unique genetic information which gives plants their individual characteristics and links generations of living plants to one another. The genetic diversity of plants, developed by evolution, hybridization, natural selection, and manipulation by humans, provides the basis for the food production which supports the world's population. This diversity is threatened by habitat loss, development, the shift to cultivation of a small number of advanced lines, and other factors. Wilkes (1988) recognized these problems and pointed out that plant germplasm is in reality biological information passed down through generations in an unbroken chain. Once this chain is broken that unique germplasm is lost forever. This has lead to the necessity of protecting and preserving plant genetic diversity for current and future use.

Preservation of the genetic diversity represented in all the plant ecosystems throughout the world has become a major issue of international concern. The loss of increasingly large numbers of plant species through habitat destruction threatens the availability of a diverse plant germplasm base which will be needed to feed future generations (Holden and Williams, 1984; Janzen, 1988; Raven, 1988; Brown et al, 1989; Center for Plant Conservation, 1991; Holden et al, 1993; National Research Council, 1993). Similar losses have occurred in existing plant collections through inadequate maintenance. Some general aspects of plant genetic conservation are presented in Given (1994) and Chrispeels and Sadava (1994). Popular accounts, often critical and dealing more with the political aspects of genetic resource conservation, include Busch et al (1995), Fowler and Mooney (1990), and Raeburn (1995).

Ideally, genetic resources should be conserved in situ. However, the factors mentioned above, especially habitat loss, make maintenance of genetic resources in situ somewhat precarious. Consequently, ex situ conservation is often necessary to salvage genetic resources. Genetic materials may be lost through disease, weather, natural disasters, etc, and so ex situ collections should be maintained in many cases even when there is not an immediate threat of habitat loss. Ex situ collections are also more accessible for researchers and necessary for characterization and evaluation. Maintenance of germplasm in a disease-free state is also desirable, and this is often

possible only in ex situ collections.

The genus *Citrus* is one of 33 genera in the sub-family Aurantioideae of the family Rutaceae (Table 1). The taxonomy and geographic origin of the Aurantioideae have been reviewed by Swingle and Reece (1967). *Citrus* and its related genera are native to Southeast Asia (northeastern India, southern China, the Indochinese Peninsula). This is the center of diversity for these species. Tanaka (1954) proposed a theoretical dividing line (the Tanaka line) which runs southeastwardly from the northwest border of India, above Burma, through the Yunnan Province of China, to south of the island of Hainan. Citron, lemon, lime, sweet and sour oranges, and pummelo originated south of this line, while mandarins, kumquats, and trifoliates originated north of the line. The mandarins apparently developed along a line northeast of the Tanaka line, along the east China coast, through Formosa, and to Japan, while the trifoliates and kumquats are found in a line crossing south-central China in an east-west direction. More recently, Gmitter and Hu (1990) have proposed that Yunnan, China, through which the Tanaka line runs, is itself a major center of origin for citrus. Some related Aurantioideae genera are native to Asia, Africa, and Australia.

'Wild' citrus is relatively rare, mostly existing as scattered trees in remote areas rather than as pure stands. *Citrus* hybridizes readily and in some instances produces true-to-type (clonal) seedlings due to nucellar embryony. These factors, plus the ability to propagate citrus vegetatively by grafting, has led to the selection of more desirable traits by humans and the perpetuation of 'elite' germplasm lines, frequently at the expense of the progenitor wild types. *Citrus* has been domesticated since ancient times, and where 'natural' populations are located, it is often difficult to determine whether they represent wild ancestors or are derived from naturalized forms of introduced varieties.

The taxonomy of *Citrus* is not precisely established. Most researchers utilize the Swingle system (Swingle, 1943; Swingle and Reece, 1967), which recognizes 16 species, or one of its modifications which recognize 17 species (Bhattacharya and Dutta, 1956; Stone, 1994a), 36 species (Hodgson, 1961), or 31 species (Singh and Nath, 1969). The recent taxonomy of Mabberly (1997, 1998) is essentially a modification of the Swingle system, with several genera being reabsorbed into *Citrus*. In contrast, the Tanaka taxonomy recognizes up to 162 species (Tanaka, 1977). This lack of agreement reflects differences of opinion as to what degree of difference justifies species status and whether or not supposed hybrids among naturally occurring forms should be assigned species status. There is no definitive work on *Citrus* taxonomy, and many workers use a sort of ad hoc system somewhat intermediate between the two systems. The Tanaka system is used widely in most countries outside the USA, and is useful in recognizing horticulturally important cultivars and characteristics. More recently, it has been suggested that only three species (*C. medica*, *C. reticulata*, *C. maxima*) constitute valid species (Scora, 1975; Barrett and Rhodes, 1976). Interestingly, the earliest workers also believed that there were only three or four valid species of citrus (Linnaeus, 1753; Hooker, 1875).

Citron (*C. medica*), mandarin (*C. reticulata*), and pummelo (*C. maxima*) are considered to be most similar to the ancestors of modern cultivated types. These three species reproduce sexually and if different cultivars within the species are intermated, the progeny are similar to their parents. The other important types (orange, grapefruit, lemon, and lime) are believed to have originated from one or more generations of hybridization between these ancestral genera. Most of the cultivars of orange, grapefruit, and lemon are believed to have originated from nucellar seedlings or budspots. Consequently, the amount of genetic diversity within these groups is relatively low, in spite of there being many named varieties. Conversely, mandarins, pummelos, and citrons have higher levels of genetic diversity since many of the cultivars have arisen through sexual hybridization. However, these types represent only a small portion of US citrus production. The number of rootstocks currently being used is limited. Genetic diversity within the different types of rootstocks is also limited, as they generally produce a high percentage of nucellar seedlings. Table 2 summarizes the current understanding of the origin, mode of reproduction, and level of genetic diversity within certain commercially important species of the genus *Citrus*.

Aurantioideae genera related to *Citrus* are utilized much less frequently and therefore exist most often as 'wild' unselected types. These 32 genera are mostly tropical and of limited commercial importance. Therefore there has been less attention focused upon them except by local inhabitants. These remote areas are often in danger of habitat destruction, and therefore the threat of losing genetic diversity is present.

One complication in dealing with the taxonomy of citrus and especially the related genera is the lack of current information on many taxa. W. T. Swingle, US Dept of Agriculture, spent over 40 years studying the taxonomy and botany of *Citrus* and its related genera. His many publications in this area are summarized in Swingle (1943) and its slight revision as Swingle and Reece (1967). Reviewing these papers indicates that in many cases, a decision

as to whether a particular species should be established was based upon a single collection or herbarium item. Due to the lack of access to many areas in which these species are native, Swingle's classification became somewhat ossified into dogma. It is possible that at least some of Swingle's species do not currently exist and perhaps never existed. There has been only a small amount of research into these related Aurantioideae genera in recent years, as summarized in Krueger and Navarro (200_). Mabberly (1997, 1998) reabsorbed the genera *Fortunella*, *Microcitrus*, and *Eremocitrus* back into *Citrus*. Recent revisions or comments have been made for *Clausena* (Stone, 1978b; Molino, 1994), *Clymenia* (Stone 1985a), *Glycosmis* (Huang, 1987; Stone, 1978a, 1985b, 1994b), *Luvunga* (Stone, 1985c), *Monanthocitrus* (Stone, 1985c; Stone and Jones, 1988), *Murraya* (Huang, 1978; Stone, 1985c; Jones, 1995), *Oxanthera* (Stone, 1985b), and *Wenzelia* (Stone, 1985b).

Date palms (*Phoenix dactylifera*) have been cultivated and subjected to selection by man since ancient times, and, like citrus, the distinction between 'wild' and cultivated date palms is blurred (Krueger, 1995, 2001a). Although it is generally accepted that there are 12 - 13 species within the genus *Phoenix* (Chevalier, 1952; Moore, 1963; Barrow, 1998), *Phoenix* interbreeds freely, interspecific hybrids are numerous and fertile, and it is possible that all *Phoenix* species should be included in a single species (Wrigley, 1995). Wild *Phoenix* species are found in the tropics and sub-tropics of Africa and Asia, while *P. dactylifera* originated in the Middle East somewhere between western India and southern Iraq (Table 3).

Agricultural utilization of these crops involves a narrow range of genetic material, both in the US and abroad, making citrus and dates genetically vulnerable. Genetic diversity in the centers of origin is severely threatened by habitat losses caused by deforestation, population pressure, fire, hydroelectric development, clearance for agriculture or other development, tourism, etc (WWF and IUCN, 1994-1995). These factors may be especially important in countries such as India and China, which have rapidly expanding populations coupled with rapid economic/industrial development. This situation makes *ex situ* conservation of genetic resources of citrus and date palms imperative. This statement is not meant to diminish the importance of *in situ* conservation and habitat preservation, but to put into perspective the very real potential for loss of genetic resources conserved *in situ*.

Assessment of the genetic vulnerability of any crop requires knowledge of the extent and distribution of genetic diversity. This is acquired by systematic sampling and mapping of the flora of the geographical areas in which the species in question are found, as well as an assessment of *ex situ* collections. Unfortunately, information on natural and semi-natural citrus and date germplasm is limited on the international level. This is due to the remoteness of some of the material, a lack of resources devoted to assessing these areas, and political considerations. In some cases, information may be available at the local or national level, but not to the international genetic resource conservation community.

The information that is available is often simply a catalog of plants present in an area, with little more than names and phenotypic descriptions. Often even information on the frequency of occurrence is lacking. More detailed characterization and evaluation data is needed to adequately assess the actual amount of genetic diversity present. This data should include both descriptive data and molecular level genetic analysis of germplasm existing both *in situ* and *ex situ* (Albrigo et al, 1997; Gmitter et al, 1999).

The status of citrus genetic resources and their conservation has been reviewed by Reuther (1977), IBPGR (1982), Albrigo (1997), and Broadbent et al (1999). A limited amount of information is found in FAO (1996). More specific information has been presented for Southeast Asia (Mehra and Sastrapodja, 1988; Jones, 1990; Verheij and Coronel, 1991; Coronel, 1995; Osman et al, 1995; Saamin and Ko, 1997; Hor et al, 1999), Thailand (Anupunt, 1999), Philippines (Garces, 1999), Malaysia (Santiago and Sarkawi, 1962; Allen, 1967; Jones, 1985; Jones and Ghani, 1987; Jones, 1989; Jones, 1991; Saamin and Ko, 1997; Ko, 1999), Vietnam (Ca, 1999; Le et al, 1999), China (Hu, 1989; Gmitter and Hu, 1989; Zhaomin, 1989; Gmitter and Hu, 1990; Zhang et al, 1992; Zheng, 1995; Chen, 1997; Deng et al, 1997; Zhusheng et al, 1996; Zhusheng, 1999), India (Singh, 1981; Singh, 1985; Dass, 1990; Singh and Chadha, 1993; Chadha, 1995; Singh and Uma, 1995; Chadha and Singh, 1996; Rai et al, 1997; Ghosh, 1999), Nepal (Chaudhary, 1999), Japan (Nishida et al, 1981; Iwamasa, 1988; Omura, 1996, 1997; Nito et al, 1999), Australia (Forsyth, 1988; Sykes, 1997, 1999; Mabberley, 1998), Spain (Ortiz et al, 1988), Morocco (El-Otmani et al, 1990), Brazil (Machado, 1997), and the United States (Cameron, 1974; Reuther, 1988). Rouse (1988) and Bittencourt et al (1992) have summarized the world citrus collection situation identifying major and minor citrus collections. These reviews deal with both *in situ* genetic resources and *ex situ* collections.

The center of origin and diversity of citrus is in Southeast Asia. Consequently, this is where the greatest amount and diversity of citrus germplasm may be expected to be found, particularly *in situ*. However, in

developing countries such as India and China, development and habitat loss can occur quite rapidly, unfortunately resulting in a loss of genetic materials and germplasm. Recognizing this threat, efforts have been made at *ex situ* conservation, as well as habitat preservation. Unfortunately, the situation is not always as it should be. Outside of the centers of origin/diversity, collections consist mostly of advanced lines and commercial varieties. Large *ex situ* citrus collections of this sort are found in Argentina, Australia, Brazil, Corsica, Morocco, New Zealand, South Africa, Spain, Turkey, and the United States (see below). Some of the larger collections contain many selections of the same variety, and so the genetic diversity is less than might be expected from the number of accessions.

Southern PR China is one of the centers of diversity for *Citrus* and related genera, and a wide range of genetic diversity is apparently still present *in situ*. However, some (though not all) areas are threatened with habitat degradation or lack of proper management that could result in decreases in genetic diversity. In PR China, exploration and collection of indigenous citrus genetic resources began in the 1950's and 1960's, but was interrupted by the Cultural Revolution of 1967 – 1972. Governmental surveys resumed during the 1970's and 1980's and uncovered a number of new putative species, including *C. honghensis*, *C. mangshanensis*, *C. daoixianensis*, and *Poncirus polyandra*. These putative species are mostly unknown outside of PR China. Areas that have been explored include Guangxi district, Guangxi province; Shennong jia, Hubei province; Sichuan, Gansu, and Shanxi provinces; Hainan Island; and Tibet. There are also a number of indigenous Aurantioideae in southern China. There is exploitation (use) of indigenous germplasm, and some attempts at *in situ* preservation have been made. However, conservation of citrus genetic resources in PR China is mostly *ex situ* at present. Beginning in the early 1960's, a National Citrus Germplasm Repository was established at Beibei, Chongqing, Sichuan province, and regional citrus germplasm repositories in Huangyan, Zhejiang province; Guilin, Guangxi province; Zhangsa, Hunan province; Guangzhou, Guangdong province; Jiangjin, Sichuan province; Wuzhong province; and Hubei province. As of 1996, the National Citrus Germplasm Repository had 1041 accessions (decreased from 1200), of which indigenous, bud mutations, and nucellars accounted for 58 %, 5 %, and 37 %, respectively. The Huangyan, Guilin, Zhangsa, and Guangzhou regional repositories had 128, 216, 40, and 140 accessions, respectively, decreased from 215, 462, 150, and 180 accessions, respectively. The substantial decreases in accessions were due to such factors as lack of funds, disease, and weather (freezes). The exact composition of these collections is unknown, but a high percentage is indigenous germplasm, and undoubtedly represents a substantial amount of diversity, although some of the germplasm, indigenous and otherwise, consists of advanced lines or selections. The accessions at the repositories have had a limited amount of characterization and evaluation done on them. Regional Citrus Research Institutes in Shantou, Guangdong; Ichang, Hubei; Thouyang, Hunan; Ganzhou, Jianxi; Yuchi, Yunnan; and Wu, Jiangsu also maintain small amounts of citrus germplasm, as do botanic gardens such as Xithanbanna and Guanzhon. As of 2000 (personal communication), the regional and national citrus germplasm repositories established in the 1980's – 1990's were mostly no longer functional. The only one that remained open was Beibei, which served as the national collection. There were problems with diseases and weather, but the critical factor was a lack of funding for maintaining the collections. This also had a negative effect on the Beibei collection, which decreased to only over 800 accessions. There seemed to be a crisis in PRC as regards preservation of citrus germplasm. However, in 2004 (personal communication), it was indicated that the situation had improved somewhat. There had been an infusion of financial support, and the number of accessions maintained had risen to more than 900. This included about 200 accessions available as virus-tested material. They were attempting to re-establish some accessions that they had had trouble with in the past.

In India, the northeast region is the center of origin/diversity. Unfortunately, this region sometimes experiences civil unrest, making evaluation of genetic diversity and plant exploration difficult. There are apparently a few stands of 'wild' citrus in these areas, but many of the 'wild' populations consist of dooryard plantings. A long history of cultivation and selection has produced many genotypes/landraces, which are difficult to separate from 'wild' citrus. Still, a wide range of genetic diversity undoubtedly exists in these areas. There is an *in situ* gene sanctuary for citrus in the Garo Hills in the northeast, which is a field gene bank with 627 accessions. Other regions of diversity include the central and northwest Himalayas, Maharashtra, and the southern peninsula. *Ex situ* conservation of citrus germplasm began in the 1950's in India, but the number of accessions maintained has declined due to lack of collection maintenance and disease. *Ex situ* collections consist of 451 - 521 accessions (depending on the source of the estimate) at 8 sites (Chetalli, Bangalore, Rahuri, Tirupati, Abohar, Bhatinda, Yercaud, New Delhi). There are smaller collections at 14 additional sites (Akola, Barapani, Birouli, Hessaraghatta, Katol, Ludhiana, Nurpur, Parbhani, Pantnagar, Pedong, Periyakulam, Sirmour, Srirampur, Tinsukia). The *ex situ* collections in India are mostly of rootstock varieties and a few local cultivars, with not much diversity represented. Many of the indigenous types described in historical accounts such as Bonavia (1890) and later works such as

Bhattacharya and Dutta (1956) and Dutta (1958) are apparently not in any of the collections. The intention is to concentrate the various collections at the National Research Centre for Citrus in Nagpur and/or at regional research centers at Bangalore Tirupati (south), Ludhiana/Abohar (north), Rahuri (central), and Shillong and Assam (northeast). However, this is still in the planning/implementation stages, and as of January, 1996, there were only a small number of accessions planted at Nagpur.

Southeast Asia (including Malaysia) is rich in indigenous germplasm and, with chance seedlings, semi-wild, and wild types. Most indigenous types of citrus are grown in the hot lowlands. One species (*C. halimii*) is still found wild in the highlands, while the majority of the others are cultivated. Some introduced species (eg, *Aegle marmelos* and *Limonia acidissima*) have become naturalized. This genetic diversity is threatened by deforestation, development, and disease. In 1983 - 1988, four IPBGR-coordinated collecting missions to Thailand, Malaysia, Indonesia, and Brunei resulted in the addition of 391 new accessions (these are maintained in Japan, the organizer of the missions). In 1986, IBPGR invited Malaysia to accept responsibility for maintaining a field collection of Southeast Asian Aurantioideae (Rimba Ilmu).

There are a number of collections in the Southeast Asia area, which although not as large as some collections, have notable genetic diversity, particularly in the pummelos and some of the related genera, and appear to be fairly well maintained and curated. There are four collections in Malaysia, the main one being the University of Malaya (Rimba Ilmu) Botanical Garden (over 140 accessions representing 25 genera and 53 species); the others (Jerangau Station, Trengganu; Kuala Kangsar, and Cameron Highlands) are maintained by MARDI. There are also some in situ conservation efforts, such as at the Taman Negara National Park in Pahang and the Danum Valley in Sabah.

There are three collections in Thailand with 585 total accessions. The most important are Phichit Horticultural Research Center, which has a collection of mostly native pummelos; and Nan Horticultural Research Station, which has approximately 70 accessions of mandarins, sweet oranges, and citrus relatives, of which approximately 25 % are native. In the Philippines, the main collection of citrus genetic resources is maintained by the National Plant Genetic Resources Laboratory of the Institute for Plant Breeding in Los Baños, and consists mostly of commercial and imported varieties; reportedly there are two other collections with slightly over 100 accessions. There are also three collections in Indonesia (498 accessions) and at least two in Vietnam (National Institute of Agricultural Science and Technology and Phu Ho Fruit Research Center), which contain materials collected by an IBPGR-sponsored program in 1992.

Asia's largest collections, outside of the centers of origin discussed above, are in Japan. Citrus entered Japan in ancient times, compared to its appearance in countries farther away from the centers of origin, and some types became semi-naturalized. There is a limited amount of in situ preservation of these naturalized types, but as in other areas development is a threat. The Fruit Tree Research Stations in Tsukuba, Okitsu, and Kuchinotsu have large collections that have a number of citrus relatives. Total accessions were said to be over 1200 in 1996. Of interest are the large numbers of mandarin-types, especially satsumas. There are also three other collections of citrus germplasm in Nagasaki, Kagoshima, and Okinawa. Japan has been active in collecting in Southeast Asia (see above), Nepal (1983-1985), and Vietnam (1996) through IBPGR-coordinated cooperative programs. Accessions collected from these ventures are maintained in Japan.

Australia has several *ex situ* collections maintained by State Government Departments of Primary Industries and the Commonwealth Scientific and Industrial Research Organization that consist primarily of cultivated types. However, this island country is the center of origin for several related genera (most notably *Eremocitrus* and *Microcitrus*), that are included in the collections, as well as in certain botanic gardens (eg, Royal Botanic Garden, Sydney, and Brisbane Botanic Gardens) and arboreta (eg, Waite Research Institute, University of Adelaide Arboretum). Also of interest are hybrids of these native types. Australia has recently (as of 2000) been cooperating with P R China in the area of germplasm evaluation. A number of trifoliates were received and being evaluated for various traits; however, this was for evaluation only and not for maintenance or distribution.

The situation with the related Aurantioideae genera is less well known, particularly from outside the South/Southeast Asian region. Although these genera are sometimes represented in collections, there is little information available about their status in situ. However, as many of them originated in countries which are currently rapidly developing, experiencing population growth and pressure, or being bothered with civil unrest, it is probable that at least some native populations are existing in habitats which may be threatened. These factors also make assessment of the situation difficult.

The situation with date palms and other Phoenix species is similar to that for the citrus relatives. *Phoenix* species apparently originated in the middle East, Africa, the Indian subcontinent, and Southeast Asia. Little information appears to be available about the amount of 'wild' or landrace *Phoenix* species present, nor about the amount of genetic diversity represented. Assessment of these factors is complicated by political considerations, which are rapidly changing. Undoubtedly, many of the factors which make some Aurantioideae genera vulnerable also threaten *Phoenix* germplasm.

Barrow (1998) lists several *Phoenix* sp as being threatened, whereas she does not consider others to be. The status of *P. dactylifera* is not clear. Certainly the species is not threatened; however, due to its long history of domestication it is not clear whether wild populations of *P. dactylifera* exist. *P. theophrasti* is sometimes considered to be a feral or wild type of *P. dactylifera*. *P. roebelini*, *P. canariensis*, and *P. loureiri* are widely distributed as ornamentals, but wild populations may be threatened. *P. paludosa*, *P. reclinata*, and *P. sylvestris* are not considered to be threatened (due to their wide distributions). The conservation status of *P. andamanensis*, *P. caespitosa*, and *P. acaulis* are not clear.

Bettencourt et al (1992) list only about ten collections world-wide, the largest of which are found in Algeria, India, Iraq, Nigeria, and the United States. Except possibly for the Nigerian collections, most accessions appear to be elite cultivars or breeding lines, so the genetic diversity is probably rather low.

Overall, the genetic diversity of Citrus, related Aurantioideae genera, and Phoenix species is vulnerable. Habitat loss is common in areas in which these plants are endemic, and eco-geographic assessments of these areas are often lacking. Although some efforts are being made in the areas of in situ and ex situ conservation, it is probable that there has been considerable genetic erosion for these species. Due to the lack of eco-geographic information, as well as characterization and evaluation data from the *ex situ* collections, it is impossible to say to what extent this erosion has occurred. It is imperative that more resources be devoted to these areas in the future.

Due to these factors, it has become evident that more intensive interactions and coordination between the various entities dealing with citrus germplasm conservation is necessary (Albrigo, 1999; Ramanatha Rao and Arora, 1999). A proposal to establish a global network on citrus genetic resources conservation and utilization was recommended during the meeting of the FAO Intergovernmental Group on Citrus, in April 1996. Accordingly, this proposal was followed up and further elaborated during the Symposium on the Conservation of Genetic Resources of Citrus and its Relatives, held in South Africa in May 1996, where the major technical issues to be addressed by a global cooperative program were analyzed (Albrigo, 1997). The global technical cooperation network (Global Citrus Germplasm Network = GCGN) was formally constituted under the aegis of the FAO, functions on a voluntary basis, and involves national institutions as well as existing regional and inter-regional networks dealing with citrus genetic resources conservation and utilization (Global Citrus Germplasm Network, 1998). It helps link initiatives in different parts of the world dealing with citrus genetic resources exploration, conservation and utilization. The GCGN plays a role in harmonizing and strengthening on-going networking initiatives that are deal with citrus germplasm conservation and utilization, and in the promotion of new undertakings in different regions of the world. The existing regional and inter-regional citrus networks (IACNET (Americas), MECINET (Mediterranean region)) and those under constitution (Asia-Pacific and Sub-Saharan Africa) participate in the GCGN. The Global Network is guided by a Coordinating Board which is chaired by the General Coordinator of the Network and includes the coordinators of the technical working groups and representatives of the different regional and inter-regional citrus networks. Workshops were held in conjunction with the Citrus Germplasm Conservation Workshop in Brisbane in November, 1997 (Broadbent et al, 1999), and MECINET in Acireale, December, 1997 (Global Citrus Germplasm Network, 1998). A general summary of the issues and recommendations was reported by Ramanatha Rao (1999). More information is available on the internet (<<http://www.lal.ufl.edu/CONGRESS/Gcgnrept.html>>). It is hoped that this type of international cooperation will increase the efficiency of citrus genetic resource conservation efforts.

Present Germplasm Activities in USA

In the United States, the primary responsibility for the conservation of genetic diversity of crop plants is charged to the USDA-ARS National Plant Germplasm System (NPGS), which had its origins in the 1970's (Shands et al, 1988; White et al, 1989; National Research Council, 1991; Shands, 1995). Efforts towards conservation of the so-called 'clonal' crops began later, in the mid- to late-1980's (Brooks and Barton, 1977; Westwood, 1986).

The National Clonal Germplasm Repository for Citrus and Dates (NCGRCD) (see <http://www.ars-grin.gov/riv> and [http://www.ecoport.org/EP.exe\\$PassCheckStart?ID=S117](http://www.ecoport.org/EP.exe$PassCheckStart?ID=S117)) in Riverside, California, is charged with serving the needs of users of citrus and date palm germplasm. The mission the NCGRCD is to acquire, preserve, distribute, and evaluate germplasm of *Citrus*, 32 related Aurantioideae genera, and date palms and other *Phoenix* species and to conduct research related to fulfillment of its mission.

The NCGRCD is cooperative venture between the United States Dept of Agriculture – Agricultural Research Service (USDA ARS) and the Agricultural Experiment Station of the University of California, Riverside (UCR). The Repository was established in 1987 on the UCR campus and on the USDA-ARS Irrigated Desert Research Station (IDRS) in Brawley, California. In 1993, additional field collections were established at the University of California South Coast Research and Extension Center (SCREC) in Irvine, California, and the University of California Coachella Valley Agricultural Research Station (CVARS) in Thermal, California. The Irvine and Thermal locations are used for field collections of cold-sensitive citrus relatives and at Thermal, date palms. The NCGRCD was established on the Riverside campus to take advantage of existing UCR programs, particularly the Citrus Variety Collection (CVC) (Soost et al, 1977) and the Citrus Clonal Protection Program (CCPP) (see <http://ccpp.ucr.edu/>) (Reuther, 1981; Gumpf, 1996; Bash, 1999; Krueger, 1999, 2001b). Due to the strength of these existing programs and the nature of citrus germplasm exchange, the functioning of the Repository has evolved differently over the years than most other clonal repositories. It must be emphasized that the Repository only exists and functions due to the cooperation of the University of California. The Repository is served administratively by the ARS location staff, housed in the US Salinity Laboratory, also located on the UCR campus. Some aspects of the Repository's functioning have been described by Krueger (1997, 1999b) and Williams (1990, 1991, 1992a,b). The cooperative nature of this venture and its University and Federal components have been more fully described in Kahn et al (2001, 2003, 200_).

The facilities currently consist of 938 ft² of laboratory space, 400 ft² of office space, 1375 ft² of headhouse/storage space, 6048 ft² of greenhouse space, and 16,200 ft² of screenhouse space. Approximately 6000 ft² of additional greenhouse space belonging to UCR also is used by the Repository (as of 2004). The laboratory is used for pathogen testing and elimination, research, and as a general work area for order processing, seed extraction, etc. Virus-tested potted trees are maintained as the protected collection in the screenhouse. The greenhouses are used for propagation, virus indexing, and maintenance of cold-sensitive materials. A 480 ft² office trailer provides office space, a break area, and additional lab space. Current staffing level is 4.5 PFTE (Plant Pathologist, Horticulturist/Curator, 2 Biological Technicians, 0.5 Computer Assistant). An additional 3.0 FTE are currently (2004) temporary appointments, and additional personnel are employed through a Research Support Agreement with UCR, the hosting institution.

Acquisition Many important diseases of citrus are caused by viruses or virus-like organisms and are transmitted by insects or by grafting with budwood infected with the pathogen. Because of the possibility of virus transmission from the use of infected budwood, movement of citrus germplasm between different countries and domestic states is highly restricted and regulated. In most instances, national or local regulations prohibit the introduction of citrus germplasm unless it is quarantined before it is released. The quarantine procedure involves testing for the presence of viruses and eliminating them if they are present. The material will be released from quarantine only when no viruses are present. In some instances, budwood not known to be virus-free is completely prohibited from entering. In any case, virus-free budwood is highly desirable for exchange as compared to budwood of unknown disease status. This situation complicates the exchange of citrus germplasm and has led to the establishment of different collections around the world, since needed germplasm would not be readily available from a single source. Some considerations in the exchange of citrus germplasm are discussed by Frison and Taher (1991), Knorr (1977), Roistacher et al (1977), Broadbent (1999), and Krueger and Navarro (200_). More information in this area may be found in the below section 'Phytosanitary and Security Issues'.

Citrus budwood is classified as a 'prohibited' commodity by USDA-APHIS, and can be introduced only under an APHIS Departmental Permit (co-issued by the California Dept of Food & Agriculture (CDFA)). Accessions arriving in the US as budwood are quarantined either by the NCGRCD or by the CCPP after a preliminary inspection in Beltsville. Citrus is unusual in that the quarantining is performed by state rather than federal agency, and release from state quarantine is obtained before release from federal quarantine. When released from quarantine, small, virus-free trees are generally maintained in the Protected Collection and also planted in the Citrus Variety Collection in the field (see below). In the past, citrus was quarantined in Beltsville before release to the states. It would then be quarantined by CCPP before it could be released within California. It has proven more

efficient overall to quarantine incoming citrus budwood as described. Materials arriving as seed are sent directly to the Repository, as there are no seed-borne citrus viruses. Obtaining materials as budwood is normally the preferred method for obtaining well established varieties or distinctive types. Seed introductions are useful in some cases for increasing genetic diversity and for obtaining citrus relative germplasm, which generally comes true-to-type from seed. The Repository has an internal quarantine program which attempts to introduce material from the CVC into the protected collection.

Date palms are propagated clonally from offshoots that arise around the base of the tree. Incoming offshoots would be quarantined in Beltsville before release to the Repository. Special arrangements would then need to be made with the CDFA before they could be moved to the Repository and established in the field. Seeds of Phoenix species can be imported in a manner similar to that described for citrus seeds. For more information, see Carpenter (1977). Due to political considerations (both international and domestic), it has been unlikely that much additional date palm germplasm would be obtained in the near future. However, political considerations in the Middle East and North Africa are changing rapidly, and this situation could improve in the future.

Note that these regulations must be adhered to no matter what the source of new germplasm. That is, whether a new accession is introduced by request from another collection or by a plant exploration expedition, all applicable federal and state regulations must be followed. The same is true in distributing germplasm (see below). The situation is greatly complicated as compared to that for annual crops and most perennial crops. Possession of an import permit by the Curator is a programmatic enhancement that is fairly unique in the NPGS and provides a number of advantages in introducing and working with germplasm.

Preservation The NCGRCD may be thought of as a 'collection of collections'. These collections include the Protected Collection; the Citrus Variety Collection; the Citrus Relatives Collections; and the Date Palm Collections. All these collections consist of living trees. Typically, the clonal repositories preserve germplasm in the form of living trees due to the limitations associated with preservation of these crops as seed (Westwood, 1986).

The Protected Collection currently (2004) consists of approximately 350 accessions that are available for distribution to qualified individuals. There are a small number of virus-tested accessions which is not available due to non-propagation agreements, being unreleased materials, or needing to be further characterized or selected. The virus-tested trees in the Protected Collection are maintained in 5-gallon pots, typically two trees per accession. A sterilized soil mix is utilized (Baker, 1957), with fertilizer supplied through a drip irrigation system. Supplementary heat is available for cold protection. The entire collection is housed in an insect-proof greenhouse to prevent infection of insect-transmitted viruses. The trees range in age from 3 - 10 years. Although repropagation is sometimes necessary, judicious pruning and training ('buckhorning') and re-potting generally keep the trees at a manageable size.

Due to the restrictions on the exchange of citrus vegetative material, the virus-tested Protected Collection is generally the only source of budwood for distributions. Only under unusual circumstances is budwood of unknown disease status from a field growing tree distributed. This is generally only to persons possessing the appropriate permits and programs in other countries. The trees in the Protected Collection are re-tested annually for tristeza virus, which is endemic in the area and the most likely to be transmitted to the collection. Re-testing for certain other pathogens is currently being instituted, for example all trees will be tested for freedom from Citrus leaf blotch virus in 2004

The Citrus Variety Collection (CVC) (see <http://citrusvariety.ucr.edu/>) at Riverside, the origins of which date to 1910, is one of the world's largest and most diverse collections of Citrus species and related genera, containing approximately 900 accessions (2004). This collection is attached to the UCR campus and is used cooperatively by the NCGRCD. The CVC is invaluable in preserving germplasm, and also is needed for purposes of characterization and evaluation. However, the disease status of trees in a field planting is unknown and in many cases the trees are positive for tristeza or for stubborn disease, both of which are endemic in the area. Consequently, budwood should not be distributed from such a collection, although seeds collected from the CVC can be distributed since most citrus virus diseases are not seed-transmitted. Seed-source trees are being tested in 2004 for freedom from Citrus leaf blotch virus, which has recently been reported to be seed-transmitted. The CVC also serves as a source of leaves, pollen, flowers, etc that are occasionally distributed. The CVC is a heavily utilized resource with almost 20 other projects that utilize the CVC in some way.

The accessions in the CVC consist of two trees each. The CVC has been repropagated and moved several

times. The oldest trees at the current location date from 1983, while the youngest are approximately 2 years old. Management of the CVC is different than management of a commercial citrus grove due to the wide variety of types present. This presents challenges in its maintenance (Krueger, 1997; Kahn et al, 2001).

The Citrus Relative Collection consists of citrus relatives which are variable in their sensitivity to environmental factors. In general, not much is known about the culture of these species. Many of them are sensitive to cold. Consequently, field plantings were established at SCREC to complement those planted in the CVC. The first planting at Irvine was done by UCR researchers in the 1960's. A larger planting was began cooperatively by NCGRCD and UCR in 1993. This planting is being re-established at SCREC due to internal factors, beginning in 1999. Although the Irvine plantings are important backups for the Riverside planting, there are some accessions that do not flower or fruit consistently in either climate. This makes distribution of seeds (the primary form in which the citrus relatives are distributed) difficult, as well as preventing characterization and evaluation. To address some of these concerns, a field planting of certain citrus relatives was established at CVARS in Thermal (a low desert environment) in 2000. Although it is hoped that eventually all citrus relative accessions will be established in at least one field location, up to this point some of them have been able to be maintained only under greenhouse conditions. It is possible that some will never be established in the field in California conditions. Approximately 50 citrus relatives are maintained at the various sites.

The Date Palm Collection is maintained in two separate field plantings. This germplasm collection is the remnants of a larger collection established and maintained by the USDA US Date & Citrus Station in Indio. When this station was closed in 1979, the accessions deemed most valuable were propagated at Brawley. The Brawley station has been threatened with closure since about 1991, and consequently the collection was repropagated to CVARS starting in 1992. Currently all accessions have been duplicated at CVARS. These are young trees and the Brawley collection, which consists of mature trees, should be maintained as long as feasible. Due to the complicated arrangement between the Federal government, the Imperial County government, and a private committee that was made when the site was originally established. If possible, the Brawley collection will be maintained as a backup to the main collection at Thermal. The date palm collections consist of approximately 65 accessions (Carpenter, 1974a, b; Krueger 2001a), each of which is represented by at least two trees in each location. Their disease free status is maintained by a California state quarantine. This same quarantine makes importation and establishment of new accessions difficult.

Currently, NCGRCD preserves germplasm of the crops for which it is responsible solely as living trees, except for a small amount of seeds which are kept on hand for distribution and use within the Repository. Summaries of other techniques useful for clonal crops by Bajaj (1995), Sakai (1995), Towill (1989), and Towill and Roos (1989) suggest *in situ*, *ex situ*, *in vitro*, and cryogenic preservation. Cryopreservation of citrus germplasm has been reviewed by Duran-Vila (1995), and Duran-Vila et al (1999); cryopreservation of date palm germplasm has been reviewed by Engelmann et al (1995). Cryopreservation and other biotechnological techniques for long term preservation of these crops have not been established, although there are some preliminary guidelines for seeds of citrus and related genera forthcoming (C. Walters, personal communication, 1997). Consequently, backup of these materials at the National Seed Storage Laboratory (NSSL) has not been established. However, research into long-term preservation of citrus seeds and vegetative tissue is ongoing at the National Seed Storage Laboratory in Fort Collins, Colorado, and it is hoped that eventually long-term preservation can be initiated.

Distribution Annual distributions of 500 - 600 accessions to 50 - 90 cooperators is the norm for NCGRCD. Generally, approximately 70 % of the accessions distributed go to foreign requestors. Public requestors usually account for over 85 % of the domestic distributions and over 70 % of the foreign distributions. The bulk of the distributions (over 90 %) are usually citrus. For citrus, domestic distributions usually account for 25 - 35 % of the accessions distributed, with the remainder going to foreign requestors. The vast majority (usually over 90 %) of domestic distributions go to public requestors, while somewhat less than this proportion (70 - 85 %) of foreign distributions go to public requestors. The small amount of date palm germplasm distributed is quite variable as to requestors. Normally 60 - 70 % of the cooperators are foreign cooperators. Of the domestic cooperators, ARS and state universities usually account for over two thirds of the total. Of the foreign cooperators, over two thirds are normally associated with governmental agencies.

Although NCGRCD is a clonal repository, it distributes a fairly large amount of material as seeds. There are several reasons for this: many of the seeds distributed are used for virus indicators or in rootstock trials when requestors do not want to wait the years necessary for trees to start producing seeds when propagated from

budwood; requestors wish to avoid quarantine hassles associated with vegetative tissue; and most distributions of citrus relatives are in the form of seeds since quarantine requirements are not well defined and the relatives generally come true-to-type from seed.

Budwood distributions mostly fall in a few categories: production of seed sources of indicator plants for virus testing or production of rootstocks; establishment of a clean-source program; commercial trials; and, a limited amount of breeding work. Citrus germplasm is also occasionally distributed as pollen, flowers, leaves, and fruit.

Characterization, evaluation, and documentation The efficient and effective utilization of germplasm requires sound and accurate knowledge and documentation of its traits. That is, it entails a description of what is in a collection. Descriptions of a germplasm resource are conveyed by descriptors based upon passport data, evaluation, and characterization of the germplasm. Passport data includes basic information on the origin and type of the germplasm. Management data traces the history of an accession, the handling of its propagative units, its distribution, regeneration, etc. This ensures that users of germplasm are handling the materials that they believe they are. This is the responsibility of the curator. A distinction between evaluation and characterization is sometimes made: characterization in this schema refers to documentation of characters which are highly heritable, are easily identified (usually qualitative), and are expressed in all environments, while evaluation consists of documentation of additional characters (often quantitative) which are thought desirable by a consensus of users of the crop. In reality, the distinction between characterization and evaluation is somewhat arbitrary and the boundaries somewhat blurred. This is due to the profound effect that environment can have upon gene expression, the genotype x environment interaction. Responsibility for characterization and evaluation varies; the curator is usually involved with at least some aspects (usually the more basic attributes), while advanced or complex evaluations may be beyond the curator's capabilities and/or resources. Curators have the primary responsibility for documentation, which increasingly is via computerized databases, such as the Germplasm Resources Information Network (GRIN) system (GRIN, 1995; Mowder and Stoner, 1989).

The last several decades have seen the evolution of biochemical and molecular markers as tools with great potential application to the challenges of germplasm characterization. These markers have a distinct advantage over morphologically based phenotypic characterization, as they are generally unaffected by the host of factors able to influence plant or organ characteristics. This allows comparisons between accessions within a collection or among collections at different locations at any time of year, while phenotypic characteristics can be masked by environmental or cultural affects.

Molecular characterization has a number of applications in the management of germplasm collections. These include elucidating systematic relationships between accessions; assessing gaps and redundancies in the collection; development of core subsets; characterizing newly acquired germplasm; maintaining trueness-to-type; monitoring shifts in population genetic structure in heterogeneous germplasm; monitoring genetic shifts caused by differential viability in storage or in vitro culture; exploiting associations among traits of interest and genetic markers; and genetic enhancement (Bretting and Widrlechner, 1995). One of the most important potential uses of molecular markers is their use in breeding programs. Identification of genes and markers associated with quantitative traits will greatly increase the efficiency of a breeding program.

Characterization, evaluation, and documentation of the CVC have been ongoing since the original planting in the early 1900's, but there are many gaps in the data. Many of the current accessions are not well documented. The majority of the current characterization/evaluation is being done by NCGRCD and UCR personnel, but there is also data being generated by cooperators in such areas as limonoid contents; responses of seeds to desiccation; genetic analysis; etc.

NCGRCD, as a part of the NPGS, describes its crop responsibility with descriptors adopted by its Crop Germplasm Committee (CGC) (Shands, 1995; White et al, 1989). The descriptors were adopted in 1989. These descriptors are based upon the IPGRI Descriptors for Citrus (IPGRI, 1999; updated from IBPGR, 1988), which are a slightly modified and expanded version of the 'Fruit Description Outline for Citrus' developed many years ago by H.J. Webber of the University of California Citrus Research Center (Hodgson, 1967; Webber, 1943).

The descriptors are adequate for describing the basic morphology of citrus. However, they do not address some very basic characteristics (eg, growth rate) and their treatment of important physiological, pathological, horticultural, and genetic characteristics is limited. One major shortcoming is that the descriptors do not address variability over time or geographical (climatic) area. Another question about the IBPGR descriptors is their utility

for the citrus relatives. Date palm descriptors are those utilized by the US Date and Citrus Station (Carpenter, 1974a). While many of the criticisms of the citrus descriptors also apply to those for date palms, there is less of a problem since date palm culture is confined to a much more limited climatic area than is citriculture.

For most of the descriptors, about 85 – 90 % of the accessions in the CVC have been characterized. This data is available in the GRIN database as well as the local NCGRCD databases. The main weakness of this data is that it was gathered only once. The current direction in characterization and evaluation of the CVC is to collect data on seasonally variable characters (eg, shoot growth, fruit quality) several times during the season, to collect data on less changeable characters (eg, trunk diameter, number of segments) once per year, and to collect data on fixed characters (eg, type of leaf, vegetative life cycle) only once. All this is dependent upon the availability of adequate resources, of course. Although it is important to characterize all accessions, there will probably have to be a prioritization of which accessions need to receive the most attention. For instance, poorly characterized accessions or accessions with potentially useful traits should be evaluated more thoroughly than accessions which have had more attention paid to them by other researchers because of their commercial value.

More complex evaluations (disease resistance, physical properties, etc) are very important but will have to be investigated as stand alone research projects, either within the NCGRCD, with cooperators, or independently by others. These types of investigations require even more resources than the initial characterizations, since they are complex, intensive, multiyear projects in many different areas. These types of investigations are by nature open ended and often yield new questions to investigate, all requiring adequate resources. Evaluation is the 'black hole' of genetic conservation.

Molecular characterization of the citrus germplasm accessions is a valuable adjunct to morphological, horticultural, and other plant-level characteristics (Gmitter et al, 1999). Regarding the Repository/CVC holdings, various investigations have been carried out. Fang et al (1997) reported the use of iSSR markers to evaluate the genetic diversity and phylogenetic relationships of the trifoliolate accessions, and found that most accessions fell into only a few groups. Phylogenetic relationships between various accessions were investigated by Fang et al (1998); their findings mostly corroborated previous classifications but revealed a number of new relationships. Federici et al (1998) investigated phylogenetic relationships between 32 accessions of Citrus and 3 of Microcitrus using RFLP and RAPD. This analysis showed, among other things, that some accessions were probably misidentified as to their species. Gulsen and Roose (2001) studied lemon accessions and found relatively little diversity, with nearly 70 % of the lemon accessions have nearly identical marker phenotypes.

An extensive survey of the citrus germplasm holdings was recently completed by Barkley (2003) (see also Barkley et al., 2003, 200_). It involved evaluation of the genetic diversity present in the CVC via evaluation of approximately 380 sexually-derived accessions by simple-sequence-repeat 24 (SSR) markers, 15 of which were developed de novo (<http://www.plantbiology.ucr.edu/people/faculty/rooselink2.html>). All 24 markers were mapped in a Sacaton x Troyer mapping population. Analysis revealed that there were 296 alleles detected, with an average of 11.84 alleles per locus. The average PIC value was 0.633. The accessions were divided into five main groups: trifoliate, citrons, mandarins, pummelos, and kumquats. Other accessions were probabilistically assigned to populations or multiple populations if their genotypes indicated admixture by using a model-based clustering approach. One of the most interesting analyses was that utilizing the Structure program, which assigns individuals to populations and infers the population structure based upon the genotype. This indicated that, indeed, many of the accessions in the CVC are apparent hybrids. In some instances, this information supported previous data based upon morphology and biochemical markers, or deduced from curatorial investigations of arcane archival annals. In other cases, it showed a hybrid ancestry where none was previously suspected. In addition, ~70 microsatellite allele fragments from 3 different markers were sequenced. The purpose of this was to determine how the microsatellite fragments were evolving. This was needed because of assumptions made in programs that evaluate microsatellite data. It was found that the different allele sizes were due to changes in the microsatellite repeat (step-wise mutation model) as opposed to changes in the flanking region (indels etc.).

The molecular data was also used to designate a 'core' collection (Barkley, 2003). A 'core' is a subset, usually including 10 – 15 % of the accessions, which represents the majority of the genetic diversity present in the entire collection (Hodgkin et al, 1995). The core collection will help prioritize accessions in the CVC for inclusion in the virus-free collection and for cryogenic backup, additionally it will assist in prioritizing accessions for more complete characterization and evaluation. Adequate passport data is missing for many accessions in the CVC, so it was not possible to designate a 'core' based upon geographical data. The core collection contains approximately 50

accessions (depending upon the sampling strategy) representing 13.5 % of the accessions studied and containing more than 90 % of the genetic diversity in the entire collection. Several different sampling strategies were evaluated (random sampling, proportional and constant stratified random samplings, and selection-based sampling). These strategies were compared to determine which methodology yielded a core collection representing the greatest genetic diversity. Although all strategies had similar numbers of alleles maintained and thus represented similar proportions of total alleles, the subset constructed from the proportional stratified sampling strategy retained slightly more alleles than the other subsets and had allele frequencies more similar to those found in the CVC. The stratified sampling strategy was therefore utilized to designate the core collection.

Molecular markers have been utilized to reduce redundancies and evaluate genetic diversity in materials received as seed (Krueger and Roose, 2003). ISSR markers were used to screen 1340 seedlings from 88 received seed lots for nucellar types. This allowed the number of seedlings maintained to be reduced. In some cases, zygotic seedlings of interest were also maintained. The technique did not work for monoembryonic types. This information will make conservation of the new accessions more efficient and potentially indicate which accessions are more likely to contain unique genes.

In citrus, molecular markers have been reported for various important traits, including cold-acclimation-responsive loci (Cai et al, 1994), nematode resistance (Ling et al, 1994), citrus tristeza virus resistance (Gmitter et al, 1996; Fang et al, 1998; Fang and Roose, 1999), and fruit acidity (Fang, Federici, and Roose, 1997). As citrus germplasm becomes more completely characterized both molecularly and horticulturally, more markers will be identified. This will increase the efficiency of evaluation of the remaining accessions and new additions, and will also increase the efficiency of their utilization in breeding programs.

There has been a small amount of molecular characterization of the date palm holdings. Cao and Chao (2002) used AFLP to evaluate the genetic relationships of various accessions. Devanand and Chao also used the AFLP system to investigate within-variety variation.

Databases and documentation The NCGRCD uses several local databases as well as the national Germplasm Resources Information Network (GRIN) database maintained by the Database Management Unit (DBMU) of the National Germplasm Resources Laboratory (NGRL) in Beltsville. Review of the local databases is ongoing; this is necessary before corrections to the GRIN database can be made. Corrections to GRIN are generally made shortly after they are made in the local databases. In addition to the review process, there have been several issues with the local databases that have needed resolution. These issues are somewhat inter-related and need resolution at more or less the same time. A relational database must be developed and implemented. The current database(s) are not relational and this results in inefficiencies. In order to implement a relational database, an inventory number must be devised. In the past, there have been inventory numbers in use, but they are not compatible with the GRIN format. In 2002, a new format for inventory numbers was devised and was partially implemented in 2003. This format is compatible with GRIN, and when fully implemented, will simplify overall functioning and use of the databases. After complete implementation of the new inventory number, conversion to a fully relational database can proceed. In addition, this will allow implementation of a bar coding system for inventory items. The NCGRCD portion of GRIN is generally in good condition. Most accessions are loaded in GRIN, and PI assignment is current. Descriptor data for approximately 10 – 15 % of the accessions need to be taken and loaded into GRIN. Images for a portion of the collection are currently being edited and will be available online when ready and when resources permit more time to be devoted to the website.

Other germplasm related activities

Although the Riverside group holds the largest and most diverse collection of citrus germplasm in the US, there are several other collections of interest. These are smaller and more specialized collections, and can not supply virus-free budwood.

The largest collection of citrus germplasm in Florida is located at the USDA-ARS AH Whitmore Foundation Farm (WFF) in Groveland. There are currently approximately 250 accessions maintained at WFF, about half of which are not duplicated in Riverside. The WFF was attached to the USDA-ARS US Horticultural Research Laboratory in Orlando, and served for many years as a breeding facility. The collection was started in the late 1950's as a consolidation of several other USDA collections (primarily in Florida, but also including Indio) to support citrus breeding programs at Orlando. The collection was incorporated into the NPGS in 1987 as a repository to 'complement' the NCGRCD in California. However, the WFF was de-commissioned as a Repository in 1992 and

reverted to being a collection attached to the Orlando location, used chiefly by breeders. When the Orlando laboratory moved to Fort Pierce, Florida, a portion of the WFF collection was relocated to the new facility. It is possible that the WFF may eventually be abandoned, however it is the opinion of the writers that this facility should be maintained. This collection has some unique and valuable accessions. However, the materials can not be considered disease-free, and some of the breeders are not amenable to open exchange of materials, even those having rather ancient PI numbers.

The Fort Pierce location has been designated as the National Citrus Genomics Center by ARS, and a high through-put sequencer has been purchased for the facility. The area of genomics is one in which collaborative efforts are essential. It is expected that this will help put this location on the forefront as far as elucidating the citrus genome.

The USDA-ARS National Clonal Germplasm Repository, located at the Subtropical Horticulture Research Laboratory in Miami, formerly maintained a limited number of Aurantioids. This collection was notable for the age and size of some of the accessions of related genera. However, these accessions had to be removed due to the recent state of Florida efforts in canker eradication. The USDA-ARS Tropical Horticulture Laboratory in Mayagüez, Puerto Rico maintains a limited number of accessions. The accessions maintained at these locations are few in number and are not available as virus-free materials.

In addition to these ARS resources, there are several other collections of note in Florida. The Florida State Department of Plant Industry maintains the Florida Citrus Arboretum at Winter Haven. This is a well-maintained and attractive collection of over 250 accessions, and includes a good representation of citrus relatives. Breeding collections are maintained by the citrus breeders at the University of Florida Citrus Research and Education Center in Lake Alfred. There is also a small collection of Citrus and Aurantioideae germplasm maintained at the University of Florida's Tropical Research and Education Center in Homestead. These contain some unique and valuable accessions; however, these collections are not generally accessible, cannot supply virus-free material, and the materials therein are not always available for free exchange.

The Texas A&M University, Kingsville, Citrus Center (TAMUK) at Weslaco has a collection of over 200 accessions. The Rio Farms Citrus Variety Collection is located approximately 10 miles from Weslaco in Monte Alto. This collection was originally established by the USDA in the 1960s and was taken over by Rio Farms, a private concern, when the USDA stopped doing citrus research in Texas in the 1970s. There are over 100 accessions in the Rio Farms collection, some of which are not present in Riverside. This collection has some unique and valuable accessions. However, Rio Farms is decreasing its involvement in citrus and it is possible that they will discontinue supporting this collection. Some of the accessions are being incorporated into the Riverside collections via the courtesy of Rio Farms.

There are two small collections of citrus in Arizona. One, at the University of Arizona Yuma Mesa Agricultural Center, has approximately 120 accessions. The other, located in Phoenix, has approximately 60 accessions.

Most uses of germplasm involve breeding (enhancement), genetic studies, evaluation, or production oriented trials. For citrus, the bulk of this work is done in either California or Florida, with a lesser amount done in Texas, Arizona, and other areas.

In California, the majority of citrus-related research is done by UCR. This institution currently supports one citrus breeder and one museum scientist working primarily and directly on germplasm-related activities. Other researchers in Botany/Plant Science, Plant Pathology, Entomology, and Cooperative Extension conduct research that may be considered evaluation activities. ARS involvement in citrus in California is limited to the Repository and a few positions at the Parlier Location. Only the NCGRCD has its primary emphasis on germplasm-related activities. Some evaluation data is generated by the Western Regional Research Center/Plant Gene Expression Laboratory in Albany.

In Florida, the University of Florida at Gainesville and Lake Alfred supports four positions involved primarily with citrus genetics and breeding. As in California, there are a number of other individuals from various disciplines involved in evaluation and production-trials. The Fort Pierce location of USDA ARS supports citrus breeding efforts. The geographic detachment of the breeding efforts from the germplasm conservation efforts creates some problems, especially since the Repository cannot currently send clonal materials to Fort Pierce due to Florida state restrictions. However, efforts are being made to resolve this problem, and it is hoped that interchange

of materials will be possible within the year. There is already cooperation between Florida breeders and the Repository; often controlled pollinations are made in the CVC and the resultant seeds sent to the breeders. The two ARS citrus-breeding positions in Florida are supposed to be national in scope. There are other individuals in Florida involved with evaluation and trials, some to a large degree, others to a lesser degree. Other ARS locations in Florida have citrus-related research being performed, a small portion of which may be considered evaluation.

There is one citrus breeding position at Weslaco, Texas, and some involvement by other researchers in Weslaco and at Texas A&M proper. There is also some citrus-related research done by such institutions as the University of Arizona, the University of Hawai'i and ARS in Hawaii, and possibly a few other institutions.

There is hardly any date palm research done in the US, and the Repository is apparently the only entity doing any germplasm-related activities.

Germplasm Needs

Collection The current holdings comprise one of the largest and most diverse collections of Citrus and related genera in the world. However, there are several specific areas that need to be strengthened. The highest priorities for acquisition are those genera not currently represented, followed by genera with single species representation, 'wild' types, and elite germplasm lines. Non-represented genera include *Luvunga* (12 species), *Merope* (1 species), *Monanthocitrus* (1 species), and *Oxanthera* (4 species) (this latter's identification in the collection is unresolved). Genera represented by a single species include *Micromelum* (9 species), *Oxanthera* (4 species), *Triphasia* (3 species), and *Wenzelia* (9 species). 'Wild' types include *Citrus halimii* (peninsular Malaysia), *Poncirus trifoliata* and *Poncirus polyandra* (southern China), papedas and rough lemons from Northeast India, mandarins from China, recently described putative Citrus species (*C. daoianensis*, *C. mangshanensis*, etc), and *Phoenix* species other than *P. dactylifera*. The virus-free collection needs to have the genetic diversity therein broadened as well.

Characterization and Evaluation Preliminary characterization and evaluation have been done for a large portion of the collection. As mentioned above, there are several weaknesses in the data thus far: most of it represents only one measurement or observation made at one location on one date and most data so far is of fruit quality characteristics, which are not necessarily the traits of interest for breeders. While characterization and evaluation of the accessions is ongoing, there are not enough resources to do a thorough job. The main resource needed is also the most expensive: personnel. Data from other geographical areas are likely to differ from those obtained at Riverside due to well-documented genotype X environment interactions. This will have to come from cooperative efforts with researchers in other environments. Thorough evaluations of such important traits as disease and pest resistance, adaptation to soil conditions, and cold tolerance need to be made. Some types of evaluations in areas of expertise not represented in the Repository will have to be made by cooperating researchers; these areas also require more resources devoted to them. Documentation for most of the evaluations made is available from the GRIN database. As more data becomes available there will have to be modifications made for such things as molecular data and imaging.

Enhancement When resources are scarce enough to limit evaluation, germplasm enhancements based upon those evaluations will not be possible. This area is also outside the range of expertise in the NCGRCD as currently staffed. Therefore any efforts towards enhancement will have to be cooperative at best.

Preservation Germplasm accessions are currently maintained in the field (CVC), with a portion of the collection maintained as virus-tested materials under screen (Protected Collection). The field planting is vulnerable to pests and weather conditions. Thus far, there have been no accessions lost to cold or disease in Riverside. Some of the most cold-sensitive accessions are maintained or duplicated in greenhouses in Riverside and as a field planting in the more moderate coastal environment of Irvine, California, and some commercial varieties are backed up in other California collections maintained by the University of California. Some of the accessions are also present in Florida collections. Long-term preservation of materials under cryogenic conditions has thus far not been possible. Investigations into this area are currently being done in cooperation with the National Seed Storage Laboratory, so cryopreservation may be possible in the future.

Resources For most of its existence, the Repository has been under-funded and under-staffed, and has not had adequate facilities. Recent budget increases have allowed the recruitment of a Plant Pathologist and two Technician positions; pending is the recruitment of an SY-level geneticist and possibly more support staff. However, facilities for existing personnel and program are inadequate. Expansion of the program will require expansion of the facilities

as well. Additional laboratory, office, screenhouse, greenhouse, and storage space are needed.

An expanded laboratory area is needed to accommodate a larger number of people working in the laboratory as well as more equipment. This need has become more apparent with the addition of the Category I Plant Pathology position to the Repository staff. Office space is needed to accommodate additional SYs as well as Technicians and support staff. In this context, office space also refers to a break/meeting area, a 'dry' work area for order preparation, computer work, etc.

A 'Facilities Expansion Plan' addressing these needs is on file at the PWA Office, but no date has been scheduled to bring these plans into reality. Therefore, additional temporary facilities may have to be erected until such time as permanent structures can be erected. The hosting University (UC Riverside) has strongly suggested temporary buildings rather than mobile units (trailers).

Recommendations

Priorities The highest priorities are to increase the representation of those species and genera not currently in the collection; increase the amount and genetic diversity of virus-free materials available; and conduct additional evaluations of the germplasm. Increasing the overall size of the collections should be the result of filling in of gaps in the collection and increasing the genetic diversity thereof rather than simply obtaining whatever germplasm is available. A possible bottleneck is the quarantining procedure, which is necessary when material is obtained as budwood. Introduction of new materials as seed is easier, but is not adequate in some cases. However, much of the non-elite germplasm comes true-to-type as seed and so can be successfully obtained as seed. Obtaining new genotypes as seed can also increase the genetic diversity in the collections. Therefore, introduction of new materials as both budwood and seed is appropriate depending upon the specific goal. The virus-tested materials are necessary for exchange of germplasm. Seeds can be exchanged more readily than budwood. However, many types do not come true-to-type and there is a long juvenile period of up to 8 years. Evaluation is necessary to utilize the germplasm efficiently. Areas that need increased attention are: genetic (molecular) characterization; disease resistance; and adaptation to environmental conditions.

The current level of support is greatly improved as compared to five years ago. If a more thorough job is to be done, more resources need to be allocated. These resources would include both monies for capital improvements (increased facilities) and salary and support for additional personnel. Priority should be the addition of a Geneticist as a Category 1 research scientist, along with appropriate additional support. Additional support personnel also is needed, such a full-time IT position. The current IT position also supplies administrative support, which detracts from fulfilling the primary function of the position. The addition of a Category 3 support scientist would be appropriate to provide support for the Research Leader.

The current greenhouse space should be adequate in the short term for a larger indexing/therapy program aimed at increasing the size and diversity of the virus-tested collection. However, it is our intention to biological index the plants in the Protected Collection so that every plant has been re-indexed every 4-5 years which will require additional greenhouse space. Additionally, if germplasm from the Protected Collection can be expedited in Florida for introduction into the State, there will be an increased demand on greenhouse facilities. An increased area under screen will be necessary to house the virus-free germplasm. Increased laboratory space is a high priority need to support programs in the areas of tissue culture, disease testing, and development of laboratory-based diagnostics for backup of biological indexing. In general, office space is too small for the current staff. Any (needed) additional staff would put even more pressure on these facilities. Consideration must be given to use of temporary facilities until permanent facilities can be built.

Many of the projects undertaken by the Repository are cooperative, primarily with the UCR, but also with other researchers. Adequate monies should be allocated to support these projects via Specific Cooperative Agreements and other means. The CCP and CVC in particular should have adequate monies allocated to support their involvement with NCGRCD activities. These monies should be allocated in a manner that will not impinge on NCGRCD activities that are performed 'in house'. Additional resources should also be devoted to extra-mural projects in California, Florida, and elsewhere in order to increase the amount of evaluation and the range of traits that could be evaluated. More support should also be allocated to plant exploration in order to increase the genetic diversity present in the collections.

Phytosanitary and Security Issues

Citrus (at least vegetative tissue) is a 'prohibited' commodity as per USDA-APHIS (CFR, 1993). This is because citrus has a number of graft-transmissible pathogens that have the potential to become economically important if introduced into susceptible scions and/or rootstocks. Many of these pathogens also are designated quarantine pests. Citrus propagative materials are not distributed unless they meet the phytosanitary requirements of the requestor's country or state. Accessions are indexed and, if needed, therapied using the procedures outlined in Roistacher (1990), supplemented with selected laboratory-based tests. The protocol followed is on file with USDA-APHIS and CDFA, along with other materials associated with the departmental permit.

Graft-transmissible pathogens known to be present in the United States include *Citrus psorosis virus* and *Citrus ringspot virus* (both caused by Ophiovirus viruses [Garcia et al, 1994]), concave gum (caused by a non-characterized virus), *Citrus variegation virus* (an ilarvirus [Timmer et al, 2000]), *Citrus tatterleaf virus* (a Capillovirus [Ohira et al, 1995]), *Citrus tristeza virus* (CTV) (a Closterovirus [Bar-Joseph et al, 1989; Bar-Joseph and Lee, 1990]), *Citrus vein enation virus* (non-characterized but probably caused by a luteovirus [DaGraca and Maharaj, 1991]); citrus viroids (Duran-Vila et al, 1988) including *Citrus exocortis viroid*, *Citrus cachexia viroid* (caused by Citrus viroid IIb), and dwarfing factor (caused by Citrus viroids III [Semancik et al, 1997]), and Citrus viroids I and IV. A fatal yellows disease, caused by an uncharacterized virus-like agent, has been reported in California occurring on lemons on *C. macrophylla* rootstock (Timmer et al, 2000). *Citrus leaf botch virus* (CLBV) has been reported from California and Florida (Guerri et al, 2004). Stubborn disease of citrus, caused by *Spiroplasma citri* occurs in the arid regions of California and Arizona (Timmer et al, 2000). Of the virus and viroid pathogens of citrus, CTV has aphid vectors with *Toxoptera citricida*, commonly called the brown citrus aphid, being the most efficient. *T. citricida* was introduced into Florida in 1995 (Halbert et al, 2000) and is not present yet in other citrus areas of the US. While CTV is present in most citrus areas in the US, strains that cause stem pitting of scions are not usually present in commercial groves. *Citrus psorosis virus* is 'naturally spread' in Argentina and Brazil, but the means of this spread has never conclusively been determined (Roistacher, 1993). *Citrus vein enation virus* is spread by several aphid species (de Mendoza et al, 1993). The remainder of the virus and viroids pathogens already in the US are reported to be mechanically transmitted and without vectors. Citrus blight is a serious disease of citrus in Florida and other areas having a similar climate (Timmer et al, 1987). The disease has been shown to be graft-transmissible using roots from an infected tree (Tucker et al, 1984), but the pathogen has not been characterized. Brlansky and Howd (2002) reported a virus associated with citrus blight, and more recently Derrick et al (2003) reported a Closterovirus associated with citrus blight. Stubborn is vectored by leaf hoppers (Garnier et al, 2001).

There are a number of graft-transmissible pathogens of citrus which are exotic to the US. These pathogens must be considered when germplasm accessions come from areas where the diseases occur. Citrus greening, or Huanglongbing, is caused by *Candidatus Liberobacter asiaticus* or *Candidatus Liberobacter africanus* for Asian or African greening, respectively. Asian or African greening is vectored in a persistent manner by psyllids; *Diaphorina citri* and *Trioza erytreae*, respectively (Garnier and Bove, 1993; Halbert and Manjunath, 2004). *D. citri* is already established in Florida, Texas, and most countries in the Caribbean Basin. Citrus variegated chlorosis (CVC) caused by a strain of *Xylella fastidiosa* became a 'new' disease in Brazil in the late 1980s (Lee et al, 1991; Hartung et al, 1994). It is vectored by xylem-feeding sharpshooter insects; once a vector acquires the bacterium, the insect retains the ability to transmit *X. fastidiosa* until the insect molts or dies if an adult (Redak et al, 2004). CVC has been reported to be present in Costa Rica (Moreira et al, 2002), and recently has been reported to be seed transmitted, increasing the risk of introduction into new areas (Hartung et al, 2003). There are a couple of apparently 'new' diseases of citrus recently reported from Brazil: Citrus sudden death which has been reported to be caused by CTV but for which Koch's postulates are unfulfilled (Renato et al, 2003; Roman et al, 2004); and a new disease in the southern citrus area of Sao Paulo State which apparently is a strain of citrus greening (J. Bove, personal communication). Another group of prokaryotic pathogens is the phytoplasmas causing witches' broom diseases. Witches' broom disease of lime, caused by *Candidatus Phytoplasma aurantifolia* and spread by leaf hoppers, has almost eliminated acid lime production in Oman and surrounding countries (Garnier et al, 1991; Zreik et al, 1995). Recently this phytoplasma has been reported to be seed transmitted (Khan and Lee, 2003). Other phytoplasmas causing witches' brooms in mandarins and other citrus varieties have been reported from Jamaica, and India (Lee et al, 2003; Ghosh et al, 1999). The phytoplasma diseases of citrus are vectored by phloem-feeding leaf hoppers. Citrus chlorotic dwarf is an emerging virus-like disease of citrus found in Turkey and vectored by the barberry whitefly (Kersting et al, 1996). Citrus yellow mosaic (caused by a badnavirus), present in India, is spread by the citrus mealy bug, *Planococcus citri* (Ahalawat et al, 1996; Huang and Hartung, 2001). *Satsuma dwarf virus* (caused by a plant picorna-like virus) and spread by an unknown soil-borne vector, is present in Japan, China and other

countries where infected budwood was imported (Miyakawa and Yamaguchi, 1981; Karasev et al, 2001). This virus has been found in Florida, but apparently the vector was missing as the virus has not spread and the infested area is now a housing development (Lee, unpublished). Cristacortis and impietratura, both caused by non-characterized viruses (Timmer et al, 2000), are present in most old-line budwood coming from Europe and Northern Africa. Biological indexing for psorosis virus also would reveal the presence of these viruses (Roistacher, 1990). *Citrus leprosis virus* (caused by a rhabdo-like virus) and vectored by *Brevipalpus* species mites, is increasing in importance as it is spreading northward through Central America (Dominguez et al, 2001; Guerra-Moreno, 2004). While reported to be present and causing major economic losses in Florida in the early 1900s, then the disease incidence declined and caused only minor damage. Leprosis has not been found in the US since the 1960s (Childers et al, 2003). Of the exotic graft transmissible pathogens of citrus, the ones having insect vectors pose the greatest risks. The vectors of Huanglongbing (Halbert et al, 2000), CVC (Damsteegt et al, 2003), leprosis (Childers et al, 2003), citrus chlorotic dwarf—the bayberry whitefly *Parabemisia tabaci* (Kersting et al, 1996), and CYMV—*Panococcus citri* (Ahlawat et al, 1996) are already present in the USA, thus these pathogens would have a means to spread if introduced.

The basis of detection of graft-transmissible pathogens of citrus begins with biological indexing on plant hosts that express distinct symptoms due to the pathogen (Roistacher 1990). In many cases, laboratory tests are also available to provide a relatively quick verification of presence or absence of the pathogen if needed, and to verify the biological test results (Roistacher, 1990). Laboratory tests are essential for diagnosis and identification of exotic graft-transmissible pathogens; they pose too great a risk to keep cultures in planta as positive controls. For the graft-transmissible pathogens present in the USA, laboratory assays are not developed for *Citrus vein enation virus*, concave gum, and fatal yellows because of the lack of information on the causal agent. Better information is needed for sampling protocols and time of year for sampling. For exotic graft-transmissible pathogens, laboratory assays are needed for cristacortis, Impietratura, citrus chlorotic dwarf, and citrus sudden death.

The seriousness of some of these diseases is attested to by the fact that of the ten plant pathogens considered to be potential biological agents and toxins, three are citrus pathogens (*X. fastidiosa* pv *citri*, *C. Liberobacter africanus*, *C. Liberobacter asiaticus*; canker was formerly included but later removed) (CFR, 2002). In addition to these pathogens, FAO (2003) adds *Citrus leprosis virus*, citrus black spot (caused by a fungal pathogen, *Guignardia citricarpa*), and citrus canker to their list of ‘examples of emerging diseases of citrus which interfere with trade and limit production’. They cite the importance of quarantine procedures, detection methodologies, and control strategies in dealing with these diseases. Although a discussion of these issues as pertains to trade is beyond the scope of this review, it should be noted that the Repository has a quarantine system in place, is active in developing and implementing detection methods, and promotes the use of healthy citrus germplasm through its distributions of ‘clean stock’ material. The utilization of ‘clean stock’ becomes increasingly important as the vectors for these diseases are inadvertently introduced or colonize new areas. For instance, *T. citricida* has recently moved into Mexico from Belize and has been reported in the southern portion of Veracruz state. This poses a potential threat to US citriculture that is best dealt with by the use of ‘clean’ propagative materials. Our interactions with the Mexican government in this area help assure the continued healthy status of US citriculture.

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Germplasm Collections

The NCGRCD may be thought of as a 'collection of collections*'. These collections include the Protected Collection (PC); the Citrus Variety Collection (CVC); the Citrus Relatives Collections; and the Date Palm Collections. All these collections consist of living trees due to the limitations associated with preservation and distribution of these crops as seed.

The overall state of the citrus germ plasm collections is shown in Table 1. Currently, 29 of the 33 genera in the subfamily *Aurantioideae* are represented in the various collections. However, some of these genera are represented by only one species. Another weakness is that due to the confused state of *Aurantioideae* taxonomy, it is unclear exactly how much of the genetic diversity is represented. A complete listing of Repository holdings may be found at the GRIN website: <http://www.ars-grin.gov/>.

Protected Collection

The Protected Collection consists of small potted trees that are propagated from pathogen-tested budwood. These trees are the source of budwood for distributions. Except under unusual circumstances, budwood is not distributed from other sources. The virus-tested collection is maintained under screen to prevent infection via insect vectors. Stringent precautions are also taken as far as sanitation, etc. There are currently over 350 accessions under screen, represented by over 800 individual trees. Most of the accessions maintained in the screenhouse are available for distribution. The rest are under non-propagation agreements, have not been released, or are being evaluated prior to official accessioning.

Accessions maintained in the Protected Collection are re-tested annually for CTV by ELISA. Trees that will be distributed from are also re-tested for CTV by ELISA prior to budwood cuts. This is probably somewhat redundant given the protected status of the collection. In addition, we are implementing re-testing every 4-5 years for exocortis and psorosis (and possibly stubborn). This will put the Protected Collection in accordance with California state regulations for registered nursery trees. In 2004, the trees in the PC were tested for *Citrus leaf blotch virus*.

Accessions are added to the protected collection after being pathogen tested or quarantined at the Repository or the UC Citrus Clonal Protection Program (CCPP). If an accession originates from outside the US, it must be quarantined and released from quarantine by USDA-APHIS and CDFA officials before it can be added to the virus-tested collection (or planted outside the quarantine facilities). Accessions originating domestically from outside of California (and from some areas within California) must be released from quarantine by CDFA but not APH IS.

Pathogen testing has been ongoing at NCGRCD for some time and a number of accessions are being held under quarantine. A quarantine report is being prepared and will be posted on the website as well as being sent to CGC members. Several years ago there were some problems with the soil mix used, which compromised the results of the indexing. These particular accessions were consequently re-indexed the following year. Since 1999, nearly 150

accessions have been indexed and are listed in Table 1. This includes some that are, strictly speaking, non-quarantine items (for instance, accessions which were received as seed). These are tested (often using the 'shotgun methodology) as a precaution and in the interest of completeness. Most of the accessions indexed have tested as apparently pathogen-free. There are still a few pending tests (mainly sPAGEfor viroids), after which quarantine release will be applied for. It is anticipated that by summer of 2005, the majority of the trees in the indexing program will be added to the Protected Collection. Not all of these will initially be available since in some cases (those received as seed), they have not flowered nor fruited. These may be available by special request, however.

Citrus Variety Collection

The UCR Citrus Variety Collection (Dr Tracy L Kahn, UCR Dept of Botany & Plant Science, Curator) is used cooperatively by the NCGRCD for characterization/evaluation, as a seed source, and occasionally as a source of miscellaneous other materials for distribution (leaves, flowers, pollen, etc). The Repository maintains a Specific Cooperative Agreement (SCA) with Dr Kahn to support maintenance and evaluation of the CVC. Cooperation with Dr Kahn is ongoing in a number of areas.

There are currently over 900 accessions maintained in the CVC. This includes accessions only planted in the CVC, not those maintained only in greenhouses or at SCREC — see below. In 2004, 1 new accession was planted in the CVC and several trees needing replacement were replanted. Forty-five seedling trees of monoembryonic types were planted at CVARS. This increases the number of seedlings maintained there to nearly 150.

Because of concern of the UCR budget, Dr. Kahn has started an endowment campaign for the CVC. The long range goal is to generate sufficient annual funds to cover the costs associated with the maintenance and preservation of this important source of citrus genetic information.

During 2004, the avocados were removed from near the east gate of Ag Ops, and this area has been designated for the CVC. Because of freeway interchange construction (Route 60/215) and widening of surface streets, several plants in the CVC will need to be relocated this coming year (2005).

Citrus Relatives

There are approximately 80 accessions of species of Aurantioideae genera other than *Citrus* that are maintained in greenhouses, the CVC, SREC, or CVARS. Eleven new citrus relative trees were planted at SCREC in 2004. Some somatic hybrids were accessed from Dr. Jude Grosser, University of Florida, which contain a citrus relative as one of the parents. While these citrus relatives may not be directly sexually compatible with *Citrus*, the somatic hybrids should be sexually compatible, thus providing a mechanism in the future to utilize citrus relative germplasm using conventional breeding methods.

The citrus relatives often are more sensitive than citrus to factors such as cold, heat, pesticides, fertilizers, etc. For these reasons, several plants of each accession are maintained in NCGRCD or UCR greenhouses. Because of these factors, citrus relatives have long been

maintained under the more moderate coastal temperatures of the SCREC. The planting at CVARS was established in order to determine if the higher heat load in this low desert environment would produce more flowering and fruiting than at the more moderate locations, even if the environmental conditions are subtropical rather than tropical.

The SCREC is located adjacent to the El Toro Marine Air Base (ETMAB) which was recently closed. It is likely the ETMAB will be utilized as a recreational/cultural/residential area. This will increase the value of the land where SCREC is located. Although SCREC has many important uses in addition to preservation of citrus and avocado germplasm, it is possible that UC may at some point cease to maintain SCREC. To prepare for this eventuality, accession found exclusively at SCREC are being repropagated in Riverside. At Riverside, these may have to be maintained in greenhouses.

Date palms

The date palm collection currently consists of approximately 68 accessions. Their disease-free status is maintained by a California state quarantine for the desert areas. Due to the difficulty of obtaining and quarantining new date palms, it is unlikely that this collection will grow very quickly in the future.

These date palms were originally a collection developed by the USDA Date and Citrus Station in Indio. When that station was closed in 1979, the dates were moved to the USDA irrigated Desert Research Station (IDRS) in Brawley, and came under NCGRCD responsibility shortly after the establishment of NCGRCD in 1987. In the early 1990's, it was apparent that the Brawley station would be closed; the date palms were then repropagated to CVARS beginning in 1993. This location (Thermal) is better suited for the growth of date palms due to soils, weather conditions, etc.

Currently the date palms exist in both Thermal and Brawley. The status of the Brawley station is unclear, due to the complicated arrangement between the Federal government, the Imperial County government, and a private committee that was made when the site was originally established. It is currently maintained by the Imperial County Farmer*s Committee (and is now called Imperial Valley Agricultural Research Station), with some Federal input for salaries. If possible, the Brawley collection will be maintained for several years, until the palms at CVARS are established.

In CY 2004, no new date palm accessions were planted. However, several new potential accessions have been received as tissue-cultured plantlets from INRA, Antibes and are presently in quarantine. These will eventually be planted in the field for evaluation and possible accessioning. Repropagation of the main collection at CVARS has been partially completed. Due to the nature of date palms, this is a rather involved and intensive activity.

Germplasm Distributions

Distributions of germplasm from NCGRCD are shown in Table 3. Distributions for the year 2004 totaled 235 accessions, 98 % of which were citrus. There were 56 requestors. This is

less than the normal number of annual distributions, but distributions through March 2005 are well ahead of the average number. Foreign requestors accounted for 60 % of the requestors and 71 % of the accessions distributed, which is about normal for annual NCGRCD distributions. Most foreign requestors, as usual, were public sector employees (two-thirds). The public sector accounted for 70 % of the total distributions made (foreign and domestic).

The above figures are for all distributions, and include budwood, seed, pollen, leaves, etc. Although NCGRCD is a clonal repository, it continues to distribute a fairly large amount of material as seeds. There are several reasons for this: many of the seeds distributed are used for virus indicators or in rootstock trials when requestors do not want to wait the years necessary for trees to start producing seeds when propagated from budwood; requestors wish to avoid quarantine hassles associated with vegetative tissue; and most distributions of citrus relatives are in the form of seeds since quarantine requirements are not well defined and the relatives generally come true-to-type from seed. Budwood distributions mostly fall in a few categories: production of seed sources of indicator plants for virus testing or production of rootstocks; establishment of a clean-source program; commercial trials; and a limited amount of breeding work.

During recent years, there have been increased numbers of domestic requests from researchers not affiliated with the major citrus-producing states, non-profit organizations (ie, botanic gardens), and backyard or hobbyist growers. The NCGRCD tries to accommodate hobbyist growers (in contrast to many other Repositories), but this group accounts for a disproportionately large amount of effort considering the amount of germplasm actually distributed to them. This is primarily because they request small amounts of seeds of accessions from which seeds are not normally collected.

As usual, dates accounted for a low percentage of distributions. Most were pollen or seeds sent to foreign requestors, but some offshoots were distributed to local growers.

Databases

The NCGRCD uses several local databases as well as the national Germplasm Resources Information Network (GRIN) database maintained by the Database Management Unit (DBMU) of the National Germplasm Resources Laboratory (NGRL) in Beltsville. Review of the local databases is ongoing (see discussion in *Citrus Relatives* section above); this is necessary before corrections to the GRIN database can be made. Corrections to GRIN are generally made shortly after they are made in the local databases.

In addition to the review process, there have been several issues with the local databases that have needed resolution. A new format for inventory numbers has been instituted and most in house databases have been converted from dBase format to Access format. Training of all Repository personnel in Access is anticipated in the coming year (2005). In December 2004, the IT specialist resigned from the Repository position to take a full time position at USSL; at the time of this writing, that position was recently filled. We have recently converted from a DOS-based dBase program to Access. Access is a relational database and the above mentioned efficiencies can be realized. This conversion is only partially implemented as the flat structure of

the existing dBase files needs some modification. It is hoped that at sometime in the future, a bar-coding or RFID system will be implemented to increase the efficiency for the inventory process.

The NCGRCD portion of GRIN is generally in good condition. Most accessions are loaded in GRIN, and PI assignment is current. Descriptor data for approximately 10 — 15 % of the accessions need to be taken and loaded into GRIN. Images for a portion of the collection are currently being edited and will be available online when ready and when resources permit more time to be devoted to the website. There is also SSR marker data that is pending entry into GRIN.

The Repository web page < <http://www.ars-grin.riv> > saw little change in 2004 due to the pending movement of a new standardized web format and due to lack of time by the IT specialist to make modification. A second webpage is available at the FAO Ecoport site [http://www.ecoport.org/EP.exe\\$PassCheckStart?ID=117](http://www.ecoport.org/EP.exe$PassCheckStart?ID=117). This should help spread the message that the Repository is here to serve the national and international research community.

Citrus Germplasm Activities

The NCGRCD is a service unit, with its primary focus on providing others with the materials necessary to do research. Much of the research conducted at the Repository is in cooperation with University and USDA-ARS cooperating scientists. Research and other activities supporting and enhancing the Repository*s mission are described in this section.

During CY 2004, several sources of extramural money were accessed, which has allowed an enhancement of the Repository research component. A project for the evaluation of seasonal fluctuations in N uptake and nutrient content of field-grown Valencia receiving funding from CRB was brought to a conclusion. Another project for the development of a sampling method for detection of *Spiroplasma citri* in field trees received continued funding from CRB and the California Citrus Nursery Advisory Board.

One of the real strengths of the Riverside Repository, as compared to some of the other repositories, is the breadth of its program, as indicated here. Due to our status as a quarantine facility, we are able to directly introduce new accessions, and we also enjoy excellent cooperation with the CCPP. This is in contrast with other clonal repositories, which must introduce new materials through the National Plant Germplasm Quarantine Office in Beltsville. Our pathogen testing program and maintenance of materials under screen also allows us to offer pathogen-tested ‘clean stock’ material, which other repositories are unable to do. In the area of evaluation and research, the Riverside repository is active in several areas. Our molecular characterization efforts are on par with those commonly found at other repositories. In addition, we are active in the areas of horticulture and plant pathology. It is not common for a repository in the NPGS to be active in all three of these areas. Some of these projects have both basic and applied components. The Repository also is closely involved in state and national phytosanitary issues due to participation in several committees.

Overall, this Repository, despite our small size and budget, has a program of which we are proud and which I believe can be favorably compared to other similar programs with greater resources and support.

Molecular Characterization of the CVC

This project was cooperative with Dr Mikeal Roose, UCR Dept of Botany & Plant Science, and is supported by a SCA. Most of the work was performed by a graduate student, Dr Noelle A Barkley. It involved evaluation of the genetic diversity present in the CVC via evaluation of approximately 380 sexually-derived accessions by simple-sequence-repeat (SSR) markers.

This project concluded in 2003. Please refer to the 2003 report for more details on the results. Dr Barkley accepted a position with ARS in Griffin, Georgia. During 2004, a manuscript was prepared based upon a portion of her dissertation. It is anticipated that it will be submitted during 2005 and additional manuscripts prepared. The first manuscript deals with the phylogenetic relationships between the accessions studied.

A core collection, which is a small subset containing the majority of the genetic diversity, was constructed utilizing SSR marker data. Several different sampling strategies were compared in the construction of the core subset. In relation to the CVC, these different sampling strategies retained 82.4% to 85.8% of the alleles found in the CYC. The subset identified from a proportional stratified sampling strategy retained the largest number of alleles and was most similar in allele frequency to the studied portion of the whole collection. The core collection has been flagged as such in GRIN, and additional information from the analysis will be added to GRIN when format issues are resolved. Information regarding the primers developed is posted at <http://www.plantbiology.ucr.edu/people/faculty/rooselink2.html>. The core subset has been flagged in the GRIN system. However, data from the SSR markers needs to be entered.

Seasonal Variations in N Uptake and Mineral Content

Contamination of ground water is increasing. One source of contamination is leaching and runoff from fertilization of crops, including citrus with its high N use. Good management practices can contribute towards reduction of this type of contamination. Application of excess N can contribute to the N load of the soil water. In order to reduce excess N application, it is necessary to have an accurate estimate of the amount of N actually utilized by the tree, and of the actual uptake of N from the soil. Previous studies have not addressed the actual N uptake vs remobilization in contributing towards N present in various organs of the tree. Other elements may also contribute towards pollution of ground water. Seasonal variations in some of these elements has not been measured, including the important minor elements. Although with current technology it is not possible to assess uptake as directly as with N, measurement of the concentration and mass of other elements in various organs of citrus can contribute towards understanding their seasonal dynamics. This information will also be useful in estimating seasonal requirements for various elements, thereby allowing more efficient and economical application. Previous studies have either not assessed the concentrations of all elements or have

been done with small, potted trees.

As mentioned, this is a new project initiated late in 2002, although actual work will begin in early 2003. It is cooperative with Dr MaryLu Arpaia, UC Riverside/Kearney Agricultural Center, and has been carried out under field conditions at the UC Lindcove Research and Extension Center in Tulare County. Mature 'Valencia' trees on 'Carrizo' will be the basis for the study. The trees were fertilized normally during the course of the experiment. In addition, non-radioactively labeled ^{15}N (0.25 lb of 5 atom %) was applied in the spring.

The trees were sampled six times during the growing season: winter, bloom, drop, color break, maturity, following winter. At each date, eight trees were removed and divided into the following organs: fruit, young leaves, mature leaves, branches, scaffold limbs, trunk, large roots, and small roots. The dry mass of these organs was measured (or estimated in the case of leaves). Concentrations of N, P, K, S, Ca, Mg, Zn, Mn, Fe, Cu, B, Mo, Na, and Cl were measured in subsamples of the various organs. The isotopic ratio for N was determined for these samples. Measurement of the concentrations of the elements and the mass of the organs allow an estimation of the total amount of the elements in the tree. This gives a more accurate representation of the amount of nutrient removed from the soil than would a measurement of concentration alone. This combined with the harvest of the entire tree allows an estimation of the amount of the elements actually removed from the soil by the tree. It will not account for losses by leaching etc which have to be assessed separately.

Some of the N found in the tree is taken up directly from the soil, while another portion is derived from pools in other parts of the tree. The use of the labeled N allows the actual uptake of N from the soil to be assessed. Uptake of ^{15}N by the tree will alter the ratio of $^{15}\text{N}:^{14}\text{N}$, thus allowing an estimation of the amount of N contributed by soil uptake as opposed to translocation or remobilization from other organs. This will help establish the seasons in which N is actually taken up from the soil as opposed to being translocated from other organs. Leaf sampling or measurements of N concentration does not account for this. In addition, soil samples from the 1 ft and 4 ft depths will be taken at each sampling date. Measurement of the N content and the $^{15}\text{N}:^{14}\text{N}$ ratio allows an estimation of the amount of N removed from the soil. Combined with information gained from the tree measurements, this allows an estimation of the amount of N lost through leaching or other movement in the soil.

This project concluded in 2004, except for soil extraction and analysis of the isotope ratio. Statistical analysis will be concluded following this final analysis. Preliminary examination of mineral content of the trees is in line with that from older studies. As expected, there is not much variation between sampling dates due to tree-to-tree variability. However, the elemental masses will be derived and delivered to the industry.

Phytopathological Activities

Development of a Method for the Detection and Sampling of Spiroplasma citri under Field

Conditions

Stubborn disease of citrus, caused by *Spiroplasma citri*, is an important disease of citrus in the hot, arid inland areas of California and Arizona, as well as other areas throughout the world with similar climates. Assaying for stubborn has recently been suggested as an additional required test for registered nursery trees in California. The most common current method of diagnosis of stubborn, and the only one accepted by most regulatory bodies, is the culture method. However, this method is not well-suited for nursery testing, since it is cumbersome and time consuming. In addition, introduction of new varieties by the Repository and the Citrus Clonal Protection Program (CCPP), as part of the complete indexing protocol of quarantine trees, requires testing for stubborn. These and similar programs would benefit from a reliable additional or backup testing method.

The two methods most commonly used in California for the detection of stubborn have been biological indexing and culture in a cell-free medium. Indexing for stubborn disease of citrus is more difficult than for most other pathogens, since it requires the somewhat tricky side-graft or leaf vein method, and it takes several months under warm temperatures to obtain results. Culturing is also somewhat time consuming, sometimes requires several attempts, and can produce false positives from contamination. Other methods include PCR. These techniques are not well-established at this point. Commercial ELISA kits have been tested at both CCPP and NCGR and do not work with California isolates of stubborn. Development of suitable antibodies might make ELISA a useful technique. A method involving a short increase of *S. citri* by culturing, followed by PCR detection using DNA obtained from a 2-3 day incubated culture has improved detection of *S. citri*. Other techniques which show promise are PCR using DNA extracts from fruit collumena without culturing. Stubborn is unevenly distributed within an infected tree, so several fruit need to be collected from field trees to be sampled.

We have been able to utilize pre-culture PCR to speed up the detection of *S. citri* from various organs of the trees. We have also recently successfully utilized PCR for direct detection of the pathogen from field-grown fruit and budwood. Evaluation of this advancement is continuing. Polyclonal antibodies have also been developed for *S. citri*, and this antisera may be useful for development of immunocapture PCR methods for detection of *S. citri*, or for use in ELISA assays. Research is continuing on testing sampling methods to ensure detection of stubborn infected field trees. This includes evaluation of the seasonal fluctuations in titer in the San Joaquin Valley and Southern California areas. Apparent genetic variability in *S. citri* from the different locations is being examined using AFLP. The actual work is being carried out by Dr Benjamin Rangel at the Repository facilities.

Development of stable, uniform controls for use in ELISA assays for Citrus tristeza virus (CTV)

In cooperation with Dr. Bar-Joseph, Volcani Center, Israel and M. Dekkers, University of Florida, a method has been developed to prepare standardized positive and negative non-infectious controls for ELISA for detection of CTV which are stable over a long period of time, even without refrigeration. The use of such standards can now permit better direct comparisons among laboratories when comparing different ELISA systems for detection of CTV and offer the

potential of internal and external quality control. Forty-three aliquots of these controls have been distributed to 10 requestors in CY2004.

Molecular characterization of the Cytoplasmic-type Citrus leprosis virus

Citrus leprosis has been present in several South American countries for many decades. The disease is transmitted by *Brevipalpus* mites. The recent emergence of citrus leprosis in Central American countries presents a serious threat to the US citrus industry where it does not presently occur. In cooperation with a graduate student, Abby Guerra, and Drs. R. Brlansky and M. Keremane, University of Florida, progress has been made on the molecular characterizations of CiLV which has enabled the development of sensitive RT-PCR based detection methods and production of CiLV-specific antibodies. Cyto- and nucleo-rhabdovirus-like particles have been known to be associated with citrus leprosis. Screening and sequencing of clones from a cDNA library made from cytoplasmic-leprosis infected tissue has led to the identification of some putative viral sequences. Northern hybridizations have shown consistent association of this sequence with the cytoplasmic-rhabdovirus-like leprosis from several countries, but not with samples from the nuclear rhabdovirus-like or healthy citrus. Northern hybridizations by using DIG-labeled DNA and RNA (sense and antisense) probes of different clones have shown two different patterns. Probes of clones from RNA 1 hybridized with two RNAs of approximately 10 kb and 1.2 kb, while probes of clones from RNA 2 hybridized with four RNAs of approximately 4.7, 2.5, 1.6, and 1.0 kb. These results clearly suggest that at least two species of RNAs are associated with Citrus leprosis virus and that the cytoplasmic- and that nuclear-type of leprosis are caused by two distinct viruses.

When this research was begun, the identification of leprosis was based mainly on visual symptoms followed by confirmation of presence of virus particles using transmission electron microscopy. Early identification of the disease from plant materials and mites is important for management of the disease and to minimize spread into new areas. An RT-PCR method of detection of the cyto-type CiLV has been developed which permits sensitive detection of the virus in samples from Brazil, Venezuela, Panama and other Central American countries. To develop antibodies specific for CiLV, a highly expressed protein from the putative cytoplasmic leprosis virus genome was cloned in a bacterial expression vector, pET 27b (Novagen). The leprosis protein with a carboxy-terminal fusion of a HSV and 6x Histidine tags was expressed in the expression host, *Escherichia coli*, strain BL21. The expressed protein was purified by two methods; the insoluble inclusion protein was purified by using Bugbuster (Novagen), and the soluble fraction protein was purified using an Ni-NTA agarose column. The expressed protein was monitored through different steps of purification by Western blotting using an anti-HSV monoclonal antibody. Both the expressed protein preparations were used as inject antigens in rabbits and chickens for producing antibodies to CiLV. ELISA and dot-immuno binding assays are being developed using these CiLV-specific antibodies.

Citrus leaf blotch virus (CLBV) and Dweet mottle virus (DMV)

CLBV has been reported to be seed transmitted at rates of up to 2.5% from several citrus varieties. Using primer pairs reported for the detection of CLBV, RT-PCR assays were used to determine if CLBV was present in reported commonly infected hosts in the Protected Collection

and in plants in the Citrus Variety Collection at UCR used for seed distributions. Additionally, rootstock varieties, Fukomoto navel sweet orange, and kumquats in the Lindcove Foundation block were tested for CLBV in cooperation with CCPP. No RT-PCR positive plants have been found using the CLBV primers. However, DMV gives a positive PCR product when tested. DMV was first found in the early 1960s when Cleopatra mandarin selections from Florida were first biologically indexed on Dweet tangor indicator plants. The mottle symptom was different from symptoms produced by concave gum and psorosis, thus the virus was named Dweet mottle virus. Earlier introductions into the CVIP were then reindexed on Dweet tangor indicator plants, but no additional DMV isolates were found on these earlier introductions. There has been a second interception of DMV on material originating from New Zealand..All citrus germplasm being introduced into California have been biologically tested on Dweet tangor, and this has probably prevented introduction of either DMV or CLBV into California

Research is underway at the Repository, in collaboration with Dr. Allan Dodds and Mr. Subhas Hajeri, graduate student, to determine the molecular relationship between CLBV and DMV (which has already been done in Spain), to produce an antibody specific for DMV for use in large scale assays for the detection of DMV (and CLBV), and to determine the mechanism of the +1 frameshift for the expression of the RNA dependent RNA polymerase gene of DMV/CLBV.

Research on Witches' broom diseases of *Citrus*

Small acid limes are an important fruit crop in the Near East. They are used as refreshment, nutrition, and a source of vitamins, dried limes are pulverized and used as an important food seasoning in the Near East. Witches' Broom disease of acid limes (WBDL) was reported in the Northern Coast of Oman in the early 1980s. WBDL has spread and has virtually eliminated acid lime production in the affected areas, killing trees 3-5 years after the appearance of the first broom. The disease is caused by a phytoplasma, *Candidatus* Phytoplasma aurantifolia. The pathogen is phloem limited, graft transmissible, and probably vectored by the leaf hopper *Hishimonus phycitis* (Distant). Recent evidence indicates that WBDL is seed transmitted, thus the threat of WBDL is widespread.

El-Kharbotly *et al.* (2000) first reported that WBDL may be seed transmitted. Following that report, seed was collected from fruit of WBDL affected trees, washed in 20 percent bleach solution, dried in Oman and then shipped under quarantine permit to the Beltsville, USDA Maryland Quarantine Research Collection of Exotic Citrus Diseases (QRCECD) in cooperation with Dr. J. Hartung, Fruit Lab., BARC, Beltsville, MD. Once received in the QRCECD, the seeds were germinated in sterilized soil and allowed to grow. After six months, seedlings were sampled, DNA extracted and the presence of WBDL phytoplasma was tested for using a PCR assay. Thirty seven of 42 seedlings were positive for the WBDL phytoplasma. The seedlings are being monitored for appearance of witches' broom diseases symptoms.

Eight somatic hybrids and crossed with somatic hybrids with acid lime-like parents are being evaluated in QRCECD for resistance or tolerance to WBDL. All lines being tested produce acid lime-like fruit and, if tolerant or resistant to WBDL, could be potentially useful in re-establishing acid lime production in areas where WBDL is a limiting factor in their production.

Additional witches' broom diseases of citrus occurring in India, Jamaica, and California are being studied to determine the genetic relationships, based on the sequence of the interspace region of the 16S rRNA gene.

Date Palm Activities

The Arizona State University (ASU) Arboretum in Tempe (Mr Richard Harris, Coordinator) has a collection of date palms that includes a few varieties not in the NCGRCD collection. The ASU collection has been threatened by development of a sports stadium (a familiar scenario to those acquainted with UCR politics). This could potentially mean the loss (in the US) of those varieties not in the Repository collection. There are difficulties in obtaining these varieties due to California state quarantines. Date palms from outside the desert areas are restricted due to Palm Lethal Yellows mycoplasma, *Fusarium*, and *Ozonium*. The first two are not present in Arizona and would not prevent bringing date palms to the Cochella Valley. *Ozonium* is not known to occur in the Tempe area; however, it is difficult to certify this due to the complexity of the *Ozonium* assay. Therefore, the varieties can not be brought into the Repository collection at least in the short term. Although the ultimate fate of the ASU collection is not known at this time, discussions with Mr Harris and Dr Glenn Wright, University of Arizona Yuma-Mesa Ag Center, suggested that should the collection's current location be lost, the threatened varieties will be propagated at Yuma in order to preserve them. It is hoped that eventually these varieties can be incorporated into the NCGRCD. Consequently, an application is being prepared for submission to CDFA for importation of these varieties.

Research on a project sponsored by the US-Egypt Joint Board for Scientific and Technological Cooperation in cooperation with A El-Assar of Horticultural Research Institute and Dr Chao for phenotypic and molecular characterization and comparison of Egyptian and USA cultivars has been completed. Dr Chao has conducted genetic analysis on samples using the AFLP system that his lab developed. Samples have also been sent to Dr Adel Kader at UC Davis for fruit quality analysis and to Dr El-Assar's laboratory for additional analysis.

Tissue cultured date palms were received from Jean-Paul Onesto of INRA, Antibes. These will be planted in the field after quarantine release and their phenology compared with those growing in France and with US-derived date palms of the same varieties. In the future, plantlets derived from tissue supplied to INRA will be imported and compared molecularly and phenotypically with plants derived from offshoots from the same mother palm. This is also a potential source of increased genetic diversity in the date palm collection.

Facilities and Resources

At the end of 2004, Repository facilities consisted of 538 ft² of lab space, 400 ft² of office space, 1375 ft² of headhouse space, 5948 ft² of greenhouse space, 16,200 ft² of screenhouse space, and 280 ft² storage space. Additional greenhouse space belonging to the University (three greenhouses and shared space in two additional greenhouses) is also used by the Repository. The lab is used for pathogen testing and elimination, research, and as a general work area for order processing, etc. Virus-tested potted trees are belonging to our Protected Collection

are maintained in the screenhouse. Greenhouse space is used for propagation, virus indexing, and maintenance of cold-sensitive materials. A 480 ft² office trailer provides office space and laboratory space for incubators, freezers, and transfer hood

The screening on the new addition of the screenhouse (completed in 2002) was defective. This screening was replaced by the manufacturer in 2004 and replaced in December at a cost of \$31,000. While the upgrade of the greenhouses (2001-2002) and enlargement of the screenhouse (2002-2003) has been completed, we have already outgrown the capacity of these facilities.

Several additional upgrades of the facilities were carried out during 2004. The growth chamber was moved from the headhouse area and relocated in the breezeway between the Trailer and the Lab building. A storage shed was installed on the north side of the Trailer. This higher quality storage area has enabled storage of plastic ware and other items not affected by temperature extremes to be stored out of the laboratory, thus providing more useable laboratory work area. The space where the growth chamber was located is being converted (in CY2005) to a dark room, and additional benches are being installed as a work area and to provide a work space for extraction of samples. Additional lighting was installed in the headhouse to facilitate budding, grafting and other close work. A roll-up awning was installed over the pot wash sink on the west side of the laboratory building. Shelving was upgraded and cabinet space improved to provide more work area in the laboratory. The emergency back-up generator, purchased at a price of approximately US\$30,000 in FY 2001, was finally installed in CY2004. Three notebook computers with Windows XP OS were purchased in CY2004. The Repository took possession of a Ford Excursion in CY2004, and the aging Ford Aerostar van was transferred to the USSL.

Personnel

During 2004, Repository (permanent, full-time) staffing was 2.0 SY: Research Leader/Research Plant Pathologist and Horticulturist/Curator and, 2 FTE Biological Technicians, and 0.5 FTE Computer Assistant (shared with US Salinity Laboratory). These positions were supplemented with 3 'term' (2 year) positions, and approximately 2.0 FTE student and casual positions hired through the University via the RSA, which is the appropriate organ for these types of hires. Additionally a graduate student from Plant Pathology, Subhas Hajeri, has taken a thesis research project relating to the mission of the repository, and does most of his research in the Repository. In addition, a post-doctoral researcher, Dr Benjamín Rangel, has been employed for several years and greatly increased the laboratory diagnostic capabilities.

CY2004 was the first full year for the Research Leader, Richard Lee. His position is a category 1 scientist, and his research has been directed at development of new or improved diagnostic techniques for citrus pathogens to strengthen the ability of the Repository to provide the highest quality pathogen-tested germplasm.

The Location support for the Repository is provided by the Location personnel housed in the US Salinity Laboratory. Administrative support from the PWA Area Office has improved with the arrival of a new Area Director, Dr D Buxton.

Major Issues

The most critical issue facing the Repository at the end of CY2004 is space. The laboratory space is very crowded, and will become more crowded in the foreseeable future. We have been dealing with this by moving the growth chamber to the breezeway and converting the previous space in the headhouse to a dark room, work benches and cabinets, and creating a space for use for sample extractions to free up an equivalent area in the laboratory. One transfer hood has been moved to the trailer which also houses two freezers and two incubators. At time moves on, the ELISA plate reader and possible the spectrophotometer will be moved to the trailer; these moves reduce the amount of office space available for use by Repository personnel. We have been able to borrow storage space from UCR, and the installation of the storage building (14' X 20') on site has helped.

While the screenhouse which houses our Protected Collection was completed recently (CY2002), we will reach capacity in this expanded space within CY2005. The shortage of greenhouse space has been temporarily helped by being able to borrow the use of greenhouse space from UCR, these have the disadvantage of not being able to make improvements to the structures because they do not belong to the USDA, and they are not adjacent to the core collection of buildings which are serviced by the back-up generator in case of prolonged power failure. This puts our collection of citrus relatives at risk of loss because they are almost totally housed in borrowed greenhouses.

Instead of about 20-25 accessions/year which has been about average, this last year about 70 accessions were realized. To continue the increased activity on accessions, especially therapy and testing for freedom of the graft-transmissible pathogens, requires increased greenhouse space and growth chamber space. Crowding the accessions in the screenhouse make distributions more difficult and greatly increases the chance of cutting budwood from the wrong plant.

Additionally, the Florida Department of Agriculture and Consumer Affairs now recognizes the high quality of the germplasm maintained in the Repository's Protected Collection. They can now access germplasm from the Protected Collection, once received in Florida it may be fast track introduced which involves a thermotherapy cycle, followed by biological indexing for freedom from psorosis and psorosis-like pathogens and from viroids, and verification of freedom from CTV by ELISA. This permits introduction into the Florida Budwood Certification Program in less than a year. Because of this, the Repository can now be truly a National Clonal Germplasm Repository, and can help with the clean up of accessions which are requested by Florida. This status enables the Repository to better fulfill our mission, but at the same time highlights the lack of physical facilities at the repository. Our goal is to be able to release 70 accessions from quarantine on a yearly basis.

Publications

Guerra-Moreno AS, Manjunath KL, Brlansky RH, Lee RF. 2004. A multi-partite RNA virus

associated with leprosis disease of citrus. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

Krueger RR, Bash J, Lee RF. 2004. Phytosanitary status of California citrus. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

Febres VJ, Moore GA, Lee RF. 2004. Development of pathogen-derived transgenic *Citrus* for resistance against *Citrus tristeza virus*. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

Manjunath KL, Chandrika R, Warren C, Niblett CL, Lee RF. 2004. Complete nucleotide sequence of a new genotype of *Citrus tristeza virus* from an isolate having a mixed infection. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

Putter T, Putter R, Lee RF, Roistacher CN. 2004. EcoPort slide shows on the internet related to citrus and citrus diseases. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

Ramos C, Castillo J, Fernandez O, Rangel B, Lee RF. 2004. Molecular characterization of *Citrus tristeza virus* isolates from Panama. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

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Garnsey SM, Civerolo EL, Gumpf DJ, Paul C, Hilf M, Lee RF, Brlansky RH, Yokomi RK, Hartung JH. 2004. Biocharacterization of an international collection of *Citrus tristeza virus* (CTV) isolates. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

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Lee RF, Dekkers MGH, Bar-Joseph M. 2004. Development of stable, uniform controls for use in ELISA assays for *Citrus tristeza virus*. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

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Rangel B, Krueger RR, Lee RF. 2004. Current research on *Spiroplasma citri* in California. Abstract in program, 16th Conf. International Organization of Citrus Virologists, Monterrey, Mexico, Nov. 2004.

Halbert SE, Genc H, Cevik B, Brown LG, Rosales IM, Manjunath KL, Pomerinke M, Davison DA, Lee RF, Niblett CL. 2004. Distribution and characterization of *Citrus tristeza virus* in South Florida following establishment of *Toxoptera citricida*. Plant Disease 88:935-941.

Villalobos, W., Moreira, L., Derrick, KS., Beretta, MJG., Lee, RF., Rivera, C. 2004. Occurrence of citrus blight in Costa Rica. Pg 181 In: Program and Abstracts of the XVI Conference of the International Organization of Citrus Virologists, Monterrey, Mexico, Nov 7-13, 2004.

Biswas KK, Manjunath KL, Marais LJ, Lee RF. 2004. Single aphids transmit multiple genotypes of *Citrus tristeza virus*, but often with changed population dynamics. Phytopathology 94:S8.

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- 1.
2. Barkley, NA, Roose, ML, and Krueger, RR. Diversity and phylogenetic relationships of the Citrus Variety Collection (International Society for Citriculture, Agadir: February)
- 3.

Kahn, TL, Bier, OJ, Semancik, JS, Bash, JA, Roose, ML, and Krueger, RR. Cooperation in the

conservation of genetic resources: Riverside, California (International Society for Citriculture, Agadir: February)

El-Assar AM, Krueger RR, Devanand PS, Chao CT. 200_. Genetic analysis of Egyptian date (*Phoenix dactylifera* L.) accessions using AFLP markers. Genetic Resources and Crop Development (in press).

Barkley NA, Roose ML, Krueger RR, Federici CT. 200_. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Genetics (manuscript under revision prior to submission).

Rangel B, Krueger RR, Lee RF. 200_. Current research on *Spiroplasm citri* in California. Proceedings, XVI Congress, International Organization of Citrus Virologists (manuscript submitted and accepted).

4. Krueger RR, Bash JA, Lee RF. 200_. Phytosanitary status of California citrus. Proceedings, XVI Congress, International Organization of Citrus Virologists (manuscript submitted and accepted).

Table I. Material held under State and/or Federal Quarantine, and other material being indexed at NCGRCD.

Index No	Name	Accession No	10
12000-01	C ichangensis	CRC 2327	749
12000-02	Rough lemon	CRC 400	757
12000-03	C sunki	CRC 3143	3169
12000-04	Brazilian sour orange	CRC 1689	3399
12000-05	Chinotto bud sport sour orange	RQ-1993-10	3166
12000-06	Entre Fina blood orange	CRC 3650	2930
12000-07	Hamlin sweet orange	CRC 3862	3270
12000-08	Dungan satsuma	RQ-1993-02	2769
12000-09	Road 164 satsuma	RQ-1993-03	2993
12000-10	McEwen satsuma	RQ-1993-01	1041
12000-11	Hamlin X Flying Dragon somatic hybrid	CRC 4025	2610
12000-b 2	(Little) Sweetie sweet lemon	RQ-I989-01	3398
12000-13	Bahman Persian sweet lemon	CI 995-I 5-02	3396

12000-b 4	Brawley mandarin	RQ-1997-07	760
12000-15	Pera sweet orange	CRC 3864	3371
12000-16	Hung Kat mandarin	CRC 2331	3449
12000-17	Tavareslimequat	RQ-1999-03	752
12001-01	Jaffasweetorange	CRC 71	3286
12001 - 02	Wheeney grapefruit	CRC 2885	747
12001-03	Ugh mandarin	CRC 2780	3492
12001-04	Tieu mandarin	RS-1995-17-02	3493
12001-05	C limon (actually a lime)	RS-1996-07-02	3494
12001-06	Kagzi kalan lime	RS-1996-bI-01	3495
12001-07	Musambi	RS-1996-08-02	3496
12001 - 08	Sathgudi	RS-1 996-09-01	3472
2001 -09	Koster tangor	CRC 3958	3497
12001-10	Sweet lime	RS-b996-18-Ob	3498
12001-11	Soh Myn Dong	RS-b996-12-04	3499
12001-12	Soh Sar Khar	RS-b996-13-03	3500

12001-13	Soh Sar Khar zygotic seedling	RS-b996-b3-b5	3501
12001-14	Karun Jamir	RS-b 996-b 7-01	17
12001-15	Hunan big leaf zygotic seedling (trifoliolate 1-91)	RRUT 22	b6
12001-15	Hunan big leaf zygotic seedling (trifoliolate 2-gb)	RRUT 23	b 9
12001-15	Hunan little leaf zygotic seedling (trifoliolate 3-gb)	RRUT 24	20
12001-b 5	Guangdong little leaf zygotic seedling (trifoliolate 4-	RRUT 25	3b 56
12001-b 6	Little leaf trifoliolate	CRC 4007	3b57
1200b-16	Little leaf trifoliolate	CRC 4007	3158
12001-16	Littleleaftrifoliolate	CRC4008	3b59
12001-16	Little leaf trifoliolate	CRC 4008	3144
12001-17	Big leaf trifoliolate	CRC 4006	3160
12001-17	OPS trifoliolate	CRC 4009	3b6b
1200b-b7	OPS trifoliolate	CRC 4009	3491
12001-b 8	Limnocitrus hittorahis	RRUT 26	2981
12001-27	C benikoji	CRC 3149	2957

12001-28	C pennivesiculata	CRC 2434	2969
12001-29	Csulcata	CRC 3257 CRC3274	3359
12001-30	Cfunadoko		766
12001-3b	Lunario	CRC 3159	2944
12001-32	Arajon	CRC 2596	3622
12001-33	Limeira	RQ-1998-48	3594
i2001-34	Sunstar	RQ-200b-32	
12001-35	Tresca	RQ-200b-51	3617, 3618
12001-36	Bebeiyou	RQ-2001-08	3553
12001-37	Shantianyou	RQ-200b-09	3554
12001-38	Tamurana	RQ-2001-28	3580, 3581
12001-39	Iwaikan	RQ-200I-29	3582, 3583
12001-40	Ling mung	RQ-2001-30	3584, 3585
12001-41	Red Ling Mung	RQ-2001-32	3587, 3588
12001-42	Baishashui	RS-1995-20-06	3623

12001-42	Rangpur Srirampur •	RS-1996-2b-06	3624
12001-42	Rangpur Kirumakki	RS-1996-23-10	3625
12001-43	Rangpur Poona	RS-b996-33-01	3626
12001-43	Rangpur Poona Srirampur	RS-1996-34-03	3627
12001-43	Lima criolla brasilia	RS-1996-32-Ob	3628
12001-44	Rangpur Knorr	RS-1996-28-09	3629
12001-44	C limonia indica	RS-1997-08-05	3630
12001-44	Honglingmeng	RS-1 998-05-03	3631
12001-44	Tu ngingmeng	RS-b 998-06-54	3632
12001-45	Kaisumwampee	CRC 3967	3412
12002-09	Clementine X Orlando (C54-4-2)	RQ-2001-21-PLOI	3566
12002-10	Clementine X Orlando (C54-4-2)	RQ-200b-2b-PLO2	3567
12002-Ib	Variant Citradia	RQ-2001-26-PLO1	3576
12002-12	Variant Citradia	RQ-200b -26-PLO2	3577
12002-13	Tung kum	RQ-2001-43-PLO1	3602

12002-14	Tung kum	RQ-200b-43-PLO2	3603
12002-15	Swingle tangelo	RQ-200b -47-PLO1	3609
12002-b6	Swingle tangelo	RQ-2001-47-PLO2	3610
12002-17	Gioia Tauro	RQ-2001-48-PLOb	36bb
12002-18	Gioia Tauro	RQ-200b-48-PLO2	3612
12002-19	M*guerqueb	RQ-2001-49-PLO1	3613
12002-20	M*guerqueb	RQ-200b -49-POL2	3614
12002-21	Citrus daoxianensis	RQ-2001-54-PLO1	3933
12002-22	Citrus daoxianensis	RQ-2001-54-PLO2	3935
12002-23	Malta blood	RQ-2001-62-PLOI	3956
12002-24	Malta blood	RQ-2001-62-PLO2	3957
12002-25	Dayap	RQ-2001-61 (RRUT 35) -- PLO1	3874
12002-26	Dayap	RQ-2001-61 (RRUT 35) — PLO2	3875
12002-27	Honju	RS-b 997-01-01-PLO I	none
12002-28	Suanju	RS-b 998-08-b 2-PLO 1	none

12002-29	Huangguogan	RS-1998-1 b-25-PLO1	none
12002-30	Bahman #2	RS-b 998-37-O3-PLO1	3403
12002-31	Xiangchen	RS-I 998-03-01 -PLO1	none
12002-32	Etonia	RS-b997-09-25-PLO1	none
12002-33	Baiju	RS-1 997-03-b 5-PLO1	none
12002-34	Gaojiantou	RS-b997-O4-07-PLO1	none
12002-35	GuI-guI	RS-1 996-05-08-PLO1	none

Table 2a. Germplasm Distributions, by Accession, CY 2002, NCGRCD

Domestic	Foreign			
Public	Private	Public	Private	
Citrus Accessions	83	57	256	90
Date Accessions	1	0	9	0
Total Accessions	84	57	265	90

Table 2b. Germplasm Distributions, by Requestor Category, CY 2002, NCGRCD

Domestic	ARS	2
	AID	0
	Other Federal	1
	State Government	17
	Commercial	7
	Non-Profit	2
	Private Individual	5
	Total	34
International	CGIAR Centre	0
	Genetic Resource Conservation Program	0
	Commercial	0
	Non-commercial	34
	Private Individual	17
	Total	51
Total	85	