Journal of the American Pomological Society 74(2): 111-116 2020

*Fruit and Nut Germplasm Collections: Treasuries of Genetic Diversity:*

# The USDA-ARS National Clonal Germplasm Repository for Tree Fruit, Nut Crops, and Grapes, Davis, CA

John E. Preece[1]

**Additional index words:** Crop wild relatives, genebank, genetic resources, pomology


## Abstract

The National Clonal Germplasm Repository Davis, CA (NCGR) curates the national collections of the following 14 Mediterranean fruit and nut crops: almond, apricot, cherry, fig, grape, kiwifruit, mulberry, olive, peach, persimmon, pistachio, plum, pomegranate, and walnut. The overarching goal is to preserve germplasm for current and future generations. The challenge is that these crops do not breed true and must therefore be maintained as plants in the field, making preservation of clonal crops considerably more expensive than annual crops that can be stored as seeds. The mission of the Repository is to acquire additional accessions to fill gaps in the collections, maintain the plants in the collections, freely distribute germplasm (typically as dormant scionwood) to scientists worldwide, and to evaluate the collections and make those data available online on the Germplasm Resources Information Network (GRIN-Global,https://npgsweb.ars-grin.gov/gringlobal/search.aspx). Because of free distribution of the germplasm, there is nothing modern and under patent or proprietary protection in the collections. Rather, they consist of older cultivars; breeder lines; and the genetically richest portion of the collections, the crop wild relatives. All are available for scientific study. Challenges with managing an expanding collection are discussed.


The National Clonal Germplasm Repository in Davis, CA (NCGR) is one of 19 national genebanks in the USA. It is located in the Sacramento Valley of California and has a Mediterranean climate with low relative humidity and typically no rainfall during the growing season from April to Nov. The winter is the wet season. During the growing season, there is about a 22ºC diurnal temperature fluctuation, leading to vibrant fruit color and good sugar accumulation. The low relative humidity provides conditions for low disease pressure, compared to wetter climates. The climate is well adapted for the 14 crops maintained by the NCGR: almond, apricot, cherry, fig, grape, kiwifruit, mulberry, olive, peach, persimmon, pistachio, plum, pomegranate, and walnut.

All of the National Genebanks have a similar 4-part mission: germplasm acquisition to fill gaps in collections, maintenance of existing accessions, free distribution of germplasm, and evaluation and characterization of the collections. Data generated about the collections are uploaded to GRIN-Global (https://npgsweb.ars-grin.gov/gringlobal/search.aspx) so that the scientific community can understand the collections and make informed decisions about which accessions will be useful to their research. This guides their ordering.

## Acquisition

It is important to take advantage of opportunities to acquire new plant material, especially if it fills gaps in a collection. Many of the existing gaps in the NCGR collections are crop wild relatives that arose and reside abroad. This creates a challenge because federal law in many countries prevents native plant materials from leaving that country. People have been arrested when attempting

[1] Supervisory Research Horticulturist, USDA-ARS National Clonal Germplasm Repository, One Shields Drive, University of California, Davis, VA 95616-8607 email: John.Preece@ars.usda.gov

to illegally transport plant material out of some countries. This restriction saves natural resources for local scientists and others; however, because of loss of habitat, crop wild relatives are being lost along with the opportunity for *ex situ* preservation of ecotypes. When plant material enters the National Plant Germplasm System it requires that there be no restrictions in regards to propagation, distribution to scientists, and that it have public domain status, similar to the other germplasm in the system. This agreement is possible with some, but not all countries.
The majority of the new germplasm being planted each year at the NCGR-Davis is seedlings of various *Prunus* spp. (almond, apricot, cherry, peach, and plum), and some *Juglans* spp. (walnut). The walnuts are mainly *Juglans regia* collected from the wild in the Republic of Georgia. The new *Prunus* is from Azerbaijan and the Republic of Georgia (Caucasus Mountain region) and throughout Kyrgyzstan in Central Asia. There is a very high level of diversity in the Caucasus Center of Diversity between the Black Sea and Caspian Sea (Rafiqpoor et al. 2005), especially in temperate fruit and nut trees (Hellier, 2011; Hummer and Hancock, 2015). Vavilov identified Central Asia a center of origin of useful plants of economic significance (Barthlott et al., 2005). There is also new native *Prunus* spp. collected from throughout the eastern and southern USA. Previously, this germplasm was poorly represented in the collections.

Additions of new germplasm requires field space and labor, which are limited at the NCGR-Davis. Therefore, the new germplasm is planted on 1 m centers within the rows and is maintained by mechanical hedging. Lack of space means that decisions on acquisition of new germplasm must be made carefully. The focus of the NCGR-Davis is to increase the amount of new crop wild relative germplasm, not heirloom cultivars. Crop wild relatives have rich genetics that are being used to help solve disease and other problems (Greene et al., 2018a and 2018b). Adding these wild accessions is greatly enriching the diversity of the collections. Additional land will allow this to continue.

**Distribution**

Ordering is done on the public GRIN-Global page (https://npgsweb.ars-grin.gov/gringlobal/search.aspx) simply by clicking on "Add to Cart." Clicking on "Descriptors" on the banner near the top will give access to data on the collections that will inform which accessions should be ordered. This can also be accessed by clicking on the Plant ID number in the first column after a species has been searched. The NCGR distributes vegetative propagative material to scientists and institutional educators who need these specific genetics to meet their goals.

Deadlines for ordering vary with repository, and sometimes with crop. The deadline for NCGR-Davis orders is 1 Nov. of each year. Germplasm is primarily distributed as dormant cuttings or scionwood that are harvested in Jan. and early Feb. After harvesting, the scionwood is stored in black plastic trash can liners with moist paper towels at 4ºC until they are shipped. The cuttings are organized and sorted into orders. A typical order has requested up to 100 different accessions, so this sorting is time and labor consuming. Orders are then shipped in March and April. Summer orders for leaves (for DNA), or cuttings of olive and apricot are routine. Olive and apricot are susceptible to pathogens if cut during the wet weather of the winter. When the order is received, the customer must be ready for propagation or DNA extractions upon arrival.

The NCGR-Davis is not set up for collecting seeds or fruit because of lack of staff. The Repository has worked with scientists who have paid for a student worker, or collaborated with a colleague at the University of California Davis to collect fruit/seeds from the collections.

Clonal germplasm has restrictions on its movement. Where those restrictions occur, special permission must be obtained for the

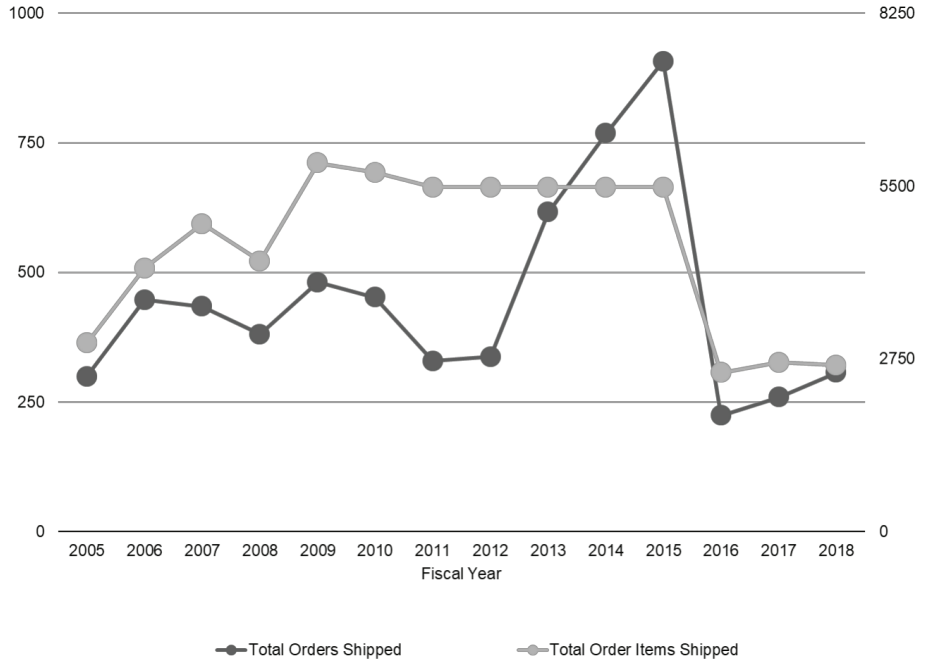


**Figure 1.** Total orders shipped from 2005 – 2018 (NCGR-Davis). There are 3-5 cuttings/item shipped.

shipment to be legal. These restrictions are not limited to international orders that require an Import Permit. Orders of some items, such as walnut (*Juglans*) to Indiana from California requires a permit because of the threat of thousand cankers disease.

Through 2015, the NCGR shipped scionwood to anyone who ordered, including hobbyists. Word got out about free plant material and the NCGR collections became more popular than there was staff to sort and fill the orders. Therefore, beginning in 2016, the NCGR restricted orders that were filled to the scientific community and institutional educators who need these materials to meet their research objectives. Thus in 2016, order numbers fell from nearly 900 to 200, and have grown to about 300/year (Fig. 1). This has been a manageable number and allows the staff the space to respond more quickly to queries from those who use the germplasm. This is an example of shrinking a unit's mission so that it matches the resources.

The majority of the items distributed in 2018 (93.5%) were to domestic customers (Fig. 2.), and most of the domestic orders are to individuals, U.S. state agencies and universities, and U.S. commercial companies (Fig. 3). The majority of the individual germplasm recipients are private plant breeders, as are the researchers at the commercial companies.

### Collection Maintenance

Collection maintenance is the most important function of the genebank and the one that requires the most resources. Fourteen fruit and nut collections is a lot to maintain. The Repository has two facilities that support the collection: a container nursery in Davis, CA and the field collection at Wolfskill Experimental Orchard in Winters, CA. The nursery is used for propagation and to produce young plants for field planting. It is also used to sup-

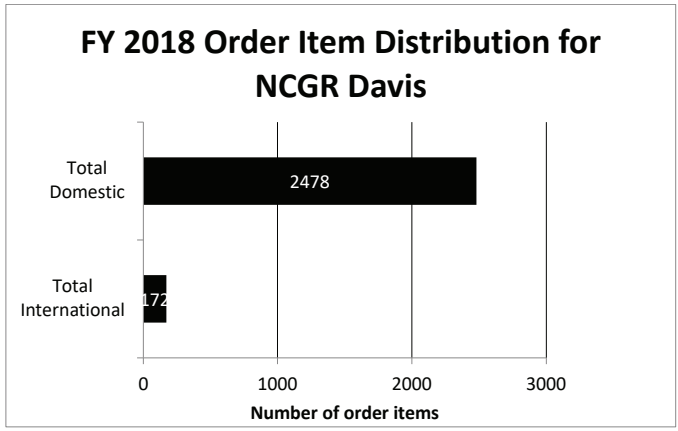


**Figure 2.** Number of items (cuttings, seed, pollen, etc.) distributed domestically and internationally during 2018.

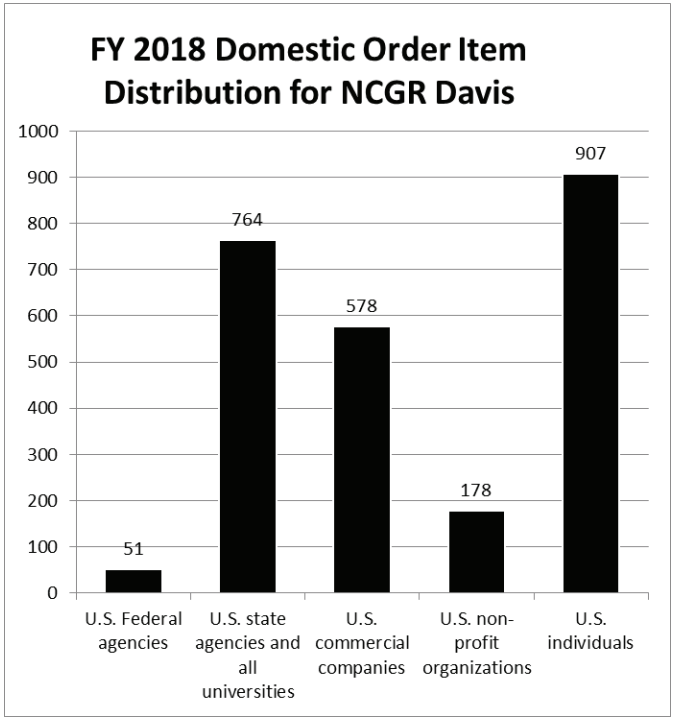


**Figure 3.** Number of items distributed to domestic customers during 2018.

port characterization and evaluation research on the collections. There is a small collection of grapes and *Prunus* maintained in insect-proof screenhouses that were sourced from pathogen indexed plant material. Distributions from these plants are cleaner than from the field collection at Wolfskill.

The nursery work is done by NCGR-Davis staff and student workers. Much of the field work is accomplished by the staff, with the exception of much of the pruning. The grape collection is pruned and tied each March by a contractual crew. Several of the other collections and the field nursery block are mechanically hedged by a contractor. Hedging is typically during the summer to avoid stimulating too much new growth.

Herbicides and other pesticides are applied by the staff. The staff also does the mowing, some pruning, fertilizing, irrigating and maintenance of the irrigation system, woody weed control, and other plant care.

There are challenges, including suckering of the collections, woody weeds (often of plants in the collections), and slow turnover of land for new planting. The field space is nearly full. The solution will be to plant the accessions closer than previously and maintain by hedging. It does not allow for as much phenotyping as properly spaced trees, but does provide scionwood for distribution.

Armillaria is killing trees in the almond collection. Persimmon (*Diospyros kaki* and *D. virginiana*) are resistant to this pathogen (Davidson and Byther 1994). Therefore, the almond and persimmon collections are being repropagated and their field locations flipped. Because there is no extra land, the persimmons are being planted where the almonds are located and vice versa. This is a slow process, but a way to save the valuable almond collection.

## Evaluation and Characterization

The scientists at the NCGR-Davis have active research programs on evaluation and characterization of the collections. Recently published evaluations papers are as follows: Various genomics methods have been used to characterize the collections, including black cherry (*Prunus serotina*, Guzmán at al., 2018), fig (Knap et al., 2018), grape (Riaz et al., 2018), and walnut (Potter et al., 2018; Zhu et al., 2019). Several of the pomegranates from the collection have been evaluated in various studies, ranging from fruit quality to consumer preference to punicalagin quantification (Chater et al., 2018a; 2018b; 2018c; 2018d; Mathon et al., 2019). Likewise, the persimmons have been evaluated for astringency and sensory attributes (Milczarek et al.; 2018; 2019). Because distributions are mainly from the field collection at Wolfskill, the phytosanitary status of the collection is important. Therefore, the grapes (Diaz-Lara et al., 2018) and pistachios (Al Rwahnih et al., 2018) were evaluated for their viruses.

## Conclusions

The majority of the human and supply resources go toward collection maintenance. The collections are primarily maintained by Repository staff, however, it has been more cost effective to use contractors to prune the grape collection and to hedge the orchard collections. Distribution is another important priority for the NCGR; however, this is mainly a seasonal activity. Orders are received by 1 Nov., and dormant cuttings are harvested in Jan. and sorted and distributed in March and sometimes into April if there are many orders. Acquisition is another focus of resources. After *Prunus* material from abroad clears quarantine with APHIS, it is shipped bare-root to the Repository, where it is planted in the field at 1 m spacing within rows. It is then maintained by hedging. It is important to evaluate and characterize the collections and make this information available to the public via GRIN-Global; however, this is not the focus of the Repository, rather it is maintenance, distribution, and acquisition before characterization.

## Literature Cited

Al Rwahnih, M., A. Rowhani, N. Westrick, K. Stevens,

A. Diaz-Lara, F.P. Trouillas, J. Preece, C. Kallsen, K. Farrar, and D. Golino. 2018. Discovery of viruses and virus-like pathogens in pistachio using high throughput sequencing. Plant Dis. https://doi.org/10.1094/PDIS-12-17-1988-RE

Barthlott, W., J. Mutke, D. Rafiqpoor, G. Kier, and H. Kreft. 2005. Global centers of vascular plant diversity. Nova Acta Leopoldina. 92(342):61-83.

Chater, J., D. Merhaut, Z. Jia, M.L. Arpaia, P. Mauk, and J. Preece. 2018. Effects of Site and Cultivar on Consumer Acceptance of Pomegranate. J. Food Sci. 83:1389-1395.

Chater, J., D. Merhaut, Z. Jia, P. Mauk, and J.E. Preece. 2018. Fruit quality traits of ten California-grown pomegranate cultivars harvested over three months. Scientia Hortic. 237(2018): 11-19.

Chater, J.M., L.S. Santiago, D.J. Merhaut, J.E. Preece, and Z. Jia. 2018. Diurnal Patterns of Photosynthesis and Water Relations for Four Orchard-Grown Pomegranate (Punica granatum L.) Cultivars. J. Amer. Pomol. Soc. 72:157-165.

Chater, J.M., L.S. Santiago, D.J. Merhaut, Z. Jia, P.A. Mauk, and J.E. Preece. 2018. Orchard establishment, precocity, and eco-physiological traits of several pomegranate cultivars. Scientia Hortic. 235: 221-227.

Davidson, R.M. and R.S. Byther. 1994. Armillaria (Shoestring) Root Rot. Plant Diseasees. Coop. Ext., Washington State Univ.. EB1776. 4 pp.

Diaz-Lara, A., V. Klaassen, K. Stevens, M.R. Sudarshana, A. Rowhani, H.J. Maree, K.M. Chooi, A.G. Blouin, N. Habili, Y. Song, K. Aram, K. Arnold, M.L. Cooper, L. Wunderlich, M.C. Battany, L.J. Bettiga, R.J. Smith, R. Bester, H. Xiao, B. Meng, J.E. Preece, D. Golino, and M. Al Rwahnih. 2018. Characterization of grapevine leafroll-associated virus 3 genetic variants and application towards RT-qPCR assay design. PLoS ONE, 13(12), p.e0208862.

Greene, S.L., K.A. Williams, C.K. Khoury, M.B. Kantar, and L.F. Marek. (eds.). 2018a. North American Crop Wild Relatives, Vol. 1: Conservation Strategies. Springer.

Greene, S.L., K.A. Williams, C.K. Khoury, M.B. Kantar, and L.F. Marek (eds.). 2018b. North American Crop Wild Relatives, Vol. 2: Important Species (Vol. 2). Springer.

Guzmán, F. A., S. Segura, M. Aradhya, and D. Potter. 2018. Evaluation of the genetic structure present in natural populations of four subspecies of black cherry (*Prunus serotina* Ehrh.) from North America using SSR markers. Scientia Hort. 232: 206-215.

Knap T, M. Aradhya, A.B. Arbeiter, M. Hladnik, and D. Bandelj. 2018. DNA profiling of figs (Ficus carica L.) from Slovenia and Californian USDA collection revealed the uniqueness of some North Adriatic varieties. Genetic Resources and Crop Evolution. 65:1503-16.

Hellier, B.C. 2011. Collecting in Central Asia and the Caucasus: U.S. National Plant Germplasm System Plant Explorations. HortScience 46:1438-1439.

Hummer, K.E. and J.F. Hancock. 2015. Vavilovian centers of plant diversity: Implications and impacts. HortScience 50:780-783.

Mathon, C., J.M. Chater, A. Green, D.J. Merhaut, P.A. Mauk, J.E. Preece, and C.K. Larive. 2019. Quantification of punicalagins in commercial preparations and pomegranate cultivars, by liquid chromatography-mass spectrometry. J. Sci. of Food and Agr. 99:4036-4042. (https://onlinelibrary.wiley.com/doi/full/10.1002/jsfa.9631).

Milczarek, R.R., P.S. Liang, T. Wong, M.P. Augustine, J.L. Smith, R.D. Woods, I. Sedej, C.W. Olsen, A.M. Vilches, R.P. Haff, and J.E. Preece. 2019. Nondestructive determination of the astringency of pollination-variant persimmons (Diospyros kaki) using near-infrared (NIR) spectroscopy and nuclear magnetic resonance (NMR) relaxometry. Postharvest Biol. and Technol. 149. pp.50-57.

Milczarek, R.R., R.D. Woods, S.I. LaFond, A.P. Breksa, J.E. Preece, J.L. Smith, I. Sedej, C.W. Olsen, and A.M. Vilches. 2018. Synthesis of descriptive sensory attributes and hedonic rankings of dried persimmon (Diospyros kaki sp.). Food Sci. Nutr. 6(1):124-136.

Potter, D., H. Bartosh, G. Dangl, J. Yang, R. Bittman, and J. Preece. 2018. Clarifying the Conservation Status of Northern California Black Walnut (Juglans hindsii) Using Microsatellite Markers. Madroño, 65(3): pp.131-141.

Rafiqpoor, D., G. Kier, and H. Kreft. 2005. Global centers of vascular plant diversity. Nova Acta Leopoldina NF, 92(342): 61-83.

Riaz, S., G. De Lorenzis, D. Velasco, A. Koehmstedt, D. Maghradze, Z. Bobokashvili, M. Musayev, G. Zdunic, V. Laucou, M.A. Walker, O. Failla, J.E. Preece, M. Aradhya, and R. Arroyo-Garcia. 2018. Genetic diversity analysis of cultivated and wild grapevine (Vitis vinifera L.) accessions around the Mediterranean basin and Central Asia. BMC Plant Biol. 18:137.

Zhu T, L. Wang, F.M. You, J.C. Rodriguez, K.R. Deal, L. Chen, J. Li, S. Chakraborty, B. Balan, C.Z. Jiang, P.J. Brown, C.A. Leslie, M.K. Aradhya, A.M. Dandekar, P.E. McGuire, D. Kluepfel, J. Dvorak and M.C. Luo.(2019) Sequencing a Juglans regia× J. microcarpa hybrid yields high-quality genome assemblies of parental species. Hort. Res. 6(1):55.