Crop Vulnerability Statement – Citrus
V2022.1

Summary of key points (1 p. maximum)

The genus *Citrus* has traditionally been considered the basis of the subfamily Aurantioideae of the family Rutaceae. Traditional taxonomic treatments have considered the Aurantioideae to consist of 33 genera, mostly tropical and subtropical in origin. *Citrus* itself is believed to have originated in Southeast Asia, from Central China through Northeastern India. From there, it spread to the east and northeast (eastern China and Japan), and south and southeast through the "Indo-China" peninsula, through the East Indies, and into Australia. Later, after domestication, citrus spread westward through South Asia, the Middle East, North Africa, and Europe. From Europe, it was spread to other areas of the world having suitable climates for its production. Today, citrus is one of the world's most cultivated fruit crops in these areas. Although not considered a staple food in the same way that, for instance, grains are, citrus does provide important dietary inputs.

Due to its long history of domestication, it is somewhat questionable as to whether truly "wild" citrus still exists. Its areas of origin and diversity are threatened by development and population pressures and whatever wild or semi-wild citrus may still exist is threatened with genetic erosion. In addition, various pests and diseases may threaten citrus at the local level. As a subtropical or tropical crop, citrus is adapted to high temperatures and is not threatened by climate change as much as some other crops may be.

Due to these threats of genetic erosion, *ex situ* conservation of citrus genetic resources is critical. In the United States, conservation of citrus genetic resources is the responsibility of the USDA-ARS National Clonal Germplasm Repository for Citrus & Dates (NCGRCD), located on the campus of the University of California at Riverside (UCR). Actual conservation activities are cooperative between NCGRCD and UCR. NCGRCD facilities are located on leased land in the Agricultural Operations area of UCR. Facilities consist of protected collections of sanitized and unsanitized accessions in screenhouses and greenhouses; greenhouse facilities for maintenance of accessions, quarantine, and pathogen-testing; laboratory facilities; and office space. Additional greenhouse space is rented from UCR. Current facilities are inadequate, and upgrades are needed. Field plantings are shared with UCR; additional field plantings are located at UC field stations in Irvine and Thermal, California.

The current composition of the overall collection includes a good representation of the edible groups of *Citrus* in both the field planting and the sanitized collection. Representation of the allied taxa is not as extensive. Although some phenotypic and genotypic characterization has been done on the accessions, more is needed. There are apparent redundancies in the collection that should be eliminated as more information becomes available. Conversely, the gaps in the collection need to be identified and filled if possible.

Acquisition of new accessions in the United States is complicated by phytosanitary and diplomatic considerations. *Citrus* and related genera are "prohibited" from entry by USDA-APHIS and can only be introduced under a Controlled Import Permit (held by the Curator) that requires therapy and extensive pathogen testing. Only about a third of the entire range of accessions is currently available as sanitized budwood. Approximately the same proportion are backed up as cryogenically stored meristems at the USDA-ARS National Laboratory for Genetic Resource Preservation (NLGRP) in Fort Collins, Colorado. Long-term goals are to have all accessions sanitized and cryogenically backed up.

Various collections of citrus genetic resources are maintained in other countries. There is need for better coordination and interaction between these collections. A Global Citrus Germplasm Network was established in the late 1990's, although its current status is not clear and it may be inactive. After the designation of citrus as an Annex 1 crop under the FAO International Treaty on Plant Genetic Resources for Food and Agriculture, additional work is needed in this area.

In addition to facilities upgrades, changes in staffing structure should be considered as staff retire in the immediate future. Programmatic enhancements are needed in the areas of pathogen testing, sanitation, and tissue culture/micropropagation. Changes in staff composition may contribute towards these enhancements.

1. Introduction to the crop (3 pp. maximum)

1.1 Biological features

The taxonomy of citrus has always been unclear and currently is particularly fluid due to advances in molecular systematics and comparative genomics. W.T. Swingle, US Dept of Agriculture, spent over 40 years studying the taxonomy and botany of *Citrus* and its related genera. His many publications in this area are summarized in Swingle (1943) and its slight revision as Swingle and Reece (1967). (Note: Since the revision of Swingle and Reece (1967) only slightly altered the original of Swingle (1943), reference will be made henceforth to Swingle (1943) with the understanding that the information is available in both sources.) Swingle (1943) placed *Citrus* into the subfamily Aurantioideae of the family Rutaceae, comprised 33 genera further divided into tribes and subtribes (Table 1).

Aurantioideae other than *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. Review of the taxonomy of Swingle (1943) indicates that in many cases, new species were named based upon a single collection or herbarium specimen; at least some are probably best treated as synonyms. Research into these related Aurantioideae genera has been limited in recent years, as summarized in Krueger and Navarro (2007) and Krueger (2010). The classic taxonomic treatments have been updated for *Clausena* (Stone, 1978b; Molino, 1994; Lu *et al*., 2016; Mou *et al*., 2018, 2021a), *Clymenia* (Stone 1985a), *Glycosmis* (Brizicky, 1962; Huang, 1987; Stone, 1978a, 1985b, 1994b; Mou and Zhang, 2009; Mou *et al*., 2012; Toyama *et al*., 2016), *Luvunga* (Stone, 1985c; Ling *et al*., 2009; Tagane *et al*., 2020), *Monanthocitrus* (Stone, 1985c; Stone and Jones, 1988), *Murraya* (Huang, 1978; Stone, 1985c; Jones, 1995; Kinoshita, 20134 Astuti and Rugayah, 2016; Mou *et al*., 2019, 2021b; Nguyen *et al*., 2019), *Oxanthera* (Stone, 1985b), *Paramignya* (Phi *et al*., 2020), and *Wenzelia* (Stone, 1985b). However, more work is undoubtedly needed in this area, particularly with genera and species that are rare or difficult to acquire.

Swingle (1943) recognized 16 species of *Citrus* (Table 2). The basic system of Swingle has been modified to recognize 17 species (Bhattacharya and Dutta, 1956; Stone, 1994a), 31 species (Singh and Nath, 1969), or 36 species (Hodgson, 1961). The taxonomic treatments of Mabberley (1997, 1998, 2004, 2022) and Zhang *et al.* (2008) recently modified Swingle (1943) by pulling *Poncirus*, *Fortunella*, *Microcitrus*, and *Eremocitrus* back into the genus *Citrus*. In contrast with the Swingle (1943) classification, the Tanaka classification recognizes up to 162 species (Tanaka, 1977). This lack of agreement reflects disagreements as to what degree of difference justifies species status and whether supposed hybrids among naturally occurring forms should be assigned species status. 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 or four species (*C. medica*, *C. reticulata, C. maxima, C. hystrix*) constitute valid species of *Citrus* sensu Swingle and that the other species are the results of admixture or reticulation events (Figure 1) (Scora, 1975; Barrett and Rhodes, 1976; Wu *et al*., 2014, 2018; Luro *et al*., 2017; Ollitrault *et al*., 2020). Interestingly, the earliest workers also believed that there were only three or four true species of *Citrus* (Linnaeus, 1753; Hooker, 1875). Genome sequence data are yielding new insights into citrus classification and genetic relationships (Wu *et al*., 2014, 2018). The evolution of *Citrus* classification has been reviewed recently by Luro *et al.* (2017) and Ollitrault *et al.* (2020); the latter group also proposes a new taxonomic treatment recognizing 42 "phylogenomic classifications."

The following botanical description of *Citrus* is taken from Ollitrault *et al.* (2020) (Figure 2). Note that the references to figures below are to the figure numbering of the source. "The fruits of *Citrus* are berries, that is, fleshy, indehiscent, many-seeded fruits containing no hard parts except the seeds (Fig. 4.1A). More specifically, *Citrus* fruits are hesperidia, in which the fleshy parts of the fruit are divided into segments and are surrounded by a separable skin (Fig. 4.1A and B). Hesperidia are confined to the fruits of Aurantioideae (Webber, 1943). The obovoid or flattened seeds (Fig. 4.1C and D) are attached adaxially (near the central axis or core, Fig. 4.1A), have smooth or ridged seed coats, and contain one to many embryos (Fig. 4.1E–J). The segments are filled with stalked fusiform pulp vesicles, which contain very watery, large-celled tissue (Fig. 4.1A and B); this is the economic part of the fruit. The segments are surrounded by a white endocarp, outside of which is the peel, which contains numerous oil glands (Fig. 4.1A, K, and L). The peel is generally green during the early stages of fruit development and turns yellow or orange at maturity. The fruit arises from the fragrant flowers, which are borne singly or in small racemes in the axils of the leaves. The flowers of *Citrus* are perfect or staminate, the latter condition being due to abortion of the pistil. The calyx is cup shaped with three to five lobes and is subglabrous. There are four to eight petals (usually five), which are white (Fig. 4.1M) or pink (Fig. 4.1N) outside, imbricate, and thick. There are usually four times as many free or basally coherent stamens as petals (Fig. 4.1M, O, and P), although there may be up to 10 times as many. The disk is annular or short, with nectary glands. The ovary contains 3–18 locules (generally 10–14), each of which contains two to eight ovaries in two collateral rows (Fig. 4.1Q–S). The style is large and cylindrical, expanding abruptly into the subglobose or oblate spheroid stigma (Fig. 4.1T).

"Members of *Citrus* are evergreen shrubs or small trees, generally 3–10 m in height. Young branches are often flat and angled, becoming cylindrical with age, usually with solitary (rarely paired) spines at the axils. Leaves are generally unifoliolate, with petioles that are usually articulated at the base of the blade and conspicuously winged (Fig. 4.1U). The leaf blade is subleathery to leathery with crenulate (rarely entire) margins and contains numerous fragrant oil glands."

1.2 Ecogeographical distribution

Citrus and related genera are native to Southeast Asia (northeastern India, southern China, the Indochinese Peninsula), which is the center of diversity for these taxa. 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 (Figure 3). 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 Sri Lanka, 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 Africa and Oceania. At least the latter migrated from the Asian landmass no earlier than the late Oligocene (Pfeil and Crisp, 2008).

From its centers of origin and diversity, citrus spread throughout the world in areas having a suitable tropical, semitropical, or subtropical climate (Figure 4) (Webber, 1967). The first type of citrus to diffuse westward was apparently the citron, arriving in Europe in the Roman era. Other acid citrus arrived in Europe near century XII, via Spain during the Arab occupation. Sweet oranges were a later introduction circa century XV, with mandarins not arriving until the centuries XVIII or XIX. *Citrus* was taken to the Western Hemisphere circa century XV by the Spanish and Portuguese during the initial colonial era due to their colonies having the most suitable climates for citrus production.

1.3 Plant breeding and its products

In the United States, modern structured citrus breeding apparently began near the beginning of century XX at both the State and Federal levels. USDA breeding began in Florida in 1893 and later expanded into California and Texas before reverting to being Florida based (Traub and Robinson, 1937; Cooper *et al.*, 1962, 1964; Hearn, 1987; Cooper, 1995). The University of California began citrus breeding in 1914 (Cameron and Frost, 1968). In Florida, citrus breeding began in the 1920's at a low level, increased somewhat in the 1950's in response to nematode threats, and greatly increased in the 1980's (Cameron and Frost, 1968; Kender, 2003). Efforts at the international level mostly began in the 1920's and 1930's (Caruso *et al.*, 2020). The many specific achievements of the various breeding programs are too extensive to be summarized here but some general areas of investigation can be cited. General traditional breeding objectives and techniques are presented by Traub and Robinson (1937), Cameron and Frost (1968), Cameron and Soost (1969), Soost and Cameron (1975), Soost (1987), Soost and Roose (1995), Roy (2002), Ollitrault *et al* (2008), Gmitter *et al.* (2009), Ollitrault and Navarro (2012), and Caruso *et al.* (2020). Newer technologies are reviewed in Khan (2007), Roose (2015), Shimizu (2020), and Germaná (2020).

Early investigations centered on selection of bud lines with superior characteristics and nucellar bud lines for disease-free status, and on hybridization. Later techniques included triploid production, mutation breeding, somaclonal variation, somatic hybridization, transformation, gene editing, etc. Along with the evolution of these tools, molecular and genomic advances have allowed more precise knowledge of the germplasm used and the resulting new genotypes. Whatever techniques are used, objectives have remained more or less the same over the last century or so: superior fruit quality and improved adaptation to biotic and abiotic stresses. Fruit quality improvements have emphasized seedlessness but also include overall flavor (sugar:acid ratio and aromatic compound profiles), season of maturity, and general appearance, (including peel and flesh color). Biotic stressors include pathogens (most notably *Phytophthora*, Citrus tristeza virus, and *Candidatus* Liberibacter spp.), insects, and nematodes, whereas abiotic stressors include soil conditions (particularly salinity and pH) and climatic conditions (mostly cold). Although work in all areas is continuing, the majority of breeding efforts in recent years have focused on dealing with Huanglongbing (Anonymous, 2018).

1.4 Primary crop products and their value (farmgate)

In the United States, the primary crop product of citrus is fruit, either fresh or processed. The most recent statistics (2019) for domestic citrus production is shown in Table 3 and state production for the major producing states are shown in Table 4. For additional information and trends, please refer to the USDA National Agricultural Statistics Service (http://www.nass.usda.gov). There are also minor industries (scents, flavors) for which statistics are not available.

1.5 Domestic and international crop production

1.5.1 U.S. (regional geography)

As per the above statistics, the major United States citrus-producing states are Florida, California, Texas, and Arizona. Florida produces mostly fruit for processing, while the other states produce primarily fruit for fresh consumption. Types of fruit vary by region. Florida produces mainly sweet oranges for processing, with additional grapefruit production. California produces mainly sweet oranges and mandarins for direct consumption. Arizona and the desert areas of California produce mainly lemons. Texas produces mostly grapefruit. The so-called “minor citrus-producing states” are located mainly on the Gulf Coast and specialize in satsuma mandarins.

1.5.2 International

Currently, citrus is produced in most areas with suitable tropical, semitropical, or subtropical climates, resulting in a “citrus belt” between approximately 40 ºN and 40 ºS and comprising 140 countries according to FAO (2022). The main constraint for most citrus production is sufficiently warm temperature during the growing season and a lack of frost during the winter. Although citrus grows well in the tropics, subtropical climates are better for production and most commercial production is between 20 ° and 40 ° in both hemispheres (Burke, 1967; Spiegel-Roy and Goldschmidt, 1996). As with domestic production, production of specific types of citrus varies with geography (Figure 6). For instance, China produces large amounts of mandarins, sweet oranges, and pummelos, while Brazil produces mainly sweet oranges.

2. Urgency and extent of crop vulnerabilities and threats to food security (4 pp. maximum)

2.1 Genetic uniformity in the “standing crops” and varietal life spans

The three or four “true” species reproduce sexually and if different genotypes within the species are intermated, the progeny are similar to their parents. The other important edible types (orange, grapefruit, lemon, and lime) are believed to have originated from one or more generations of hybridization between these ancestral species. Most of the cultivars of orange, grapefruit, and lemon are believed to have originated as apomictic seedlings or bud sports. Consequently, the amount of genetic diversity within these groups is relatively low, in spite of there being many named cultivars. Conversely, mandarins, pummelos, and citrons have higher levels of genetic diversity since many of the cultivars have arisen through sexual hybridization. The number of rootstocks currently being used is limited but increasing. Genetic diversity within the different types of rootstocks is also limited, as they generally produce a high percentage of nucellar seedlings.

The varietal life span of citrus is changing. For most of the time citrus has been cultivated, traditional local cultivars have been grown and varietal life spans were decades or centuries. Even in modern industrial citrus production, varietal life spans have been long. For instance, the original ‘Parent Washington’ navel has been produced continuously since 1873 and the cultivar is still widely planted. Traditionally, citrus orchards would be planted and not removed or top-worked for generations. More recently, orchard and varietal turnover has been more frequent. The development of seedless mandarin-type fruit has prompted somewhat of a shift from sweet oranges in traditional fresh fruit-producing areas. In addition, grapefruit planted area has decreased and been replaced with other types of fruit in recent decades.

2.2 Threats of genetic erosion *in situ*

Assessment of the genetic vulnerability of any crop requires knowledge of the extent and distribution of genetic diversity, particularly wild species or populations. 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 and herbaria specimens. Unfortunately, information on natural and semi-natural citrus 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* situations (Albrigo *et al.*, 1997; Gmitter *et al.*, 1999).

Genetic diversity in the centers of origin and diversity of citrus 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.

Southern 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 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 several putative new species, including *C. hongheensis*, *C. mangshanensis*, *C. daoxianensis*, and *Poncirus polyandra*. These putative species are mostly unknown outside of China. Areas that have been explored include Guangxi District, Guangxi Province; Shennong Jia, Hubei Province; Sichuan, Gansu, and Shanxi provinces; Hainan Island; and Tibet. There are also a number of indigenous Aurantioideae in southern China. There is exploitation (use) of indigenous germplasm, and some attempts at *in situ* preservation have been made. This information was compiled from notes made during the meeting of the Global Citrus Germplasm Network held during the International Citrus Congress of 2000 in Orlando, Florida.

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 likely exists in these areas. An *in situ* gene sanctuary for citrus with 627 accessions was established in the Garo Hills in the northeast (Singh, 1981). Other regions of diversity include the central and northwest Himalayas, Maharashtra, and the southern peninsula.

Southeast Asia (including Malaysia) is rich in indigenous germplasm, 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 (e.g., *Aegle marmelos* and *Limonia acidissima*) have become naturalized. This genetic diversity is threatened by deforestation, development, and disease.

2.3 Current and emerging biotic, abiotic, production, dietary, and accessibility threats and needs

2.3.1 Biotic (diseases, pests)

As is the case for all crop and non-crop plant species, citrus is attacked by a spectrum of pests and diseases. Reviewing all pests and diseases is beyond the scope of this Statement. Mention will be made of a few of the more important pests and diseases.

Citrus pests include vertebrate, arthropod, and nematode pests. These are present in all citrus-growing areas and can and do produce economic damages and adverse health effects and, if not managed, can result in tree death.

However, the authors do not believe that any biotic pest represents an existential threat to worldwide citrus at this time.

Numerous viroid, viral, bacterial, and fungal pathogens can infect citrus and result in economic damage, tree health decline, and sometimes tree death. Most of these diseases are managed in part by the development of certification and registration programs mandating the use of clean propagative stock (Navarro, 1993; Vidalakis *et al.*, 2010 a,b). Nevertheless, mention will be made of three serious diseases of citrus: *Phytophthora*-caused root rot; Citrus tristeza virus; and Huanglongbing (HLB). The first two are now mostly managed using a combination of cultural (fungicide treatment) and genetic (tolerant/resistant rootstocks) practices. HLB, however, does not at this time have established management options of these types. Observations have shown that, once introduced to a citrus-producing area, HLB becomes established and widespread (Gottwald, 2010). Given its level of potential destructiveness, HLB must be considered the current existential threat to citrus production. Consequently, most citrus research is currently aimed at HLB-related topics. However, at this time even countries severely affected by HLB, such as Brazil, continue to have a viable citrus industry. This situation could change, however, as potentially more destructive or infectious strains emerge. Therefore, continued research into the biology and management of HLB and its vector is necessary, and vigilance on the part of citrus producers and researchers is warranted.

2.3.2 Abiotic (environmental extremes, climate change)

Probably the greatest abiotic threats to citrus are those development-related factors discussed in section 2.2. The greatest environmental threat to citrus is probably salinity. Soil salinity significantly limits citrus production in many areas worldwide. Although data on fruit yields in response to salinity are limited, they indicate that grapefruit, lemons, and oranges are among the most sensitive of all agricultural crops. Fruit yields decrease about 13% for each 1.0 dS m^{-1} increase in electrical conductivity of the saturated-soil extract (ECe) once soil salinity exceeds a threshold ECe of 1.4 dS m^{-1}. Accumulation of excess Cl^- and Na^+ can cause specific ion toxicities, but this problem can be minimized by selecting rootstocks that restrict the uptake of these ions (Maas, 1993). Although citrus is sensitive to salinity and may in some cases decline or even die, salinity cannot be considered an existential threat to citrus on a global scale.

Climate change is modelled in various ways with differing assumptions and conclusions. See, for instance, CCSP (2008) and USGCRP (2017). Most likely scenarios project increases in average and extreme temperatures, but the magnitude of these changes varies from slight to large, depending on the model. Conversely, the effect on precipitation is not as well understood, and varies depending on region of the earth. CCSP (2008) concludes that production of forage and grain crops will be affected less by climate change than will production of “many” horticultural crops. Annual crops and weeds will likely change their geographic range and lifespans of annual crops may be shorter. CCSP (2008) deals mainly with staple agronomic crops with little said regarding perennial crops other than to note that there will likely be fewer chill hours under most models.

As a crop adapted to relatively high temperatures and little or no chilling requirement (Krajewski and Rabe, 1995), citrus may be less threatened by modelled climate changes than some other crops. However, changing temperature conditions may shift the areas capable of citrus production to the north and south of traditional cultivation areas in the Northern and Southern Hemispheres, respectively. There have been a few reports on observed and predicted effects of climate change on citrus growth and production. In growth chambers, Baker and Allen (1993) observed increases in growth and photosynthesis and decreases in water use by citrus when CO_2 concentrations increased above the then ambient. Water used increased with increasing temperature, however. Martinez-Ferri *et al.* (2013) modelled increased irrigation requirements of 6 –16 % for citrus in Spain under various climate projections. In contrast, Fares *et al.* (2017) modelled decreases in evapotranspiration and irrigation requirements of up to 12 % and 37 %, respectively, resulting from CO_2 increases under a number of temperature and precipitation models. Canopy light interception and subdrainage were modelled to increase under these models. In contrast to Martinez-Ferri *et al.* (2013), Fares *et al.* (2017) modelled on a global level and reported great variability from region to region and month to month. Downton and Baker (1993) described changes in cold temperatures in Florida in response to climatic oscillations (CO_2 was not included in this particular report). Since many of these climate models predict that the increases in average temperature will be associated with increases in minimum temperature, it is possible that climate change may result in fewer cold losses in citrus production. However, warmer nights during fruit maturation may result in lower sugar levels and other negative changes in fruit quality parameters.

Rosenzweig *et al.* (1996) estimated citrus production at 22 simulated sites under 9 different temperature/CO_2 scenarios. Results of the simulations without CO_2-induced yield improvement indicated that production may shift slightly northward in the southern states, but yields may decline in southern Florida and Texas due to excessive heat during the winter. CO_2 effects tended to counteract the decline in simulated citrus yields. Tubiello *et al.* (2002) simulated 5 different climate change models at 8 current citrus-producing areas and 5 areas that may become suitable for citrus production. Yields increased 20 – 50 % with decreased water use and decreased freeze losses in areas currently suitable for citrus production. However, increases were much less in areas currently marginal for citrus production and the northward expansion of production was little. Similarly, Du *et al.* (2010) and Duan *et al.* (2010) assessed the possibilities of climate change affecting citrus production in China. They predict more threats to citrus production from high temperatures in certain regions of the country and increased "adaptability" towards the Northeast.

It therefore appears at this time that citrus production will not be too adversely affected physiologically by climate change as it is currently modelled. However, there may be effects on pest pressure from climate change. Narouei-Khandan *et al.* (2016) modelled the global distribution of HLB and its vector, the Asian Citrus Psyllid (ACP), finding a good correlation between the models and existing presence data. The model identified areas having suitable climates for the expansion of HLB and ACP ranges, which were not always the same. Jesus Junior *et al.* (2009) predicted increases in vector populations and disease severity for Citrus variegated chlorosis, Huanglongbing, and Citrus leprosis, as well as increased severity of Citrus black spot and Citrus floral rot, under conditions predicted by climatic change models in Sao Paulo State, Brazil. Conversely, Aurambout *et al.* (2009) modelled decreased ACP activity in Australia with climate change due to decreases in the flushing period in the spring; however, they predicted an increase in the area suitable for ACP presence. Urbaneja-Bernat *et al.* (2019) predicted possible increased presence of two-spotted mites under warmer temperatures due to increased reproductive capacity and possible losses of predator species. Although HLB has received the most attention as a citrus disease that may be affected by climate change, additional pathogens, insect pests, and other biotic stressors may increase pressure on citrus and other crops due to climate change (Juroszek *et al.*, 2020).

It appears that pest pressure as well as plant physiology may be affected by predicted climate changes, but these should not be considered existential threats to citrus culture or genetic resources at this time. However, this situation could change in the future as many pest problems have erupted without warning in the past.

2.3.3 Production/demand (inability to meet market and population growth demands)

Although citrus is widely grown and consumed, it is not a staple food and therefore will probably be able to meet market and population growth demands.

2.3.4 Dietary (inability to meet key nutritional requirements)

Although citrus is a nutritious food, it is not a source of a large amount of calories for meeting daily energy requirements. Therefore, an inability to meet key nutritional requirements is not associated with citrus.

2.3.5 Accessibility (inability to gain access to needed plant genetic resources because of phytosanitary/quarantine issues, inadequate budgets, management capacities or legal and bureaucratic restrictions)

US phytosanitary regulations are discussed in 3.1.3 below. The main constraint of acquiring new and interesting germplasm is the inability to identify these unique genotypes and find persons willing and able to provide them (see 3.1.2 below). Although individual scientists may be willing to exchange germplasm, in some cases this is prohibited by national governments. Examples of countries with potentially interesting germplasm that do not permit the exchange of some or all citrus germplasm include China and Vietnam. Continuing engagement between governments regarding mutually beneficial germplasm exchange may open up these resources in the future. Recently (2021), progress in this area has been made with respect to Vietnam. In addition, certain international treaties and agreements limit or constrain the exchange of plant genetic resources. Notable are the Conventional on Biological Diversity (CBD) (https://www.cbd.int/ accessed 2020-03-29) and the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) http://www.fao.org/plant-treaty/en/ accessed 2020-03-29). The United States official policy is for the free and open exchange of plant genetic resources. This policy is in conflict with the CBD, which mandates benefit sharing and other restraints on the sharing of plant genetic resources. The United States is not a party to the CBD; however, the United States is a party to the ITPGRFA, which is less restrictive. Citrus is an "Annex 1" crop in the ITPGRFA, so it has fewer restrictions than non-Annex I crops. However, these treaties overall make accessing new accessions more difficult than was the case prior to their implementation. In some cases, acquisition of new accessions is limited by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (https://www.cites.org/, accessed 2020-03-29), to which the United States is a party.

3. Status of plant genetic resources in the NPGS available for reducing genetic vulnerabilities (5 pp. maximum)

3.1 Germplasm collections and *in situ* reserves

3.1.1 Holdings

The holdings of the NCGRCD are summarized in Table 5. Note that this includes accessions associated with the UCR Citrus Variety Collection (CVC) (see 3.4.2 below). The locations at which they are held (see 3.4.2 below) are shown in Table 6. A long-term goal is to incorporate more material into the sanitized (pathogen-tested), protected collection maintained under screen (see 3.4.2 below).

3.1.2 Genetic coverage and gaps

The citrus genetic resources maintained in Riverside have diverse origins (Kahn *et al.*, 2001). Collection of these resources began in the early years of the 20th century primarily by the USDA Office of Crop Physiology and Breeding (CPB). Since citrus originated outside the United States, it was necessary to import materials from outside the country. The original emphasis was on edible citrus or accessions that had apparently useful traits, such as cold tolerance (Cooper, 1995). The original USDA work on citrus began in Washington D.C. and in Florida. Other states, most notably California, had robust citrus industries, and breeding and varietal development programs in California began importing USDA-sourced materials from Florida as well as sourcing their own materials from various sources. By the middle and later years of the 20th century, researchers from USDA, the University of California, and other institutions had brought in many accessions, mostly with the aim of developing palatable varieties but also looking for disease resistance and other useful traits, and in some cases obtaining botanical curiosities when available. Many of these after circuitous routes became incorporated into the CVC and later the NCGRCD.

Given the development of the collection, there is a good representation of the major edible types of citrus. Although this collection contains one of the largest if not the largest representations of the wild relatives, it is these related taxa that are most in need of increased numbers of accessions. However, the actual status of these related taxa *in situ* is somewhat unclear. Many of the species described by Swingle (1943) were based upon a single herbarium specimen and it is somewhat doubtful that some still exist or should be recognized. Although the last several decades have seen some additional research on related genera (see 1.1 above), identifying sources of these and persons willing and able to exchange them is challenging. In addition, there are international treaties and agreements that constrain acquisition of new germplasm (see 2.3.5 above). Thus, expanding the representations of the wild relatives presents challenges. A final consideration is that traditionally, the wild relatives acquired were mostly in the subfamily Aurantioideae of the Rutaceae as defined by Swingle (1943). Perhaps it is time to reexamine this strategy and consider genera more widely dispersed within the Rutaceae.

The collection as it currently stands contains redundancies as well as gaps. Redundancies should be removed to allow more efficient management of the collection. ARS and UC cooperate in identifying redundancies and errors based upon morphology and passport data. The availability of genomics tools should increase the efficiency of redundancy removal (see 3.2.2, 3.2.3, 3.2.4 below).

3.1.3 Acquisitions

Citrus budwood has been a prohibited commodity as per USDA-APHIS for some time and recently seeds of *Citrus* and other Aurantioideae from most countries have also been prohibited (USDA-APHIS, 2020). Thus, all new acquisitions must come under a Plant Controlled Import Permit (PCIP). There are currently three permits allowing introduction of new citrus germplasm: one held by NCGRCD, one by the University of California Citrus Clonal Protection Program (CCPP), and one by the Florida Department of Primary Industries (DPI). State of California regulations are such that even germplasm introduced by DPI must undergo more or less the same treatment as if it were introduced from international sources when introduced into California either by CCPP or NCGRCD. The terms of the PCIP mandate extensive pathogen-testing and therapy of new introductions, along with various restrictions on handling and disposal of plant material. The protocols approved by USDA-APHIS and counter-signed (for NCGRCD and CCPP) by the California Department of Food and Agriculture (CDFA) are submitted along with the PCIP application. NCGRCD and CCPP are generally harmonized regarding the protocol, and DPI is also more or less harmonized at this time. The general flow of plant introductions is shown in Figure 7. Quarantine release is granted first by the CDFA and subsequently by USDA. The same procedures are used to sanitize existing but unsanitized accessions.

Acquisitions have the general goal of increasing genetic diversity available to the user community. These may be identified by the Curator, the CGC, or other interested parties. The same parties contribute towards prioritization of sanitation of existing accessions.

3.1.4 Maintenance

Germplasm at Riverside is maintained in three ways: field plantings; protected plantings; and cryopreservation. The first two represent the active collection and consist of living trees.

The CVC represents the main field planting, with a smaller satellite planting for evaluation prior to official incorporation into the CVC. Both plantings consist of two trees propagated conventionally. Due to CDFA regulations, the CVC planting is under a state permit. Vegetative propagative materials cannot be taken from the field plantings, but seeds of *Citrus*, *Poncirus*, and their hybrids can be distributed. Other materials are prohibited except under permit. These restrictions have increased in recent years due to the establishment of an HLB quarantine zone that encompasses Riverside.

Protected trees are propagated conventionally and maintained as potted trees in protective structures that are approved and inspected by USDA-APHIS. The largest sized pots used are # 7 (24 L). This, as well as the protective structure dimensions, limits the size of the trees it is possible to maintain. Trees are pruned as needed to meet the size limitation. There are two categories of protected trees: sanitized and unsanitized. The sanitized trees are maintained in a separate screened structure, have passed through the above (3.1.3) sanitation process, and meet the USDA-APHIS standards for interstate movement of nursery stock (https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/citrus/nursery-stock, accessed 2020-03-30). The sanitized trees are re-tested annually for CTV and CLas, and otherwise meet CDFA standards for citrus nursery stock testing (https://www.cdfa.ca.gov/plant/pe/nsc/nursery/citrus.html, accessed 2020-03-30). Sometimes new diseases or pathogens are identified and, in these cases, the entire protected sanitized collection may be tested. Accessions that are protected but unsanitized are basically maintained the same as the sanitized accessions but are held in separate structures. These unsanitized accessions may be “clean” but do not meet the current regulatory standards, which require a therapy step. The unsanitized accessions are not typically sources of material for distribution except when the recipient has the appropriate permits.

The majority of sanitized accessions (current count 534) are also backed up as cryopreserved meristems (buds) at the National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, Colorado (https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/, accessed 2020-03-30). Certain accessions are also backed up as seed or pollen. Long-term plans are for cryopreservation of all accessions.

3.1.5 Regeneration

Plants in the active collections are currently regenerated by conventional propagation. Since citrus is a perennial crop, regeneration is not needed on a regular basis but rather on an ad hoc basis. Trees are surveyed by the curator, technicians, or UCR personnel and regenerated as needed.

3.1.6 Distributions and outreach

Most distributions are budwood or seed, although other plant parts (leaves, pollen) and nucleic acids are also distributed. Distributions are mostly for research, plant introductions, or to establish clean propagative sources in areas where they are not available. NCGRCD is recognized as a source of clean propagative materials (budwood) by FAO (Frison and Taher, 1991) and the USDA National Clean Plant Network (NCPN) (https://www.nationalcleanplantnetwork.org/citrus-1, accessed 2022-10-08). As such, it distributes budwood internationally to establish clean propagative materials and domestically to the minor citrus-producing states that do not have a state-level citrus clean stock program. Seeds are distributed for several reasons: non-availability of budwood or regulatory restrictions, for use in screening, for use in biological indexing, and for specific research purposes.

The number of distributions varies from year to year. Generally, 400–700 items are distributed to about 100 requestors. In years past, approximately 65 % of all distributions were international. The past 10 years has seen a reversal of this pattern, and current approximately 65 % of all distributions are domestic. This shift has been due to the large increase in HLB-related research in the US in the past decade. In addition, some of the distributions are considerably larger in quantity than previously. Domestic distributions of budwood have also increased recently. NCGRCD does not distribute to the major citrus-producing states of California and Florida since these states have their own citrus budwood programs. However, distributions to the minor citrus-producing states has increased due to their inclusion in the NCPN and the emphasis on using clean source propagative materials.

3.2 Associated information

3.2.1 Genebank and/or crop-specific web site(s)

NCGRCD does not maintain a separate website. Information on specific accessions is available from the USDA Germplasm Resources Information Network (GRIN, now GRIN-Global [GG]) public website (https://npgsweb.ars-grin.gov/gringlobal/search.aspx, accessed 2022-10-08) and administrative information is available on the ARS website (https://www.ars.usda.gov/pacific-west-area/riverside-ca/national-clonal-germplasm-repository-for-citrus/, accessed 2022-10-08). The CVC has a separate website with high-quality photographic images (https://citrusvariety.ucr.edu, accessed 2020-10-08), as does the CCPP (https://ccpp.ucr.edu, accessed 2022-10-08). These three Riverside-based programs work closely together, and the websites try to link each accession page to the pages maintained by the other programs. As far as "crop-specific web sites," there are many websites relating to citrus, and these are best found by querying with respect to the specific topic of interest.

3.2.2 Passport information

Passport information is available for most of the accessions. Due to the way the collection developed over the last 100-plus years (3.1.2 above), some of the oldest accessions have limited passport information and this is sometimes secondary ("John Carpenter's backyard") rather than primary. Not all the passport information is well incorporated into GRIN currently.

3.2.3 Genotypic characterization data

To this point, there has been limited genotypic characterization of Riverside citrus germplasm. Perhaps the most extensive to this point was done by Dr. Noelle Anglin as a PhD candidate with Prof. Mikeal Roose in the early 2000's using SSR markers (Barkley, 2003; Barkley *et al.*, 2006, 2009). More recently, SNPs have been studied by Dr. Yoko Hiraoka (unpublished results), also under Prof. Roose. Sequence-related characterization is also being generated by Prof. Danelle Seymour. These data do not yet appear in GRIN. In addition, a limited amount of specific genotypic data (for instance, identifying apparent nucellar seedlings) done in cooperation with Prof. Roose is sometimes referred to in GRIN but the actual data do not appear. Additional genotypic information would facilitate rationalization of the collection and prioritization of various activities. Whole genome sequencing data for a limited number of accessions is housed outside the GRIN system on platforms such as citrusgenomedb.org (accessed 2022-10-08) and https://phytozome-next.jgi.doe.gov (accessed 2022-10-08).

3.2.4 Phenotypic evaluation data

Basic morphological characterization was performed on existing accessions in the late 1980's to early 1990's. A subset of the accessions that also existed in Florida were also evaluated in the early 1990's. These data appear in

GRIN. The UC partner, the CVC, has documented basic fruit quality parameters for a limited number of accessions. These data are available on the CVC website (URL in 3.2.1 above). A limited amount of non-morphological phenotypic data has been collected. Collection of this type of data was facilitated by the development of a “core” collection by Barkley (2003). This core collection was used to evaluate *Fusarium* reactions (Krueger and Bender, 2015) and, in Florida, colonization by *Diaphorina citri* (Westbrook *et al.*, 2011; Hall *et al.*, 2017, 2019) and *Phyllocnistis citrella* (Richardson *et al.*, 2011); canker incidence and severity (Stover *et al.*, 2014); cold (Inch *et al.*, 2014); and HLB resistance/tolerance (Ramadugu *et al.*, 2016; Miles *et al.*, 2017). Most of this information is available in GRIN.

3.3 Plant genetic resource research associated with the NPGS

3.3.1 Goals and emphases

During the early years of NCGRCD functioning, research focused on basic characterization of accessions. Research from 2003 - 2014 emphasized general plant pathology rather than plant genetic resources. For most of the previous and current 5-year plans, NCGRCD was financially constrained from achieving more than the minimum programmatic service goals. With an increase in base funds in FY 2019, increased research should be possible in the near future. This research should emphasize better phenotypic and genetic characterization of accessions, and appropriate use of specific accessions. It should be noted that due to germplasm distributions, NCGCRD contributes to a wide range of research efforts domestically and internationally, especially those associated with HLB and basic genomic/metabolomic/phenomic characterization.

3.3.2 Significant accomplishments

Germplasm accessions were screened and a “core” collection designated (see 3.2.3 above). Specific phytopathological and entomological traits were screened (see 3.2.4 above). Over one hundred accessions have been sanitized and made available to the user community (see 3.1.3 above).

3.4 Curatorial, managerial and research capacities and tools

3.4.1 Staffing

Current (2022-10-01) overall staffing of NCGRCD is 6.5 permanent FTE with 2 SY FTE (Category 1 Research Leader; Category 4 Horticulturist/Curator, currently vacant); 4.0 FTE technical support staff (Support Plant Pathologist; Biological Science Technicians (BST) (3 FTE: 2 greenhouse-based, 1 laboratory-based); Program Support Assistant (1.0 FTE); Maintenance Mechanic (0.5 FTE)). Support is also currently supplied by approximately 2.0 FTE UCR cooperative employees as well as several UCR Student Assistant part-time positions.

Two of the BST are responsible for maintenance and propagation of plant material maintained in structures, as well as shoot-tip grafting and thermotherapy, recordkeeping (database work), most of the distribution-related activities, and a portion of the cryopreservation activities. One of the BST serves as Collateral Duty Safety Officer for the entire Location. This is insufficient and the NCGRCD is too reliant on UCR employees. The base funds increase of FY 2019 should allow hiring of additional staff (see section 5 below).

3.4.2 Facilities and equipment

The main NCGRCD facility is co-located on the University of California, Riverside (UCR) campus (Figure 8). Approximately 1 ha of leased land houses approximately 130 m^2 of lab and office space; 185 m^2 of protected plant work area; 560 m^2 of greenhouse space; and 1,500 m^2 of screenhouse space. In addition, approximately 1,000 m^2 of UCR greenhouse space is rented or utilized (Figure 9). With the exception of small amount of greenhouse space devoted to quarantine of tissue culture date palms held under a PCIP, the greenhouse and screenhouse space are used for maintenance of citrus and supporting activities. The laboratory is used for pathogen testing and elimination and research. 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 45 m^2 office trailer and a recently installed 91 m^2 modular building provide office space, a break area, and additional lab space.

Field plantings are all on University of California-owned land. The CVC (see 3.1.1 and 3.1.4 above), approximately 9.0 ha, and a smaller planting, approximately 1.0 ha, are on the Riverside campus. A smaller planting of citrus relatives, approximately 1.0 ha, is located on the UC South Coast Research and Extension Center in Irvine, a milder coastal climate than Riverside (which is considered an Inland Valley). Citrus germplasm and some citrus relatives are maintained on the UCR Coachella Valley Agricultural Research Station in Thermal, a low desert climate. The Riverside and Irvine plantings technically belong to UC, but USDA utilizes them as field genebanks, sources of specific tissues for distributions, and provides advice as requested to UC in management. The Thermal plantings are USDA plantings on leased land but are available to UC as needed.

3.5 Fiscal and operational resources

The NCGRCD budget was static for well over a decade. Salary and other inflation gradually eroded operational financial resources and operations were severely constrained. An FY 2019 increase to base funds increased the total base funds to approximately USD 1.7 million. This should greatly improve NCGRCD function. Extramural funds are sometimes obtained by NCGRCD personnel. However, these are awarded with specific goals in mind and contribute little to the core service functions.

USDA mandates that 4 % of base funds be allocated to repairs and maintenance. In addition, NCGRCD proportionately support the ARS Riverside Location staff, which provides administrative and IT support. NCGRCD allocates approximately USD 100,000 per year to UCR in the form of a Research Support Agreement. This money is used for cultural practices (mostly date palm-related), pest control, soil mix, facilities maintenance, communications and network, utilities, environmental health and safety, student salaries, and miscellaneous other infrastructure support. Non-Assistance Cooperative Agreements are established with UCR as appropriate to support specific programmatic activities.

4. Other genetic resource capacities (germplasm collections, *in situ* reserves, specialized genetic/genomic stocks, associated information, research and managerial capacities and tools, and industry/technical specialists/organizations) (2 pp. maximum)

Citrus is an important crop world-wide, and many countries have at least some assemblages of citrus genetic resources. There have been a number of reports summarizing the status of these collections over the years, which are summarized in Krueger and Navarro (2007). More recent information on a country level can be found at the FAO Second Report on the State of the World's Plant Genetic Resources (http://www.fao.org/agriculture/crops/core-themes/theme/seeds-pgr/sow/sow2/en/, accessed 2020-03-31) and at the taxon level at Genesys (https://www.genesys-pgr.org, accessed 2020-03-31).

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. However, conservation of citrus genetic resources in PR China is mostly *ex situ* at present. Beginning in the early 1960s, a National Citrus Germplasm Repository was established at Beibei, Chongqing. Regional citrus germplasm repositories were also established. Huazhong Agricultural University also maintains a large collection. Smaller collections are maintained at regional universities and botanic gardens such as Xishuangbanna Tropical Botanical Garden in Yunnan Province and South China Botanical Garden in Guangdong Province.

In India, the northeast region is the center of origin/diversity. Unfortunately, this region has sometimes experienced 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 apparently consist of dooryard plantings. There is an *in situ* gene sanctuary for citrus in the Garo Hills of the northeast. *Ex situ* conservation of citrus germplasm began in the 1950s in India, and there are now collections at eight sites, with smaller collections at other sites.

Southeast Asia (including Malaysia) is rich in indigenous germplasm and, with chance seedlings, semi-wild and wild types. In 1983–1988, IBPGR coordinated four collecting missions to Thailand, Malaysia, Indonesia, and Brunei. The materials are maintained in Japan. There are four collections in Malaysia, the main one being the University of Malaya (Rimba Ilmu) Botanical Garden. This was established at the request of IBPGR in 1986. There are also some *in situ* conservation efforts, such as at the Taman Negara National Park in Pahang and the Danum Valley in Sabah. In Asia, there are also collections in Thailand, the Philippines, Vietnam, Korea, and Japan.

Australia has several *ex situ* collections that consist primarily of cultivated types. However, this island country is the center of origin for several taxa formerly considered related genera (most notably *Eremocitrus* and *Microcitrus*), which are included in the collections, as well as in certain botanic gardens.

Large *ex situ* citrus collections are found in major citrus-producing countries such as Argentina, Brazil, Corsica (France), Morocco, New Zealand, South Africa, Spain, Turkey, and the USA.

It became evident in the latter decades of the 20th century that more interaction and coordination between the various entities dealing with citrus germplasm conservation was necessary (Albrigo, 1999; Ramanatha Rao and Arora, 1999). Establishment of a global citrus genetic resources network was proposed at the FAO Intergovernmental Group on Citrus in 1996 and further developed at the Symposium on the Conservation of Genetic Resources of Citrus and its Relatives in 1996 (Albrigo, 1997).

The Global Citrus Germplasm Network (GCGN) was formally constituted under the aegis of the FAO in 1997, although its current status is unknown and it may be inactive. The GCGN was to function on a voluntary basis and involve national institutions as well as existing regional and inter-regional networks dealing with citrus genetic resource conservation and utilization (Global Citrus Germplasm Network, 1998). It was to link different initiatives in different parts of the world dealing with citrus genetic resource exploration, conservation, and utilization. The GCGN was also to play a role in harmonizing and strengthening ongoing networking initiatives that deal with citrus germplasm conservation and utilization, and in promoting new undertakings in different regions of the world.

After Citrus was recognized as an Annex 1 crop under the ITPGRFA, a global conservation strategy needs to be established. A workshop was held at the Congress of the International Citrus Congress in 2012 to plan the development of that strategy. The meeting was attended by more than 60 delegates from 20 countries and included reports on the status of citrus germplasm collections in 6 countries. The meeting resolved to establish a global conservation strategy for citrus as required by the ITPGRFA (Roose *et al.*, 2015a). To characterize the current state of citrus germplasm collections, a survey of citrus genebanks was developed and distributed worldwide (Roose *et al.*, 2015b). Although the response rate was low, a few tentative conclusions were drawn. Although collections in developed countries are active in distributing germplasm and information, some collections were not well supported nor able to do these activities. Backup was also lacking in many cases. Better support of these collections is needed. It is also important to establish a common database for citrus germplasm collections, and to provide DNA sequence-based tools that can be used to efficiently characterize genetic diversity in each collection and be used to compare accessions between collections. Although these are useful conclusions, there was a need for more complete responses. A follow-up meeting was held in Riverside in 2016 with a Bioversity representative, during which various new initiatives were discussed. In 2021, USDA-ARS and University of Florida personnel formed an *ad hoc* committee funded by the Global Crop Diversity Trust (https://www.croptrust.org, accessed 2022-10-08) to develop the global conservation strategy. The strategy is expected to be completed by 2023.

Although Riverside on its own has top citrus geneticists who can provide insight into accessions and their relationships, the citrus world is a small one. Most persons working with citrus genetics, genomics, and germplasm are known to each other and can be communicated with as needed.

5. Prospects and future developments (2 pp. maximum)

The NCGRCD has infrastructure and programmatic needs in several areas. The budget augmentation of FY 2019 provided relief from a financial standpoint and will help alleviate some of the needs. Others are longer term in nature.

Regarding infrastructure, current facilities are “maxed out” as Gen X phrases it. The screenhouse protecting the sanitized accessions is at capacity. Trees are too close together and there is no room for additional accessions to be added. A planned expansion of approximately 550 m^2 was planned, starting with FY 2019 money. However, due to problems in the acquisition process, this project was abandoned in FY 2022. This remains a critical need but needed funds are not currently available (FY 2023).

As demonstrated by the use of greenhouse space rented from UCR, this too is a critical area. The greenhouses belonging to UCR are old, sometimes unreliable, and are also at capacity. There was thought to be room for a greenhouse of approximately 550 m^2 available on the land leased from UCR. This has not been budgeted for at this time (FY 2022). In addition, the UCR Fire Marshall has designated this area as being a fire lane, so it is doubtful that it can be used for facilities expansion at this time. Addition of reliable, secure, up-to-date greenhouse space is critical.

"People space" is also limited. Office and library space are limited or lacking. An office trailer (modular building) of approximately 75 m^2 was installed in FY 2021. This addition will provide additional office and storage space, and a break area separate from the work area (a safety requirement).

The CVC is currently vulnerable to HLB. A UCR project has the goal of protecting a portion of the collection in a screenhouse. This project was initiated in 2021. It needs to be considered whether accessions maintained in this structure would still need to be maintained in their current greenhouses as well.

Personnel-wise, resources are also inadequate. The unit will potentially experience up to 3.5 FTE retirements in the next few years, including an SY position and the Support Scientist position. Succession planning the next few years is critical in maintaining program delivery.

The two plant-related Technician positions have taken on added responsibilities and rely heavily on student help to fulfill basic plant maintenance and care. Prior to about 2007, there were three Technician positions dealing with plant care. A third plant care BST should be recruited, or an additional Category 3 position established. Consideration of a database-related support position should also be considered. Finally, a shared secretary position with the other unit at the Location should be considered in order to devote more resources toward programmatic goals. Facilities maintenance may also be disrupted by retirement and restructuring of these responsibilities should be considered.

Programmatically, there are also some issues to resolve. As stated above (3.1.4), most of the sanitized accessions have been backed up cryogenically at NLGRP. As additional accessions are sanitized, they will be added to the backed-up accessions. A few efforts are being made to cryopreserve pollen and seeds of non-sanitized accessions, but efforts should also be made to cryopreserve meristems of non-sanitized accessions. As non-sanitized accessions are sanitized, they should replace the non-sanitized versions in cryopreservation. To reduce the workload in this area, a longer-range prioritization of sanitation activities should be developed. Currently, USDA-APHIS considers accessions regenerated from cryopreservation to be contaminated, even though there is a documented chain of custody, and they are never exposed to possible contamination. This policy should be changed.

Regarding the sanitation program, currently 15 – 20 accessions are sanitized per year. Efforts should be made to increase this number. Current resources are probably inadequate for this but when staffing turnover occurs, this should be taken into consideration. Current testing procedures are mostly qPCR-based, with additional biological indexing and culturing. Next generation sequencing-based techniques should be incorporated into the testing regime, when appropriate.

NCGRCD is currently working on establishing micropropagation of rootstocks and indicator plants, but this has not been completely implemented. This effort needs to be expanded as the use of seedlings may become restricted further in the future. Tissue culture capability is also desirable. A suitable position responsible for tissue culture, micropropagation, and shoot-tip grafting would be an important addition to the staff (see previous paragraph in this section).

As stated in 3.2.3 above, abilities to genotypically characterize accessions have increased in recent years. A strong effort needs to be made between USDA and UC to eliminate redundant accessions. The genomic tools will be a strong supplement to morphological and passport data in making decisions. Increased knowledge will also make management of the collection more efficient.

Efforts also need to be made to fill gaps in the collection. At this time, passport data (geographic origin, taxonomy) is the primary tool being used to identify these gaps. As knowledge of the genomes of *Citrus* and related taxa becomes more extensive, it may be possible to identify actual genes or genetic structures missing in the collection. Efforts should be made to deal with the diplomatic issues that might impede these efforts (2.3.5 above).

Older records in GRIN need to be improved. In general, more information needs to be added to GRIN for public display. This might require a dedicated position. Use of the GRIN Curator Tool to replace the current local MS Access database is planned. However, the local database is also used for quarantine tracking, propagations, and other activities not currently compatible with GG as it stands. Working with the Dev Team to implement these areas may or may not be possible. It is therefore not clear whether the local database can ever be completely eliminated, as would be desirable.

A final thought regarding the long-term sustainability of agricultural research on the UCR campus must be raised. Currently, the agricultural/environmental departments at UCR are strong and bring in grant money. UCR is close to Coachella Valley and Imperial County, which are currently strong agricultural producers. It is closer to Kern County and about equidistant from Tulare County as compared to UC Davis. Therefore, it is reasonable to believe that agricultural/environmental research will remain strong at UCR. However, there are various pressures against maintaining the AES (field) areas of UCR. Currently, these areas seem relatively safe and are mostly preserved in the most recent long-range plan. However, this could change with future events and UC administrations. In the event that the AES area is diminished or eliminated, the prospects for NCGRCD and other citrus-related activities is unclear. Siting NCGRCD at Parlier or elsewhere in the San Joaquin Valley is not necessarily a viable option. Although this elimination of AES activities is not an immediate or medium-term concern, some thought should be given to the long-term viability of agricultural research (and agriculture) in California, and the future of the NCGRCD.

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7. Appendices (number and lengths at the CGC's discretion)

Table 1. The Aurantioideae (Orange) subfamily of the plant family Rutaceae (2019-12-31) (Swingle, 1943).

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	Philippines
			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

Table 2. The genus *Citrus* (Swingle, 1943).

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	1765	hybrid	China	nucellar	low
C. maxima	Pummelo	2000 (?)	1917	true species	China	sexual	high
C. limon	Lemon	800	1765	hybrid	India	partly sexual	moderate
C. reticulata	Mandarin	2000 (?)	1837	true species	China	variable	high
C. aurantiifolia	Lime	700	1913	hybrid	Malaya	partly sexual	moderate
C. paradisi	Grapefruit	200	1830	hybrid	Barbados	nucellar	low
C. tachibana	Tachibana	2000 (?)	1924	unknown	Japan	sexual	moderate (?)
C. indica	Indian wild org	2000 (?)	1928	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 (?)

Table 3. US annual citrus production, 2019 (source: nass.usda.gov, accessed 2020-03-10).

Year	Geo Level	Data Item	Value
2019	NATIONAL	CITRUS TOTALS - ACRES BEARING	686,200
2019	NATIONAL	CITRUS TOTALS - PRODUCTION, MEASURED IN $, PHD EQUIV	3,353,837,000
2019	NATIONAL	CITRUS TOTALS, FRESH MARKET - PRODUCTION, MEASURED IN TONS	3,458,000
2019	NATIONAL	CITRUS TOTALS, PROCESSING - PRODUCTION, MEASURED IN TONS	4,486,000
2019	NATIONAL	CITRUS TOTALS, UTILIZED - PRODUCTION, MEASURED IN TONS	7,944,000

Table 4. US annual citrus production by state, 2019 (source: nass.usda.gov, accessed 2020-03-10).

Year	State	Data Item	Value
2019	ARIZONA	CITRUS TOTALS - ACRES BEARING	7,300
2019	ARIZONA	CITRUS TOTALS - PRODUCTION, MEASURED IN $, PHD EQUIV	41,706,000
2019	ARIZONA	CITRUS TOTALS, FRESH MARKET - PRODUCTION, MEASURED IN TONS	36,000
2019	ARIZONA	CITRUS TOTALS, PROCESSING - PRODUCTION, MEASURED IN TONS	18,000
2019	ARIZONA	CITRUS TOTALS, UTILIZED - PRODUCTION, MEASURED IN TONS	54,000
2019	CALIFORNIA	CITRUS TOTALS - ACRES BEARING	267,000
2019	CALIFORNIA	CITRUS TOTALS - PRODUCTION, MEASURED IN $, PHD EQUIV	2,106,051,000
2019	CALIFORNIA	CITRUS TOTALS, FRESH MARKET - PRODUCTION, MEASURED IN TONS	3,048,000
2019	CALIFORNIA	CITRUS TOTALS, PROCESSING - PRODUCTION, MEASURED IN TONS	1,024,000
2019	CALIFORNIA	CITRUS TOTALS, UTILIZED - PRODUCTION, MEASURED IN TONS	4,072,000
2019	FLORIDA	CITRUS TOTALS - ACRES BEARING	387,100
2019	FLORIDA	CITRUS TOTALS - PRODUCTION, MEASURED IN $, PHD EQUIV	1,116,283,000
2019	FLORIDA	CITRUS TOTALS, FRESH MARKET - PRODUCTION, MEASURED IN TONS	233,000
2019	FLORIDA	CITRUS TOTALS, PROCESSING - PRODUCTION, MEASURED IN TONS	3,235,000
2019	FLORIDA	CITRUS TOTALS, UTILIZED - PRODUCTION, MEASURED IN TONS	3,468,000
2019	TEXAS	CITRUS TOTALS - ACRES BEARING	24,800
2019	TEXAS	CITRUS TOTALS - PRODUCTION, MEASURED IN $, PHD EQUIV	89,797,000
2019	TEXAS	CITRUS TOTALS, FRESH MARKET - PRODUCTION, MEASURED IN TONS	141,000
2019	TEXAS	CITRUS TOTALS, PROCESSING - PRODUCTION, MEASURED IN TONS	209,000
2019	TEXAS	CITRUS TOTALS, UTILIZED - PRODUCTION, MEASURED IN TONS	350,000

Table 5. NCGRCD holdings (as of 2019-12-31).

	Accessions	**Species**	**Inventory**
Rutaceae	1518	169 (89)	5413
Aurantioideae	1513	164 (84)	5401
Citrus	1233	96 (16)	4312
Poncirus and hybrids	148	4	516
Fortunella and hybrids	30	6	113
Microcitrus and hybrids	30	7	99
Aegle	2	1	13
Aeglopsis	1	1	8
Afraegle	2	2	12
Atalantia	6	5	43
Balsamocitrus	1	1	8
Bergera	4	1	25
Citropsis	4	4	28
Clausena	6	6	26
Clymenia	1	1	5
Eremocitrus and hybrids	3	2	10
Feroniella	1	1	3
Glycosmis	5	5	20
Hesperethusa	1	1	11
Limnocitrus	1	1	1
Limonia	1	1	9
Merrillia	1	1	4
Micromelum	1	1	1
Murraya	5	3	27
Oxanthera	1	1	4
Pamburus	2	1	9
Paramignya	2	2	6
Pleiospermium	2	2	7
Severinia	12	2	56
Swinglea	2	1	11
Triphasia	2	1	5
Wenzelia	1	1	1
×*Coleara*	1	1	4
Unknown	1	1	4
Ruta	2	2	4
Zanthoxylum	1	1	2
Vepris	1	1	5
Esenbeckia	1	1	1
Citrus pathogens	115	23	156

Table 6. NCGRCD Accessions & Inventory by Location (as of 2019-12-31).

Location	Accessions	Inventory
CVC	1065	1939
Riverside_Field_Not_CVC	118	201
CVARS	78	232
SCFS	46	79
SH (clean)	603	1197
GH_CVC_etc	808	1754
GH_50_(quarantine)	69	71
NLGRP (cryo)	534	*

Figure 1. Phylogenetic origins of major secondary Citrus species with the maternal and paternal ancestors (dotted lines are hypothetical cross) (Luro *et al.*, 2017.)

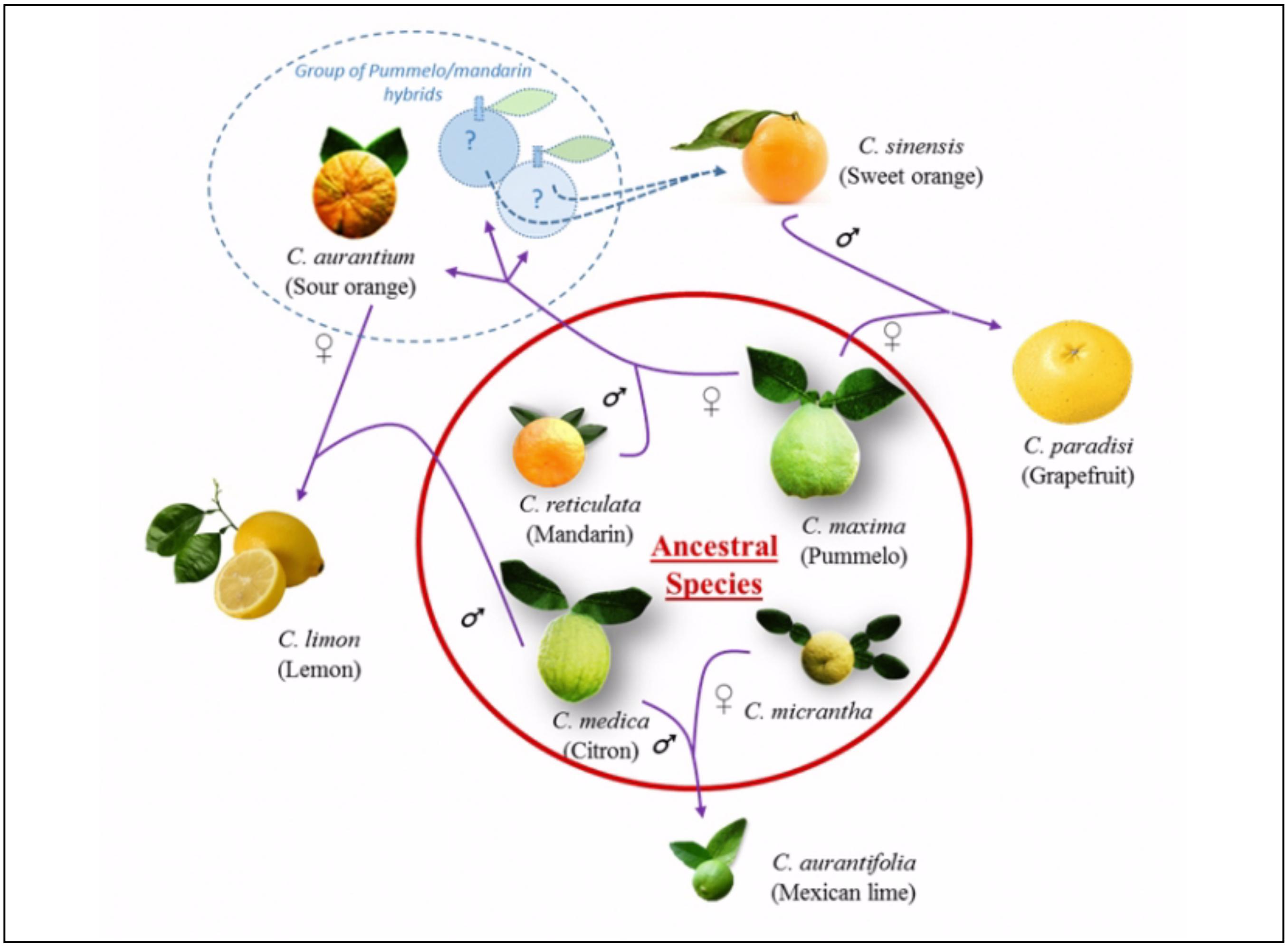


Figure 2. Botanical traits of citrus (Ollitrault *et al.*, 2020).

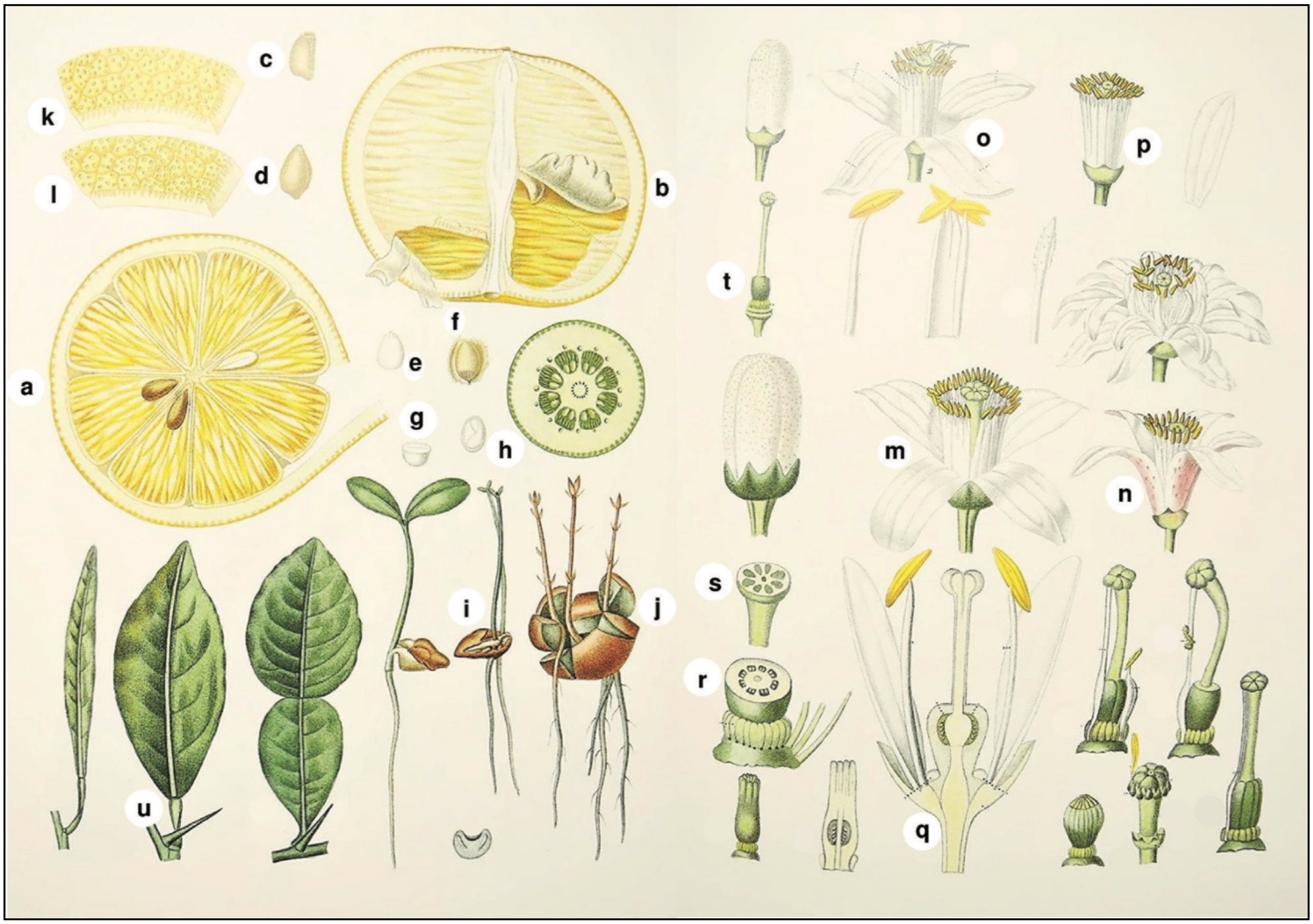


FIG. 4.1 Botanic traits of the *Citrus* species. A: cross-section through a citrus fruit, B: longitudinal section through a citrus fruit; C: semi-deltoid citrus seed; D: obovoid citrus seed; E: longitudinal section through a citrus seed; F: citrus seed with seed coats, G: cross-section through a citrus seed; H: poly- embryonic citrus seed; I and J: polyembryonic citrus seedling; K and L: outside citrus peel section with oil glands; M: open citrus flower; N: open lemon flower; O: open orange flower; P: citrus flower stamens; Q: longitudinal section through a citrus flower; R and S: cross-section through a citrus ovary; T: pistil (ovary, style and stigma) of a citrus flower; U: unifoliate citrus leaves. *Modified from "Histoire Naturelle des orangers" (Risso A, Poiteau A. Histoire Naturelle des Orangers. Paris: Imprimerie de Mme Hérissant Le Doux, Imprimeur ordinaire du Roi et des Musées Royaux; 1818).*

Figure 3. Geographical distribution of the origin areas of the Asian *Citrus* species divided by Tanaka's line (Luro *et al.*, 2017).

Figure 4. The areas of origin of the major citrus varieties and their paths of distribution (Davies and Albrigo, 1994).

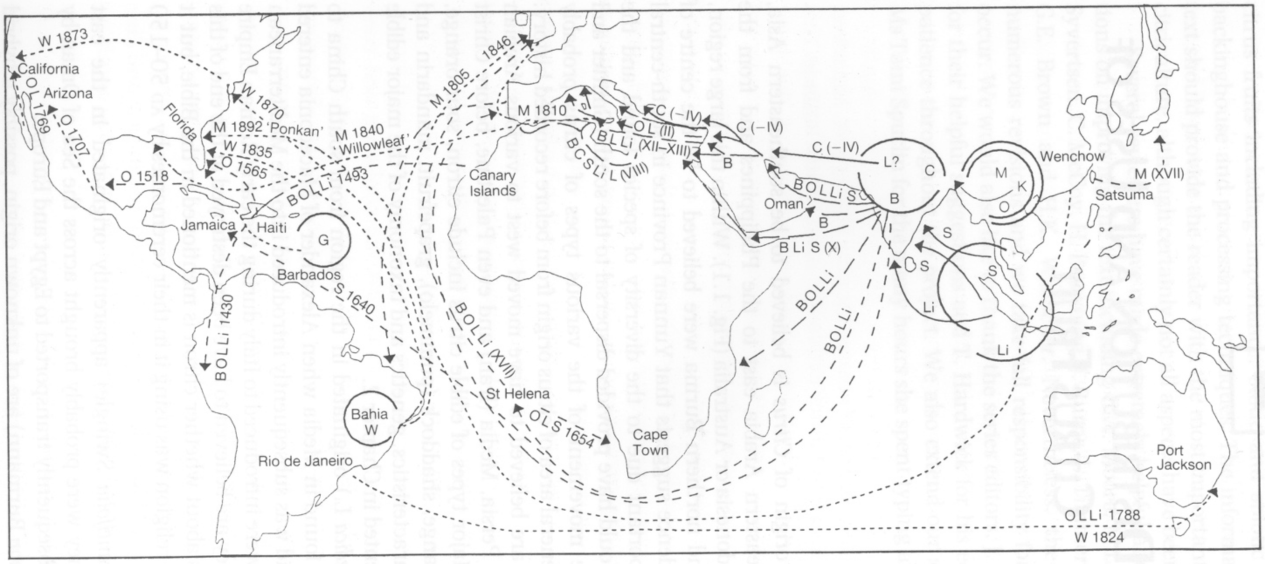


Fig. 1.1. The areas of origin of major citrus species and their paths of distribution (Chapot, 1975). Abbreviations: B, bigarade (*Citrus aurantium*); C, citron (*Citrus medica*); G, grapefruit (*Citrus paradisi*); K, kumquat (*Fortunella margarita*); L, lemon (*Citrus limon*); Li, lime (*Citrus aurantifolia*); M, mandarin (*Citrus reticulata*); S, shaddock (*Citrus grandis*); W, 'Washington' navel; ——, BC; — —, AD 1–700; ———, AD 700–1492 (711: Arab occupation of Spain); – – –, AD 1493–1700 (1493: second expedition of Christopher Columbus); --------, after AD 1700 (first appearance of grapefruit). Centuries are given in roman numerals (minus sign indicates BC); years are given in arabic numerals.

Figure 5. World citrus production areas (Burke, 1967).

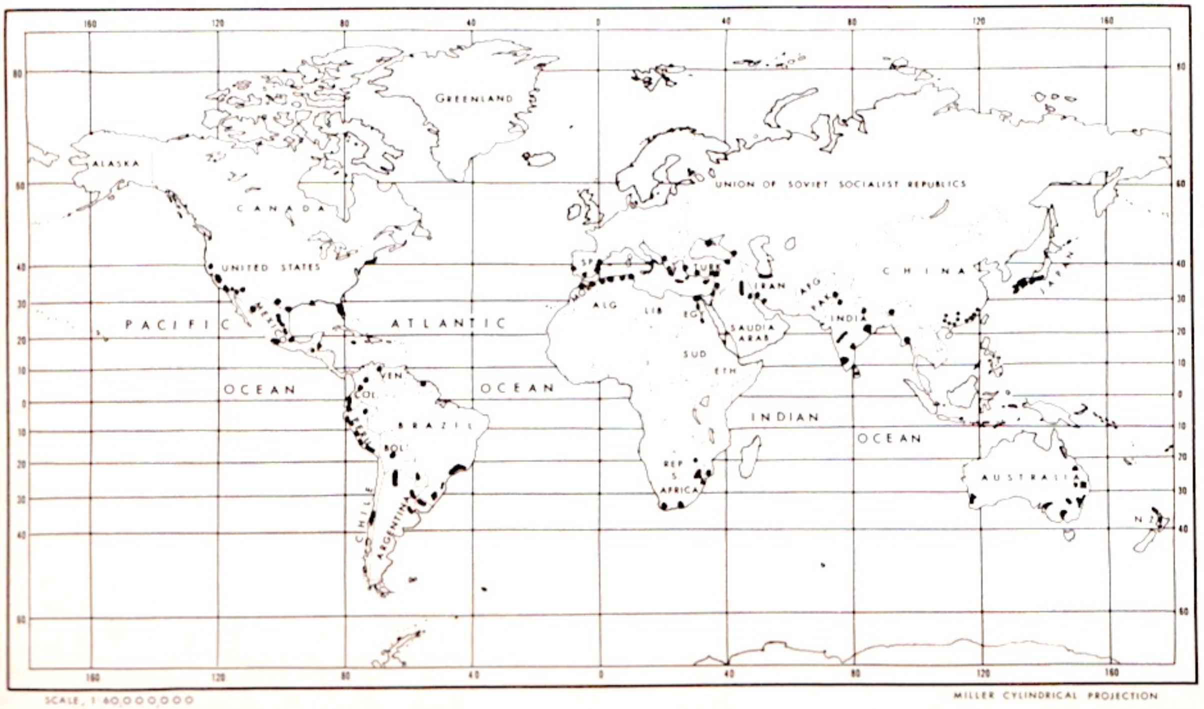
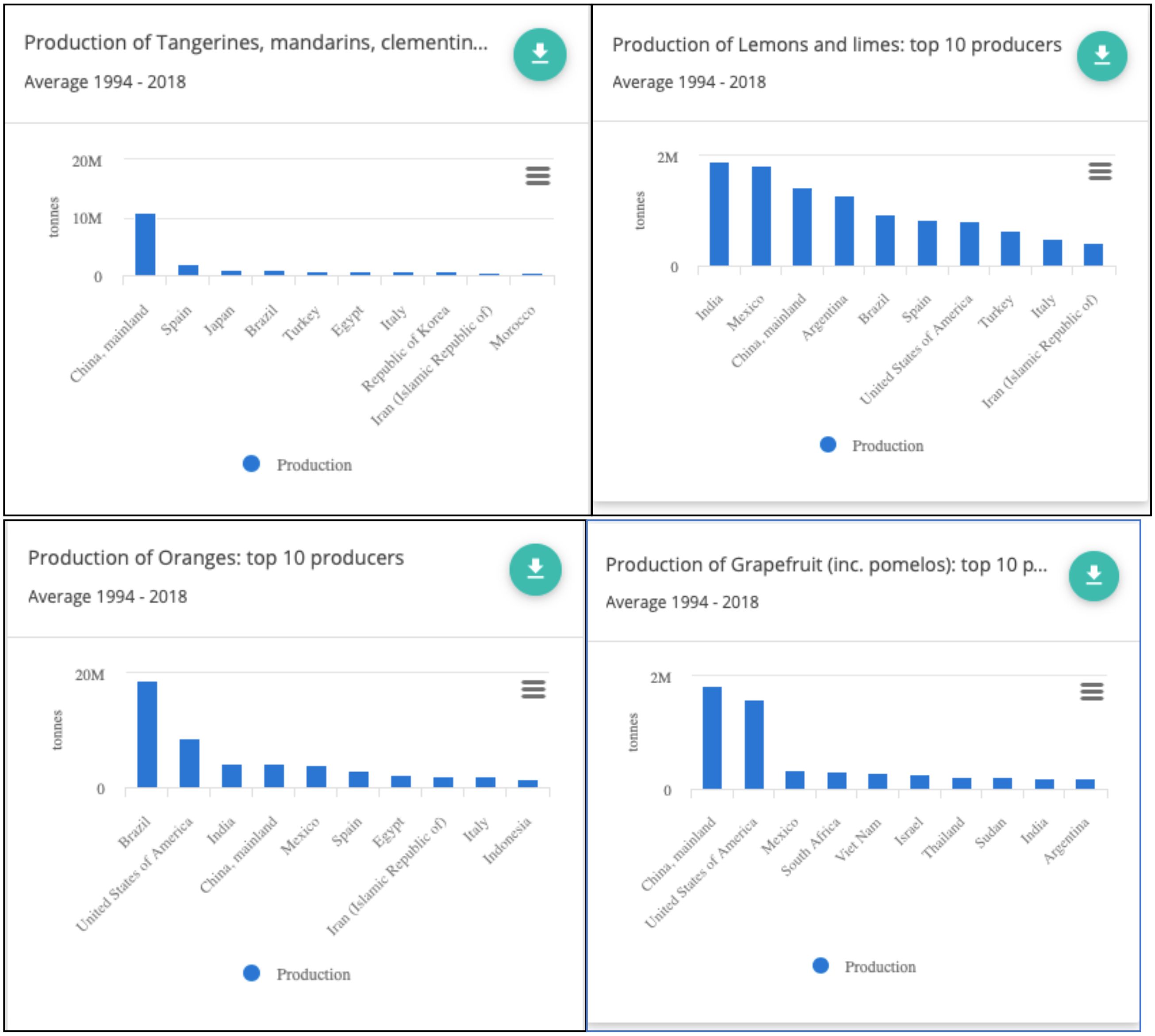


Fig. 2–1. The citrus belt of the world.

Figure 6. Top international producers of citrus, 1994–2018 (source: http://www.fao.org/faostat, accessed 2020-03-11).



Production of Tangerines, mandarins, clementin...

Average 1994 - 2018

Production of Lemons and limes: top 10 producers

Average 1994 - 2018

Production of Oranges: top 10 producers

Average 1994 - 2018

Production of Grapefruit (inc. pomelos): top 10 p...

Average 1994 - 2018

Figure 7. Flow of introduction under the Plant Controlled Import Permit.

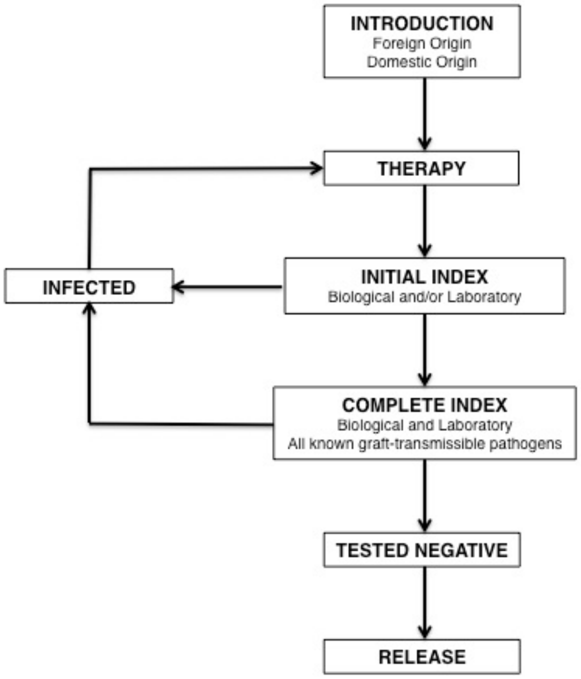


Figure 8. NCGRCD Location in Agricultural Operations are of University of California, Riverside.

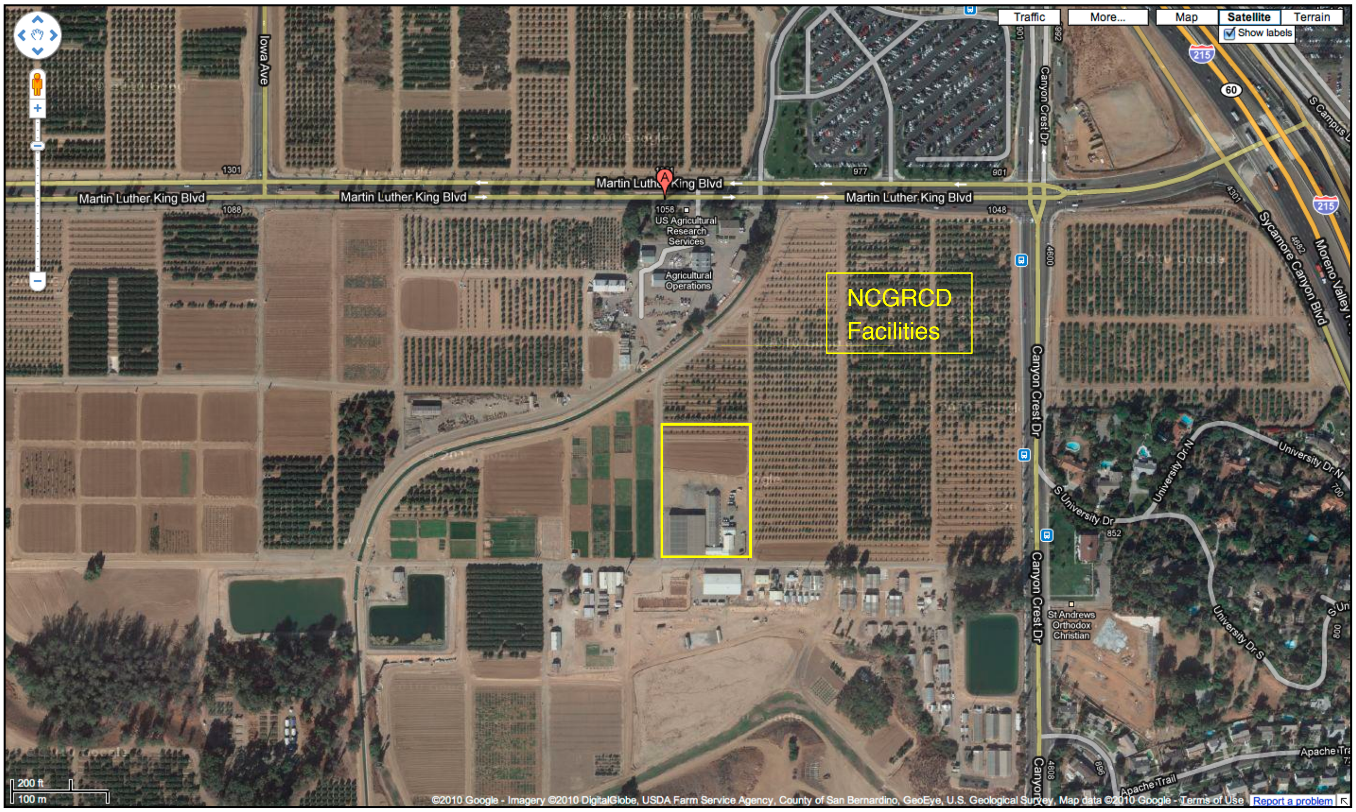


Figure 9. NCGRCD Facilities.