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Fruit and Nut Germplasm Collections: Treasuries of Genetic Diversity:

Dynamic Use of the National Collection of Genetic Resources for Pecans and Hickories

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Abstract

Our unit was initially designated as a USDA Agricultural Research Service National Clonal Germplasm Repository based on an existing collection of grafted cultivars developed for use as parents by the USDA ARS Pecan Breeding Program. From its inception, the NCGR-*Carya* has pursued a dynamic collection strategy with three distinct targets: 1) collection of named cultivars from culturally distinctive growing regions to represent selections made over the 170+ year history of grafting pecans; 2) provenance collections of self-rooted seedlings to represent genetic diversity in the context of geographic origin; 3) representative collections of *Carya* species to provide the broadest base of potentially compatible genetic diversity. The entire collection is maintained as living plant inventories at two worksites in Texas, where 5 ARS workers maintain trees on 242 hectares. Graftwood distributions have historically been requested from only a small subset of accessions, primarily the most recently released cultivars, while seed distributions have been from a small subset of primarily standard seedstock sources. Graftwood exchange is impacted by the presence of disease caused by the bacterial pathogen *Xylella fastidiosa*. The broad genetic diversity available in the collection is being used by multiple international teams for developing improved methods of molecular characterization based on genome sequencing. Inventory specific records of NCGR-*Carya* provide the “type” collections for genomic profiles of taxonomic species and are a particularly valuable laboratory for studying interspecific hybridization. Phenotypic records linked to pecan provenance inventories in replicated test configurations provide evidence of regional adaptation related to genetic diversity that varies between geographic subpopulations. Historically documented passport records of regional pecan cultivars linked to their phenotypic and genomic characterizations provide the foundation for identity verification procedures and tools for development of marker assisted selection. This accessible, diverse, living collection of trees is contributing to the development of the next generation of *Carya* scientists, as they work toward the refinement of improved genomic techniques. The next generation of breeders will use these tools to select cultivars with traits targeted for improved regional performance from broad foundations of genetic diversity available within the collection. Long-lived test systems on NCGR-*Carya* worksites will provide foundations for future development of improved techniques such as remote imaging. As this generation of breeders works cooperatively within these diverse collections, we hope to facilitate continued coordinated use. Cryopreservation of pollen from selected inventories is being pursued in conjunction with scientists at the USDA National Center for Genetic Resources Preservation, Fort Collins, CO.

The USDA Pecan Breeding Program began in July 1930, when appropriations were made through the efforts of Congressman James Paul Buchanan of Brenham, Texas (Romberg, 1968). First located on the “Little Campus” of the University of Texas in Austin, the US Pecan Research Laboratory found a more appropriate home on Pecan Bayou in Brownwood, Texas, where the first trees were planted in 1931. Louis D. Romberg (1898-1989) joined the program in 1931 and served as the first USDA pecan breeder until he retired in 1968. Romberg selected pecan cultivars from across the United States for use as parents in breeding. George Madden, who succeeded him as USDA pecan breeder from 1968-1978 added to that collection. The “breeder’s collection” associated with the

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USDA ARS Pecan Breeding Program was selected as the foundation of the National Clonal Germplasm Repository for Pecans and Hickories when first envisioned (Brooks and Barton 1977).

Brooks and Barton (1977) noted that success in breeding many crops was due to accessibility of diverse germplasm, often "brought in from other countries since most fruit and nut crops are not indigenous to the U.S." When breeding programs were terminated by federal and state breeding programs, breeder's collections were often lost. The primary function of repositories would be to collect, maintain, evaluate, preserve and distribute valuable genetic material, both domestically and internationally. Brooks and Barton (1977) observed that, "suggested locations may not provide optimal plant growth and performance of fruit and nut clones but should provide for optimal conditions for plant survival" (p 299). In 1979 the staff at the USDA Pecan Station in Brownwood purchased 32 hectares of land adjacent to the 62.7 hectares along Pecan Bayou, named for its indigenous pecan trees, which the City of Brownwood had made available to USDA in a long-term Memorandum of Understanding.

From the beginning, the effort to represent genetic diversity of *Carya* has been a cooperative, multi-disciplinary, multi-institutional, international effort. The first meeting of the *ad hoc* Crop Germplasm Committee was in San Antonio, Texas, July 10, 1984, and elected a dynamic strategy of provenance and species collections in addition to augmenting existing cultivar collections. Ruben Castro y Medina from Mexico's Comisión Nacional de Fruticultura (CONAFRUT), shared information on the distribution of Mexican native pecans and invited a joint collection. Plans were immediately made for provenance collections across the pecan's range. Bruce Wood (Horticulturist, ARS, GA), trained in forestry, worked with Gene Namkoong (U.S. Forest Service) to establish methods. I (then Horticulturist at the LSU Pecan Research-Extension Station in Robson, LA) reviewed previous botanical collections from Mexican species of *Carya* and worked with Jerry Payne (Entomologist, ARS, GA), Wood and Castro to establish the itinerary. While plans were under review, NPGS made recommendations to include collection from native *Juglans* species. Our teams made collections from native pecan populations across the U.S. in 1986 and, from native *Carya* and *Juglans* across Mexico in 1987. Mexican collections were facilitated by Castro and were joined by Dan Parfitt (*Juglans* Curator, NCGR-Davis) (Grauke et al., 1989). From those collections, replicated orchards were planted at the Southeastern Fruit and Tree Nut Lab (SEFTNL) in Byron, Georgia with partial plantings in Burleson and Brown County, Texas in what are now the two worksites of the National Collection of Genetic Resources for Pecans and Hickories (NCGR-*Carya*) (Grauke et al., 1989; Wood et al., 1998). Additional provenance plantings were made to represent regional native pecan populations across the U.S. in 1989, Arkansas in 1994 and coastal Louisiana in 1997. Among the most valuable pecan provenance collections maintained at the Burleson County worksite is one grown from nuts obtained by Tommy Thompson (ARS Geneticist, TX and third USDA pecan breeder) without the effort of a plant collection. Thompson judged the 1994 Third Mexican National Pecan Show in Piedras Negras, Coahuila, Mexico and requested the nut samples that had been submitted for competition in the Native category. They were provided, along with origination and nut quality records, and were planted by maternal families in greenhouses in Brownwood. For representation of North American pecans in the same provenance test, nuts were planted from 16 pecan cultivars used as regional seedstocks in the U.S. Seedlings were evaluated for growth while still in greenhouses and were planted in a randomized block test having eight blocks to control variation in soils and to accommodate the 1,400 seedling trees.

Asian *Carya* was targeted by collections made in 1990, from Vietnam and China

(Grauke et al., 1991). Limited numbers of seedlings were established of *C. tonkinensis* and *C. sinensis* from seed collected in Vietnam. No living accessions were established from Chinese *Carya* populations. However, herbarium vouchers collected and maintained at NCGR-*Carya* contributed to the treatment of *Juglandaceae* in the Flora of China (Lu et al., 1999). Seed of Asian *Carya* species have been received subsequently in small lots. Seedlings of *C. cathayensis* are currently maintained in a greenhouse. Available methods of molecular characterization have been used to characterize the Asian accessions, only one of which (now expired) have survived to maturity.

The current *ex situ* collections of the NCGR *Carya* are maintained at two worksites in Texas. Priorities for management are outlined in the most recent Crop Vulnerability Report for *Carya* (Grauke et al., 2016). Those collections are maintained by staff that has been much reduced over the life of the program, from 5 to 2 scientists (0.5 SY in the Repository project) and from a maximum of 14 support staff to the current 3. Unlike other fruit and nut repositories, breeding continues to be the primary focus of the pecan program and is pursued by the same staff in a sister project (CRIS 3091-21000-039-OOD). This program is unusual within fruit and nut repositories by internationally distributing germplasm from a U.S. native species that the same staff is actively breeding.

We are currently at the close of the first generation of effort in the collection, characterization and distribution of genetic diversity for the *Carya* genus. The ability to distribute graftwood of *Carya* has been impacted by the identification of *Xylella fastidiosa* broadly within the U.S. commercial pecan industry and specifically within NCGR-*Carya* inventories (Hilton et al., 2017; Bock et al., 2018). To whom, in what form, and to what purpose distributions are made are contentious issues on which domestic producers of commercial pecans differ from their federal and state research partners. Decisions on those questions have implications for the future of the collection. For perspective, it is helpful to examine how the existing collection has been used.

Materials and Methods

NCGR-*Carya* Inventories

Cultivar collections: Pecan culture by grafting began in the mid-1800s, and excellent records of geographic origin occur in various cultivar registries (e.g., Corsa, 1896; Taylor, 1905; Crane et al., 1937; Brooks and Olmo, 1944). Vouchers from the original ortets of many pecan cultivars from which our living inventories descend are maintained at the McKay Nut Collections of the National Arboretum) (Grauke, 2019a, 2019b). Over 300 grafted accessions of pecan are maintained at the two NCGR-*Carya* worksites. These include the first generation of selections made from native trees in several states, propagated as cultivars by the developing U.S. pecan industry, and used as parents in controlled crosses for the next generation of cultivars (Thompson and Young, 1985). Identification of cultivars has traditionally relied on morphological patterns of nuts and kernels. NCGR-*Carya* inventories have been characterized by measurement and photography of nut vouchers, with photographs from verified inventories being available on the program website (https://cgru.usda.gov/carya). Native selections have been georeferenced by assigning coordinates for latitude and longitude based on documented passport information.

Provenance collections: A small provenance collection is maintained at Brownwood, comprised of only 120 pecan seedlings growing on their own roots, representing 63 mother trees from 49 populations. The planting consists of self-rooted seedling trees maintained in a low maintenance planting of 2.8 Ha (7 acres) of 9.7 Ha available (24.1 A) on the southwest of the Santa Fe Railroad line. The orchard is challenging to maintain with tractors and equipment since implements must be driven through town, under a narrow overpass, and through a residential area to get to the site. The majority of provenance orchards are maintained at the Burleson County worksite: over 2000 self-rooted trees growing on about 12 Ha (30 acres) represent native pecan populations from Oaxaca, Mexico to Missouri, with detailed collections from Arkansas and coastal Louisiana. A small seedling population from a noteworthy putative native stand in Alabama is also maintained.

Species collections: The recent *Carya* crop vulnerability report noted the need to conserve and characterize indigenous species in Mexico and Asia (Grauke, Wood and Harris, 2016). That goal will require international cooperation and could contribute to conservation of threatened germplasm as well as development of improved tools of genetic analysis. In existing NCGR-*Carya* collections, the majority of *Carya* species collections are maintained at the Burleson County worksite. Mortality of seedlings grown on their own roots has led to grafting accessions onto pecan seedling rootstocks. A range-wide collection of the Florida endemic, *C. floridana* is currently maintained in containers in a greenhouse, along with same-aged seedlings of *C. glabra.*

Secondary collections: Significant collections of pecan cultivars, provenance collections (Grauke et al., 2011) and species collections (Wood and Grauke, 2011) are maintained at the USDA ARS Southeastern Fruit and Tree Nut Research Lab in Byron, GA.

Results and Discussion

Grafted inventories at our worksites are in decline, with some inventories in Brownwood dating to the 1930s, and others on rootstocks planted in the 1970s. Trees have been lost during recent severe drought conditions. Burleson County inventories are grafted onto rootstocks that were planted in unirrigated orchards during the 1980s. They have received minimal pesticide protection in order to observe patterns of disease susceptibility. As a result, all inventories could be regenerated into more accessible, replicated plantations of selected, highly valued accessions (a core collection) and could be held under better management in order to facilitate distribution. Alternatively, existing reproductively mature inventories are an accessible source of nuts and pollen that can currently be distributed while graftwood distributions are curtailed by phytosanitary restrictions. Decisions concerning the management of NCGR-*Carya* holdings and their integration into breeding and cooperative research with both domestic and international partners are necessary from federal managers of both breeding and repository projects.

Patterns of use

Distributions

Distributions made from NCGR-*Carya* since 1995 were evaluated in relation to germplasm identity, form and receiver. Of the 1805 total distributions made, 1078 (60%) have been to recipients within the United States, with 31 states receiving germplasm with most going to recipients in Texas and Louisiana. Forty six percent of domestic distributions have been in the form of seed. Germplasm has been sent to twenty-one countries, with China (224), India (149), Argentina (86), and Brazil (66) receiving the greatest number of distributions. Each of those countries except Brazil received germplasm in the form of seed as well as graftwood. Like Brazil, many countries (France, Mexico, Netherlands, Pakistan and South Africa) have requested and received germplasm only as graftwood. the countries (Croatia, Georgia, Germany, Guatemala, Jordan, Laos, Romania, Slovakia and Turkmenistan) have requested and received only seed distributions, as a necessary first step to establishing rootstocks and as a valuable first evaluation of germplasm adaptation. Belgium, Italy, and Sweden have received both seed and graftwood distributions.

Over 400 germplasm accessions have been distributed, with selected cultivars (‘Kanza’ and ‘Pawnee’) being distributed more than any others. The top twenty cultivars distributed include 10 standards of the commercial pecan industry [‘Elliott’(FL), ‘Peruque’ (MO), ‘Giles’ (KS), ‘Colby’ (IL), ‘Riverside’ (TX), ‘Moore’ (FL), ‘Posey’ (IN), ‘Curtis’ (FL), ‘Desirable’ (MS) and ‘Major’ (KY)] (state of origin in parentheses, listed in decreasing distribution frequency). These cultivars represent native and seedling selections from a broad geographic range, manifest diversity in their phenology, nut morphology and disease resistance, and are used within the industry as both grafted cultivars and as seed stocks. Also among the top twenty distributions were 10 that are more recent releases from the USDA Pecan Breeding Program (‘Kanza’, ‘Pawnee’, ‘Caddo’, ‘Oconee’, ‘Lakota’, ‘Osage’, ‘Nacono’, ‘Mandan’, ‘Barton’ and ‘Choctaw’)(listed in decreasing distribution frequency).

‘Major’ is an interesting entry in the above list. It has been reported to be a cryptic hybrid between pecan and other *Carya* species, based on microsatellite profiles (Grauke et al., 2015). It is also a parent of three of the USDA cultivars in the above list (‘Kanza’, ‘Lakota’, and ‘Osage’) and is a grandparent of ‘Mandan’, through ‘Osage’.

The primary interest of both domestic and international cooperators is recently released cultivars. The top ten distributions made as graftwood included 8 USDA cultivars (‘Kanza’, ‘Pawnee’, ‘Oconee’, ‘Caddo’, ‘Lakota’, ‘Nacono’, ‘Mandan’ and ‘Osage’), along with only ‘Desirable’ and ‘Peruque’ to represent older standard cultivars. Among commonly distributed standard cultivars was ‘Cape Fear’ which was the original cultivar on which Pecan Bacterial Leaf Scorch was described (Sanderlin and Heyderich-Alger, 2000) and was among the inventories recently found positive for *X. fastidiosa* (Hilton et al., 2017).

The top ten distributions made as seed included eight standard cultivars used by the pecan industry as seed stocks (Grauke, 2010) (‘Elliott’, ‘Moore’, ‘Riverside’, ‘Giles’, ‘VC-168’, ‘Peruque’, ‘Posey’ and ‘Colby’) (listed in decreasing distribution frequency). ‘Apache’ was the only USDA cultivar in the top ten seed distributions and is a popular seed stock. One seedling selection from Mexican provenance collections made in Jaumave, Tamaulipas (87MX5-1.7) was also commonly distributed. Although these distributions may indicate the bias introduced by curatorial recommendations, they also affirm that an important function of germplasm distribution is accomplished by sending appropriate seed for rootstock development. In seed distributions made to Laos in 2006, conventional seed stocks ‘Elliott’ and ‘Moore’ were accompanied by seed from several selections from the Mexican provenance orchard, grown from seed collected south of the Tropic of Cancer. In subsequent distributions in 2007 and 2009, only Mexican seedlings were sent, as requested by the cooperators. Research indicates that phenology of open-pollinated seed collected from our diverse germplasm orchards reflects the geographic origins of the maternal parent (Grauke and Starr 2014). Distribution of seed is not a standard function of the “National Clonal Germplasm Repositories”, whose accessions must be maintained by vegetative propagation to be true to type. Open-pollinated seed is an effective way to distribute genetic diversity. At its inception, NCGR-*Carya* initiated internationally cooperative provenance collections based on forestry practice. The distribution of that material as seed has been practiced and could become even more important under phytosanitary limitations to the distribution of vegetative material due to *X. fastidiosa*. Research is currently underway to determine if the association between pecan and *X. fastidiosa* is endemic across *Carya* species throughout their geographic ranges, to better understand genetic resistance and susceptibility that could impact both seed stock and scion development, and to elucidate methods of remediation of infected materials.

Distribution of seed necessitates bearing trees, which requires extensive time in

Carya. The extent and maturity of the current collection argues in favor of its maintenance, although increased management will be required. Existing NCGR-*Carya* orchards representing diverse geographic populations manifest patterns of phenology in which consistent, season-long monitoring will benefit by developing techniques of remote sensing. Those orchards will be excellent locations for refinement of remote sensing techniques for those purposes. Cooperative research on remote sensing is currently being pursued with Dr. Sorin Popescu (Texas A&M University, Dept. of Ecosystem Science and Management).

Evaluations

Development of molecular genetic profiling methods has been an objective of the NCGR-*Carya* since the use of isozymes in the 1990's (Grauke et al., 1995, Marquard et al., 1995) and has contributed to our ability to verify cultivar identity and parentage. Success in the effort depends on availability of samples from verified inventories. Since many USDA cultivars are the result of controlled crosses between cultivars represented in the collection by verified inventories, there are multiple levels of confirmation (Marquard et al., 1995). 'Kiowa' was reported to be a controlled cross between 'Mahan' and 'Odom' (McCracken, 1976; Brooks and Olmo, 1978). Its isozyme profile was compared with both parents, revealing that 'Odom' could not be the pollen parent (Marquard, 1987). With very few markers to point to the true parent, 'Desirable' had a consistent profile. Since not every tree is accurately labeled, confirmation of cultivar identity is significant for an individual inventory. The Pecan Breeding & Genetics program has always labeled its trees but saw the value in reporting the source trees used in molecular profiling efforts (Marquard et al., 1995, Table 2). Documentation linking the tissue source by tree inventory to the molecular profile creates a valuable "type" specimen. Previously verified specimens increase confidence in the results of additional testing and propagations. Quality control in the breeding process is provided by using molecular markers. Quality control in tree labeling and at each stage of propagating is necessary for nursery producers as well. Molecular methods of cultivar verification are valuable for certification of stock tree identity. Although reliable methods of cultivar verification are now available, the certification process should be an industry driven effort.

Isozyme analysis was also used within provenance collections maintained by NCGR-*Carya* (Grauke et al., 1995) and at the SEFTNL (Ruter et al., 1999). Those studies revealed genetic diversity in provenance collections not found in the cultivar collections. Genetic diversity is often recognized without understanding its function. One hint is geographic distribution. One form of the important enzyme phosphoglucose isomerase (PGI2, EC 5.3.1.9) was clearly concentrated in native populations from West Texas (Grauke et al., 1995). Three forms, or alleles, of the enzyme (A, B and C) are commonly found in pecan cultivars (Marquard et al., 1995). The D allele was clearly concentrated in native populations from West Texas where it was found in 40 trees in the Lipan Creek area of Tom Green County, and in the Devil's River area of Val Verde County, Texas (Grauke et al., 1995). The geographic coordinates of the mother trees from which original seed was collected are associated with geographic coordinates of living inventories in our orchards, and with isozyme profiles linked to those individual seedlings. Inventory specific association of genetic information of progeny, within the context of their current site and culture, linked to the geographic origin of their progenitor, connects accessible research orchards to the native forest and can impact developing conservation and utilization strategies.

Microsatellite markers have increased the resolution provided by isozymes, and inclusion of maternally inherited plastid markers offers evidence of the direction of a cross

(Grauke et al., 2015; Wang et al., 2016). As resolution has increased in molecular profiles, the distinctions between inventories thought to represent the same genotype has improved: 'Jackson' inventories in NCGR-*Carya* that were inseparable using 8 nuclear microsatellite primers are distinct with the 14 primers currently in use (Grauke, unpublished). 'Jackson' is reported to be a controlled cross ('Success' × 'Schley') made by Theodore Bechtel, Ocean Springs, Jackson County, Mississippi and released in 1917 (Thompson and Young, 1985). Microsatellite profiles of one accession of 'Jackson' in NCGR-*Carya*, when compared with profiles of 'Success' and 'Schley', has consistent alleles to confirm that parentage while the other accession does not.

The diversity found in wild populations of pecans and other hickories justified their inclusion in polymorphic panels used in developing microsatellite or simple sequence repeat (SSR) markers for *Carya* (Grauke et al., 2003). Plastid as well as nuclear SSR markers were developed and used across provenance collections (Grauke et al., 2011) demonstrating different patterns of genetic diversity related to geographic origin revealed in the maternally inherited plastids as compared to the nuclear profile.

Across all *Carya* genotypes profiled by SSRs, only one individual (87MX3-2.11) grown from seed collected in Oaxaca, Mexico in 1987 was found homozygous at all 14 nuclear SSR loci, possibly due to topographic isolation and inbreeding. That genotype was further evaluated by cooperators at HudsonAlpha Genome Sequencing Center and found to be "an excellent choice from which to produce a reference genome." (Jenkins et al., 2015). This single individual from Oaxaca was compared to 'Pawnee', which was significantly more heterozygous, and both assemblies were made available as preliminary scaffolds for continued sequencing efforts at the 2013 ISHS Symposium where the research was reported to the pecan research community.

The National Plant Germplasm System requires periodic analyses of challenges to crop genetic diversity from the Crop Germplasm Committees that represent them. The most recent *Carya* crop vulnerability report highlighted the need to cooperate domestically to develop genomic tools for use in breeding (Grauke et al., 2016). That goal was accomplished with the successful Specialty Crop Research Initiative proposal "Coordinated development of genetic tools for pecan". The SCRI project (2016-51181-25408) is led by Jennifer Randall (New Mexico State University) with participation from Texas A&M University (Patricia Klein), University of Georgia (Patrick Conner), Noble Research Institute (Charles Rohla, Maria Monteros), HudsonAlpha Genome Sequencing Center (Jeremy Schmutz, Jerry Jenkins, Jane Grimwood), and USDA-ARS units working with pecans in both Georgia (Bruce Wood (retired) and Clive Bock) and Texas (LJ Grauke and Xinwang Wang). Using the template genome for sequence alignment has allowed genome-by-sequencing (GBS) analysis of pecan diversity by multiple teams. (e.g. Bentley et al., 2019). The 87MX3-2.11 template genome is complete with chromosome level resolution and will be published and made publicly available soon.

Teams of researchers from NMSU and TAMU working in the SCRI grant sampled 498 NCGR-*Carya* inventories that originated across the U.S. and Mexico in May 2019. DNA from those samples was immediately extracted and has been sent to HudsonAlpha Genome Sequencing Center for resequencing. Those genomic profiles will characterize *Carya* diversity in relation to geographic distribution across North America. The association of genomic markers in *Carya* with phenotypic traits such as seasonal phenology, vigor, nut quality and disease expression linked to the individual tree records found in the Repository and Breeding Program inventories will contribute to the rapid development of marker assisted selection.

The initial HudsonAlpha draft sequence

of 'Pawnee' was made available to Chinese cooperators in 2013. Their team completed the reference genome sequence for 'Pawnee', along with a reference sequence from the Chinese hickory, *Carya cathayensis*, and reduced-coverage genome sequence profiles for many U.S. *Carya* species provided by NCGR-*Carya* (Huang et al., 2019). Comparison of gene families that differ between pecan and the Chinese hickory highlighted key genes in biotic and abiotic tolerance, oil, polyphenols, essential amino acids, and B vitamins, and provides valuable genetic perspective on the members of the genus *Carya*. An important, historical botanical question relates to the relationships between species in several important plant families that are found in both Eastern North America and in Asia. Since the genus *Carya* has valuable species used as nut crops in the U.S. and in China, the Chinese team was interested in exploring their genetic relationships over the extended period of time these species have been geographically separated. The Chinese teams were interested in exploring diversity of all U.S. species of *Carya*, both diploid (2n = 2x = 32, as in pecan) and tetraploid (2n = 4x = 64). All diploid hickories make fertile hybrids with pecans but crossing across ploidy levels is more difficult. In their research, Wood and Grauke (2011) noted that tetraploid *Carya* accumulated rare earth elements. This trait was suspected to be related to adaptation to dry sites and merited further research but is currently not being pursued by any domestic researchers. Sequencing the genomes of multiple tetraploids facilitates their evaluation and possibly their incorporation in breeding. For instance, *Carya floridana* is a tetraploid species that is well represented by collections made across the limited geographic range of the species in Florida in 2009 (Grauke and Mendoza-Herrera, 2012). It maintains the smallest tree size of any *Carya* species and will be valuable to characterize as reduced tree size is pursued in breeding.

With a team of researchers lead by Jennifer Randall (NMSU) and Hiroyoshi Iwata (University of Tokyo), we sampled 1,000 accessions across all orchards at the College Station worksite in 2015. DNA from those samples was extracted by the NMSU team and analyzed using genotyping by sequencing (GBS) procedures by the Japanese team. Preliminary data is consistent with previous analyses of genetic diversity in relation to geographic distribution (Grauke et al., 2011), highlighting the wealth of diversity available in native, especially southern, pecan populations. Although the information from those analyses has been reported at scientific meetings, publications are still in process (Ishimori et al., in press). It is valuable to have multiple teams, whose goals for selection are diverse, actively working cooperatively within these collections. International teams from China and Japan sought access to genetic diversity, but brought their expertise, focus and resources to the problem. The results of their work are being shared with U.S. researchers, who have contributed to the work but did not have to finance it.

Cryopreservation of pollen from selected inventories is being pursued in conjunction with scientists at the USDA National Center for Genetic Resources Preservation, Fort Collins, CO. Diverse, fruiting inventories of NCGR-*Carya* are a safe and immediately available source of genetic diversity that could be available to domestic and international cooperators for use in breeding.

Conclusions

When plans for the National Fruit and Nut Repositories were being developed, pecan was an atypical crop. Unlike most agricultural crops grown commercially in the U.S., pecan is native to North America and is therefore surrounded by co-evolved insects and diseases. Access to native populations impacted the dynamic strategy of making provenance and species collections. Excellent documentation of cultivar origin across the short history of horticultural selection of pecan has contributed to increased regional cultivar collections. Unlike other Repositories, our collections are actively used for breeding by the same team charged with developing and maintaining the genetically diverse collections. The close association between the USDA Pecan Breeding Program and the U.S. pecan production industry has been beneficial, providing motivation to develop methods for verifying cultivar identity that have steadily improved and developed, on the foundation of verified inventories. Methods of population biology and forestry have been adopted and have broadened our base of diversity and increased the precision of tools available to dissect and select from that diversity. Long-term test systems have been established in order to control site variability that could have obscured phenotypic resolution of genetic differences. The scales we use to measure diversity have benefited by the inclusion of the morphologically and genetically diverse wild relatives.

When pecan growers view the list of cultivars released by the USDA Pecan Breeding Program (Table 1), they see cultivars such as 'Pawnee', 'Wichita', 'Oconee' and 'Cheyenne' that they know and value in their orchards, despite known problems in all of them. They may not be concerned that the records of parentage for 'Caddo', 'Kiowa', and 'Creek' have been corrected using molecular markers. However, the excellent historical records of the breeding program, coupled with the extensive origination records of selections made from the forest by pecan growers over time, are an asset to developing markers for improved genetic selection of the next generation of cultivars.

When pecan growers look at the list of cultivars in Table 1 they may not notice that there are many parents, like 'Schley', 'Success', 'Mahan' and 'Evers' that occur several times, and that the offspring of those cultivars are further used as parents. Since 'Mahan' is a 'Schley' progeny, 21 of the 29 USDA releases trace back to 'Schley.' In more recent crosses made in the USDA Pecan Breeding Program, 'Pawnee' is the parent of over 20,000 progeny and 'Wichita' the parent of over 18,000. Although thousands of 'Pawnee' × 'Wichita' and reciprocal crosses have been evaluated in USDA nurseries and orchards in Texas and other states, none have been released. 'Wichita' by 'Pawnee' is the parentage of four cultivars ('Byrd', 'Morrill', 'Cunard' and 'Treadwell') patented by UGA Research Foundation (Sparks, 2010, 2013, 2014, 2015b). An additional four UGA patented cultivars ('Huffman', 'Tom', 'Whiddon' and 'Tanner') are 'Pawnee' by 'Desirable' (Sparks, 2015a, 2016, 2017, 2018).

Genetic diversity is the foundation of population survival under the challenges of local environment, allowing for adaptation over time. Genetic diversity is the raw material used for breeding. My generation entered our work with the mandate, initiated by the federal NPGS, to collect, maintain, characterize, conserve, and distribute the diversity of our crop. We have confirmed that genetic diversity in geographically distributed populations of native pecans can contribute to understanding adaptation of both rootstocks and scion cultivars. It is obvious from Repository records that most distributions have been from a small subset of select cultivars and seedstocks, effectively subsidizing international competition rather than distributing adapted diversity.

Breeding and selection target increasing uniformity. Crop-wide genetic uniformity can create situations like the Southern Corn Leaf Blight Epidemic of 1969-70 that helped inaugurate the National Plant Germplasm System. It takes time to develop resistance through breeding. The temptation in commercial production is to rely on chemical protection that masks emerging problems. The U.S. pecan industry has known since 2000 that pecans become chronically infected with Pecan Bacterial Leaf Scorch (Sanderlin and Heyderich-Alger, 2000), causing yield loss (Sanderlin and Heyderich-Alger, 2003). Methods of reducing transmission of PBLS by heat treating graftwood had been reported and recommended (Sanderlin and Melan-

Table 1. Pecan cultivars released by the USDA ARS Pecan Breeding Program in chronological order by breeder, with parentage, cross number, year of release and years from cross to release.

No.	Cultivar name	Nut Parent	Pollen Parent	Tested as Cross number	Breeder, Year released	Years from cross to release
					Romberg	
1	'Barton'	'Moore'	'Success'	1937-03-0020	1953	16
2	'Comanche'	'Burkett'	'Success'	1938-07-0022	1955	17
3	'Choctaw'	'Success'	'Mahan'	1946-15-0276	1959	13
4	'Wichita'	'Halbert'	'Mahan'	1940-09-0193	1959	19
5	'Apache'	'Burkett'	'Schley'	1940-04-0017	1959	19
6	'Sioux'	'Schley'	'Carmichael'	1943-04-0006	1962	19
7	'Mohawk'	'Success'	'Mahan'	1946-15-0195	1965	19
8	'Caddo'	'Brooks'	'Schley'	Philema 1175	1968	46
9	'Shawnee'	'Schley'	'Barton'	1949-17-0166	1968	19
					Madden	
10	'Cheyenne'	'Clark'	'Odom'	1942-13-0002	1970	28
11	'Cherokee'	'Schley'	'Evers'	1948-22-0027	1971	23
12	'Chickasaw'	'Brooks'	'Evers'	1944-04-0101	1972	28
13	'Shoshoni'	'Odom'	'Evers'	1944-15-0059	1972	28
14	'Tejas'	'Mahan'	'Risien #1'	1944-10-0293	1973	27
15	'Kiowa'	'Mahan'	'Desirable'	1953-09-0191	1976	23
					Thompson	
16	'Pawnee'	'Mohawk'	'Starking Hardy Giant'	1963-16-0125	1984	21
17	'Houma'	'Desirable'	'Curtis'	1958-04-0061	1989	31
18	'Osage'	'Major'	'Evers'	1948-15-0003	1989	41
19	'Oconee'	'Schley'	'Barton'	1956-07-0072	1989	33
20	'Navaho'	'Apalachee'	'Wichita'	1974-01-0011	1994	20
21	'Kanza'	'Major'	'Shoshoni'	1955-11-0011	1996	41
22	'Creek'	'Mohawk'	'Western'	1961-06-0067	1996	35
23	'Hopi'	'Schley'	'McCulley'	1939-05-0050	1999	60
24	'Nacono'	'Cheyenne'	'Sioux'	1974-05-0055	2000	26
25	'Waco'	'Cheyenne'	'Sioux'	1975-05-0006	2005	30
26	'Lakota'	'Mahan'	'Major'	1964-06-0502	2007	43
27	'Mandan'	BW-1	'Osage'	1985-01-0002	2009	24
28	'Apalachee'	'Moore'	'Schley'	1948-13-0311	2009	61
29	'Lipan'	'Cheyenne'	'Pawnee'	1986-03-0624	2011	25

son, 2008; Melanson and Sanderlin, 2015). However, the disease is not usually noticed in well-managed orchards, and efforts to recognize or reduce it were not pursued. Sanderlin (2015) screened several pecan seedstocks and found that susceptibility to infection differed after mechanical inoculation with *X. fastidiosa*, simulating typical insect transmission. The most susceptible seedstocks observed were progeny of 'Schley', which as noted above, is the foundation parent of the U.S. pecan industry. The identification of *X. fastidiosa* in NCGR-*Carya* collections was precipitated by the necessity of phytosanitary certifications required for international distributions, and by the inadequacy of existing diagnostic methods (Hilton et al., 2017; Bock et al., 2018). Research to improve diagnostic methods for detection of *X. fastidiosa* was funded in part by grants from the National

Plant Germplasm System. National leaders who planned the original Repository system expected multi-disciplinary teams to oversee the collections, in order to safeguard international germplasm exchange. Although *X. fastidiosa* has curtailed international distribution of *Carya* graftwood from this collection, the dynamic use of these collections by international teams is facilitating cooperative use and will result in improved methods to safely share genetic diversity. There is value in recognizing disease susceptibility of both rootstocks and scion cultivars in any breeding program.

Nuts from selected NCGR-*Carya* inventories can be distributed to state forestry teams for establishment of regionally adapted plantations facilitating continued screening of diversity under reduced chemical protection. Nuts from diverse inventories in our collections can be internationally distributed with less phytosanitary oversight than graftwood and will carry much greater genetic diversity. However, the production, collection and distribution of viable nuts requires maintenance and management of orchards that is currently not possible with existing NCGR-*Carya* personnel. Distribution of pollen from fruiting inventories in Repository collections can make genetic diversity swiftly and safely available. People with necessary skills and resources to collect and process diverse pollen and ship it for cryopreservation are available in this program, if the demand for access to that diversity exists. Past distributions, and the parentage of cultivars being patented by other programs suggests that diversity is not highly valued.

Using the genetic tools that have been developed from these diverse collections and are currently in hand, identity and parentage of seedlings can be determined. With continued effort, marker assisted selection can be developed and pursued for traits that have not previously been under selection, such as tree size control. Success in those efforts will require active breeding as well as knowledgeable access to the diversity on which breeding depends. This program has been responsible for the collection, maintenance and evaluation of diversity and its use in breeding. Decisions must be made on how, and by whom, these collections will be used in the future.

Original National Plant Germplasm System plans chose sites where the target species would survive (Brooks and Barton, 1977). Trees in our collections are resilient and are surviving even though we provide them with minimal care, in order to observe their expression of disease without the filter of multiple fungicidal sprays. The local database of NCGR-*Carya* links the GPS coordinates of each tree to its phenotypic and genetic characterizations over time and will be valuable to foresters as well as to the U.S. improved pecan industry, and to international users who have access. These collections were envisioned as long-term assets whose dynamic use would continue. On these sites, these resilient collections will survive and can be found using existing documentation if anyone looks for them. Hopefully their maintenance and strategic utilization will be continued and increased.

Cohen et al. (1991) reviewed the status of international plant germplasm conservation efforts and categorized four “eras” of *ex situ* genetic resource conservation and use. Their timeline extended from 1850 to 2030. The period from 2010-2030 was predicted to be one of more efficient utilization of genetic resources with closer linkage between diverse collections and breeders, facilitated by accessible, well-documented material. The National Collection of Genetic Resources for Pecans and Hickories has arrived at that point. Where the next generation goes from here will require coordination of the federal program, domestic and international research partners, and the industries that use the genus *Carya*. The next generation can use the genetic resources, living test systems and molecular tools developed by this generation to more responsibly use and possibly domesticate pecan.

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Correction:

On the web page (at http://www.pubhort.org/aps/73/v73_n4_a3.htm) for volume 73(4), for the article by Yu Zou, Mind Zhao, Peng Wu, Haiwang He, and Fang Long "Grape Cultivation delays Soil Desertification and produces Economic Benefits in the Karst Region", the second author's name was misspelled as Mind Shao and should be Ming Zhao.