

Vulnerability of *Carya*
L. J. Grauke
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We have been asked to update the vulnerability statement for the materials represented in the National Clonal Germplasm Repository for Pecans and Hickories. The analysis, as stated in the memo of July 8, 2004 from Drs. Dwayne Buxton and Peter Bretting, should include "up-to-date assessments of the degree of genetic uniformity of the "standing crop," identification and ranking of the highest impact crop diseases, and identification of the crop genetic diversity "in reserve" that might be mobilized in a crop disease-associated emergency. " The request for an update of the vulnerability assessment of *Carya* affords the opportunity to evaluate the status of our efforts to collect, characterize and conserve the genetic resources of this valuable nut genus that is native to North America.

For general information on the evolution, history, taxonomic organization, identification, and distribution of the U.S. *Carya* species, see Grauke, 2003. For general information on the identification and distribution of Asian *Carya*, see Grauke et al. 1991 and Lu et al. 1999. Other sources of information used for this report include the very valuable forest inventory records made available on the internet (Miles, 2004), the National Report on Sustainable Forests-2003, and national pecan yield records of the National Agricultural Statistics Service. Estimates of genetic diversity are based on interpretation of unreported results from microsatellite analysis using primers developed by research at the NCGR for Pecans and Hickories (Grauke et al., 2003a).

The hickories are a component of the broadleaf forests. According to the National Report on Sustainable Forests-2003, oak-hickory (*Quercus/Carya* spp.) is the largest single forest cover type in the U.S, with 132 million acres. It constitutes more than 17 percent of all U. S. forest land and nearly half of all broadleaf forests. Several species are individually identified within the Forest Inventory. The most recent estimates of the number of live trees of those species is given in Table 1, along with the number reported on public lands. Trees on public lands (federal, state, and local municipalities) can be considered a form of reserve. The number of trees of *C. tomentosa*, *C. glabra*, *C. texana*, *C. ovata* and *C. cordiformis* is adequate for the purposes that those trees fulfill. Although *C. aquatica* has significantly fewer trees in the total inventory, trees on public lands exceed those of *C. cordiformis*. Maps of the distribution of each species were also produced using the data on total tree numbers within the Forest Inventory Mapmaker 1.7 program (only *C. texana* shown, Fig. 1). Those maps have resolution to the county level and provide useful indications of the areas of maximum species density for these species. For instance, *C. tomentosa* and *C. texana* occur in very high numbers throughout the Ouachita and Ozark mountain regions of Arkansas, Oklahoma, and Missouri, while *C. aquatica* shows high density of along some regions of the Mississippi River in Louisiana. Despite the lack of threat to these species, areas of high species occurrence should be well represented in Repository ex situ collections to ensure appropriate characterization of diversity. Two of the species addressed individually by Forest Inventory records are poorly represented in the inventory; pecan and shellbark hickory. The inventory of pecan includes Texas populations only from the eastern part of the state, and therefore misses both the greatest numbers of native trees

as well as those that are most genetically unique. Although thirteen million pecan trees on public lands in the U.S. may seem adequate, we have additional information to direct the establishment of specific regional in situ reserves that will better represent the diversity of the species to meet future needs. The poor representation of *C. laciniosa* in this inventory elevates the need to better collect and characterize that species.

Establishment of ex situ collections will be from judiciously selected public lands, so that their characterization may serve as the initial description for future in situ reserves of the species.

Eight microsatellite or simple sequence repeat (SSR) primers, producing a total of 92 polymorphic bands, were demonstrated by canonical discriminant analysis to be capable of clearly distinguishing between genera of the Juglandaceae (*Carya*, *Juglans*, and *Pterocarya*) (Fig. 2) (Grauke et al., unpublished data). The amplification of DNA from both walnut and hickory accessions suggests the strategy of developing a suite of informative SSR primers to be shared between *Juglans* and *Carya* repositories. That strategy is being pursued by cooperative research with Dr. Malli Aradhya (NCGR-Davis).

The 8 SSR primers were capable of clearly distinguishing between sections *Sinocarya*, *Apocarya* and *Carya* of the genus *Carya* (Fig. 3). Section *Sinocarya* is made up entirely of species that are native to Asia and is relatively poorly represented in Repository collections. Information concerning the distribution of those species in Asia is limited, but previous collections in Vietnam and China suggest that few undisturbed native populations exist. Although the representation of genetic diversity within the Repository may be increased by acquisition of additional accessions of Asian species, care must be taken to avoid the introduction of associated insects and diseases that could devastate native species. Asian species might harbor diseases to which they have resistance, but to which our native North American species are susceptible. The native American chestnut [*Castanea dentata* (Marsh.) Borkh.] was a dominant tree in the eastern forests of North America until introduced diseases (ink disease caused by *Phytophthora cinnamomi* and chestnut blight disease, caused by *Cryphonectria parasitica*) devastated the species (Anagnostakis, 2001). The Oriental Chestnut Gall Wasp (*Dryocosmus kuriphilus*) is an introduced insect pest that is also damaging remaining native chestnut species (Anagnostakis, 2001). Ink disease evidently came in to the U.S. on cork oak trees from Portugal, while chestnut blight was introduced with Japanese chestnut planting stock. The gall wasp is thought to have been introduced into Georgia in 1974 on scion wood that did not pass through proper quarantine (Anagnostakis, 2001). The Formosan subterranean termite (*Coptotermes formosanus*) is an introduced pest with the potential to cause severe damage to native hickory populations, since it has the ability to live in living wood and chooses moist habitats such as occupied by species such as pecan. Careless introductions have been responsible for tremendous damage to the native forest in the past, and should be guarded against in the future. Continued vigilance against such self-inflicted wounds, by guarding points of entry and by monitoring stands for evidence of infestation, will be critical safeguards.

Interspecific hybrids between species of different sections were shown by SSR analysis to be intermediate between sections (Fig. 3). Introgression of genes by interspecific hybridization between sympatric populations may have contributed to the

regional genetic diversity of hickory populations, an idea that can be tested by more thorough analysis of the accessions from Repository collections. Two types of collections are maintained in the Repository; the Cultivar Collection represents named pecan selections used as grafted cultivars by the pecan industry; the Provenance Collection represents wild populations from throughout the range of the species. The Cultivar Collection is maintained as grafted trees, while the Provenance Collection is maintained as seedling trees growing on their own roots. Within the Cultivar Collection, some cultivars represent selections from the native forest, while others represent either controlled crosses between selected parents (often of distant origin) or seedling selections of often unknown origin (Thompson and Young, 1985). The accessions demonstrated to be intermediate between sections (Fig.3) are named cultivars of hickory that had been selected (often from artificially cultivated populations) and were known to be hybrid. Natural populations from different geographic regions are represented in the Provenance collection and include hybrid swarms between different species: e.g., accessions of pecan, water hickory (*C. aquatica*), and their interspecific hybrid *C. X lecontei*, from the Gulf Coast of Louisiana; accessions of pecan and *C. X brownii* (*C. cordiformis* X pecan) from northwest Arkansas. A broader application of SSR analysis within the Provenance Collection may confirm regionally significant introgression of genes between particular species. In this limited analysis, genetic similarity of pecan was found to be greatest with *Carya laciniosa* (shellbark hickory) and *C. ovata* (shagbark hickory)(Table 2). Those three species have been the most widely used by humans since prehistoric times. They are all diploid species, with $n=16$ and cross naturally to form interspecific hybrids.

Pecan is the most economically important member of the *Carya* genus and is the most valuable native North American nut crop. In the ten years between 1993 and 2002, U.S. pecan production averaged 120 million kg per year with a mean total value of 220 million dollars. Production is erratic, with years of low production often followed by very heavy crops (Grauke and Thompson, 2004). Pecan is the primary target of collection for the Repository, and is the target of improvement through breeding. Previous analysis of annual yield records reported by the National Agricultural Statistics Service indicated significant reductions in genetically diverse native pecan stands in general, with dramatic decreases in some states (Grauke et al., 1995) possibly associated with increased soybean planting in the 1970's. At the same time, production from grafted trees, comprised of large acreages of relatively few genotypes, is increasing. Assessment of vegetative traits of seedling trees in the Byron Provenance Orchard led to the recognition of major differences between Mexican and U.S. pecan populations (Wood et al., 1998). Canonical discriminate analysis of microsatellite data from native pecans (from both the Cultivar Collection and a portion of the Provenance Collection) showed distinct clustering on the basis of state origin (Fig. 4). Native pecans arising in Kentucky or Illinois and Kansas showed the greatest genetic distance from the major grouping of native pecans. When pecans from those states were eliminated from analysis, pecans arising from Texas, Louisiana, Arkansas and Mexico were all distinguishable as clusters. External confirmation of the genetic differences between regional pecan populations is found in the recent evaluation of leaf morphology of seedlings growing in the Byron Provenance Orchard. Leaflet area was found to be significantly greater in seedlings grown from seed collected in the northeast, decreasing

to the southwest (Grauke et al., 2003a)(Fig. 5). Leaflet density (weight per unit area) was greatest in the seedlings with southwestern origin, decreasing to the northeast (Fig. 6). Zinc content varied between regional populations, with the highest levels of zinc associated with seedlings arising from regions of alkaline soil in the southwest (Fig. 7). These patterns may reflect genetic adaptations to decreased light intensity in the northeast as compared to the southwest, and are consistent with the adaptation of native plants to arid conditions and alkaline soils. Observations in Repository collections confirm that cultivars with eastern origins have the most resistance to pecan scab disease. Furthermore, seedling accessions grown from materials originating in humid regions show higher levels of resistance in Provenance Orchards, consistent with relatively high heritability for resistance to scab (Thompson and Grauke, 1994). Finally, the seedling trees from different regions exhibit variation in patterns of growth that are consistent with their geographic origin, indicating genetic adaptation to climatic patterns that may make them unfit in some regions (Grauke, 1998)(Fig. 8). Demonstrably different regional native pecan populations provide justification for in situ conservation across diverse regions. Target pecan populations have been selected from the western edge of the species range in Texas, and are being sought from the northeastern provenance in Kentucky as well as the Illinois and Kansas region. The northeastern portion of pecan's range is where it is sympatric with *C. laciniosa*, shown in Table 2 to be genetically closest to pecan. The observed patterns may reflect introgression.

The most serious disease currently threatening pecan is pecan scab, caused by *Fusicladosporium effusum* (G. Winter) Partridge & Morgan-Jones. Within its area of affect, cotton root rot [*Phymatotrichum omnivorum* (Shear) Duggar] is the most destructive, since it is not restricted to pecan, kills trees quickly and remains resident in the soil. There are no resistant rootstocks, and no prophylactic or curative measures. Pecan is susceptible to *Xylella fastidiosa*, which incites bacterial leaf scorch (Sanderlin and Heyderich-Alger, 2003), a condition that can be chronic, reducing growth and nut quality. Although *Xylella* is devastating to a wide range of species, including grape and almond, it is not considered a major pest of pecan at this time. Like many forest diseases, *Xylella* can be vectored by insects; Pierce's disease of grape, caused by this organism, is vectored by the glassy winged sharpshooter. Methods of *Xylella* spread in pecan are not known. Pecan is not known to be susceptible to *Phytophthora* or other wide-spread root diseases that have devastated walnuts, oaks and chestnuts, and is not susceptible to any known virus. Many of the hickories are used mainly for wood products, and their primary disease enemy is canker and rot caused by *Poria spiculosa*.

The most critically challenged *Carya* species are not addressed by the Forest Inventory, since they occur in such low numbers that they are not recognized and counted. Those species include the nutmeg hickory (*C. myristiciformis*), and scrub hickory (*C. floridana*). Microsatellite analysis of nutmeg hickory accessions should contribute to our understanding of the extent to which forest fragmentation endangers that species, which is the most threatened of the North American hickories. The most geographically restricted hickory species in the U.S. is *C. floridana*, which is endemic to central Florida. The species is not commercially valuable for either nut or wood production, but is an ecologically important species, providing high energy food and habitat in a very selective environment. Scrub hickory is adequately represented on the Ocala National Park for that public holding to constitute a de facto in situ reserve.

SUMMARY

The genetic diversity of pecan (and by extension, related hickories) reveals long term adaptation to climatic and edaphic variation across its habitat. Evaluation of our collections using morphological, phenological, and molecular genetic indices confirms that regional adaptation is complex, but is often clinal across sources of variation. Maintenance of adequate levels of genetic diversity to meet future regional needs will require regional in situ reserves. Currently, de facto reserves exist for most species of *Carya*, due to the prevalence of the oak-hickory forest in the Southeastern U.S. In situ reserves should be designated on selected public lands for target species. Those species include pecan, nutmeg hickory, and shellbark hickory. The de facto in situ reserve for scrub hickory at Ocala National Park should be inventoried and monitored.

The greatest threat to the long term sustainability of the genetic diversity of these trees is forest elimination associated with agriculture and urbanization. That process is suspected to proceed through increasing levels of fragmentation and isolation to debility and demise, and has already proceeded to different extents in different species. The process should be studied within these important native nut species, even as we seek to prevent it.

The introduction of exotic pests poses a real threat that can be countered in part by routine vigilance in the introduction of foreign materials, and in part by the maintenance of robust populations in their native habitats.

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Table 1. Total number of trees, and number on public lands, for several *Carya* species, based on the most recent Forest Service Inventory data (Miles, 2004).

Species	total trees	on public lands
<i>C. tomentosa</i>	2,314,183,185	271,073,666
<i>C. glabra</i>	1,822,196,831	221,605,890
<i>C. texana</i>	1,055,790,444	192,098,595
<i>C. ovata</i>	960,750,534	81,139,169
<i>C. cordiformis</i>	617,791,055	40,456,312
<i>C. aquatica</i>	149,590,277	45,359,955
<i>C. illinoensis</i>	103,949,793	13,312,849
<i>C. laciniata</i>	41,916,764	2,634,849

Table 2. Squared distance from species*. Number of observations per species in parenthesis.

Squared Distance to Species											
From	ovt	tom	aqu	flo	gla	ill	ton	lac	myr	pld	tex
ovt (11)	0.0	504.4	419.1	849.4	401.6	125.3	649.0	107.3	436.7	1657.0	1548.0
tom (7)	504.4	0.0	785.9	568.9	893.4	642.1	1045.0	587.9	1008.0	1085.0	971.3
aqu (3)	419.1	785.9	0.0	851.7	811.2	295.7	694.8	329.5	618.5	1802.0	1542.0
flo (2)	849.4	568.9	851.7	0.0	1232.0	732.4	1369.0	634.6	935.9	998.6	976.2
gla (3)	401.6	893.4	811.2	1232.0	0.0	551.5	1172.0	481.0	936.3	1963.0	2057.0
ill (280)	125.3	642.1	295.7	732.4	551.5	0.0	668.0	93.8	381.9	1600.0	1535.0
ton (6)	649.0	1045.0	694.8	1369.0	1172.0	668.0	0.0	701.9	804.1	2499.0	1368.0
lac (12)	107.3	587.9	329.5	634.6	481.0	93.8	701.9	0.0	328.8	1519.0	1479.0
myr (8)	436.7	1008.0	618.5	935.9	936.3	381.9	804.1	328.8	0.0	1576.0	1743.0
pld (2)	1657.0	1085.0	1802.0	998.6	1963.0	1600.0	2499.0	1519.0	1576.0	0.0	1640.0
tex (3)	1548.0	971.3	1542.0	976.2	2057.0	1535.0	1368.0	1479.0	1743.0	1640.0	0.0

*ovt=ovata, tom=tomentosa, aqu=aquatica, flo=floridana, gla=glabra, ill=illinoisensis, lac=laciniosa, myr=myristiciformis, pld=pallida, tex=texana.

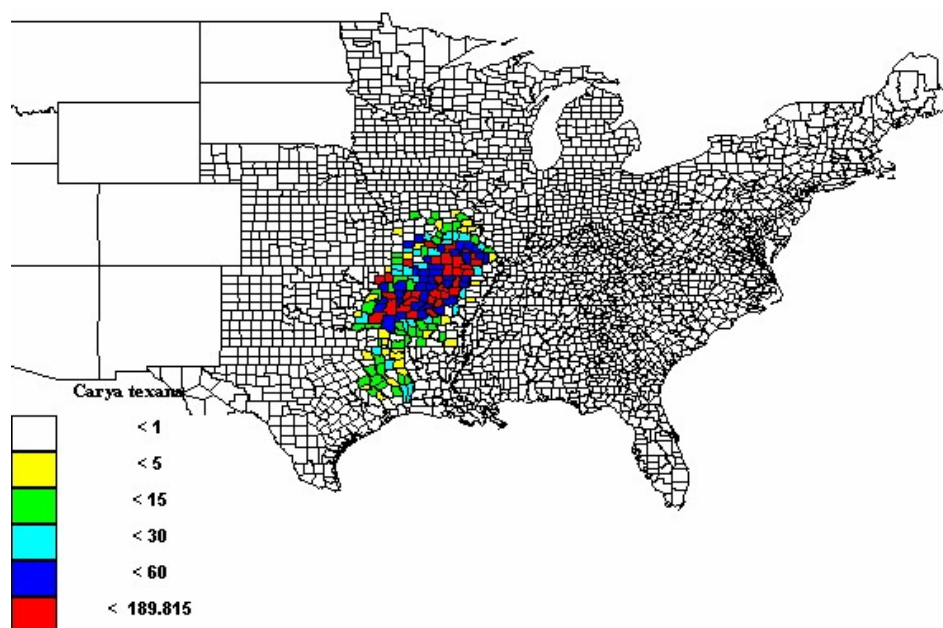


Figure 1. Density distribution of *Carya texana*, based on Forest Inventory records (Miles, 2004)

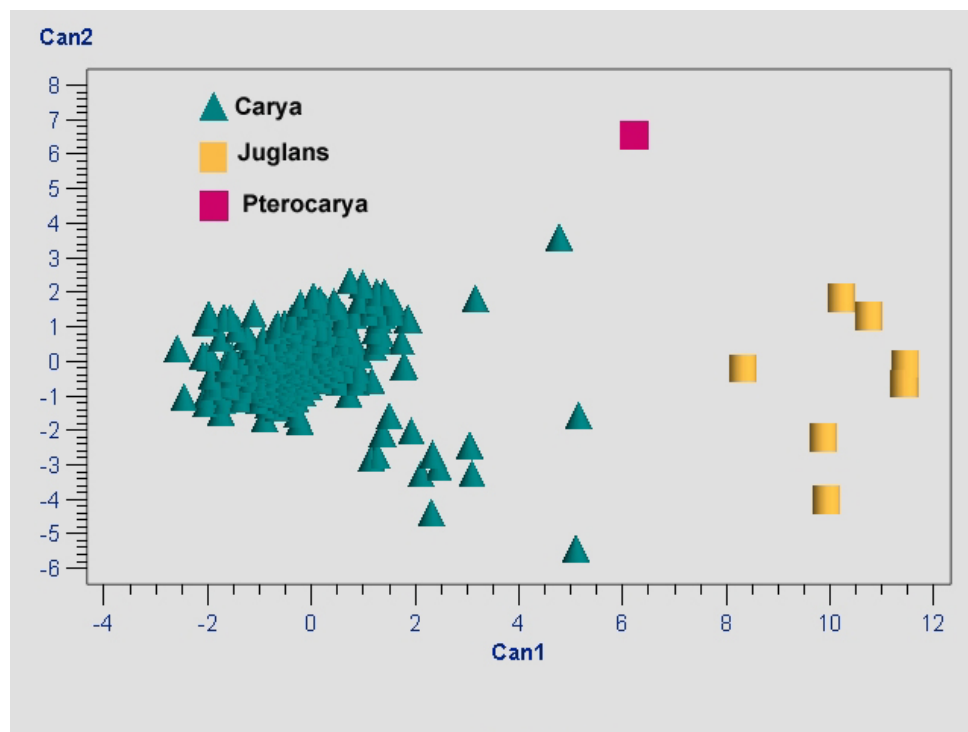


Figure 2. Cluster analysis of genera of Juglandaceae based on 8 SSR loci developed from pecan.

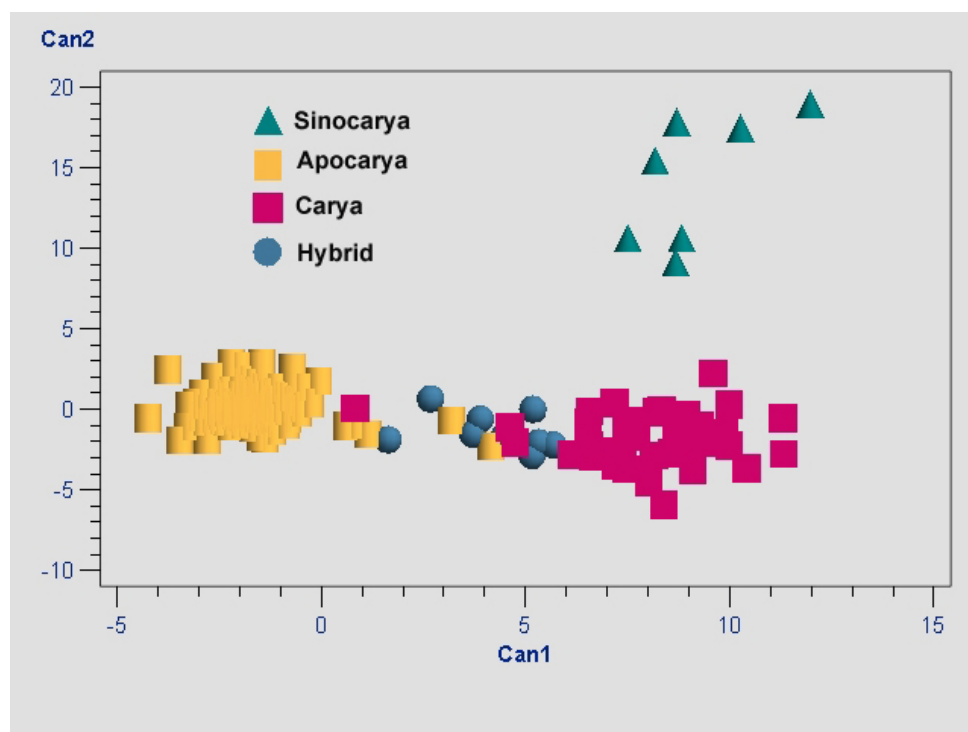


Figure 3. Cluster analysis of *Carya* sections based on 8 SSR loci.

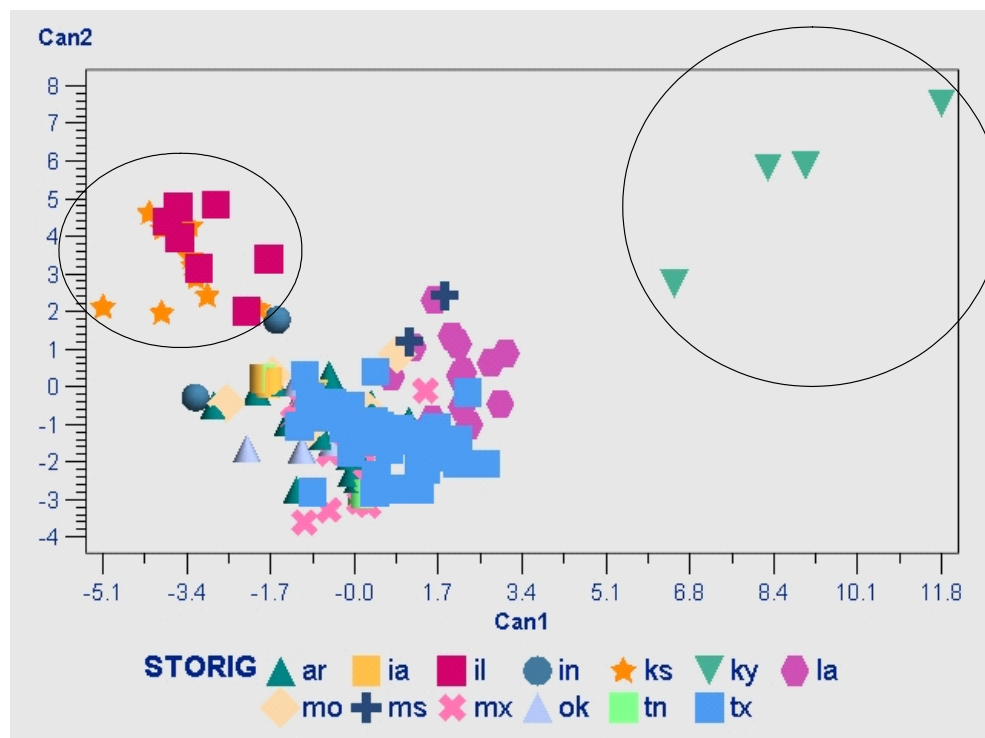


Figure 4. Cluster analysis of native pecan accessions in relation to state of origin, based on 8 SSR loci.

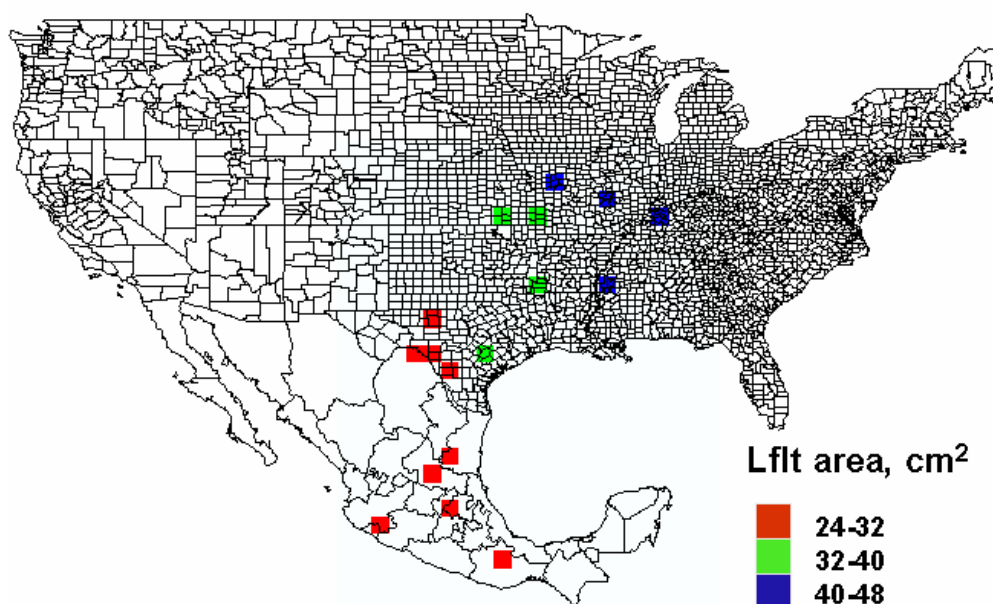


Figure 5. Leaflet area of 967 pecan seedlings grown in Provenance Orchard, Byron GA, varied in relation to geographic region of seedstock origin. (Grauke et al., 2003b)

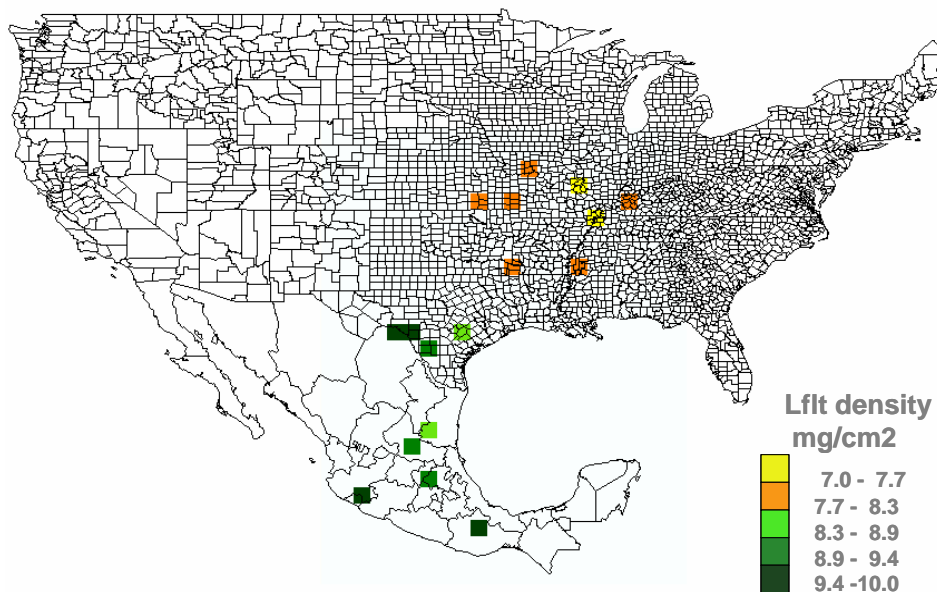


Figure 6. Leaflet density of 967 pecan seedlings grown in Provenance Orchard, Byron GA, varied in relation to geographic region of seedstock origin. (Grauke et al., 2003b)

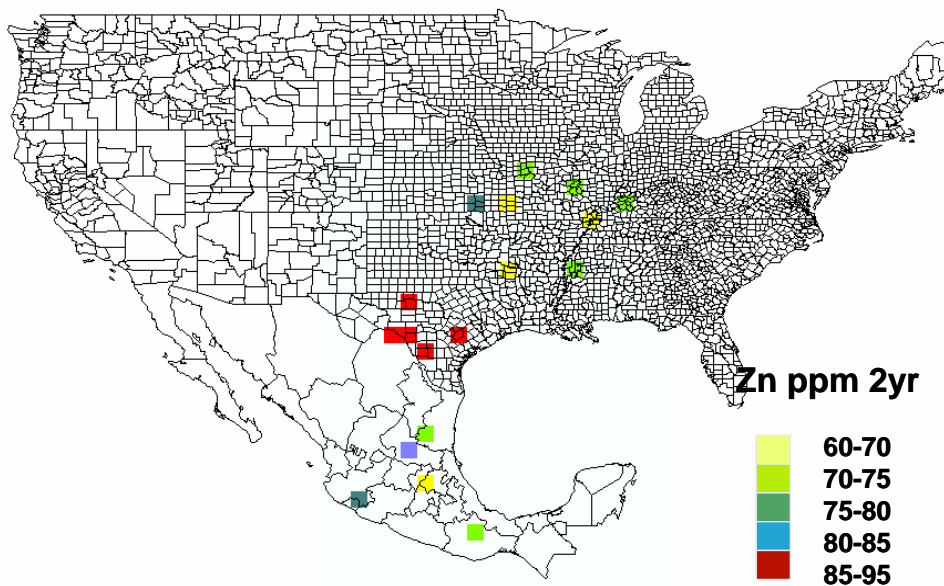


Figure 7. Leaflet Zn content of 967 pecan seedlings grown in Provenance Orchard, Byron GA, varied in relation to geographic region of seedstock origin. (Grauke et al., 2003b)

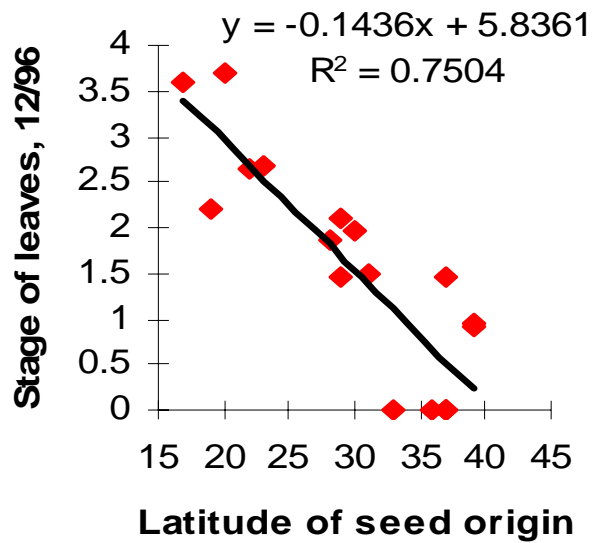


Figure 8. Pecan seedling phenology measured in Burleson County, Texas, as related to latitude of seed origin. Rated 12/4/96 using scale: 1= fully defoliated, 2=>50%leaf drop, 3=<50% leaf drop, 4= active growth