Citrus & Date Germplasm: Crop Vulnerability, Germplasm Activities, Germplasm Needs

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 (see http://www.fao.org/es/ESC/common/ecg/28189_en_FinalBull2003.pdf; additional information may be accessed at http://www.fas.usda.gov/htp/Hort_Circular/2004/08-04/8-31-04 Citrus Feature.pdf). Brazil and Florida, USA produce primarily citrus fruit destined for the juice or concentrate market, while China, México, Spain, and California, USA 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 lead 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 budsports. 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. WT 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*. *Ex situ* collections are also important as they make germplasm more readily available for distribution to users; make possible the collection of characterization and evaluation data; and help reduce possible errors in documentation.

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 Bettencourt *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 daoxianensis*, and *Poncirus polyandra*. These putative species are mostly unknown outside of PR China. Areas that have been explored include Guangxi district, Guangzi province; Shennon 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; Guiling, Guangxi province; Zhangsa, Hunan province; Guangzhou, Guangdong province; Jiangjin, Sichuan province; Wuzhung 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, Guiling, 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 so 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 are, 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 Statio, 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 PR 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* spp 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 dactlyifera*. *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.

Current Citrus & Date Germplasm Conservation 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) (http://www.ars-grin.gov/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) in Riverside, California (http://www.ars-grin.gov/riv), is charged with serving the needs of users of citrus and date palm germplasm users. Additional information and a different perspective on the Repository is available at http://www.ecoport.org/perl/ecoport15.pl?SearchType=slideshowView&slideshowId=117. The mission of 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) (http://www.ars.usda.gov/) and the Agricultural Experiment Station of the University of California, Riverside (UCR) (http://www.ucr.edu). 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 (http://danrrec.ucdavis.edu/south_coast/home_page.html), 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) (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 ft^2 of laboratory space, 400 ft^2 of office space, 1375 ft^2 of headhouse/storage space, 6048 ft^2 of greenhouse space, and 16,200 ft^2 of screenhouse space. Approximately 6000 ft^2 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 ft^2 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 lead 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 at the Repository level rather than by the National Plant Germplasm Quarantine Office in Beltsville. This is a very important and valuable programmatic enhancement. 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 not required to be quarantined, as until recently there were no reports of seed-borne citrus viruses. However, materials received as seeds routinely undergo indexing for certain viruses. With the recent reports of seed transmission of a few citrus viruses (see below), this indexing increases in importance and at some point seeds may also become prohibited. 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. Development of a protocol for introduction of date palms is critical, as this expertise does not currently exist in Beltsville.

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 corps 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 screenhouse 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 form 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 trifoliate 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 24 simple-sequence-repeat (SSR) markers, 15 of which were developed de novo by ARS-UCR cooperation (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: trifoliates, 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 Citrus & Date Germplasm Related Activities in USA

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, which is attached to the USDA-ARS US Horticultural Research Laboratory, Fort Pierce, Florida (http://www.ushrl.saa.ars.usda.gov/). There are currently approximately 250 accessions maintained at WFF, about half of which are not duplicated in Riverside. The WFF served for many years as a breeding facility for breeders at the USHRL (then located in Orlando). 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.

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 Station in Miami, Florida (http://www.ars-grin.gov/ars/SoAtlantic/Miami/homeshrs.html), 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, many accessions were destroyed by various hurricanes, and the remainder 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 (http://www.ars-grin.gov/may/) 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 (http://www.doacs.state.fl.us/pi/budwood/arb.html). 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 (http://primera.tamu.edu/kcchome/) 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 (http://ag.arizona.edu/aes/yac/yumamesa.htm), 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 (http://www.ucr.edu). 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 portions of 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 Alban.

In Florida, the University of Florida at Gainesville (http://www.ufl.edu/) and the associated Citrus Research and Education Center in Lake Alfred (http://www.lal.ufl.edu/) support 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 daoxianensis*, *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).

Critical Issues

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 intent to increase the amount of biological indexing carried out, to possibly include reindexing of the screenhouse collection for select pathogens. This will necessitate additional greenhouse space. Increased area under screen will be necessary in the near future 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 CCPP 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 vegetative tissue is a 'prohibited' commodity as per USDA-APHIS (7CFR319, 1993, http://frwebgate4.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=04671626660+79+0+0&WAISaction=retrieve 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 illarvirus [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 removed) (CFR, 2002, http://frwebgate6.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=036428146723+7+0+0&WAISaction=retrieve, 2004 http://frwebgate4.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=03755916458+20+0+0&WAISaction=retrieve). In addition to these pathogens, FAO (2003, http://www.fao.org/DOCREP/MEETING/006/Y9061E.HTM) 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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Table 1. The Aurantioideae (Orange Subfamily) of the plant family Rutaceae

Subfamily	Tribe	Subtribe	Genus	Species	Species in Collections	Origin
Aurantioideae	Clauseneae	Micromelinae	*Micromelum*	9	1	SE Asia, Oceania
		Clauseninae	*Glycosmis*	35	4	SE Asia, Oceania
			Clausena	23	4	S Asia, Oceania
			Murraya	11	2	S & SE Asia, Oceania
		Merrilliinae	*Merrillia*	1	1	SE Asia
	Citreae	Triphasiinae	*Wenzelia*	9	1	Oceania
			Monanthocitrus	1	0	Oceania
			Oxanthera	4	1	Oceania
			Merope	1	0	SE Asia, Oceania
			Triphasia	3	1	SE Asia, Oceania
			Pamburus	1	1	S & SE Asia, Oceania
			Luvugna	12	0	S & SE Asia, Oceania
			Paramignya	15	2	S & SE Asia
		Citrinae	*Severinia*	6	2	S China, SE Asia
			Pleiospermium	5	2	S Asia, Oceania
			Burkillanthus	1	0	SE Asia, Oceania
			Limnocitrus	1	1	SE Asia
			Hesperethusa	1	1	S & SE Asia
			Citropsis	11	4	Central Africa
			Atalantia	11	5	S & SE Asia
			Fortunella	5	5	S China
			Eremocitrus	1	1	Australia
			Poncirus	2	1	Central & N China
			Clymenia	1	1	Oceania
			Microcitrus	7	5	Australia
			Citrus	16	16	S & SE Asia, S China
		Balsamocitrinae	*Swinglea*	1	1	Phillipines
			Aegle	1	1	India
			Afraegle	4	2	West Africa
			Aeglopsis	2	1	W Africa
			Balsamocitrus	1	1	Uganda
			Limonia	1	1	S & SE Asia
			Feroniella	3	1	SE Asia

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Table 2. The genus *Citrus*: a summary

Species	Common name	Known age (yrs)	Year named	Probable origin	Probable native habitat	Seed reproduction	Genetic diversity
C medica	Citron	2300	1753	true species	India	sexual	moderate
C aurantium	Sour orange	900	1753	hybrid	China	nucellar	low
C sinensis	Sweet orange	500	1757	hybrid	China	nucellar	low
C maxima	Pummelo	2000 (?)	1765	true species	China	sexual	high
C limon	Lemon	800	1766	hybrid	India	partly sexual	moderate
C reticulata	Mandarin	2000 (?)	1837	true species	China	variable	high
C aurantifolia	Lime	700	1913	hybrid	Malaya	partly sexual	moderate
C paradisi	Grapefruit	200	1930	hybrid	Barbados	nucellar	low
C tachibana	Tachibana	2000 (?)	1924	unknown	Japan	sexual	moderate (?)
C indica	Indian wild org	2000 (?)	1931	unknown	India	sexual	moderate (?)
C hystrix	Mauritius papeda	2000 (?)	1813	unknown	SE Asia	sexual	moderate (?)
C macroptera	Malesian papeda	2000 (?)	1860	unknown	SE Asia	sexual	moderate (?)
C celebica	Celebes papeda	2000 (?)	1898	unknown	Celebes	sexual	moderate (?)
C ichangensis	Ichang papeda	2000 (?)	1913	unknown	China	sexual	moderate (?)
C micrantha	Papeda	2000 (?)	1915	unknown	Philippines	sexual	moderate (?)
C latipes	Khasi papeda	2000 (?)	1928	unknown	Assam	sexual	moderate (?)

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Table 3. The genus *Phoenix*: a summary

Species	Common name	Distribution	Notes	Synonyms
P acaulis	--	N India, Burma	stemless; fruit edible; sometimes confused with *P loureiri*	--
P andamanensis	--	Bay of Bengal	single trunk; semi-dwarf; species status somewhat questionable	--
P caespitosa	--	Somalia, Arabian peninsula	habitat: wadis; stemless; fruit edible; species status somewhat questionable	*P arabica*
P canariensis	Canary (Island) date palm	Canary Islands	wide range of habitats within distribution; single trunk; fruit edible; widely cultivated as ornamental	*P cycadiflora, P Jubae*, *P tenuis*
P dactylifera	Date palm	Middle East to W India, N Africa	habitat: wadis, oases; widely cultivated in suitable climates for fruit; many other plant parts utilized	*P atlantica*
P loureiri	--	India, China, Indochina, Philippines	dwarf; fruit edible; other plant parts utilized; taxonomy somewhat confused: 2 varieties (*loureiri*, *humilis*)	*P formosana, P hanceana, P humilis*, *P ousleyana*
P paludosa	--	Bay of Bengal, Indochina, Malaysia	habitat mangrove swamps and estuaries; semi-dwarf	*P siamensis*
P pusilla	--	S India, Sri Lanka	fruit edible; other plant parts utlilized	*P farinifera, P zeylanica*
P reclinata	Senegal date palm	tropical & subtropical Africa, Madagascar, Comoro Islands	habitat and morphology variable; fruit edible; other plant parts utilized; widely cultivated as ornamental	*P abyssinica, P baoulensis, P comorensis, P madagascariensis*, *P senegalensis*, *P spinosa*, *P zanzibarensis, etc*
P Roebelenii	Pygmy date palm	Laos, Vietnam, S China	rheophytic; dwarf; widely cultivated as ornamental	--
P rupicola	Cliff date palm	N India	single trunk; semi-dwarf; fruit eaten by animals but not humans	--
P sylvestris	Indian date palm	India & Pakistan	wide range of habitats; utilized for sugar, fruit	--
P theophrasti	Cretan date palm	Crete, Turkey	habitat: coastal areas; species status questionable	--

After Barrow SC. 1998. A monograph of *Phoenix* L (*Palmae*: *Coryphoideae*). Kew Bull, 53:513-575.

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