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The Tropical and Subtropical Germplasm Collections at the National Germplasm Repository in Miami, Florida

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Abstract

The Subtropical and Tropical USDA, ARS, National Germplasm Repositories (NGR) in Miami, FL, Mayaguez, PR, and Hilo, HI are responsible for the collections of subtropical and tropical fruits, nuts, grasses, and ornamentals for the USDA, ARS, National Plant Germplasm System (NPGS). The NPGS is responsible for managing plant genetic resources in the United States. This paper reviews primarily the fruit and nut collections held by the NGR in Miami, FL. The Subtropical Horticultural Research Station in Miami, FL houses the repository which manages approximately 5,000 accessions of fruit, nuts, ornamentals, sugarcane and their relatives. Research efforts are dedicated to answering questions that help curators conserve and manage genetic resources in a more effective and cost-efficient manner. The repository is managed by a curator/horticulturist, three research geneticists and a research molecular biologist. Ongoing research, management strategies for safeguarding these collections, and the procedures for requesting and distributing germplasm are discussed here.

The USDA/ARS National Plant Germplasm System (NPGS) is responsible for managing plant genetic resources in the United States. The NPGS provides a continuous flow of genes from source to end use, a continuum that keeps high-yielding cultivars on the market, improves the quality of agricultural products, minimizes production costs, reduces dependence on pesticides, enhances the quality of the environment, and minimizes the vulnerability of agriculturally important germplasm to pests and environmental stresses (21). The system houses more than 511,000 accessions comprising over 2100 genera and more than 13,100 species (23).

The maintenance of germplasm collections for plants has become the primary means for preserving genetic diversity. As environmental degradation continues, the associated loss of species diversity makes *ex situ* collections, in some cases, the last pool for genetic variation. Moreover, these collections are the only place where material is catalogued and evaluated, thus providing basic material in an organized manner for plant enhancement programs. Controlling the number of genotypes, by careful evaluation and characterization, guarantees that the maximum amount of genetic variation is preserved, with the least number of individuals. If duplicate accessions with different names can be removed through the use of molecular and/or phenotypic markers, the costs of field gene bank maintenance can be reduced. Genetic mechanisms can be used to solve a large number of horticultural problems, thus making a significant contribution to sustainable agriculture while also safeguarding our botanical treasures. The maintenance, evaluation, and enhancement of subtropical/tropical fruit crops [mango (*Mangifera indica* L.), avocado (*Persea americana* L.), banana and plantain (*Musa* hybrids), passion fruit (*Passiflora edulis* Sims and hybrids), annona (*Annona squamosa* L. and hybrids), carambola (*Averrhoa carambola* L.), longan (*Euphoria longan* Steud.), lychee (*Litchi chinensis* Sonn.), sapodilla (*Manilkara zapota* Van Roy.), mamey (*Pouteria sapote* (Jacq) More & Stem), jujube (*Ziziphus* spp.), jackfruit (*Artocarpus heterophyllus* Lam)], sugarcane (*Saccharum* spp.) and related grasses, tropical ornamentals, and *Tripsacum* germplasm are

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the major objectives for the NGR at Miami.

Facilities

The Subtropical Horticulture Research Station (SHRS) in Miami is located at latitude N25°38'33.76" and longitude W80°17'37.86. The SHRS is an 85 ha farm located in southeastern Miami-Dade County, adjacent to Biscayne Bay. Of the 85 ha, 50.6 are equipped with drip irrigation. The major soil type is a Krome calcareous soil (loamy-skeletal, carbonatic, hyperthermic Lithic Udorthents) and is typical for this part of Florida. Forty-two ha are currently planted in germplasm collections with another 7 ha in experimental field trials. The SHRS has all the necessary cultivation equipment and state permits for maintaining germplasm and experimental research plots in Miami Dade County.

The plant genetics group at the SHRS is housed in a recently completed (March 2009), state-of-the-art, laboratory building of approximately 3344.5 m^2. There are eight laboratories currently used in the project. Two of these are "wet" labs where DNA extraction, PCR and cycle-sequencing reactions are conducted, one lab is dedicated for the automated sequencers, one lab is dedicated to tissue culture/cloning where recombinant DNA cultures are grown, and two are for general use. Another lab is dedicated to bioinformatics/statistical analysis, and one lab for phenotypic evaluation of tropical fruit and grasses.

All accessory equipment for successful DNA extraction, quantification, visualization, PCR amplification and cycle sequencing are available for this project. In addition, we have the full GCG software package and are licensed users of PAUP, McClade, Sequencher, Mapmaker, and JoinMap® Versions 4 and 5, and MapQTL® Versions 3 and 4 and the full complement of ABI software for fragment and sequencing analysis. We have a Linux workstation for bioinformatics, running the publicly available software packages Phred, Phrap, Consed, PolyPhred, TGICL, PolyBayes, and the BioPerl modules.

Collection Maintenance, Documentation, Distribution and Evaluation

The Miami repository contains over 5000 accessions (23) under field, quarantine, and greenhouse conditions for maintenance, distribution and genetic evaluation and characterization. All of the clonal accessions are maintained in field plots, but some new fruit crops germplasm (e.g. cacao, mango, sugarcane, and avocado) must be quarantined in greenhouses before moving to the field. The repository is responsible for over 20 important tropical fruit and nut collections in addition to sugarcane, *Tripsacum*, and tropical ornamentals (Table 1).

New germplasm accessions are continually being acquired from plant explorations, botanical gardens, gene banks, foreign exchange programs, research and breeding programs, and private and government organizations. The objective of the NPGS distribution policy is to provide quality germplasm to both domestic and foreign scientists in a timely manner, and to develop strong relationships with the requestors for future exchanges and research. Plant germplasm is distributed worldwide to scientists, educators and other legitimate research and education entities from active NPGS germplasm collection sites. Germplasm of mango, banana, plantain, sugarcane, avocado, lychee, cacao, carambola, papaya, mamey sapote, sapodilla, *Annona* spp., acerola, passion fruit, coconut, longan and other tropical/subtropical fruits and nuts from this repository is available free of charge. There is an exception for clonal material due to the limited amount of material available; shipping costs are to be paid by the requestor and quantities distributed are limited. Access to the Germplasm Resources Information Network (GRIN) information is available to the general public online at www.grin-ars.gov (22). Distributions follow regulations issued by the Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) Office, USDA-ARS, and the Florida Department of Plant Industry

Table 1. Tropical and subtropical fruit and nut collections maintained by the USDA germplasm clonal repository in Miami, Florida, USA.[z]

Genus/species	Common name(s)	No. of accessions
Annona spp.	Annona, bullock's heart, custard apple, sugar apple, cherimoya, izlama, papausa	38
Artocarpus heterophyllus	Jackfruit, nangka, langka	85
Averrhoa carambola	Carambola, star fruit, yang tao, balimbing	38
Bactris gasipaes	Bactris, peach-palm, pejibaye	11
Casimiroa edulis	Mexican-apple, white sapote, pomme mexicaine	2
Cocos nucifera	Coconut, kelapa, kokosu, koko	20
Dimocarpus longan	Longan, dragon's eye	25
Garcinia spp.	Mangosteen, malabar tamarind, chikana red mango	25
Litchi chinensis	Lychee, leechee, laichi	18
Malpighia spp.	Acerola, Barbados cherry	9
Mangifera indica	Mango, mangobaum, mangopalme	335
Manilkara zapota	Sapodilla, nispero, sawo londo, chicozapote	10
Musa spp.	Banana, plantains, bungulan, bananier	100
Persea americana	Avocado, palta, aguacate	345
Pouteria spp.	Mamey sapote, mamey-colorado, zapote, marmalade-plum	40
Spondias spp.	Hog plum, Java plum, tropical plum, marapa, jobo	4
Syzygium spp.	Java-apple, bellfruit, water-apple	5
Tamarindus indica	Tamarind, tamarindo, tamarin, sampalok	35
Theobroma cacao	Cacao, cocoa	130
Ziziphus mauritiana	Jujube, Chinese date, Malay jujube	4
Saccharum spp., *Tripsacum* spp.	Sugar cane, cana, gamagrass	>1800
Ornamentals	Tabebuia, amaryllis, iris, plumeria, portlandias, palms	>1600

[z] Data from GRIN (Germplasm Resources Information Network) as of March, 2009 (NPGS, 2009).

and Agriculture. Other regulatory international treaties such as the International Treaty on Plant Genetic Resources (15), the Convention on Biological Diversity (9), and the Convention on International Trade in Endangered Species of Wild Flora and Fauna (10) controlling introduction and distribution of plant material, are also followed.

Collection, documentation and evaluation data are maintained in the GRIN which is the centralized computer database for the NPGS (37) and maintains records for all germplasm repositories. Currently the NPGS system is working on a new GRIN-Global Project which will create a new, scalable version of the GRIN to provide the world's crop genebanks with a powerful, flexible, easy-to-use plant genetic resource (PGR) information management system. The system will help safeguard PGR and information vital to global food security, and will encourage PGR use. The GRIN is designed to facilitate the management and operation of the NPGS, as well as making the germplasm and information available to scientists and other users (20, 25). Digital images are used to document many accessions on the GRIN database, as exemplified in Fig. 1 for avocado, cacao, mango and litchi accessions at Miami. Several characteristics can be obtained from these images such as fruit and seed size, fruit color, and flesh color.

The Miami repository receives policy advice from the National Plant Germplasm Committee and technical advice on collections from the Tropical Fruit and Nut Crop Germplasm Committee (24). These commit-

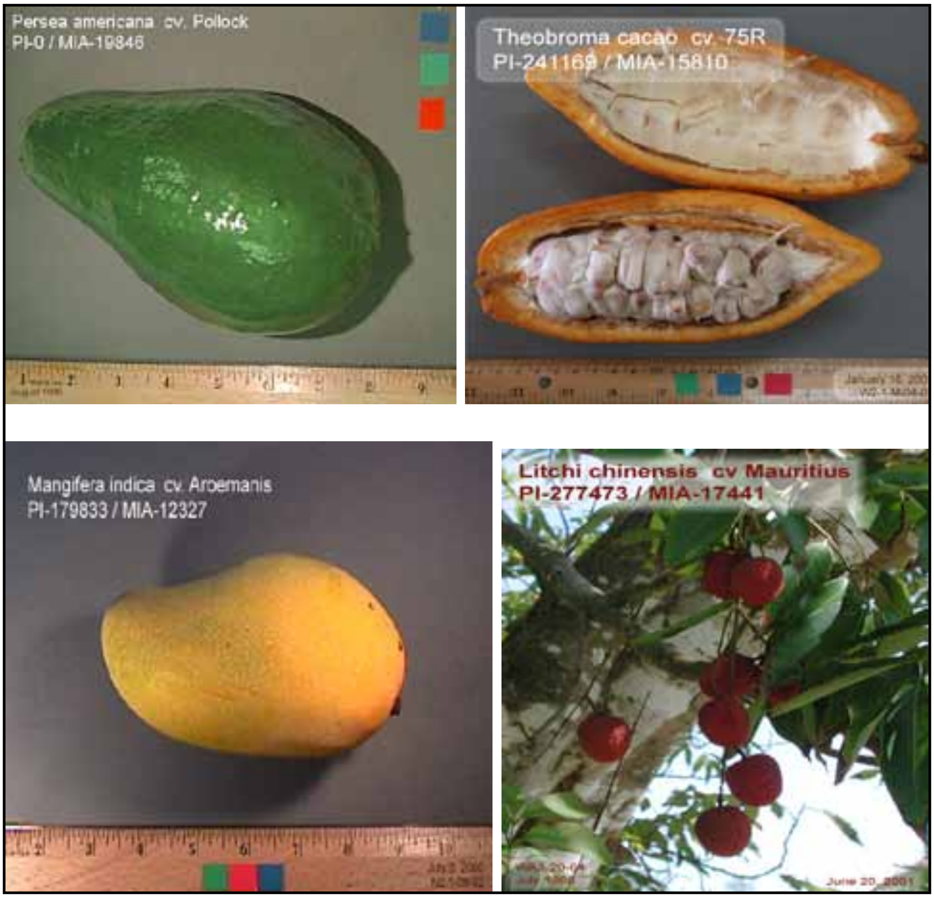

Fig. 1. Digital images (avocado, cacao, mango and litchi) generally used to document accessions on the GRIN database.

tees are national working groups composed of specialists representing various disciplines and include federal, state and private industry members. These committees help curators identify and fill gaps in U.S. collections, prioritize traits for evaluation, appraise research proposals to perform evaluations, and help ensure the accuracy of data entered to the GRIN (24).

The main objective of the repository is to maintain healthy field collections of existing accessions of tropical/subtropical fruit trees, ornamentals, grasses, and plants with medicinal, beverage, and chemurgical potential.

a. Germplasm Management for the Major Collections

Characterization and evaluation data are recorded using descriptors that were developed by the International Plant Genetic Resource Institute (IPGRI), now Bioversity, for many of these crop species. In each collection, every tree is individually labeled with the Plant Introduction (PI) number, MIA number (local number), and other important accession information. There are typically three clones maintained for each accession in the field plantings. Field maps are detailed and accurate and are stored electronically and as hard copies. All fertilization and cultural practices are recorded and maintained in a database.

Mango. The entire 50-year-old mango collection was re-propagated in 2006 into three-tree plots in a block planting. The old collection was also augmented with additional clones to fill in gaps from trees destroyed in previous hurricanes. Digital images are available on the GRIN. A core backup of this collection is established at the NGR in Mayaguez, Puerto Rico. Phenotypic evaluation includes the collection of data for the following traits: number of years to first flowering, length of flower duration, date of first fruit set and duration of fruit set, date of fruit harvest, fruit bearing intensity, fruit length, fruit diameter, fruit and seed weight, fruit shape, color of the fruit (using a Minolta CR 400 colorimeter) at the shoulder, mid section and apex, °Brix, internal breakdown (1 to 5 scale), and anthracnose resistance (1 to 5 scale).

Avocado. The avocado collection is in the process of being re-propagated and this should be completed by 2010. Accessions are being planted in three-tree plots. Many of the old accessions are infected with Avocado Sunblotch Viroid (ASBVd) and attempts thus far to remove the viroid from the germplasm have failed (35). However, the infected material is too valuable to destroy. We have established two different blocks separated by 1.3 km for isolation of infected material. Strict sanitation measures have been established for working with the avocado germplasm and the collection is monitored periodically (30). Digital images are available on the GRIN. A new backup site has been selected at the NCGR in Hilo, HI. Only non-ASBVd infected accessions are distributed from this collection. Exceptions are made for individual researchers working with viroids. Phenotypic evaluation includes the collection of data for the following traits: number of years to first flowering, flower type (A or B), length of flower duration, date of first fruit set and duration of fruit set, date of fruit harvest, fruit bearing intensity, fruit length, fruit diameter, fruit and seed weight, fruit shape, color of the fruit (using a Minolta CR 400 colorimeter), and fruit oil content. The "A" flower type is female in the morning of the first day and male in the afternoon of the second day (when the weather is warm). The "B" type is just the reverse: its flowers are female in the afternoon and male the following morning. Not all accessions are evaluated every year. Sub-sets of accessions are evaluated according to age and amount of previous data collected on each given accession.

Cacao. The NGR in Miami serves as a quarantine station and a backup site for the cacao germplasm collection in Mayaguez, PR. A quarantine period of two years is required and a successful bioassay for Cacao Swollen Shoot Virus (CSSV) must be completed. The plants are inspected and released by an APHIS pathologist. This collection has been augmented with the addition of over 100 new accessions released from quarantine in 2006 and 2007. The cacao collection has 194 accessions at the Miami location and approximately 300 in Mayaguez. The 100 new accessions will be transferred by sending scion wood to Mayaguez in 2009. Cacao is very susceptible to temperatures below 10°C and must be maintained in our cold protected area in Miami known as the walled area. Phenotypic data are not collected on this collection, but data are being collected on the primary collection in Mayaguez. The Miami collection does serve as a breeding nursery and a significant number of distributions are made every year.

Sugarcane. The sugarcane collection contains approximately 1500 accessions of each of the five species of *Saccharum,* as well as related grasses in *Miscanthus* and other potential biomass/energy crop candidates. The collection has been designated by the International Society of Sugarcane Technologists (ISSCT) as one of the two World Collections; the other World Collection is located in India. *Saccharum. officianarum* L., *S. robustum* Brandes & Jesw. ex Grassl, *S. sinense*, *S. barberi* Jesweit, and related species are maintained in 2 x 4 m field plots under drip irrigation and with cold protection. The clones are planted in a 4 ha field site with soil amended for sugarcane. The plots are rotated every three years and the old plots retained for an additional two years. In collaboration with the sugarcane breeding project in Canal Point, FL, the clones have been screened for Ratoon Stunt Disease (RSD) caused by *Leifsonia xyli* subsp. *xyli* Davis et al. 1984, leaf scald caused by *Xanthomonas albilineans* (Ashby 1929) Dowson 1943 and Sugarcane Yellow Leaf Virus (SCYLV). *S. spontaneum* is considered a noxious weed by APHIS and must be maintained in pots on trellis rows. Flowering is strictly controlled for the *S. spontaneum* plants by severe pruning in the fall. Distributions are made for all species in this collection. Requestors of *S. spontaneum* germplasm must have a current APHIS noxious weed permit for this species.

Tripsacum. The *Tripsacum* collection contains individuals of each of the 15 species in the genus and has approximately 340 accessions. The collection is one of two major collections; the other is located at the International Maize and Wheat Center (CIMMYT), Texcoco, Mexico. The accessions are maintained in 3 x 3 m field plots, under drip irrigation and with cold protection. Each clone is individually labeled with the PI number, MIA number (local number), and other important accession information. The clones are planted in a 1.0 ha field site, trenched and amended with soil and fertilizers. The collection is rotated every three years and the old plots retained for an additional two years.

b. Germplasm Maintenance for the Minor Collections

The collections of banana, annona, jackfruit, carambola, jujube, mamey, sapodilla, litchi, longan, guava, garcinia, passiflora and other miscellaneous fruit species are all small and maintained in various locations with various degrees of replication. All fertilization and cultural practices are recorded and maintained in a database. New accessions are added as they become available from foreign collaborators and breeding programs. The banana collection is a backup for the collection at Mayaguez, PR. The litchi and longan collections are a backup for the collections in Hilo, HI. All accessions are distributed upon request.

c. Molecular Characterization of the Germplasm

Developments in molecular biology have produced many new tools for germplasm management. These tools, primarily DNA markers and improved DNA sequencing techniques, both based on the polymerase chain reaction (PCR), have allowed more accurate analysis of genetic diversity, with reliability, speed, and at a dramatically lower cost than with older methods. Due to these new molecular techniques, coupled with increased computational abilities, we are providing better scientific evaluation, preservation, and utilization of genetic resources.

Avocado. Schnell et al. (28) evaluated 254 accessions and two related species from the USDA-ARS collection in Miami and the University of California collection at Irvine, using 14 microsatellite markers. Significant differences were found for each racial comparison, Guatemalan vs. West Indian, Guatemalan vs. Mexican, and Mexican vs. West Indian for 13 of the 14 loci from the Chi-square analysis. Phylogenetic analysis of the microsatellite data for the three populations and the interpopulation hybrids was in agreement with the previously reported genetic relationships that separated the Guatemalan (G), Mexican (M) and West Indian (W) races (11, 13, 18). The neighbor-joining tree, based on Cavalli-Sforza and Edwards (8) chord distance, grouped the three races into distinct clusters. The Persea spp. were distinct from any other group. The Guatemalan race and GxM hybrids clustered together with a high bootstrap value, and the West Indian and WxG clustered together again with a high bootstrap value, all other groups had little bootstrap support. Based on the microsatellite data, the racial designation of 50 (19.7%) accessions was changed. This has contributed greatly to our understanding of the genetic diversity in the avocado collection and to defining priorities for future collections of avocado germplasm. Seventy new microsatellite loci were identified from EST sequences for avocado (5). These markers will be useful for the mapping project, along with primers developed previously (1, 32). Investigations of the ASBVd pathogen continued with the discovery of persistence in nucellar cultures and variant generation while in culture (35). We also demonstrated that the viroid cannot be eliminated using micrografting (35).

Mango. Schnell et al. (31) developed a new set of 15 microsatellite markers and analyzed 59 Florida cultivars and four related species. Schnell et al. (27) evaluated 203 accessions of mango in the U.S. germplasm collection using 25 microsatellite loci. The microsatellite loci were moderately polymorphic with an average of 6.96 alleles per locus and average PIC value of 0.552. Using Principal Coordinate Analysis (PCA) a close relationship was found between the Florida, Israel, and Hawaii types and this was expected, as much germplasm was exchanged between these three areas. The analyses also support a closer association between the Indian and Florida cultivars when compared to the Southeast Asian cultivars. Among the 64 Florida cultivars evaluated in the parentage analysis, the genetic background was found to be based on as few as four Indian cultivars, and the polyembryonic cultivar 'Turpentine' (27).

Cacao. Approximately 320 microsatellite markers have been developed for cacao. These have been used to generate genetic linkage maps and to map quantitative trait loci (QTL) for disease resistance and productivity traits (6, 7). These QTL are being used in cacao breeding programs to increase the efficiency of selection for desirable traits (29). Genetic diversity within the cacao germplasm collections has been characterized using microsatellite markers. Over 1300 individuals from > 70 localities have been analyzed using 100 microsatellite markers. Bayesian statistics and computer simulations were used to infer genetic groups for population genetic analyses using the program "Structure" (26). Structure does not require prior information on the population of origin and we were able to exclude mislabeled individuals identified using this approach. At least thirteen major genetic groups were delineated based on this analysis, instead of the traditional genetic groups Criollo, Trinitario, and upper and lower Amazon Forastero. A genetic distance matrix between individuals was estimated using Shared Allele Distance coefficient. Individuals of the same population cluster together. We compared the diversity of germplasm traditionally used in breeding and the unexploited germplasm. Much higher diversity values were estimated for the unexploited germplasm than for the traditionally used parental cultivars. One parameter is of particular interest: the number of alleles exclusive to each group. The germplasm traditionally used only has 44 exclusive or "private alleles", whereas the unexploited germplasm contained 471 such alleles. Based on our results, most of the recently collected germplasm has not been used in breeding programs (19).

In the Miami laboratory we continue to increase the number of microsatellite markers for mango, avocado, cacao and other minor tropical fruit crops. We are also developing a new type of molecular marker called a Single Nucleotide Polymorphism (SNP) for all the aforementioned crops. SNPs and microsatellite markers are being used for accession fingerprinting, genetic diversity analysis, and linkage mapping. Mapping populations are in place for mango and avocado. These populations are segregating for disease resistance, yield, and other desirable horticultural characteristics. The production of saturated linkage maps will lead to the identification of QTLs for desirable traits such as resistance to *Colletotrichum gloeosporioides* (anthracnose) in mango and *Phytophthora cinnamomi* (Rands) (root rot) in avocado.

Summary

Production of many of the tropical and subtropical fruits and nuts is based on a few commercial cultivars. This reliance on select genotypes, which are usually closely related, has developed because of consumer demands for quality products and the difficulties associated with breeding long-lived crops. Genetic vulnerability is potentially a problem. The collections maintained at the NGRs in Miami, Mayaguez, and Hilo are presently major genetic pools for mitigating potential problems caused by genetic homogeneity in farmers' fields. The tropical and subtropical repositories maintain fruits and nuts with recalcitrant seed that limit options available for the conservation of genes. Research is being focused at the National Center for Genetic Resource Preservation (NCGRP) to develop long-term preservation of these tropical/subtropical species using low temperature and cryogenic storage of seeds, clonal buds, and scions. Cryopreservation is proving to be a safe, cost-effective approach to enhance management of these subtropical/ tropical fruit and nuts germplasm collections eliminating the need for costly backup field collections at the three tropical/subtropical repositories.

Biotechnology offers a broad range of techniques for the molecular characterization, disease indexing, pathogen elimination, propagation, documentation, preservation and exchanging of disease-free plant genetic resources (3). DNA techniques have become useful tools for identifying clones and for locating genes associated with valuable traits. Many publications have documented the utility of molecular markers in fruits and nuts management including hazelnut (14), pears (2, 36), guava (34), persimmon (38), macadamia (33), avocado (4, 28), cacao (12) and coconut (16, 17). Increasingly important is the exponential expansion of DNA sequence information on plant species. This information will increase in importance as sequencing costs continue to decline and our analysis abilities improve.

The investment in the conservation of tropical and subtropical fruit and nuts by USDA/ NPGS will continue to yield large economic and humanitarian benefits. It appears only reasonable to support and maintain germplasm conservation measures. The value of these genetic resources has greatly increased over time. Our ability to economically sequence the genome of selected individuals will provide genetic information heretofore unavailable. This information will greatly increase our ability to manipulate these species for human betterment. The USDA-ARS will continue to maintain and establish valuable fruit and nut collections representing world diversity of not only commercially important cultivars, but also their wild relatives.

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EPP in Two Olive Cultivars May Not Limit Fruit Set

The effective pollination periods (EPP) for 'Manzanillo' and 'Picual' olive trees were studied for two consecutive years in California and Spain. The duration of the EPP varied between years and cultivars. Fruit set declined gradually in response to sequential cross-pollination. Fertilization was first observed on day-2 after cross-pollination in both cultivars. Ovule longevity appeared to last > 14 d in both cultivars. The stigmas remained receptive for > 8 d. Estimates of the duration of the EPP, based on analyses of its components, were longer than those indicated by the declines observed in fruit set, suggesting that other factors may limit the duration of the EPP Self-incompatibility may be a more important factor than a short EPP in limiting fruit set in 'Picual' and 'Manzanillo' olive trees. Paraphrased from J. Cuevas et al. 2009. J. Hort. Sci. Biotech. 84(3):370-374.