VARIATION IN ECOGEOGRAPHICAL TRAITS OF PECAN CULTIVARS AND PROVENANCES

A Dissertation

by

MADHULIKA SAGARAM

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2007

Major Subject: Molecular and Environmental Plant Sciences

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Approved by:

Chair of Committee,	Leonardo Lombardini
Committee Members,	L.J. Grauke
	Carol Loopstra
	John Jifon
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ABSTRACT

Variation in Ecogeographical Traits of Pecan Cultivars and Provenances. (August 2007)

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M.S., West Texas A&M University

Chair of Advisory Committee: Dr. Leonardo Lombardini

Pecan [*Carya illinoinensis* (Wangenh.) C. Koch] is a species distributed over an area of varied geographic and climatic variation in the Unites States and Mexico providing a potential for anatomical and physiological adaptation within the cultivars and provenances (i.e., the area of origin of seed). An assessment of leaf anatomical traits of pecan cultivars (Pawnee, Mohawk and Starking Hardy Giant) collected from three locations (Tifton, GA., Chetopa, KS., and Stillwater, OK.) was conducted to provide an understanding of patterns of ecogeographic variation within the natural range. The stomatal density 'Pawnee' (404 stomata/mm^2) was intermediate between that of 'Mohawk' (363 stomata/mm^2) and 'Starking Hardy Giant' (463 stomata/mm^2). There were differences among the three pecan cultivars at the same location but there were no differences in stomatal density within the same cultivar grown at three distinct locations. The study suggested that differences in stomatal density in pecans are cultivar-specific rather than being determined by environmental factors. The stability of certain leaf anatomical characteristics, such as stomatal density, for pecan cultivars grown at different locations confirms that these traits can be used for screening provenances with desirable leaf anatomical characteristics for breeding and cultivar development.

To achieve the objective of studying anatomical, morphological and physiological traits, Mexican and U.S. provenances grown at the Pecan Genetics and Breeding Program facility in Somerville, Texas were used. The prominent results from the provenance study indicate the presence of intra-specific variation in pecan provenances for the morphological and anatomical traits along the east-west gradient. It is also interesting to note that western provenances displayed the least stomatal density (350 stomata/mm^2) while an eastern provenance showed the greatest stomatal density (728 stomata/mm^2). This trend may be explained with the gradient in moisture availability from the wetter conditions in the east to the arid conditions in the west in North America. Most of the physiological traits measured did not show any distinct differences between the provenances. There is a great possibility that anatomical traits like stomatal density are genetically controlled to a great extent in pecan in comparison to the physiological traits.

DEDICATION

This dissertation is dedicated with great respect and humility to my teachers Mr. T.V.R. Murti and Dr. Leonardo Lombardini for standing beside me during the darkest hours of my life

ACKNOWLEDGEMENTS

This acknowledgement is written to note my appreciation of those people who have shaped my life and my work. These people made it possible for me to be where I am, who I am, and to make my work a reflection of myself. I am grateful to all of them for their advice and encouragement. As I think about them I realize there are many that from behind the scene have encouraged and supported me and I wish to thank them for it.

I would like to express my heartfelt gratitude to my mother for standing by me in every endeavor and being a source of support even during trying times. I am thankful to my husband Uma Shankar Sagaram for his encouragement and support. He is my best critic and my most trusted friend. His eye for perfection has always helped me realize my mistakes and reach my goals. I thank him from the depth of my heart for his undying patience and moral support all through our educational pursuits together for the past 12 years. I am also fondly thankful to my sister Archana for keeping the pressure on me at all times to be a good role model.

If I have to attribute my learning in life, my growth as a human being, my strong foundation in education to one influence in my life, it is clearly Mr. T.V.R. Murti. He made me believe I could be whatever I wanted to be and always encouraged me to be my best self. My teacher Mr. T.V.R. Murti taught me the benefit of service, leadership, trust and the importance of 'living with dignity'.

I would like to thank my advisor Dr. Leonardo Lombardini for his valuable guidance and support during the course of my study and preparation of this dissertation. His strong faith in me and my capabilities allowed me to accomplish all my dreams. His

encouragement helped me convert a degree into education and for that I will always be grateful to him.

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I am forever indebted to the people of my country, India for spending public money on me and bearing the expense of my education at bachelor's level. I strongly believe that the work of some unknown person makes our lives easier and better everyday. I would like to thank all those people that I never met, but whose published work inspired me all my life.

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CHAPTER I

INTRODUCTION

Plants demonstrate enormous ecophysiological and functional diversity, which underlies variation in growth rates, productivity, population and community dynamics, and ecosystem function. The broad congruence of these variations with climatic and environmental conditions at local, regional, and global scales has fostered the concept that plant ecophysiological characteristics are well adapted to their surroundings. Natural variation patterns provide in-depth understanding of the requirements for improvement of crops (Bagley, 1980).

Pecan [*Carya illinoinensis* (Wangenh.) C. Koch] is a species distributed over an area of geographic and climatic variation extending from northern Illinois and southeastern Iowa to the gulf coast of the United States (Grauke, 1990). It is a riparian species that grows abundantly along the Mississippi River, the rivers of central and eastern Oklahoma and the Edwards plateau of Texas. The species is also distributed in the form of sporadic populations and regenerating stands throughout north-central Mexico (Fig. 1) and as far south as the state of Oaxaca (Grauke, 1990; Thompson and Grauke, 1991a). The geographic segment of the U.S. pecan-producing land area has been expanded to Georgia, California, Arizona, New Mexico and western Texas. Such a wide distribution produces exposure to varied environmental conditions providing a

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This dissertation follows the style and format of Journal of the American Society for Horticultural Science.

potential for anatomical and physiological adaptation within the cultivars and provenances (i.e., the area of origin of seed).

Provenances and Populations

A provenance has been described as "a distinct morphological or physiological form, or population, resulting from (natural) selection by a distinct ecological condition" (Arnold, 2002).The definition of a provenance has also been provided as "the original geographic area from which seed or other propagules were obtained" (Zobel and Talbert, 1984). Zobel and Talbert (1984) further defined a provenance as "a subdivision of a species consisting of genetically similar individuals, related by common descent, and occupying a particular territory to which it has become adapted through natural selection". Provenances can be distinguished by geographic source and geographic ecotypes based on various factors including latitude, altitude, precipitation, temperature, soil type, and day length (Zobel and Talbert, 1984). A spatially-distributed group of trees that are capable of cross fertilization is defined as a "population" (Grauke, 1990). Thus, based on the definitions provided, populations and provenances can be identified for a particular species.

Provenance studies have been conducted on many species including *Carya illinoinensis* (Wangenh.) C. Koch (Wood et al., 1998), *Acer* spp. (St. Hilaire and Graves, 2001; Zwack and Graves, 1998)), *Alnus maritima* (Marsh.) Muhl. ex Nutt. (Schrader and Graves, 2000), *Chamaecyparis thyoides* (L.) B.S.P. (Jull et al., 1999), *Maackia*

amurensis Rupr. (Pai and Graves, 1995), *Platanus occidentalis* L. (Shoemake, 1996; Shoemake and Arnold, 1997) and *Pinus ponderosa* Dougl. ex Laws (Cregg, 1994). An appreciation and understanding of the genetic base of the species is required to fully exploit the potential of the pecan germplasm for the commercial advancement of the crop (Grauke, 1990). However, little has been done to explore the relationship within populations and provenances across the wide distribution of the pecan, thus overlooking the use of this information for cultivar development (Grauke, 1990). It is important to use native plant populations as much as possible to select the most productive and valuable traits (Vavilov, 1992). Thus, it is imperative to understand the geographical distribution of plant resources and establishment of the enormous intraspecific diversity of pecan.

Phytogeographical methods provide avenues to elucidate and establish material useful for plant breeding (Vavilov, 1992). Exploration of diversity of natural plant populations has in the past few decades revealed a new wealth of information useful to botanists, taxonomists, geneticists and ecologists (Bradshaw, 1959). The determination of composition of populations and the inherited variation of characteristics of each species as well as the general system of inherited variability could be of utmost importance to plant breeding (Vavilov, 1992). The study of native plant populations as a consequence of their adaptation to local climatic conditions will probably yield information concerning genetic and ecological factors that affect the distribution of the populations (Bradshaw, 1959). The differentiation of a species into agroecological and

geographical groups reveals information about morphological, physiological, ecological and agronomically valuable properties (Vavilov, 1992).

Morphology and Physiology: Geographic Distribution

Studies in ecology and natural history have always focused on the spatial patterning and geographic distribution of plants and animals (Turner, 1989). An understanding of the geographical distribution or geobotany of a species provides an understanding of the relationship between plant ecology and plant geography (Rubel, 1927). Studies of variation at geographic scales have focused on two broad areas of investigation. Firstly, these studies mainly concentrated on the patterns and causes of variation in morphologically and physiologically important traits in relation to environmental gradients, such as latitude or elevation. Secondly, the focus has been on the patterns and causes of trait variation between populations at 'the center vs. the margin of a specific geographic range' (Jonas and Geber, 1999).The 'center of origin' theory and associated concept of 'gene microcenter' explaining the variation between populations at the center of geographic ranges were first suggested by Vavilov during the 1930's. Following Vavilov's suggestions numerous theories have been proposed both opposing and supporting the theory of 'center of origin' as well as the marginal variation theories.

Researchers have also explained phenotypic variation in a species in relation to its adaptation to the environmental conditions in its geographic range. Variation in sensitivity to climate may favor selection of "different phenotypes in different environments constituting various ecotypes or provenances (specialists) rather than a

single phenotype across a geographical gradient (generalists)" (Levins, 1968; Oyama, 1994). This hypothesis, however, only describes two extremes of numerous variation patterns occurring in natural conditions (Oyama, 1994). Initial studies of geographic variation more or less revolved around analyses leading to the determination of presence of either clinal patterns (gradual change of phenotype in a species over a geographical area) or discontinuous variation in ecotypes (Linhart and Grant, 1996). There were ambiguities about the concepts leading to the representation of the same results in different ways (clinal or ecotypic) by different groups of researchers. Nevertheless, detailed analyses of geographic variation of species have shown that, within the same species, some characteristics can vary gradually and others discontinuously, depending on the flow of genes across populations, selection intensity, and terrain configuration (Linhart and Grant, 1996). However, when the climate 'is a graded patchwork of different conditions', for a given species, the pattern of differentiation is similar, thus resulting in an overlapping distribution pattern (Bradshaw, 1959). Under such conditions, it is not possible to distinguish and identify intraspecific variation. There is a great possibility that a similar trend could be found in most of the outbreeding, continuously distributed plant species occupying a wide range of habitats (Bradshaw, 1959). Thus, it would be interesting to find out the variation pattern of an outbreeding species like pecan in its geographic distribution. Adaptation theories become persuasive (a) when the same pattern of trait variation in relation to an environmental gradient is found in different geographical locations, (b) when functionally-related traits vary in synchronized manner across an environmental gradient, or (c) when intra- and

interspecific patterns of trait variation are similar in relation to an environmental gradient (Jonas and Geber, 1999). On similar lines, it is of great interest to find out if anatomy, morphology and physiology of pecan follow any of these patterns.

The pattern of differentiation is immensely affected by spatial distribution of a species whether continuous or discontinuous (Bradshaw, 1959). However, discontinuous distributions result in genetically-distinct populations by preventing gene flow (Bradshaw, 1959). Intraspecific and interspecific patterns of phenotypic response to environmental variations could be very different for individual plants. Such changes in phenotypic responses to different environments are often referred to as "phenotypic plasticity", which has been defined as "the change in the expressed phenotype of a genotype as a function of the environment" (Scheiner, 1993). Plasticity in morphological and physiological traits is not a genotypic property but is specific to a trait or a complex of traits (Scheiner and Goodnight, 1984).

However, a common thread in plant ecology is the distribution of plant species along gradients of resource availability (Hoagland and Collins, 1997; Ohmann and Spies, 1998). Gradients of resource availability and climate are usually associated with each other, the most visible of the association being changes in elevation over short distances (Tilman, 1988). Hence, many studies on distribution of plant species and resource availability have relied on elevation gradients, along with other environmental factors (Martens et al., 2001).

The geographic variation of photosynthetic activity of plants is determined to a great extent by the distribution of solar energy with latitude (Monsi et al., 1973).

Latitudinal trends for numerous anatomical characteristics of wood seem to be a general phenomenon for woody dicotyledonous genera with a wide geographical distribution (Noshiro and Baas, 2000). On the other hand, the morphological and physiological characteristics of plants are largely a result of their adaptation to environmental conditions (Monsi et al., 1973). Plant populations are differentiated with respect to morphology and physiology, at both small and large spatial scales of distribution (Linhart and Grant, 1996).

Variation in leaf morphology can be found to a great extent at both interspecific as well as intraspecific levels of geographic distribution (Hovenden and Vander Schoor, 2006). Correlations between morphology and habitat have been studied but comparatively little is known about the adaptive significance of the observed differences between populations (Lewis, 1969). It has very often been assumed that morphological differences among populations are of direct adaptive significance without any experimental consideration of their physiological and survival value (Lewis, 1969). Lewis (1969) reviewed the suggestion that "any plant which survives and reproduces is adapted to its habitat" (Wilkins, 1960) and, consequently, if an argument is based on this statement, physiological adaptation is likely to be as important as morphological adaptation.

Morphological adaptations may have a direct relationship with physiological consequences or can be a simple consequence of basic metabolic adaptation (Lewis, 1969). However, wherever morphological differentiation is found to be correlated with environmental factors, it could be an indication of the existence of adaptive divergence

between populations without any reference to the physiological aspects of the differences observed (Lewis, 1969). Heslop-Harrison (1964) provided an explanation for the morphological patterns and the population behavior as the differential effect of selection in the various habitats (Lewis, 1969). Nevertheless, correlation of anatomical and physiological characteristics with climatic trends continues to generate considerable interest among ecologists and botanists. It is still very important to understand the mechanisms of adaptation and survival because of the potential for use of such information in selecting for desired characters.

Several researchers have reported the association between geographical patterns of morphological traits and the distribution of climatic parameters (Hovenden and Vander Schoor, 2006; Strickland, 2003). To assess and understand the importance of the latitudinal and altitudinal trends in anatomical characters of wood, the climatic conditions and gradients should be taken into consideration (Noshiro and Baas, 2000). On similar lines, it would be of utmost importance to include climatic gradients into the scope of the study to completely understand patterns of morphological and physiological variation. Leaf morphology varies reliably with increasing altitude due to resulting changes in temperature in many species (Hovenden and Vander Schoor, 2006).

Gradients in climatic variation are ideal characteristics to be used in studying physiological adaptation and local differentiation in confounding biogeographic situations and helpful as a tool for interpretation (Ledig and Korbobo, 1983). Along gradients of altitude, environment changes rapidly over short distances. Radiation flux density, precipitation, wind speed, and snow cover generally increase with altitude. On

the other hand, temperature, growing season, barometric soils pressure, and soil depth decrease with altitude (Ledig and Korbobo, 1983). The geographical division of plant species in relation to altitude is a well known phenomenon and has been well documented (Woodward, 1983). Leaf morphology varies to a great extent with increasing altitude in many species, and this pattern is generally considered to be associated to temperature (Strickland, 2003).

According to the review done by Woodword (1983), Geiger (1965) explained the various aspects of the microclimate of plants, including temperature changes associated with changes in altitude and that it is generally assumed that these variations control the observed patterns of distribution. There are several qualitative arguments in the literature based on the selective advantages of specific morphological traits. Such qualitative arguments have now been replaced by quantitative correlations between structural or performance characteristics of organisms and their function in relation to their habitat (Koehl, 1996). A positive correlation was observed in leaf area and absence of serrated margins with temperature and precipitation (Strickland, 2003).

Morphology and Physiology: Provenance Variation

Stebbins (1952) suggested the reasons for rapid plant evolution in arid to semiarid regions. Where moisture is limited, differences in local terrain, soil type, and other factors have a more profound effect on the vegetation than in areas with adequate moisture (Stebbins, 1952). Interspecific comparisons of populations at the hotter, more arid ends of environmental gradients indicated rapid development, presence of small

flowers and vegetative structures, and high rates of gas exchange (Jonas and Geber, 1999). However, it is possible that intraspecific investigations may or may not conform to the pattern observed in interspecific comparisons.

There is a great possibility of division of medium- to large-sized populations into smaller isolated populations capable of exchanging genes by occasional migration in semiarid climates, thus establishing populations that may give rise to new populations or taxa (Stebbins, 1952). This could be particularly true for an open-pollinated species, like pecan. Also, flora in dry regions are frequently characterized by modified vegetative patterns, such as reduced leaf size, presence of scales and trichomes, that may enable plants to withstand periods of severe drought (Stebbins, 1952).Local and regional variations in environment assume a great importance in determining the characteristics of populations. Environment played a key role as a dominating factor in determining population differentiation in *Agrostis tenuis* Sibth (Bradshaw, 1959). Sharp changes in environment were associated with sharp changes in the *A. tenuis* populations, whereas gradual changes in environment were translated into gradual population changes (Bradshaw, 1959).

Leaf morphological characters such as leaf size, thickness and specific leaf area are strongly influenced by altitude in *Nothofagus cunninghamii* (Hook.) Oersted (Hovenden and Vander Schoor, 2004), but leaf morphology is also very strongly genetically controlled (Hovenden, 2001). Although there was evidence that a clinal trend prevailed in populations of *Clarkia unguiculata* Lindl. along environmental gradients, interaction between altitude and latitude dominated patterns of variation (Jonas and

Geber, 1999). For most traits measured, latitudinal trends at the low elevations differed from trends at mid- and high-elevation areas (Jonas and Geber, 1999).

Provenance variation in morphological and physiological traits related to drought resistance have been reported in *Cercis canadensis* L. (Griffin et al., 2004), *Pinus taeda* L. (Seiler and Johnson, 1988), and *Pinus ponderosa* Dougl. ExLaws. (Cregg, 1994). Morphological and physiological traits related to drought resistance from xeric areas were reported in provenances of *Fraxinus pennsylvanica* Marsh. (Abrams et al., 1990) and *Acer saccharum* Marsh. (St. Hilaire and Graves, 2001). Griffin (2004) found that ecotypes of *C. canadensis* from xeric environments had higher instantaneous water use efficiency than mesic ecotypes because of the adaptation to the dry environment. Stebbins (1952) also indicated that populations in xeric environments can adapt to mesic conditions if favorable conditions prevail. Hence, the populations of the same region could consist of members (descendants) of the same ancestry adapted to similar conditions in different ways. Thus, as a result of their different adaptive histories, variation occurs among populations in the same region. It is of utmost importance to understand similar variation patterns in pecan and identify provenances with adaptive significance to dry environments. Such an approach will aid in the identification of specific seed sources for breeding purposes.

The native range of pecan covers latitudes from 16º 30′ to 26º N (Thompson and Grauke, 1991a), altitudes from 500 to 2500 m above seal level (USGS, 1997) and levels of precipitation between 300 and 1500 mm/year (NOAA, 2002). Thus, precipitation and elevation patterns indicate that an east-west transect exists in North America with arid

environments to the west and more humid conditions to the east. This diversity of habitats may have promoted a corresponding geographic differentiation pattern of the pecan species inhabiting these areas.

Significance of the Proposal to U.S. Agriculture

Pecan is the only major nut tree that is native to North America and is an important crop contributing to the agriculture, economy and the history of the United States. It has been used for centuries and is an important tree grown for its edible nuts and timber (Hall, 2000). In 2004, the average yearly United States pecan production was 181 million pounds deriving from stands of both native and improved cultivars (USDA-NASS, 2005). Price of inshell nuts averages $1.00/lb, with peaks of up to $1.67/lb in 2004 (USDA-NASS, 2005). Despite the encouraging trend, the multimillion dollar pecan industry is faced with several challenges such as environmental stresses, pests, diseases, and alternate bearing, which all lead to severe economic losses. Hence, it is necessary to emphasize the possibility of testing new pecan genetic material to facilitate the development of novel cultivars tolerant to adverse conditions. The establishment of differences in physiologically and horticulturally valuable characteristics may be one effective procedure of testing native populations.

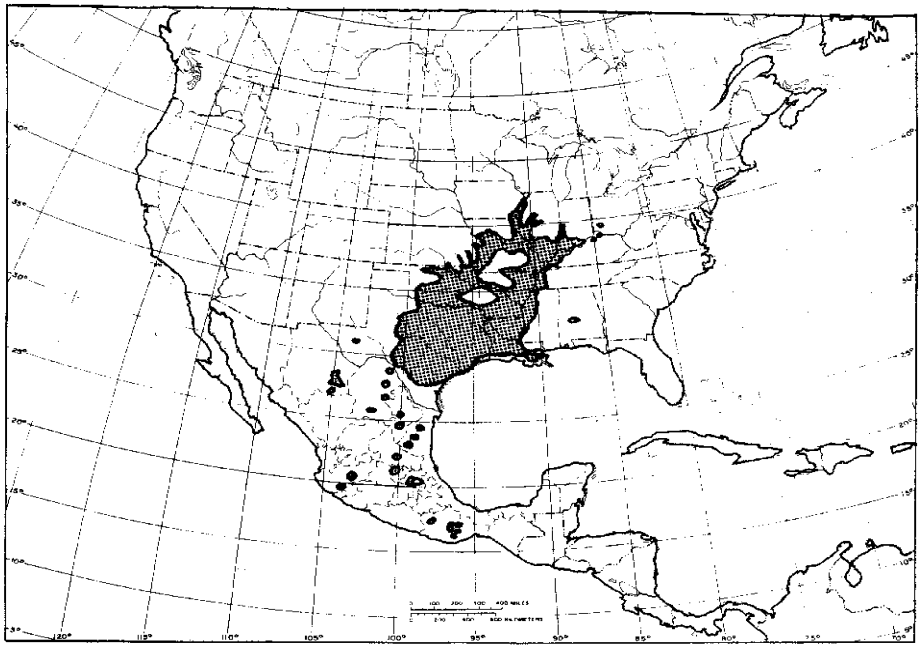

Fig. 1. Native distribution of pecan [Used from (Thompson and Grauke, 1991b)]

CHAPTER II

VARIATION IN LEAF ANATOMY OF PECAN CULTIVARS FROM THREE ECOGEOGRAPHIC LOCATIONS*

Introduction

Pecan has been known for centuries for its edible nuts and is the most valuable nut tree native to North America (Hall, 2000). It is a species distributed over an area of geographic and climatic variation extending from northern Illinois and southeastern Iowa to the gulf coast of the United States (Thompson and Grauke, 1991b). This riparian species grows abundantly along the Mississippi River, the rivers of central and eastern Oklahoma, and the Edwards Plateau in Texas. Since the species is widely distributed across varied environmental conditions, it has developed anatomical and morphological differences within the provenances (Grauke et al., 2003; Nemati and Roberts, 1968). Today, pecan is commercially produced outside its native range in Georgia, California, Arizona, New Mexico, and western Texas where environmental conditions can differ from those of its native range.

Traits affecting the use and assimilation of resources such as carbon, water, and nutrients directly influence physiological processes and plant growth and development (Ackerly et al., 2000). According to Jones (1998), features of leaf surface anatomy are

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*This chapter is reprinted with permission from "Variation in leaf anatomy of pecan cultivars from three ecogeographic locations" by Sagaram, M., L. Lombardini and L.J. Grauke. 2007. J. Amer. Soc. Hort. Sci. 132(5): 1-5.

a complex of traits defined by stomatal characteristics (density, frequency, and position) and epidermal characteristics (density, shape and size of epidermal cells).

Although flower (Amling and Amling, 1983; Wood, 2000; Wood et al., 1997), fruit (Grauke et al., 2001; Rehman et al., 1999; Rohla et al., 2005; Thompson, 2005) and leaf characteristics, such as leaflet area, specific leaf area, nutrient content (Grauke et al., 2003) and cuticular content (Chortyk et al., 1995) of pecan have received considerable attention, little information is available regarding additional leaf anatomical characteristics such as stomatal and epidermal cell density and number and types of trichomes (Grauke, 1982; Nemati and Roberts, 1968). Trichomes are hair-like structural elements of the epidermis of plants that play a role in plant defense (Levin, 1973), water use efficiency (Johnson, 1975) and temperature regulation (Ehleringer and Björkman, 1978). In juvenile pecan trees, three different types of trichomes, namely awn-like hairs, concave peltate, and bladder-like or vesicular trichomes, were observed and described (Grauke et al., 1987). Due to their importance in regulation of water loss and water use efficiency, leaf anatomical characteristics could be useful traits for cultivar development, particularly in selection for drought tolerance. This study was undertaken to characterize the leaf anatomical features of three pecan cultivars at various geographical locations and investigate the influence of cultivar and environment on stomatal density and epidermal cell density.

Materials and Methods

Plant material

Leaves from three pecan cultivars (Pawnee, Mohawk and Starking Hardy Giant) were obtained from three major pecan growing regions, namely, Tifton, GA. (lat. 31°27’48”, long. 83°30’36”W, altitude 117 m), Chetopa, KS. (lat. 37°02’15”N, long. 95°5’31”W, altitude 229 m) and Stillwater, OK. (lat. 36°07’18”N, long. 97°04’7”W, altitude 300 m) (Fig. 2). Two fully expanded leaves were selected from exterior north-facing canopy positions at 8-10 m from the ground (top third) of five 25- to 35-year-old trees. Leaf samples were collected between 25 Sept. and 2 Oct. 2005 and shipped overnight to the Texas A&M University laboratory in College Station, Texas, and acetate leaf casts were made immediately upon receipt of the material.

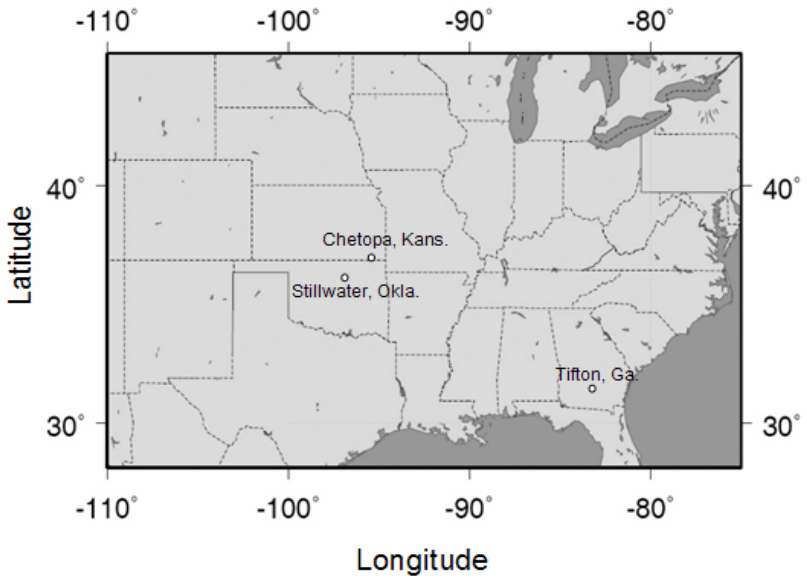

Fig. 2. Collection sites used in the study to investigate leaf anatomical features of pecan cultivars (‘Pawnee’, ‘Mohawk’ and ‘Starking Hardy Giant’).

Sample preparation

Pecan leaves are hypostomatic with anomocytic stomata (Grauke, 1982); consequently, only the leaf abaxial sides were investigated. To determine the density of stomata, epidermal cells and trichomes, the abaxial side of the distal pair of leaflets was coated with clear nail enamel (Fisher, 1985). After the enamel was allowed to dry for 10-15 min, the cast was stripped using clear tape and placed on microscope slides.

Microscopy

A microscope (model BX51, Olympus America Inc., Melville, N.Y.) was used to count epidermal cells and stomata from each cast at magnification of 200×. The microscope was attached to a digital camera (model DP70, Olympus America Inc., Melville, N.Y.) interfaced with a personal computer. Differential interference contrast (DIC) images from ten different interveinal areas of each cast were collected using DP70-BSW software version 01.01 (Olympus America Inc., Melville, N.Y.). Precautions were taken to avoid taking images in the same location by keeping a numbering system for the veins. In pecan, stomata are raised on the abaxial surface of the leaf in comparison to the epidermal cells. Hence, two DIC images were taken on each chosen area on the cast, one with the focus adjusted to highlight the epidermal cells and eliminate the stomata into the background (Fig. 3, top) and a second one with the focus on stomata and trichomes (Fig. 3, bottom). The number of stomata and epidermal cells from each image was recorded and analyzed for stomatal density [SD (stomata/mm^2)] and epidermal cell density [ED (epidermal cells/mm^2)]. Stomatal index (SI) was calculated as [SD/ (SD + ED)] x 100. Total trichome density [TD (trichomes/mm^2)] and the type of trichomes,

namely concave peltate and bladder (Fig. 4), were recorded for each cultivar at different locations.

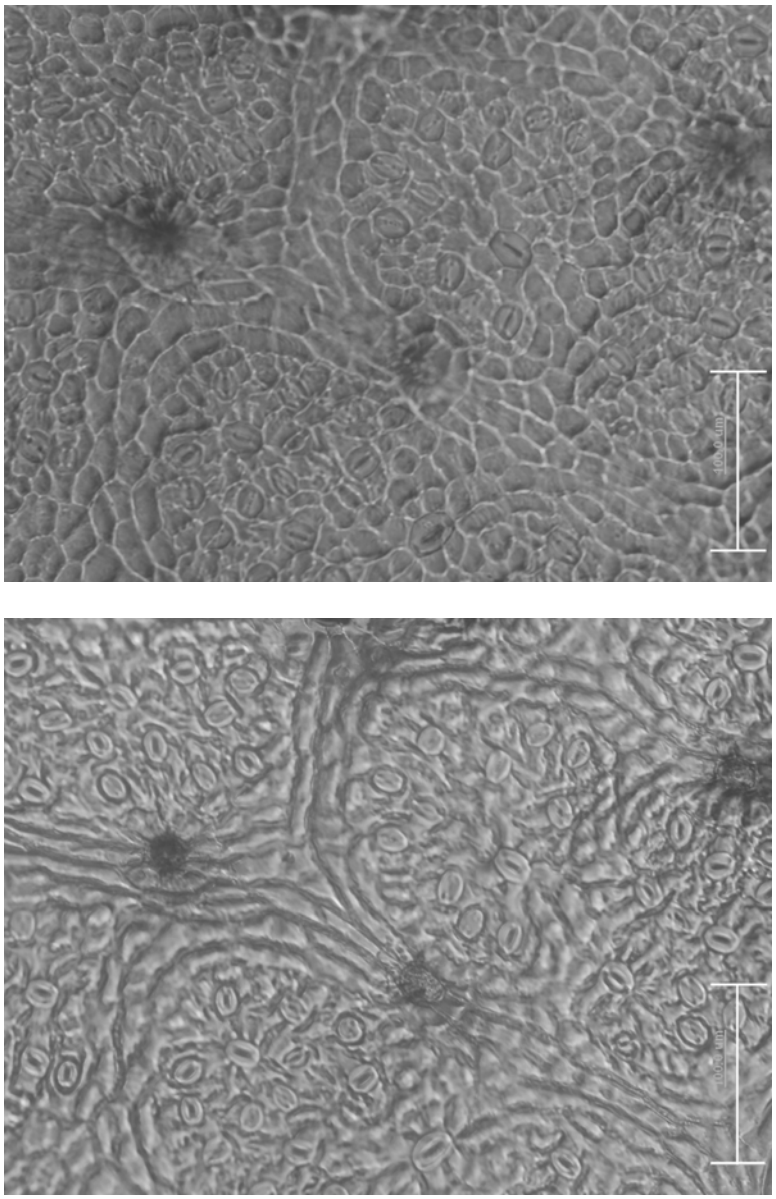

Fig. 3. Images of the abaxial surface of ‘Mohawk’ pecan leaves showing epidermal cells (top) and stomata (bottom) visible on two different focal planes of the same microscopic view (bar = 100 µm).

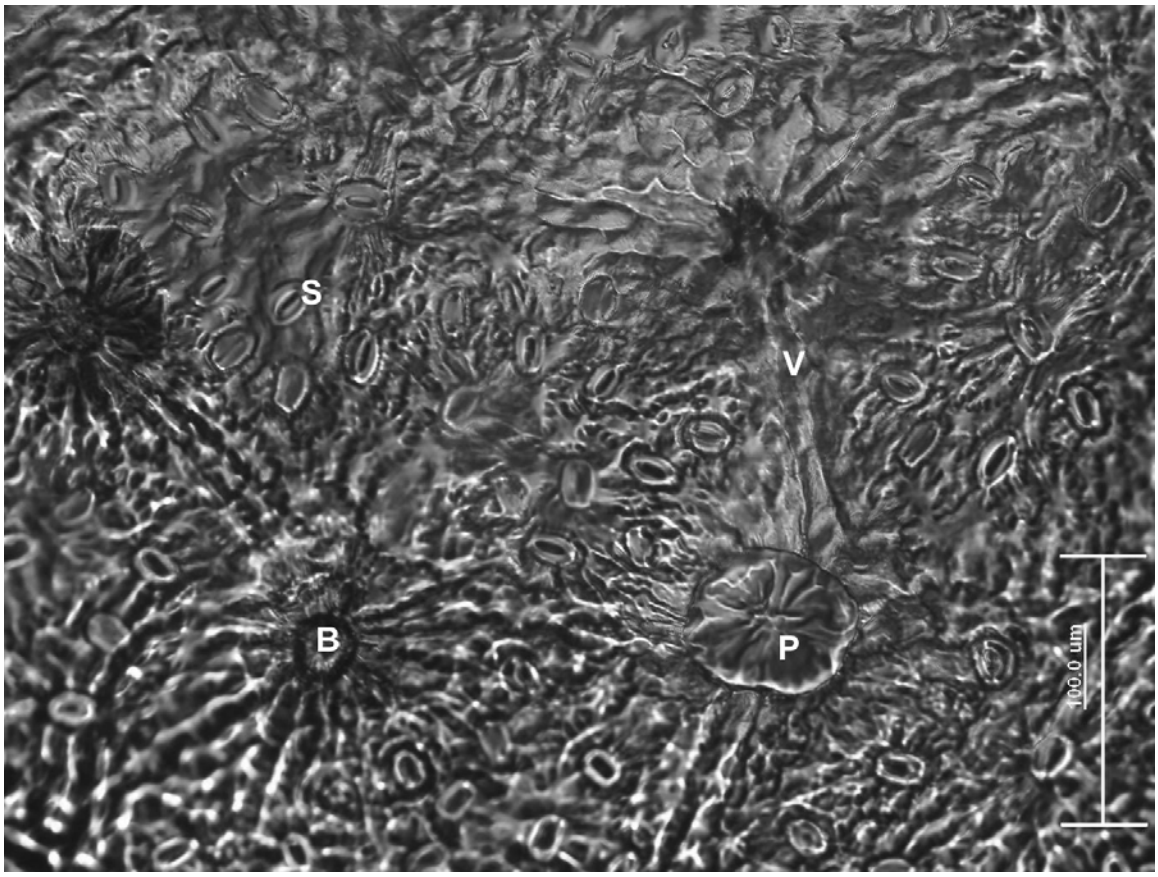

Fig. 4. Types of trichomes observed on the abaxial surface of 'Mohawk' pecan leaves (bar = 100 µm). S, stomata; V, leaf vein; B, bladder trichome; P, concave peltate trichome.

Statistical design and analysis

The experiment was setup as a 3 × 3 factorial (cultivar × location) design. Variability and cultivar differentiation was estimated via analysis of variance (ANOVA), using SAS software (SAS Institute Inc., Cary, N.C.).

Results

Stomatal density differed among the three pecan cultivars investigated (Table 1) but there were no effects of location on SD within a cultivar. ‘Starking Hardy Giant’ (463 stomata/mm^2) had 15% more stomata per leaf area than ‘Pawnee’ (403 stomata/mm^2), and 28% more stomata than ‘Mohawk’ (363 stomata/mm^2) (Table 1). Similar to SD, ED was different among cultivars grown at the same location (Table 1) but it showed no differences across locations. ‘Starking Hardy Giant’ exhibited the least ED of all three cultivars (1413 cells/mm^2), 'Pawnee’ had the greatest (2510 cells/mm^2), while ‘Mohawk’ showed an intermediate value (2210 cells/mm^2) (Table 1). There were large differences in SI between ‘Starking Hardy Giant’ (24.65%) and the other two cultivars at each location (14.06% and 13.86%, in ‘Mohawk’ and ‘Pawnee’, respectively) (Table 1). However, there were no differences across locations within cultivars.

The density of bladder type trichomes in ‘Pawnee’ and ‘Starking Hardy Giant’ was similar at all locations (Table 2). In ‘Mohawk’, the density was greater in leaves from Stillwater and Chetopa than in those from Tifton. At Stillwater and Chetopa, ‘Mohawk’ and ‘Starking Hardy Giant’ displayed the greatest and the least density of bladder type trichomes, respectively. At Tifton, the density of bladder type trichomes in ‘Pawnee’ and ‘Mohawk’ was greater than in ‘Starking Hardy Giant’. The density of concave peltate type trichomes did not change in ‘Mohawk’ across locations (Table 2). In ‘Pawnee’, the density of concave peltate type trichomes at Tifton and Stillwater differed. In ‘Starking Hardy Giant’ the density at Stillwater and Chetopa was greater than at Tifton. At Tifton and Stillwater, there were differences in TD among all three

Table 1. Stomatal density, epidermal cell density, and stomatal index recorded on leaves of pecan cultivars collected from three different locations (Tifton, GA., Chetopa, KS., and Stillwater, OK.). Data are average of 10 microscopy images from each of 10 leaves investigated per cultivar.

Location	Cultivar	Stomatal density (stomata/mm^2)	Epidermal cell density (epidermal cells/mm^2)	Stomatal index (%)
Tifton, GA.	Pawnee	404 b[z]	2,518 a	13.85 b
	Mohawk	363 c	2,201 b	14.05 b
	Starking Hardy Giant	462 a	1,417 c	24.60 a
Stillwater, OK.	Pawnee	406 b	2,501 a	13.97 b
	Mohawk	362 c	2,218 b	14.05 b
	Starking Hardy Giant	463 a	1,409 c	24.72 a
Chetopa, KS.	Pawnee	401 b	2,513 a	13.78 b
	Mohawk	363 c	2,211 b	14.10 b
	Starking Hardy Giant	463 a	1,415 c	24.65 a

[z] Means within same column for a location indicated by different letters are significantly different at $P \leq 0.05$ by Fisher's LSD.
Means within same column for a cultivar are not significantly different at $P \leq 0.05$ by Fisher's LSD and thus mean separation is not indicated.

Table 2. Type and density of trichomes recorded on leaves of pecan cultivars collected from three different locations (Tifton, GA., Chetopa, KS., and Stillwater, OK.). Data are average of 10 microscopy images from each of 10 leaves investigated per cultivar.

		Trichome density (trichomes/mm^2)		
Trichome type	Cultivar	Tifton, GA.	Stillwater, OK.	Chetopa, KS.
Bladder	Pawnee	7.60 a[z]A[y]	9.34 bA	8.83 bA
	Mohawk	6.58 aB	13.76 aA	14.26 aA
	Starking Hardy Giant	2.89 bA	3.11 cA	3.04 cA
Concave peltate	Pawnee	2.82 cB	4.20 bA	3.62 bAB
	Mohawk	6.52 aA	8.25 aA	7.39 aA
	Starking Hardy Giant	4.42 bB	7.02 aA	8.33 aA
Total	Pawnee	10.42 bA	13.54 bA	12.45 bA
	Mohawk	13.10 aA	22.01 aA	21.65 aA
	Starking Hardy Giant	7.31 cB	10.13 cA	11.37 bA

[z] Means within same column indicated by different letters (lower case) are significantly different at $P \leq 0.05$ by Fisher's LSD.
[y] Means within same row indicated by different letters (upper case) are significantly different at $P \leq 0.05$ by Fisher's LSD.

cultivars, with 'Pawnee' showing an intermediate number of trichomes (Table 2). At Tifton, TD was greatest in 'Mohawk' followed by 'Pawnee' and 'Starking Hardy Giant'. At Stillwater, TD in 'Starking Hardy Giant' was less than that in the other two cultivars

(Table 2). At Chetopa, there were no differences between ‘Pawnee’ and ‘Starking Hardy Giant’. ‘Starking Hardy Giant’ was the only cultivar that displayed differences in TD among the locations, with lesser number of trichomes recorded at Tifton than at Stillwater and Chetopa (Table 2).

Discussion

The current results suggest that SD, ED and SI are stable within a pecan cultivar despite ecogeographical differences of the growing sites. Differences between cultivars were maintained across locations, with ‘Pawnee’ showing the greatest ED of the three cultivars and intermediate SD between ‘Mohawk’ and ‘Starking Hardy Giant’. Trichome density is an anatomical characteristic that can be influenced by environmental factors, such as light intensity (Upadhyaya and Furness, 1998) and resource availability (Wilkens et al., 1996). Species might diverge in response to the selection pressure in a specific region, thus resulting in differences in trichome type and density within and between taxa in ecogeographical correlations (Levin, 1973). The types of trichomes and patterns of TD observed in this study varied at the three locations and were different between cultivars.

Glandular trichomes not only represent a physical impediment for aphid movement, but they also secrete sticky exudates (Levin, 1973). Density of trichomes could be related to gradients in abiotic components of the environment, such as solar radiation, and altitude. Glandularity of trichomes is less likely influenced by the environment, since it has negligible effects on the biophysical properties of the leaf surface (Levin, 1973). The glandularity may be a result of long-term predator pressure

and differences in predation from one region to the other (Levin, 1973). Analogous patterns for TD at Chetopa and Stillwater may be the result of similar geographical and environmental conditions or similar predator pressure (Fig. 2).

Stomatal densities have been related to tolerance to abiotic stress conditions, such as drought (Jarvis and Davies, 1998; Van Rensburg et al., 1999) and temperature extremes (Kleinhenz et al., 1995; Nayeem, 1989). However, stomatal response to elevated CO_2 had contrasting results varying from a decrease in SD (Lin et al., 2001; Woodward and Kelly, 1995) to a lack of stomatal acclimation within a single generation in wheat (*Triticum aestivum* L.) and sour oranges (*Citrus aurantium* L.) (Estiarte et al., 1994). In a survey conducted to study the influence of CO_2 concentration on SD of several species grown in controlled environment, Woodword and Kelly (1995) found that changes in SD were generally greater in samples from amphistomatous species than those from hypostomatous species, such as pecan. This indicates that certain species may not show plasticity to environmental changes in a single generation for some ecogeographical traits.

Pecan SD ranged from 363 to 463/mm^2 depending on the cultivar investigated. The values found here were similar to those reported previously for other six pecan cultivars (Giles, Gratex, Greenriver, Major, Peruque, and Western Schley) (288-462 stomata/mm^2) (Nemati and Roberts, 1968) and for walnut (*Juglans regia* L.) (250-450 stomata/mm^2) (Bongi and Paris, 2006), but greater than those reported for other temperate climate trees, such as olive (*Olea europaea* L.) (270-350 stomata/mm^2) and stone pine (*Pinus pinea* L.) (280-345 stomata/mm^2) (Woodward and Kelly, 1995).

This study illustrates distinct differences in epidermal features of the leaf in different cultivars. ‘Pawnee’ originated in 1963 from a controlled cross of ‘Mohawk’ and ‘Starking Hardy Giant’ (Thompson and Hunter, 1985); it was released in 1984 and it is now the most widely planted pecan cultivar (Thompson and Grauke, 2000). ‘Starking Hardy Giant’ is a northern cultivar propagated from a native tree grown in Brunswick, Mo., in 1950 (Grauke and Thompson, 1997). ‘Mohawk’ is a pedigreed cultivar originated in 1946 by controlled cross of two southern cultivars (‘Success’ x ‘Mahan’) by the U.S. Dept. Agr. (Grauke and Thompson, 1997).

The results of the present investigation showed that the values for SD and ED did not change for the same cultivar at different ecogeographical locations. Consequently, the SI remained constant for a cultivar grown in different locations. This indicates that SD may be linked to the long-term climatic conditions of the location where the species (or cultivar) developed, and it may not be a very plastic trait within an individual generation of trees/cultivars. It is of great interest to understand the extent of plasticity of the ecogeographical traits to determine the stability and the possible use of the traits in breeding. In *Arabidopsis thaliana*, SD has been linked to mechanisms of instantaneous water use efficiency (transpiration efficiency) indicating the importance of the trait for plant survival in drought conditions (Masle et al., 2005).

Conclusion

In conclusion, the stability of certain leaf anatomical characteristics, such as SD and ED, for pecan cultivars grown at different locations confirms that these traits can be used for screening ecotypes and provenances for breeding and cultivar development.

CHAPTER III

GEOGRAPHICAL PATTERNS FOR MORPHOLOGY AND PHYSIOLOGY: PECAN PROVENANCES IN NORTH AMERICA

Introduction

Pecan [*Carya illinoinensis* (Wangenh.) C. Koch] is a species distributed across the North American continent and is used for its nuts and timber. Its native range appears to be extending from northern Iowa (42º 20′ N. Lat.) to Oaxaca in Mexico (16º 30′ N. Lat.). While the native distribution is continuous in the United States, in Mexico the native populations are isolated and discontinuously distributed (Thompson, 1991).

An evaluation of the geographic variation of vegetative traits showed the presence of at least two distinct pecan populations in North America; one north of Texas and the other distributed throughout Texas and Mexico (Wood et al., 1998). Most researchers involved in biogeographical studies agree that populations separated by geographic barriers can evolve in different directions if they are separated for longer periods of time (Briggs, 1981). It is also believed that variation in morphology and geographical separation of the populations are required for the formation of subspecies and then species (Losos and Glor, 2003).

Variation in morphology distributed across a geographic gradient reflects phenotypic responses to genetic variation, biogeographic history of the species, and evolution among populations (Ellison et al., 2004). Vavilov (1992) theorized that an area represented by the maximum genetic diversity could be considered the center of origin of a species. This idea itself has undergone changes over the past 50 years and several

scientists have probed into the theory. Vavilov (1992) also suggested that smaller geographic regions may have potential for greater diversity than a large geographic area. These theories have been studied by Harlan (1951) who proposed that the "centers of origin" include smaller geographical regions with great diversity for a crop and can be referred to as "gene microcenters". An understanding of geographic distribution and natural variation patterns are imperative for crop improvement (Bagley, 1980). Vavilov (1992) and Harlan (1951) both stressed the need to study the native forms of cultivated crops and the gene microcenters in order to identify desirable traits, as adaptation tends to occur at a very rapid rate in these distributions.

Very little is known about the intraspecific variation and adaptability of pecan in its native range to identify patterns of natural variation. Hence, it is important to understand the correspondence between geographic patterns of divergence of plant morphological and physiological traits and the distribution of pecan over a geographical gradient.

This study focused on the patterns of geographic distribution of morphological and physiological variation in pecan. The objective/hypothesis was to determine if any variation existed between the provenances for morphological and physiological traits and if a geographical pattern could be established.

Materials and Methods

Plant material

Nineteen provenances from Mexican and U.S. locations were grown at the Pecan Genetics and Breeding Program facility in Somerville, Texas, and selected as treatments for the experiment. The 19 provenances consisted of 13 Mexican and five U.S. locations with two seed sources chosen from the same provenance. Nuts of Mexican provenances were obtained from entries considered to be "native pecans" from the Third Mexican National Nut Conference held at Piedras Negras, Coahuila, Mexico, in November of 1994 (Table 3). The nuts were obtained from open-pollinated trees grown throughout Mexico (Fig. 5).

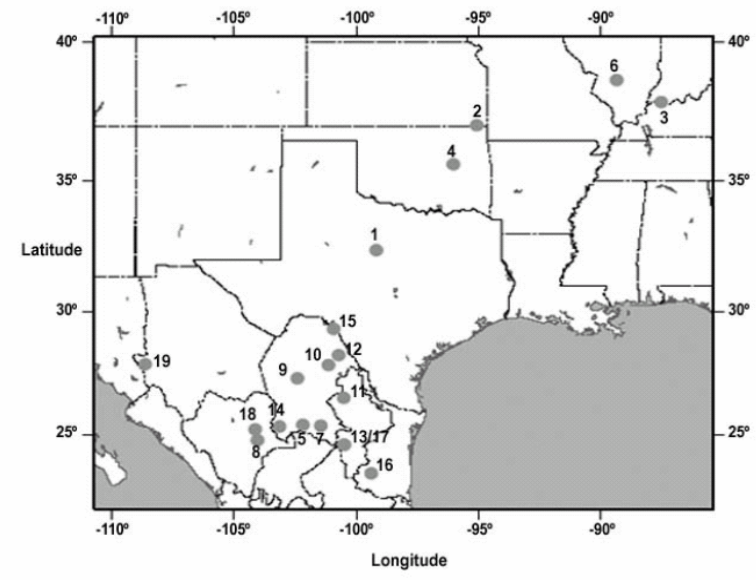

Fig. 5. Provenances of mother trees providing seeds for open-pollinated pecan provenances used in the study to investigate leaf morphological and physiological traits of pecan germplasm. Symbols represent collection sites, while values correspond to provenance numbers, as listed in Table 3.

Table 3. Sites of origin of open-pollinated pecan seeds utilized for the morphological and physiological analyses. Seeds were collected in 1995 and grown in a common orchard in Somerville, Texas.

Provenance	Longitude	Latitude	Location
1	99º16'W	32º37'N	Putnam, Texas
2	95º05'W	37º04'N	Chetopa, KS.
3	87º48'W	37º89'N	Basket, Ky.
4	96º01'W	35º62'N	Okmulgee, OK.
5	102º17'W	25º42'N	Parras, Coahuila, Mexico
6	89º30'W	38º67'N	Keyesport, IL.
7	101º45'W	25º38'N	General Cepeda, Coahuila,
8	104º83'W	24º78'N	Peñon Blanco, Durango,
9	102º40'W	27º31'N	Ocampo, Coahuila, Mexico
10	101º11'W	27º85'N	Sabinas, Coahuila, Mexico
11	100º50'W	26º53'N	Bustamante, Nuevo León,
12	100º71'W	28º25'N	Villa Union, Coahuila,
13	100º48'W	24º60'N	El Carmen, Nuevo León,
14	103º13'W	25º35'N	Zaragoza, Coahuila, Mexico
15	100º91'W	29º30'N	Acuña, Coahuila, Mexico
16	99º38'W	23º41'N	Jaumave, Tamaulipas,
17	100º48'W	24º60'N	El Carmen, Nuevo León,
18	104º13'W	25º23'N	Nazas, Durango, Mexico
19	108º63'W	27º88'N	Saucillo, Chihuahua, Mexico

Nine nuts of each Mexican entry were planted in the greenhouse in 1995, along with open-pollinated nuts collected from trees of cultivars (Burkett, Colby, Dooley, Frutoso, Giles, and Major) already present in repository orchards of the Pecan Genetics and Breeding Program in Brownwood, Texas. The cultivars from repository orchards were chosen to represent commonly-grown seedstocks used in different pecan growing

regions. Seedlings were germinated and grown in a greenhouse at Brownwood and planted at 4.5 × 5.5 m in the test orchard at Somerville in spring of 1997. The orchard was laid out with eight replicate blocks and 175 seedlings per block. At the time of the present investigation, trunk diameter at breast height (130 cm) ranged between 6 cm and 24 cm, depending on the provenance.

For the current study, only three blocks and 19 seedlings were used (the 156 seedlings not used in the present study were obtained from different seed sources). Seedlings for each provenance in the three blocks were derived from nuts obtained from the same seed source. The size of the orchard was 3.52 ha and the soil type varied between the three blocks with Ships soil in one block and Weswood soil in the other two. Ships series of soils are a very deep clay soil, with 0% to 3% slope and are moderately well drained or well drained and nearly leveled or very gently sloping (USDA, 2005). Weswood soils are loamy, well drained, very deep, moderately permeable, nearly level or very gently sloping soils with 0% to 3% slope (USDA, 2005).

Leaf morphology

Morphological measurements (leaf area, leaf fresh weight, and leaflet number) were collected on 10 leaves selected from exterior canopy positions on the north side of the tree in July 2006 at 10 m from the ground. For ease of measurement, weight and area of the leaf rachis was included in the calculations. Average leaf area was measured using a leaf area meter (model 3100; LI-COR, Lincoln, Nebr.) and the leaf fresh weight was measured immediately using a balance. Average leaflet area and fresh weight were

calculated by dividing leaf area and fresh weight, respectively, by the average leaflet number per leaf. Specific leaf area was calculated as the ratio between the leaf area of five leaves and their dry weight (Vile et al., 2005).

Gas exchange

Single-leaf net carbon dioxide assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s) were determined using an open infrared gas exchange system (model 6400; LI-COR, Lincoln, Nebr.). The selected portion of the leaves was irradiated with 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (*PPFD*), supplied by a red/blue light source (model 6400-02B; LI-COR, Lincoln, Nebr.) on the adaxial leaf surface. The ratio between A and E was calculated to indicate the instantaneous water use efficiency (WUE). Gas exchange measurements were collected from each tree on terminal leaflets on three fully-expanded leaves per tree selected from exterior canopy positions on the north side of the tree at 5m from the ground on 14 June and 13 July, 2006.

Chlorophyll fluorescence

Photochemical efficiency of PSII or quantum efficiency was estimated by the expression Fv/Fm, which is the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) (Maxwell and G.N.Johnson, 2000). The quantum efficiency relates the effect of environmental stress on the photosynthetic mechanism.

Chlorophyll a fluorescence measurements were made on two different leaves from the same plant using an open infrared gas exchange system (Model 6400, LICOR,

Lincoln, Nebr.) with an integrated fluorescence head (Model 6400, LICOR, Lincoln, Nebr.) on Aug. 21 2006. One leaf was dark adapted in lightweight cuvettes (Model 9964-061, Li-COR, Lincoln, Nebr.) for 20 minutes before the fluorescence measurement and provided the maximal fluorescence data. The second leaf was light-adapted and provided data pertaining to fluorescence under prevailing environmental conditions. The tip of two fully expanded leaves of each plant was marked with a permanent marker. The reference CO_2 concentration was set at 360μL·L^{-1} while the leaf temperature varied according to the air temperature. Each leaf was illuminated with an extremely dim light (1-2 μmol m^{-2} sec^{-1}) to measure the dark-adapted minimal fluorescence (Fo). Maximum fluorescence (Fm) was measured after illumination with a brief pulse (0.8 sec) of saturating light intensity (6000 ± 25 μmol m^{-2} sec^{-1}). Variable fluorescence (Fv=Fm-Fo) was calculated and provided further calculation of maximal photochemical efficiency or quantum efficiency (Fv/Fm). The light-adapted leaf was used to measure minimum (Fo'), maximum (Fm'), variable fluorescence (Fv'=Fm'-Fo') and steady state fluorescence (Fs) after illumination with light saturating pulse of >6000 μmol m^{-2} s^{-1} (Genty et al., 1987). We expect that the relative fluorescence measurements Fv/Fm and Fv'/Fm' will provide information about the tolerance to drought.

Leaf chlorophyll fluorescence parameters (photosynthetic efficiency, photosynthetic yield, and photochemical quenching) were determined on light-adapted leaves using the same gas exchange system used for gas exchange measurements with an integrated fluorescence chamber head (model 6400-40; LI-COR, Lincoln, Nebr.) on 20 July 2006. Chlorophyll fluorescence measurements were collected on the adaxial leaf

surface of the terminal leaflets on two fully-expanded leaves per tree selected from exterior canopy positions on the north side of the tree at 5m from the ground.

Statistical design and analysis

The test was composed of three randomized complete blocks, each having one seedling from each of the 19 entries. Variability and provenance differentiation was estimated via analysis of variance (ANOVA), using SAS software (SAS Institute Inc., Cary, N.C.). ANOVA was structured to allow unequal replications for each provenance due to tree death.

Results

All the morphological traits measured (leaflet number, leaf area, and leaf fresh weight) showed differences between the provenances (Table 4). There was a noticeable overlap in leaflet number in most provenances without any prominent differences between them. However, the number of leaflets in provenance 8 was smaller than in all other provenances. Provenances 5, 6 had the greatest and provenance 8 had the least leaflet number (Table 4). Leaf area was similar in most provenances and few differences were observed. Leaf area was the greatest in provenance 7 and the least in provenance 8 (Table 4). Leaf fresh weight was the greatest in provenance 16 and the lowest in provenances 12 and 8 (Table 4). The correlations between leaf fresh weight and leaflet number ($r= 0.31$) (Fig. 6), leaf fresh weight and leaf area ($r= 0.20$) (Fig. 7), leaf area and leaflet number ($r= 0.07$) (Fig. 8) were positive but weak.

Table 4. Mean leaflet number, leaf area, and leaf fresh weight of pecan provenances obtained from various locations in Mexico and the United States.

Provenance	Leaflet number	Leaf area (cm^2)	Leaf fresh weight (g)
1	11.6 ± 0.74 bcd[z]	35.54 ± 3.25 abc	6.40 ± 0.74 bcd
2	13.3 ± 0.74 abc	29.84 ± 3.25 bcde	6.46 ± 0.74 bcd
3	13.3± 0.74 abc	30.57 ± 3.25 bcd	7.78 ± 0.74 ab
4	12.9 ± 0.74 abc	30.72 ± 3.25 bcd	7.28 ± 0.74 abc
5	14.3 ± 0.74 a	27.22 ± 3.25 cde	6.62 ± 0.74 bcd
6	14.1 ± 0.74 a	37.89 ± 3.25 ab	8.21 ± 0.74 ab
7	12.6 ± 0.74 abc	42.44 ± 3.25 a	7.96 ± 0.74 ab
8	9.9 ± 0.90 d	18.44 ± 3.98 e	4.62 ± 0.91 d
9	12.6 ± 0.90 abc	29.92 ± 3.98 bcde	7.32 ± 0.91 abc
10	13.4 ± 0.90 abc	28.38 ± 3.98 bcde	4.95 ± 0.91 cd
11	13.7 ± 0.90 abc	31.00 ± 3.25 bcd	7.19 ± 0.74 abcd
12	12.8 ± 0.74 abc	26.46 ± 3.25 cde	4.75 ± 0.74 d
13	14.0 ± 0.90 ab	32.31 ± 3.98 abcd	6.50 ± 0.91 bcd
14	11.6 ± 0.74 cd	30.98 ± 3.25 bcd	5.31 ± 0.74 cd
15	12.4 ± 0.74 abc	33.81 ± 3.25 abc	6.45 ± 0.74 bcd
16	14.0 ± 0.74 ab	35.57 ± 3.25 abc	9.23 ± 0.74 a
17	14.0 ± 0.74 ab	29.52 ± 3.25 bcde	5.14 ± 0.74 cd
18	11.6 ± 0.90 bcd	22.76 ± 3.98 de	4.98 ± 0.91 cd
19	12.8 ± 0.74 abc	31.49 ± 3.25 bcd	7.03 ± 0.74 bcd

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

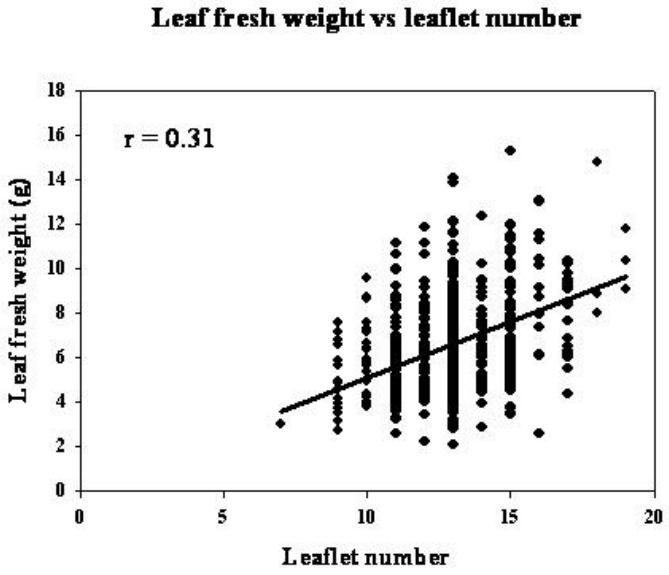

Fig. 6. Relationship between leaf fresh weight and leaflet number of pecan provenances from across Mexico and the United States grown in Somerville, Texas.

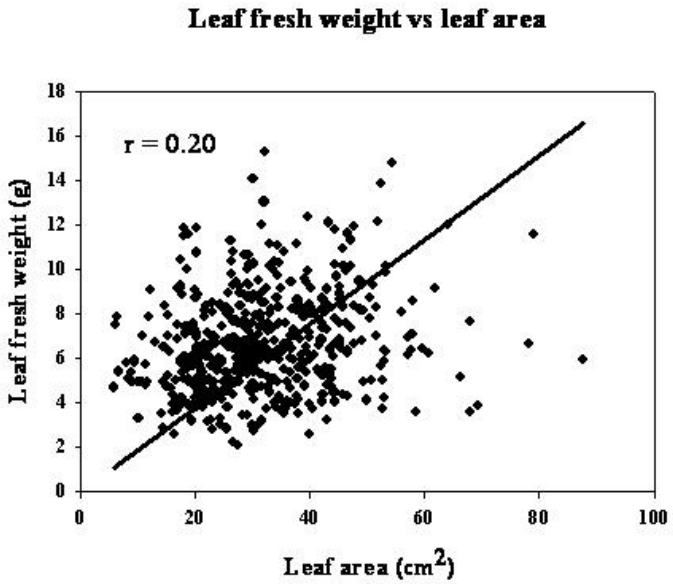

Fig. 7. Relationship between leaf fresh weight and leaf area of pecan provenances from across Mexico and the United States grown in Somerville, Texas.

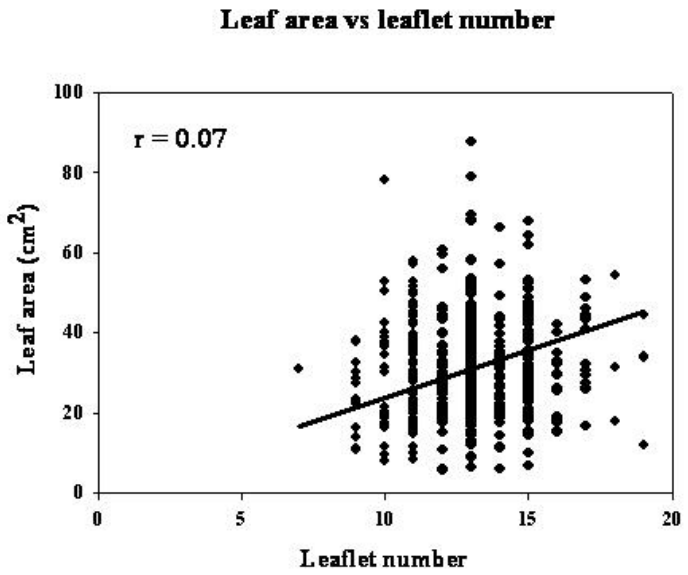

Fig. 8. Relationship between leaf area and leaflet number of pecan provenances from across Mexico and the United States grown in Somerville, Texas.

When leaf area was calculated on a leaflet basis (Table 5), provenance 7 had the greatest value and provenances 5, 8, 12, 17, and 18 had the smallest values. Calculated leaflet fresh weight was greatest in provenance 16 and smallest in provenance 17 (Table 5). There was a prominent overlap in specific leaf area, with provenances 1 and 11 exhibiting the greatest specific leaf area, while provenances 5 and 16 showed the least values (Table 6). Leaves of provenance 17 had the greatest fresh/dry weight ratio of all other provenances (Table 6). The correlations between leaflet fresh weight and leaflet area (r= 0.23) (Fig. 9), and fresh/dry weight ratio and specific leaf area (r= 0.15) (Fig. 10) were positive but weak.

Table 5. Mean leaflet area and mean leaflet fresh weight of pecan provenances obtained from various locations in Mexico and the United States.

Provenance	Leaflet area (cm^{-2})	Leaflet weight (g)
1	3.09 ± 0.30 ab[z]	0.55 ± 0.05 abc
2	2.28 ± 0.30 bc	0.47 ± 0.05 bcde
3	2.33 ± 0.30 bc	0.59 ± 0.05 abc
4	2.37 ± 0.30 bc	0.55 ± 0.05 abc
5	1.91 ± 0.30 c	0.46 ± 0.05 cde
6	2.71 ± 0.30 abc	0.58 ± 0.05 abc
7	3.43 ± 0.30 a	0.63 ± 0.05 ab
8	1.90 ± 0.37 c	0.45 ± 0.06 cde
9	2.43 ± 0.37 bc	0.58 ± 0.06 abc
10	2.13 ± 0.37 bc	0.37 ± 0.06 de
11	2.25 ± 0.37 bc	0.52 ± 0.06 abcde
12	2.08 ± 0.30 c	0.37 ± 0.05 de
13	2.41 ± 0.37 bc	0.47 ± 0.06 bcde
14	2.67 ± 0.30 abc	0.45 ± 0.05 cde
15	2.81 ± 0.30 abc	0.53 ± 0.05 abcd
16	2.58 ± 0.30 abc	0.66 ± 0.05 a
17	2.12 ± 0.30 c	0.36 ± 0.05 e
18	1.95 ± 0.37 c	0.42 ± 0.06 cde
19	2.48 ± 0.30 bc	0.54 ± 0.05 abcd

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Table 6. Specific leaf area and fresh/dry weight of leaves of pecan provenances obtained from various locations in Mexico and the United States.

Provenance	Specific leaf area ($cm^{-2} \cdot g^{-1}$)	Fresh/dry weight ($g \cdot g^{-1}$)
1	12.50± 1.44 a[z]	1.92 ± 0.18 b
2	8.33 ±1.44 ab	1.96 ± 0.18 b
3	9.16 ± 1.44 ab	1.92 ± 0.18 b
4	8.65 ± 1.44 ab	2.01 ± 0.18 b
5	7.97 ± 1.44 b	2.04 ± 0.18 b
6	9.60 ±1.44 ab	1.87 ± 0.18 b
7	11.89 ± 1.44 ab	1.94 ± 0.18 b
8	8.50 ± 1.77 ab	2.01 ± 0.22 b
9	8.78 ± 1.77 ab	2.01 ± 0.22 b
10	11.11 ± 1.77 ab	2.32 ± 0.22 b
11	12.76 ± 1.77 a	2.03 ± 0.22 b
12	12.02 ± 1.44 ab	1.98 ±0.18 b
13	10.10 ±1.77 ab	1.97 ± 0.22 b
14	11.72 ± 1.44 ab	1.97 ± 0.18 b
15	10.12 ± 1.44 ab	2.11 ± 0.18 b
16	8.00 ± 1.44 b	1.77 ± 0.18 b
17	10.80 ± 1.44 ab	2.67 ± 0.18 a
18	10.34 ± 1.77 ab	2.00 ± 0.22 b
19	8.80 ± 1.44 ab	1.96 ± 0.18 b

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

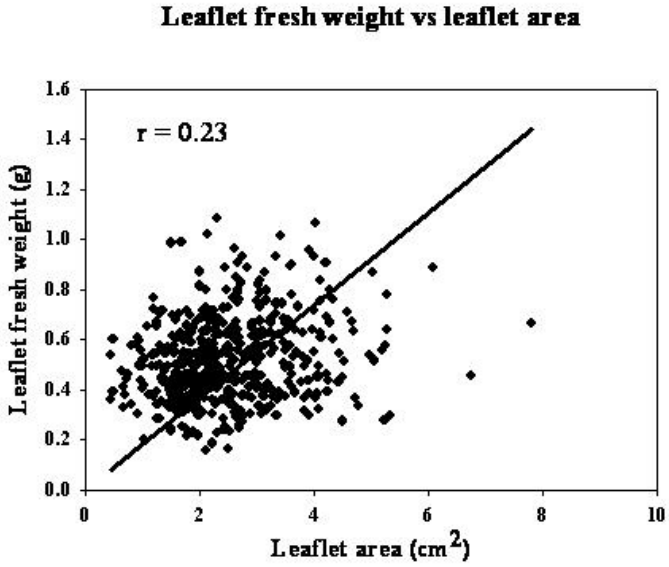

Fig. 9. Relationship between leaflet fresh weight and leaflet area of pecan provenances from across Mexico and the United States grown in Somerville, Texas.

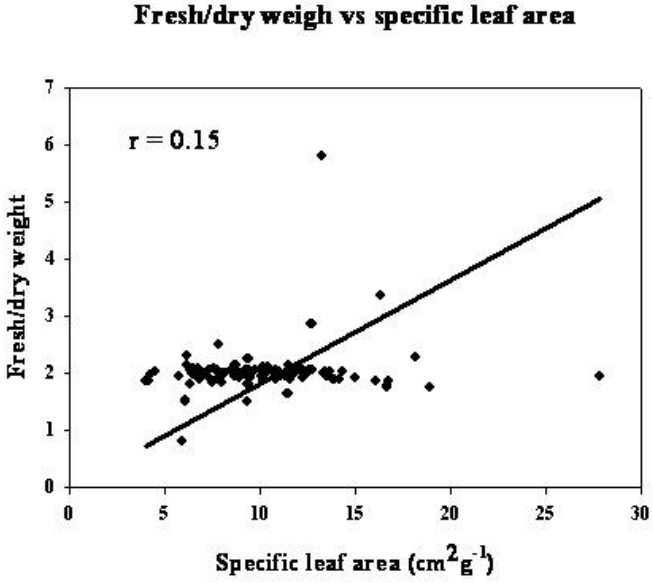

Fig. 10. Relationship between fresh/dry weight ratio and specific leaf area of pecan provenances from across Mexico and the United States grown in Somerville, Texas.

Table 7. Mean gas exchange measurements of pecan provenances from across Mexico and the United States grown in Somerville, Texas as recorded on 14 June 2006.

Provenance	Mean Net CO_2 assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)	Transpiration rate ($mmol \cdot m^{-2} \cdot s^{-1}$)	Instantaneous water use efficiency ($\mu mol \cdot mmol^{-1}$)
1	2.70 ± 1.79 a[z]	0.01 ± 0.008 a	0.85 ± 0.54 a	3.23 ± 0.76 a
2	2.25 ± 1.79 a	0.01 ± 0.008 a	1.32 ± 0.54 a	1.31± 0.76 b
3	2.82 ± 1.84a	0.02 ± 0.008 a	1.26 ± 0.54 a	2.50 ± 0.78 ab
4	5.59 ± 1.84a	0.03 ± 0.008 a	1.86 ± 0.54 a	3.30 ± 0.78 a
5	2.93± 1.79 a	0.01 ± 0.008 a	1.15 ± 0.54 a	2.18 ± 0.76 ab
6	5.20 ± 1.79 a	0.03 ±0.008 a	1.96 ± 0.54 a	2.60 ±0.76 ab
7	3.47 ± 1.84 a	0.01 ± 0.008 a	1.27 ± 0.54 a	2.00 ± 0.78 ab
8	2.00 ± 2.40 a	0.01 ± 0.01 a	0.99 ± 0.77 a	1.95 ± 0.87 ab
9	3.71 ± 2.40 a	0.02 ± 0.01 a	1.25 ± 0.77 a	2.65 ±0.87 ab
10	1.57 ± 2.94 a	0.01 ± 0.01 a	0.79 ± 0.77 a	1.37 ±1.20 ab
11	Not available	Not available	Not available	Not available
12	6.63 ± 1.79 a	0.03 ±0.008 a	2.46 ± 0.54 a	2.35 ±0.76 ab
13	5.72 ± 1.79 a	0.03 ±0.008 a	2.07 ± 0.54 a	2.63 ±0.76 ab
14	3.62 ± 1.79 a	0.02± 0.008 a	1.47 ± 0.54 a	2.36 ±0.76 ab
15	5.30 ± 1.95 a	0.01 ± 0.008 a	1.06 ± 0.54 a	3.58 ± 0.82 a
16	2.76 ± 1.79 a	0.01 ± 0.008 a	0.92 ± 0.54 a	3.05 ± 0.76 a
17	2.03 ± 1.79 a	0.01± 0.008 a	0.79 ± 0.54 a	2.76 ±0.76 ab
18	2.89 ± 2.40 a	0.01 ± 0.01 a	1.05 ± 0.77 a	2.88 ± 0.87 ab
19	6.01 ±1.84 a	0.03 ± 0.008 a	2.14 ± 0.54 a	2.32 ±0.78 ab

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Table 8. Mean gas exchange measurements of pecan provenances from across Mexico and the United States grown in Somerville, Texas as recorded on 15 July 2006.

Provenance	Mean $NetCO_2$ assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)	Transpiration rate ($mmol \cdot m^{-2} \cdot s^{-1}$)	Instantaneous water use efficiency ($\mu mol \cdot mmol^{-1}$)
1	6.95 ± 1.68 abc[z]	0.07 ± 0.03 ab	2.10 ± 0.77 a	3.62 ± 0.45 a
2	10.05 ± 1.68 a	0.11 ± 0.03 a	3.21 ± 0.77 a	3.32 ± 0.45 a
3	7.77 ± 1.68 abc	0.07 ± 0.03 ab	2.57 ± 0.77 a	3.20 ± 0.45 a
4	7.27 ± 1.68 abc	0.07 ±0.03 ab	2.29 ± 0.77 a	3.20 ± 0.46 a
5	7.48 ± 1.68 abc	0.09 ±0.03 ab	2.92 ± 0.77 a	2.75 ± 0.45 a
6	4.85 ± 1.68 c	0.03 ± 0.03 b	1.47 ± 0.77a	3.65 ± 0.45 a
7	5.35 ± 1.68 bc	0.06 ± 0.03 ab	1.87 ± 0.77 a	3.59 ± 0.45 a
8	5.81 ± 1.93 a	0.09 ± 0.03 ab	2.72 ± 0.88 a	2.59 ± 0.52 a
9	5.95 ± 1.93 abc	0.05 ± 0.03 ab	1.79 ± 0.88 a	3.67 ± 0.50 a
10	7.70 ± 1.93 abc	0.08 ± 0.03 ab	2.96 ± 0.88 a	2.56 ± 0.52 a
11	6.89 ± 1.93 abc	0.09 ± 0.03 ab	2.69 ± 0.88 a	2.88 ± 0.52a
12	6.02 ± 1.68 abc	0.05 ± 0.03 ab	1.97 ± 0.77 a	3.13 ± 0.45 a
13	7.90 ± 2.54 abc	0.09 ± 0.04 ab	3.48 ± 1.15 a	2.40 ± 0.70 a
14	5.35 ± 1.68 bc	0.05 ± 0.03 ab	1.76 ± 0.77 a	3.38 ± 0.45 a
15	6.98 ± 1.68 abc	0.08 ± 0.03 ab	2.66 ± 0.77 a	3.06 ± 0.45 a
16	7.73 ± 1.68 abc	0.08 ± 0.03 ab	2.73 ± 0.77 a	3.09 ± 0.45 a
17	4.21 ± 1.68 c	0.03 ± 0.03 b	1.39 ± 0.77 a	3.26 ± 0.45a
18	7.50 ± 1.93 abc	0.09 ± 0.03 ab	3.12 ± 0.88 a	2.39 ± 0.52 a
19	8.85 ± 1.68 ab	0.11 ± 0.03 a	3.23 ± 0.77 a	2.98 ± 0.45 a

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Net CO_2 assimilation rate, stomatal conductance, and transpiration did not show any significant differences between the provenances in June (Table 7). However, there were differences in the June WUE with the greatest values recorded in provenance 1, 4, 15 and 16, and the least in provenance 2 (Table 7). All other provenances had similar WUE values in June (Table 7). In July, net CO_2 assimilation rate in provenances 2 and 8 was greater than in provenances 6, 7, 14, and 17 (Table 8). Stomatal conductance in July was greatest in provenances 2 and 19 and least in provenance 6 and 17. In July, both net CO_2 assimilation rate and stomatal conductance were similar for majority of the provenances (Table 8). Transpiration and WUE in July did not show any differences between the provenances (Table 8).

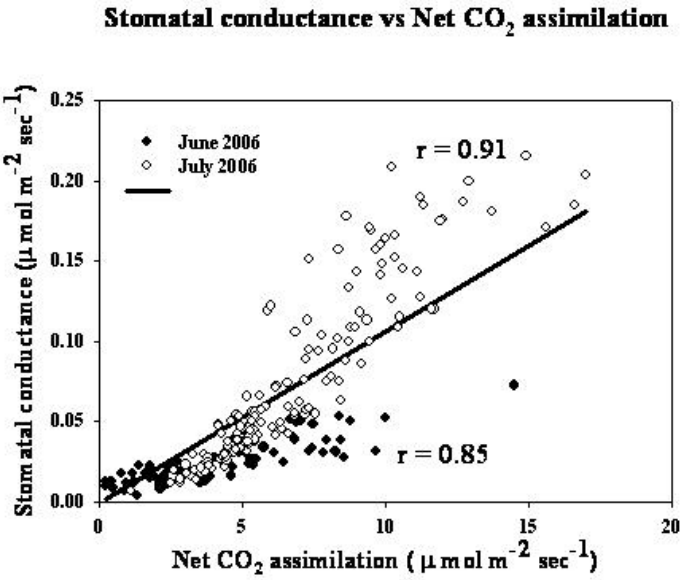

Fig. 11. Relationship between stomatal conductance and net CO_2 assimilation rate of pecan provenances from across Mexico and the United States grown in Somerville, Texas as collected on 14 June 2006 and 13 July 2006.

A positive and strong correlation existed between stomatal conductance and net CO_2 assimilation rate [r = 0.86 and r = 0.91, in June and July (Fig. 11), respectively]. The correlation between transpiration and net CO_2 assimilation was also positive and strong [r = 0.88 and r = 0.89, in June and July (Fig. 12), respectively].

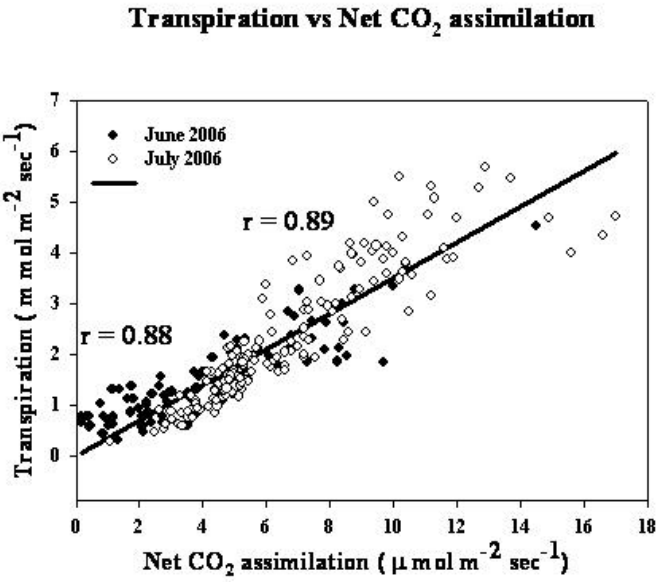

Fig. 12. Relationship between transpiration and net CO_2 assimilation rate of pecan provenances from across Mexico and the United States grown in Somerville, Texas as collected on 14 June 2006 and 13 July 2006.

The correlation between WUE and net CO_2 assimilation was positive and moderately strong in June (r = 0.52) (

Fig. 13), and it was negative and moderately weak in July (r =− 0.42) (Fig. 13).As expected, the correlation between transpiration and stomatal conductance was positive and strong [r = 0.98 and r = 0.96, in June and July (Fig. 14), respectively].The correlation between water use efficiency and stomatal conductance was positive but very

weak in June (r = 0.13) (Fig. 15), and it was negative and moderately strong in July (r = −0.60) (Fig. 15).

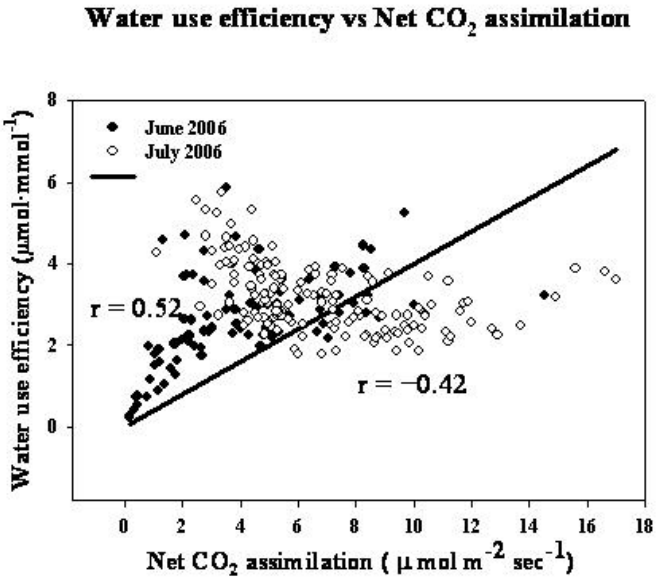

Fig. 13. Relationship between water use efficiency and net CO_2 assimilation rate of pecan provenances from across Mexico and the United States grown in Somerville, Texas as collected on 14 June 2006 and 13 July 2006.

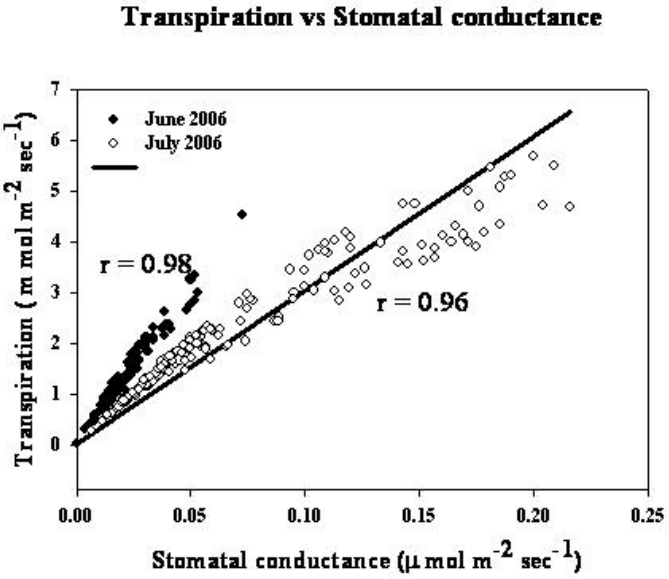

Fig. 14. Relationship between transpiration and stomatal conductance of pecan provenances from across Mexico and the United States grown in Somerville, Texas as collected on 14 June 2006 and 13 July 2006.

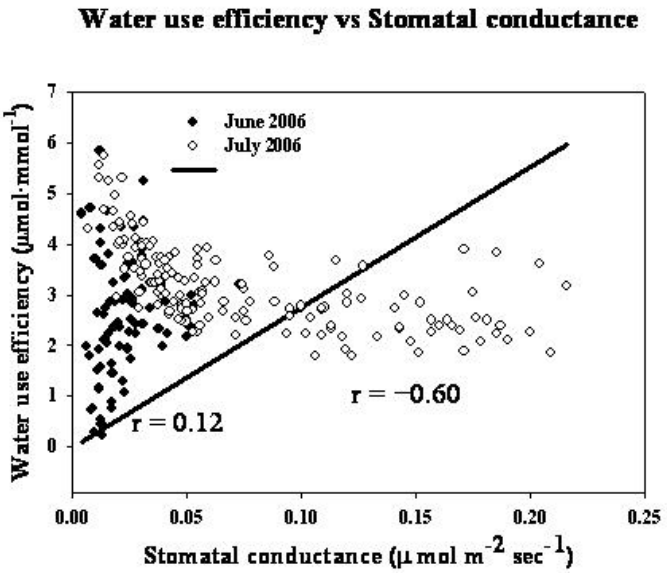

Fig. 15. Relationship between water use efficiency and stomatal conductance of pecan provenances from across Mexico and the United States grown in Somerville, Texas as collected on 14 June 2006 and 13 July 2006.

The correlation between water use efficiency and transpiration was weak but positive in June (r = 0.13) (Fig. 16) and it was negative and strong in July (r = −0.70) (Fig. 16).

Photosynthetic efficiency, as determined with the analysis of chlorophyll fluorescence measurements, differed among the provenances in July (Table 9) as well as in August (Table 10). Even though a consistent trend was not observed to coincide with the data collected in July and August, provenance 19 from the western coast of Mexico showed greater photosynthetic efficiency on both the collection dates as compared to the other provenances.

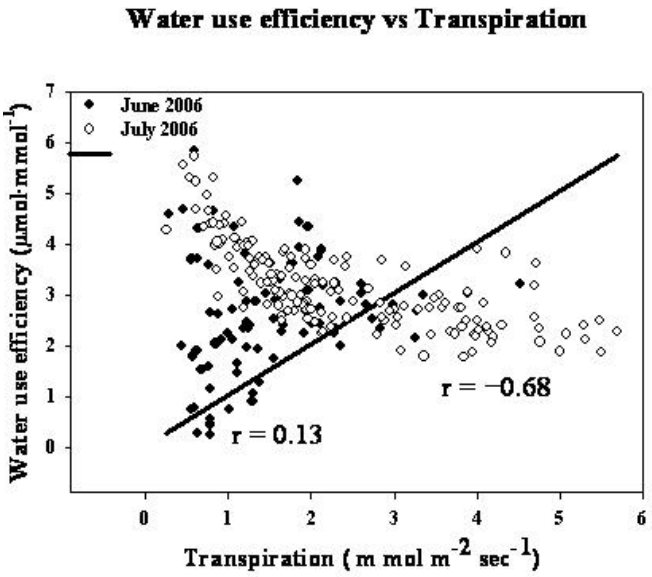

Fig. 16. Relationship between water use efficiency and transpiration of pecan provenances from across Mexico and the United States grown in Somerville, Texas as collected on 14 June 2006 and 13 July 2006.

There were no differences in the photosynthetic yield in July (Table 9); in August differences were found only between provenances 6, 9 and 17 with provenance 6 and 9 exhibiting the greatest and provenance 17 the least value (Table 10). There was an overlap in quantum efficiency for provenances 1, 3,6,9,12,15 and 17 for the measurement in August, no significant differences were observed between the other provenances (Table 10).The correlation between photosynthetic efficiency and photosynthetic yield in July (r = 0.09) was positive and very weak (Fig. 17). Photosynthetic efficiency and photosynthetic yield showed a negative but weak correlation (r = −0.04) in August (Fig. 17).

Table 9. Mean fluorescence measurements of pecan provenances from across Mexico and the United States grown in Somerville, Texas as recorded on 20 July 2006.

Provenance	Photosynthetic efficiency	Photosynthetic yield
1	0.21 ± 0.06 cd[z]	0.004 ± 0.003 a
2	0.30 ± 0.05 abc	0.005 ± 0.002 a
3	0.26 ± 0.05 bcd	0.005 ± 0.002 a
4	0.28 ± 0.05 bcd	0.004 ± 0.002 a
5	0.27 ± 0.05 bcd	0.004 ± 0.002 a
6	0.24 ± 0.05 bcd	0.004 ± 0.002 a
7	0.17 ± 0.05 d	0.004 ± 0.002 a
8	0.24 ± 0.06 bcd	0.005 ± 0.003 a
9	0.18 ± 0.06 cd	0.003 ± 0.003 a
10	0.22 ± 0.06 bcd	0.004 ± 0.002 a
11	0.21 ± 0.06 bcd	0.005 ± 0.002 a
12	0.31 ± 0.06 abc	0.004 ± 0.002 a
13	0.26 ± 0.05 bcd	0.004 ± 0.002 a
14	0.20 ± 0.05 cd	0.004 ± 0.002 a
15	0.22 ± 0.05 bcd	0.005 ± 0.003 a
16	0.34 ± 0.06 ab	0.006 ± 0.003 a
17	0.24 ± 0.05 bcd	0.011 ± 0.002 a
18	0.24 ± 0.06 bcd	0.004 ± 0.003 a
19	0.36 ± 0.05 a	0.006 ± 0.002 a

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Table 10. Mean fluorescence measurements of pecan provenances from across Mexico and the United States grown in Somerville, Texas as recorded on 21 August 2006.

Provenance	Photosynthetic efficiency	Photosynthetic yield	Quantum efficiency
1	0.22 ± 0.01 ab[z]	0.009 ± 0.003 ab	0.79 ± 0.019 ab
2	0.20 ± 0.01 abcd	0.009 ± 0.003 ab	0.80 ± 0.019 a
3	0.19 ± 0.01 abcd	0.007 ± 0.003 ab	0.80 ± 0.019 ab
4	0.21 ± 0.01 abc	0.009 ± 0.003 ab	0.80 ± 0.019 a
5	0.20 ± 0.01 abcd	0.006 ± 0.003 ab	0.81 ± 0.019 a
6	0.16 ± 0.01 bcde	0.011 ± 0.003 a	0.79 ± 0.019 ab
7	0.15 ± 0.01 cde	0.007 ± 0.003 ab	0.80 ± 0.019 a
8	0.24 ± 0.02 a	0.008 ± 0.004 ab	0.83 ± 0.028 a
9	0.18 ± 0.02 abcde	0.011 ± 0.004 a	0.80 ± 0.028 ab
10	0.16 ± 0.02 bcde	0.006 ± 0.004 ab	0.81 ± 0.028 a
11	Not available	Not available	Not available
12	0.18 ± 0.01 abcde	0.007 ± 0.003 ab	0.78 ± 0.019 ab
13	0.22 ± 0.01 ab	0.008 ± 0.003 ab	0.80 ± 0.019 a
14	0.20 ± 0.01 abcd	0.01 ± 0.003 ab	0.80 ± 0.019 a
15	0.14 ± 0.01 de	0.008 ± 0.003 ab	0.79 ± 0.019 ab
16	0.15 ± 0.01 de	0.009± 0.003 ab	0.73 ± 0.019 b
17	0.14 ± 0.01 e	0.006 ± 0.003 b	0.76 ± 0.019 ab
18	0.21 ± 0.02 abcd	0.009 ± 0.004 ab	0.81 ± 0.028 a
19	0.24 ± 0.01 a	0.008 ± 0.003 ab	0.82 ± 0.019 a

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

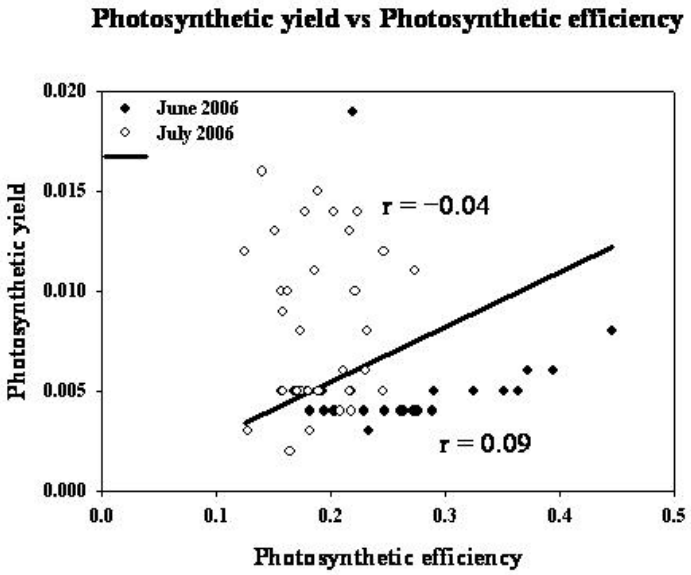

Fig. 17. Relationship between photosynthetic yield and photosynthetic efficiency of pecan provenances from across Mexico and the United States grown in Somerville, Texas. Data were collected on 20 July 2006 and 21 August 2006.

Discussion

Provenances can be represented either by complex groups, differing in both morphological and physiological characteristics, or by groups with more or less equivalent characteristics (Vavilov, 1992). According to Vavilov (1992), a species is represented by a complicated morphological system at the subspecies level which is the result of the origin of the subspecies within a specific environment and distribution area. The study of morphological differences among populations and provenances is a primary step in determining the characteristics and relative significance of the evolutionary forces promoting or inhibiting differentiation (Dominguez et al., 1998).

The morphological variation observed in the current study did not show a geographical pattern. Similar results indicating the absence of a geographic pattern were obtained in a study conducted on intra- and interpopulation variation for flower morphology in twelve populations of the red mangrove, *Rhizophora mangle* L. in Mexico (Dominguez et al., 1998). The disparity and variation in leaf weight, leaf area and leaflet number between pecan provenances in a localized area (central Mexico) can be explained by the geographical and climatic differences existing in the region. Provenance 8, which displayed the least values for leaf area, leaflet number and leaf fresh weight, was obtained from Peñon Blanco, which is located on the west coast of central Mexico. Conversely, provenance 16, which had the greatest leaf fresh weight and higher range of values for leaflet number and leaf area, was obtained from Jaumave, located on the eastern coast of central Mexico. In a previous study conducted to elucidate variation in pecan provenances, the Jaumave provenance displayed important vegetative traits such as the largest trunk diameters, longest foliation period, and tallest trees (Wood et al., 1998). It is of utmost importance to acknowledge the corroboration of the results obtained from the present study with the results obtained by Wood et al. (1998). Results from both the studies indicate that provenances from the eastern coast of Mexico are adapted to conditions where water is not limiting.

However, it is interesting to note that the contrast between provenances 8 and 16, which exhibited the two extreme ends of the variation pattern, was obtained between 23º N and 25º N of latitude. The gradient between arid, water limiting conditions on the west to the wetter conditions on the east in North America could be a possible

explanation for the trend observed. Also, there is a great possibility that the geographic area between 23º N and 25º N of latitude represents a 'gene microcenter' for morphological traits (Harlan, 1951).

Physiological and morphological adaptive responses to water deficit may cause changes in plant structure and functions resulting in variation in growth rate, water-use efficiency, osmotic potential of the tissue and stomatal conductance (Jones, 1992). In a study conducted on provenances of *Eucalyptus microtheca* F.J. Muell., seedlings from more arid environments produced leaf dry mass/turgid mass ratios favorable for drought resistance compared to provenances from mesic environments when exposed to drought conditions (Li, 1998). Li (2000) also found that a positive correlation exists in *E. microtheca* provenances between mean driest quarter rainfall and total biomass, height, transpiration, and specific leaf area. Moreover, provenances from drier environments had a larger foliage/stem area ratio, lower transpiration rates, and shorter hydraulic pathways (Li, 2000). On the other hand, the physiological traits observed in the current study neither concretely propose a trend nor indicate a microcenter population that can be put to further scrutiny. Andersen (1991) indicated that net CO_2 assimilation rate and stomatal conductance were not strongly coupled in pecan leaves. However, in the present study a very strong relationship was observed between the physiological traits on both the collection dates. It has been well documented that physiological traits are very plastic and change in response to the environment (Scheiner, 1993). The results presented here indicate that the physiological traits exhibit greater phenotypic plasticity as compared to the morphological traits. Nevertheless, provenance 19 from Saucillo on

the west coast, an arid environment, displayed greatest photosynthetic efficiency whereas provenances from humid environments on the eastern coast of Mexico displayed the lowest photosynthetic efficiency values. There were no significant differences between provenances for photosynthetic yield and quantum efficiency. This could be an indication that there are no prominent differences in relation to drought tolerance among the provenances. Dominguez et al. (1998) indicated that the lack of phenotypic differentiation among populations for a trait could be a result of (1) "a recent origin of the populations", (2) "high rates of gene flow between populations" , or (3) "natural selection favoring similar phenotypes in each population" (Dominguez et al., 1998). Since pecan is an open-pollinated species there is definitely a high rate of gene flow between populations and provenances in addition to the phenotypic plasticity of the physiological parameters.

The pattern of variation between provenances 13 and 17 from El Carmen on the eastern coast in central Mexico was very interesting. Both the provenances exhibited similar morphological features with values of leaflet number on the higher end and those of leaf area and leaf fresh weight towards the center of the distribution of the parameters. The physiological parameters however, showed phenotypic plasticity in contrast to the overlap in morphological traits. Dominguez et al. (1998) indicate that intense natural selection may favor "different phenotypes in each provenance in response to differences in selective regimes among localities".

Conclusion

It can be concluded that the physiological response for photosynthetic efficiency of provenances from drier environments on the western coast of Mexico is greater than those on the eastern coast. This can be attributed to the adaptation of the provenances to harsher and drier environments. However, the morphological traits show a promise of varied phenotypic differentiation in localized centers between 23º N and 25º N of latitude. Further scrutiny of specific provenances from the localized area using a multivariate approach may provide detailed information about the interaction of the morphological traits with each other as well as the environment. Such an intensive survey to study trends of microcenter provenances could yield very important information to plant breeders (Harlan, 1951).

CHAPTER IV

PROVENANCE VARIATION IN ANATOMY AND CARBON ISOTOPE DISCRIMINATION OF PECAN PROVENANCES

Introduction

Pecan is a species distributed over a wide area of geographic and climatic variation. The native range of pecan appears to extend about 26º in latitude from northern Iowa (42º 20′ N. Lat.) to Oaxaca in Mexico (16º 30′ N. Lat.) (Thompson and Grauke, 1991a). There is a great possibility that pecan provenances are adapted to their native habitat in their structure and function. It is important to acknowledge that understanding a simple plant function (physiology) requires a detailed study of the structure at the cellular or organ level (anatomy) (Taiz and Zeiger, 1998). It is imperative to study the anatomy and physiology of plants to completely understand the dynamics of the variation patterns at the provenance level.

Studies conducted on ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) reported intraspecific variation for anatomical and physiological traits such as stomatal density and gas exchange (Cregg, 1993; Cregg, 1994). Anatomical studies have also been conducted on leaf characteristics of several flowering plants, including species of the Juglandaceae family. According to a review done by Grauke et al. (1987), leaf surface features of pecan were studied by Parmentier (1911) and Nagel (1914), following which a detailed study on the leaf surface features of juvenile and adult pecan leaves was conducted (Grauke et al., 1987). Meyer and Meola (1978) reported singular

and multiple hairs on pecan leaf surfaces. Wetzstein and Sparks (1983) found glandular trichomes on both adaxial and abaxial surfaces of pecan leaves. They also reported that "awn-shaped hairs" were found less frequently. Hardin and Stone (1984) studied the surface characteristics of several interspecific hybrids of *Carya* spp. in North America and reported six types of trichomes. The trichomes were divided into two groups and defined as glandular and non-glandular trichomes (Hardin and Stone, 1984). The non-glandular group of trichomes consisted of acicular, fasciculate, multiradiate and solitary trichomes. On the other hand, glandular trichomes consisted of capitate glands and peltate scales (Hardin and Stone, 1984). Capitate glands were described as stalked trichomes, with the stalks consisting of three cells (Grauke et al., 1987). Grauke et al. (1987) further described the peltate scales as two different types, concave peltate scales and bladder, or vesicular scales. While the studies reported so far focused on leaf surface characteristics, Nemati and Roberts (1968) studied the stomatal density of various pecan cultivars and reported that differences existed between cultivars for the stomatal density (i.e., number of stomata per unit leaf area) (SD).

According to Jones (1998), features of leaf surface anatomy are a complex of traits defined by stomatal characteristics (density, frequency, and position) and epidermal characteristics (density, shape and size of epidermal cells). Studies conducted on several species of grasses, reported that stomatal density did not have a profound effect upon photosynthesis (Jarvis and Davies, 1998; Jones, 1998). In addition, a weak correlation between stomatal density and plant productivity was reported in wheat (Bhagwat and Bhatia, 1993), rice (Yamashita et al., 1995), *Lolium perenne* (Wilson,

1971) and in *Rhododendron simsii* Planch. azalea (Heursel et al., 1987). Stomatal densities have been related to tolerance to abiotic stress conditions, such as drought (Jarvis and Davies, 1998; Van Rensburg et al., 1999) and temperature extremes (Kleinhenz et al., 1995; Nayeem, 1989).In *Arabidopsis thaliana*, SD has been linked to mechanisms of instantaneous water use efficiency (transpiration efficiency) indicating the importance of the trait for plant survival in drought conditions (Masle et al., 2005).

Plants can avoid drought stress in several ways, such as increasing water use efficiency (WUE) and limiting stomatal opening at low water potentials (Levitt, 1980). Intrinsic WUE expresses the CO_2 assimilation rate (A) in relation to the transpiration rate (E) and can be estimated either by instantaneous measurements of gas exchange or by time-integrated measures (Ares et al., 2000). Consequently, plants with a higher WUE may need less water to produce the same amount of biomass as compared to plants with a lower WUE. Instantaneous WUE is the ratio between net CO_2 assimilation rate and transpiration rate and can be easily calculated using infrared gas analyzers. Agronomic WUE can be estimated by dividing the plant biomass produced in an entire season by the amount of water lost (or applied with irrigation) (Taiz and Zeiger, 1998). Several studies have also demonstrated variation between genotypes for carbon isotope discrimination in crops and forest trees (Farquhar et al., 1989). The isotopic ratio of ^{13}C to ^{12}C in C3 plants varies mainly because of discrimination towards the heavier isotope during diffusion of CO_2 as well as the enzymatic processes of carbon fixation. The rate of diffusion of the heavier isotopic CO_2 ($^{13}CO_2$) is lower than that of $^{12}CO_2$ by a factor of 4.4‰ (per mil) across the stomatal pore. There is also an isotope effect caused by

ribulose bisphosphate carboxylase (Rubisco) preferring $^{12}CO_2$ over $^{13}CO_2$. As a result, a discrimination against the heavier isotope ^{13}C is quite prominent (Condon et al., 1990; Donovan and Ehleringer, 1994). Furthermore, the discrimination against ^{13}C has been shown to be highly negatively correlated with water use efficiency. Several studies on cereals, millets and legumes suggest that genetic variation in isotopic discrimination could be used as a selection criterion for tolerance to drought (Condon et al., 1990; Wright et al., 1994).

The measurement of season-long integrated carbon isotopic (^{13}C) composition of a sample compared to a standard is also an indicator of the WUE of a plant (Lambers et al., 1998). Such a measurement can be reported directly as ^{13}C composition (δ_p) or as discrimination (Δ). The advantage of reporting it as Δ is that it directly indicates the "consequences of biological processes", whereas δ_p is the outcome of both "source isotopic composition and carbon isotope discrimination" (Farquhar et al., 1989). The relationship between Δ and WUE is a strong negative and inverse relationship (Farquhar et al., 1989). The carbon isotope ratio of plant tissue provides an integrated measurement of internal plant physiological and external environmental properties influencing photosynthetic gas exchange over the time when the carbon was fixed (Anderson et al., 1996). Earlier research showed that WUE is related to both the environmental (Garten and Taylor, 1992) and the genetic characteristics of the species (Lauteri et al., 1997; Zhang et al., 1993). Genetic variation in Δ can be exploited by understanding the relationship and interaction between genotype and environment. In other words, if genotype performance is stable across multiple environments then genotypic selection

can be generalized for that particular trait (Cregg et al., 2000). Species with high WUE would have an adaptive benefit in situations wherein water availability is a constraint for plant growth (Ares et al., 2000).

In a study conducted on five populations of *Eucalyptus microtheca* F. Muell. seedlings grown under different water regimes, differences were observed for WUE (Li, 2000). The study indicated that WUE may be a valuable tool for selecting genotypes with improved drought adaptation and biomass under different environmental conditions (Li, 2000). Hence, there may be a possibility of such a selection in the present study.

The objectives of the present study were: (1) to determine whether WUE and anatomical characteristics vary among 19 provenances of *C. illinoinensis* in its natural distribution; (2) to analyze the relationships between anatomical features. Such information is essential for selection of suitable genotypes of this species under different environmental conditions.

Materials and Methods

Plant material

Nineteen provenances from Mexican and U.S. locations were grown at the Pecan Genetics and Breeding Program facility in Somerville, Texas, and selected as treatments for the experiment. The 19 provenances consisted of 13 Mexican and five U.S. locations with two seed sources chosen from the same provenance. Nuts of Mexican provenances were obtained from entries considered to be “native pecans” from the Third Mexican National Nut Conference held at Piedras Negras, Coahuila, Mexico, in November of

1994 (Table 11). The nuts were obtained from open-pollinated trees grown throughout Mexico (Table 11). Nine nuts of each Mexican entry were planted in the greenhouse in 1995, along with open-pollinated nuts collected from trees of native cultivars (Burkett, Colby, Dooley, Frutoso, Giles, and Major) already present in repository orchards of the Pecan Genetics and Breeding Program in Brownwood, Texas. The cultivars from the repository orchards were chosen to represent commonly-grown seedstocks used in different pecan growing regions. Seedlings were germinated and grown in a greenhouse at Brownwood and planted at 4.5 × 5.5 m in the test orchard at Somerville in spring of 1997. The orchard was laid out with eight replicate blocks and 175 seedlings per block. At the time of the present investigation, trunk diameter at breast height (130 cm) ranged between 6 cm and 24 cm, depending on the provenance.

For the current study, only three blocks and 19 seedlings were used (the 156 seedlings not used in the present study were obtained from different seed sources). Seedlings for each provenance in the three blocks were derived from nuts obtained from the same seed source. The size of the orchard was 3.52 ha and the soil type varied between the three blocks with Ships soil in one block and Weswood soil in the other two. Ships series of soils are a very deep clay soil, with 0% to 3% slope and are moderately well drained or well drained and nearly leveled or very gently sloping (Jurena, 2005). Weswood soils are loamy, well drained, very deep, moderately permeable, nearly level or very gently sloping soils with 0% to 3% slope (Jurena, 2005).

Table 11. Sites of origin of open-pollinated pecan seeds utilized for the morphological and physiological analyses. Seeds were collected in 1995 and grown in a common orchard in Somerville, Texas.

Provenance	Longitude	Latitude	Location
1	99º16'W	32º37'N	Putnam, Texas
2	95º05'W	37º04'N	Chetopa, KS.
3	87º48'W	37º89'N	Basket, Ky.
4	96º01'W	35º62'N	Okmulgee, OK.
5	102º17'W	25º42'N	Parras, Coahuila, Mexico
6	89º30'W	38º67'N	Keyesport, IL.
7	101º45'W	25º38'N	General Cepeda, Coahuila,
8	104º83'W	24º78'N	Peñon Blanco, Durango,
9	102º40'W	27º31'N	Ocampo, Coahuila, Mexico
10	101º11'W	27º85'N	Sabinas, Coahuila, Mexico
11	100º50'W	26º53'N	Bustamante, Nuevo León,
12	100º71'W	28º25'N	Villa Union, Coahuila, Mexico
13	100º48'W	24º60'N	El Carmen, Nuevo León,
14	103º13'W	25º35'N	Zaragoza, Coahuila, Mexico
15	100º91'W	29º30'N	Acuña, Coahuila, Mexico
16	99º38'W	23º41'N	Jaumave, Tamaulipas, Mexico
17	100º48'W	24º60'N	El Carmen, Nuevo León,
18	104º13'W	25º23'N	Nazas, Durango, Mexico
19	108º63'W	27º88'N	Saucillo, Chihuahua, Mexico

Sample preparation

Terminal leaflets on five fully expanded leaves were selected from exterior north-facing and south-facing canopy positions at 5 m from the ground. Acetate leaf casts from the selected leaves were collected in July 2006 with the leaves still attached to the trees. Pecan leaves are hypostomatic with anomocytic stomata (Grauke, 1982); consequently,

only the leaf abaxial sides were investigated. To determine the density of stomata, epidermal cells and trichomes, the abaxial side of the distal pair of leaflets was coated with clear nail enamel (Fisher, 1985). After the enamel was allowed to dry for 10-15 min, the cast was stripped using clear tape and placed on microscope slides.

Microscopy

A microscope (model BX51, Olympus America Inc., Melville, N.Y.) was used to count epidermal cells and stomata from each cast at magnification of 200×. The microscope was attached to a digital camera (model DP70, Olympus America Inc., Melville, N.Y.) interfaced with a personal computer. Differential interference contrast (DIC) images from ten different interveinal areas of each cast were collected using DP70-BSW software version 01.01 (Olympus America Inc., Melville, N.Y.). Precautions were taken to avoid taking images in the same location by keeping a numbering system for the veins. In pecan, stomata are raised on the abaxial surface of the leaf in comparison to the epidermal cells. Hence, two DIC images were taken on each chosen area on the cast, one with the focus adjusted to highlight the epidermal cells and eliminate the stomata into the background and a second one with the focus on stomata and trichomes. The number of stomata and epidermal cells from each image was recorded and analyzed for stomatal density [SD (stomata/mm^2)] and epidermal cell density [ED (epidermal cells/mm^2)]. Stomatal index (SI) was calculated as [SD/ (SD + ED)] × 100. Trichome density [TD (trichomes/mm^2)] for the different types of trichomes, namely concave peltate, bladder and non glandular awn-like hair, were recorded for each provenance.

^{13}C discrimination

Three 10-15 cm long twigs per tree were collected from the previous season's shoots in January 2007. The twigs were debudded, cut into 2- to 3-cm-long sections, dried at 70 ºC for 72 h, ground to pass through the 40-mesh screen on a Wiley mill, as described by Glenn et al., (2003) .Three plant samples per tree of 2 ± 0.05 mg were loaded into tin capsules at Texas A&M University, College Station, Texas, and shipped to the Stable Isotope Facility at University of California, Davis, where they were analyzed for ^{13}C content. ^{13}C discrimination (Δ) was calculated using the formula $\Delta = (\delta_a - \delta_{p)} /(1 - \delta_p)$, where δ_p is the isotopic composition of the plant material and δ_a is that of air (assumed to be -8 ‰) (Farquhar et al., 1989)

Statistical design and analysis

The test was composed of three randomized complete blocks, each having one seedling from each of the 19 entries. Variability and provenance differentiation was estimated via analysis of variance (ANOVA) and student's t-test using SAS software (SAS Institute Inc., Cary, N.C.). ANOVA was structured to allow unequal replications for each provenance due to occasionally missing trees.

Results

A t-test conducted to verify the difference between the leaf anatomical features (SD, ED and TD) revealed that there were no differences between SD and SI on either side of the

trees ($p = 0.60$) and, consequently, the data from the north and south side were pooled (Table 12). On the other hand, ED and TD for all the different types of trichomes showed a difference between the north and south sides of the trees ($p < 0.0001$). Hence, ED and TD data for north and south sides of the tree were analyzed separately (Table 3, 4 and 5).

The greatest pooled SD was recorded in provenance 16 and the least in provenance 19 (Table 12). There were no differences between provenances 3, 9, 11, and 15 and also between 6 and 17 for SD (Table 12). Pooled stomatal index followed the same pattern as SD and was greatest and least in provenances 16 and 19, respectively (Table 12). There were no differences between provenances 4, and 5 or between 1, 3, 9, 14 and 15 for pooled SI (Table 12). The greatest and the least ED for the north side was observed in provenances 19 and 12, respectively (Table 12). There were no differences in ED between provenances 2, 3, 14, and between 10, 11, and 17 (Table 12). Similarly, provenances 9 and 16 did not show any differences for ED on the north side of the trees (Table 13). The ED was greatest in provenance 19 and least in provenance 16 on the south side of the trees (Table 13). There was a very prominent overlap in the epidermal cell densities for provenances 5, 6, 7, and also between 9, 10, and 11 on the south side of the trees (Table 13).

Table 12. Pooled stomatal density and stomatal index recorded on leaves of pecan provenances obtained from various locations in Mexico and the United States. Data are an average of five microscopy images from each of five leaves collected from the north and south side of the trees.

Provenance	Pooled stomatal density (stomata/mm^2)	Pooled stomatal index (%)
1	451 ± 9.44 gh[z]	21.83 ± 0.47 hi
2	435 ± 9.44 hij	20.48 ± 0.47 i
3	468 ± 9.44 efg	21.64 ± 0.47 hi
4	544 ± 9.44 c	30.40 ± 0.47 c
5	604 ± 9.44 b	29.60 ± 0.47 c
6	492 ± 9.44 de	24.94 ± 0.47 de
7	485 ± 9.44 def	26.37 ± 0.47 d
8	417± 11.56 ij	17.09 ± 0.58 j
9	461 ± 11.56 fgh	22.71 ± 0.58 fgh
10	466 ± 11.56 efhg	23.40 ± 0.58 efg
11	476 ± 11.56 defg	23.97 ± 0.58 ef
12	556 ± 9.44 c	31.89 ± 0.47 b
13	509 ± 11.56 d	26.38 ± 0.58 d
14	437 ± 9.44 hi	20.55 ± 0.47 i
15	466 ± 9.44 efg	22.24 ± 0.47 gh
16	728 ± 9.44 a	48.98 ± 0.47 a
17	485 ± 9.44 def	24.46 ± 0.47 e
18	406 ± 9.44 j	16.36 ± 0.58 j
19	350 ± 9.44 k	13.61 ± 0.47 k

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Table 13. Epidermal cell density recorded on leaves of pecan provenances obtained from various locations in Mexico and the United States. Data are an average of five microscopy images from each of five leaves collected from the north and south side of the trees.

Provenance	North epidermal cell density (Epidermal cells/mm^2)	South epidermal cell density (Epidermal cells/mm^2)
1	1603 ± 13.36 de	1792 ± 48.66 c
2	1684 ± 13.36 c	1694± 48.66 cde
3	1683 ± 13.36 c	1702 ± 48.66 cd
4	1242 ± 13.36 j	1248 ± 48.66 ij
5	1440 ± 13.36 gh	1433 ± 48.66 gh
6	1470 ± 13.36 fg	1488 ± 48.66 gh
7	1347 ± 13.36 i	1361 ± 48.66 ih
8	2037 ± 16.36 b	2006 ± 59.55 b
9	1575 ± 16.36 e	1557 ± 59.55 defg
10	1504 ± 16.36 f	1541 ± 59.55 efg
11	1497 ± 16.36 f	1521 ± 59.55 fg
12	1187 ± 13.36 k	1188 ± 48.66 j
13	1414 ± 16.36 h	1428 ± 59.55 gh
14	1691 ± 13.36 c	1688 ± 48.66 cde
15	1626 ± 13.36 d	1634 ± 48.66 def
16	753 ± 13.36 l	763 ± 48.66 k
17	1505 ± 13.36 f	1488 ± 48.66 gh
18	2068 ± 16.36 b	2081 ± 59.55 ab
19	2231 ± 13.36 a	2213 ± 48.66 a

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Table 14. Type and density of trichomes recorded on leaves of pecan provenances obtained from various locations in Mexico and the United States. Data are an average of five microscopy images from each of five leaves collected from the north side of the trees.

Provenance	Concave peltate (trichomes/mm^2)	Bladder-like scales (trichomes/mm^2)	Awn-like hair (trichomes/mm^2)
1	4.44 ± 2.57 cd[z]	3.67 ± 1.80 a	4.25 ± 6.82 bcd
2	7.14 ± 2.57 abcd	6.18 ± 1.80 a	10.71 ± 6.82 abcd
3	8.01 ± 2.57 abcd	4.34 ± 1.80 a	21.81 ± 6.82 abcd
4	4.15 ± 2.57 d	5.21 ± 1.80 a	20.75 ± 6.82 bcd
5	8.88 ± 2.57 abcd	6.27 ± 1.80 a	5.40 ± 6.82 abcd
6	10.03 ±2.57 abcd	4.24 ± 1.80 a	17.08 ± 6.82 abcd
7	4.63 ±2.57 cd	7.81 ± 1.80 a	25.96 ± 6.82 a
8	15.23 ± 3.15 a	8.40 ± 2.21 a	0 ± 8.35 d
9	12.43 ± 3.15 abc	8.54 ± 2.21 a	24.17 ± 8.35 abc
10	14.60 ± 3.15 ab	6.95 ± 2.21 a	24.33 ± 8.35 ab
11	6.98 ± 3.15 abcd	3.62 ± 2.21 a	19.26 ± 8.35 abcd
12	8.69 ± 2.57 abcd	9.07 ± 1.80 a	16.89 ± 8.35 abcd
13	7.81 ± 2.57 abcd	5.79 ± 2.21 a	14.04 ± 8.35 abcd
14	9.26 ± 2.57 abcd	7.72 ± 1.80 a	11.10 ± 6.82 abcd
15	9.26 ± 2.57 abcd	7.24 ± 1.80 a	18.05 ± 6.82 abcd
16	6.76 ± 2.57 bcd	8.39 ± 1.80 a	15.44 ± 6.82 abcd
17	9.07 ± 2.57 abcd	7.62 ± 1.80 a	6.47 ± 6.82 abcd
18	13.35 ± 3.15 ab	6.37 ± 2.21 a	0.14 ± 8.35 cd
19	4.25 ± 2.57 d	4.15 ± 1.80 a	21.52 ± 6.82 abcd

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Table 15. Type and density of trichomes recorded on leaves of pecan provenances obtained from various locations in Mexico and the United States. Data are an average of five microscopy images from each of five leaves from the south side of the tree investigated per provenance in each of the three blocks.

Provenance	Concave peltate (trichomes/mm^2)	Bladder-like scales (trichomes/mm^2)	Awn-like hair (trichomes/mm^2)
1	6.27 ± 1.78 de[z]	5.50 ± 1.57 ab	4.05 ± 5.93 bc
2	8.68 ± 1.78 bcde	7.04 ± 1.57 ab	10.23 ± 5.93 abc
3	9.65± 1.78 abcde	5.79 ± 1.57 ab	22.97 ± 5.93 a
4	5.59 ± 1.78 e	5.98 ± 1.57 ab	18.62 ± 5.93 abc
5	7.84 ± 1.78 abcde	7.52 ± 1.57 ab	4.44 ± 5.93 bc
6	10.23 ± 1.78 abcde	6.75 ± 1.57 ab	18.33 ± 5.93 abc
7	7.52 ± 1.78 cde	9.36 ± 1.57 ab	23.26 ± 5.93 a
8	14.67 ± 2.18 a	9.01 ± 1.91 ab	0 ± 7.27 c
9	11.75 ± 2.18 abcd	10.34 ± 1.91 a	24.03 ± 7.27 a
10	13.63 ± 2.18 ab	7.30 ± 1.91 ab	22.58 ± 7.27 ab
11	8.01 ± 2.18 bcde	6.40 ± 1.91 ab	19.11 ± 7.27 abc
12	8.49 ± 1.78 bcde	10.13 ± 1.57 a	16.98 ± 5.93 abc
13	8.02 ± 1.78 bcde	7.28 ± 1.91 ab	13.75 ± 7.27 abc
14	9.36 ± 1.78 abcde	7.36 ± 1.57 ab	12.45 ± 5.93 abc
15	10.13 ± 1.78 abcde	7.62 ± 1.57 ab	16.98 ± 5.93 abc
16	7.33 ± 1.78 cde	9.07 ± 1.57 ab	13.51 ± 5.93 abc
17	9.45 ± 1.78 abcde	9.65 ± 1.57 a	6.56 ± 5.93 abc
18	12.94 ± 2.18 abc	7.13 ± 1.91 ab	0 ± 7.27 c
19	5.40 ± 1.78 e	5.30 ± 1.57 b	18.62 ± 5.93 abc

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

Many provenances had similar numbers of concave peltate scales on both sides of the trees, with the most abundant on provenance 8 and least on 4 and 19 (Table 14 and Table 15). There were no differences in number of bladder-like scales measured on the north side (Table 4), but differences were observed on the south side where the number of this type of trichomes in provenances 9, 12, and 17 was greater than in provenance 19 (Table 5). Provenances 8 and 18 did not have any awn-like hair on either the north or the south side of the trees (Table 14 and Table 15). There was a prominent overlap in the awn-like hairs for the other provenances on either side of the tree (Table 14 and Table 15).

A t-test conducted to verify the difference between Δ on north and south sides of trees revealed that there were no differences ($p = 0.80$). Hence, the data were pooled to obtain Δ values (Table 16). The pooled Δ values also indicated that there were differences between provenances 1, 5, 10 and 4, 15, 16 and 17 (Table 16). Provenances 1, 5 and 10 displayed Δ values towards the greater side and hence are associated with low WUE. Whereas, the Mexican provenances 15, 16 and 17 and the U.S. provenance 4 that displayed lower Δ values are associated with greater WUE. The correlation between instantaneous and integrated WUE indicated that there is no strong correlation between the parameters (Fig. 18).

Table 16. Isotopic ^{13}C discrimination (Δ) recorded on leaves of pecan provenances obtained from various locations in Mexico and the United States and grown in Somerville, Texas. Data are an average of three samples from the twigs on north and south side of the tree investigated per provenance in each of the three blocks.

Provenance	Δ pooled
1	22.45 ± 0.33 a
2	22.04 ± 0.33 abc
3	21.68 ± 0.33 abcd
4	21.37 ± 0.33 cd
5	22.21 ± 0.33 ab
6	21.70 ± 0.33 abcd
7	21.57 ± 0.33 bcd
8	21.38 ± 0.39 bcd
9	21.74 ± 0.39 abcd
10	22.56 ± 0.39 a
11	21.68 ± 0.39 abcd
12	21.68 ± 0.33 abcd
13	21.86 ± 0.39 abcd
14	22.10 ± 0.33 abc
15	21.28 ± 0.33 cd
16	21.11 ± 0.33 d
17	21.27 ± 0.33 cd
18	22.10 ± 0.39 abc
19	21.78 ± 0.33 abcd

[z] Means within same column indicated by different letters are significantly different at $P \leq 0.05$ by conservative t grouping for least squares means.

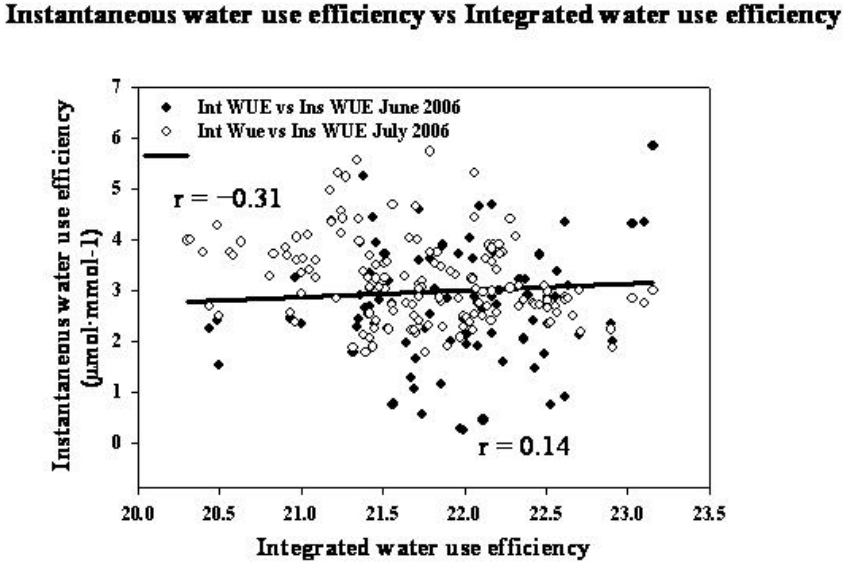

Fig. 18. Relationship between instantaneous water use efficiency and integrated water use efficiency of pecan provenances from across Mexico and United States grown in Somerville, Texas.

Discussion

The SD results indicated that there is a prominent trend observed across the east-west gradient. Provenance 16 from Juamave on the east coast of Mexico had the greatest SD whereas provenance 19 from the west coast displayed the least value. The trend presented here matches with the theories presented on optimal stomatal regulation elaborated by Cowan (1977) and Mäkelä (1996), who proposed that aggressive water use strategies are associated with plants from wetter climates. In a study conducted on several species of *Opuntia* in Mexico, the greatest variation between the subspecies was reported for stomatal frequency and stomatal index (Conde, 1975). On similar lines, the anatomical features in the present study, SD and SI displayed the greatest variation between the provenances indicating that these traits could be of adaptive value to pecan.

The other anatomical features studied were the density and types of trichomes and both the parameters varied on either side of the tree. Provenances 8 and 18 did not have any awn-like hairs on leaves collected on the south side of the tree. Previous research revealed that the non glandular awn-like hair were found in greater numbers in juvenile trees as compared to adult pecan trees (Grauke et al., 1987). Also, mature leaves had a lower trichome density than immature leaves (Wetzstein and Sparks, 1983). Hence, both the age of trees and their maturity, along with biotic and abiotic stress conditions, could have played a role in determining the density of trichomes, thus resulting in an unclear separation between provenances for TD. Grauke et al. (1987) reported that pecan had both the types of glandular hairs, namely peltate scales and capitate glands, and that the latter are shriveled and can only be observed at high

magnifications (700×.). Since the observations for the present study were made at 200× it is possible that the capitate glands went unnoticed.

When the Δ values found here are compared with the typical Δ values reported for C_3 species (15‰ to 28‰), it is clear that this species is associated with low water use efficiency (Lombardini, 2006). Pecan stomata are insensitive to high temperatures and do not close under unfavorable temperature conditions (Andersen and Brodbeck, 1988). Perhaps this can be attributed to unusually high photosynthetic rates and low WUE for the species among temperate fruit trees (Andersen and Brodbeck, 1988). The observed insensitivity of pecan stomata to high temperatures and light intensities also translates into high season long ^{13}C isotopic discrimination values (21‰ to 22‰) (Lombardini, 2006). A study done to describe the influence of irradiance on short-term fluctuations indicated that pecan does not adjust stomatal aperture in response to intermittent cloud cover thus behaving like a non-sun tracking species (Andersen, 1991). This may result in a more rapid loss of soil moisture and reduction in water use efficiency. However, an unclear pattern for Δ translates into the absence of a pattern of differences between provenances for WUE in the present study.

Conclusion

This study reiterated the earlier findings of low WUE for pecan and showed that a pattern for WUE is absent in the species along a geographic gradient. It can be concluded that the anatomy of provenances from drier environments on the western

coast of Mexico is different than those on the eastern coast. This can be attributed to the adaptation of the western provenances to harsher and drier environments.

CHAPTER V

EXPLORING RELATIONSHIPS BETWEEN PECAN PROVENANCES FOR ANATOMY, MORPHOLOGY AND PHYSIOLOGY ACROSS THEIR GEOGRAPHIC DISTRIBUTION

Introduction

Genotypic variation in the physiology and morphology of tree species can often be related to the habitat from which the plants originate (Teklehaimanot et al., 1998). Hence, it is useful to understand patterns of similarities between the provenances in relation to their geographic distribution.Pecan is a species widely distributed in the form of sporadic populations and regenerating stands throughout north-central Mexico (Fig. 1) and as far south as the state of Oaxaca (Grauke, 1990; Thompson and Grauke, 1991a; Thompson and Grauke, 1991b).

An exploration into the patterns of clustering of provenances and populations across the geographic distribution will provide insight into the morphological and physiological behavior of the species. This analysis is being looked upon with an exploratory approach rather than as an experimental study because of the limitations and requirements associated with cluster analysis. Provenances 1, 5, 6, 7, and 16 were closely related to each other while provenances 8 and 18 were grouped with the other cluster based on leaf fresh weight, leaflet number and leaf area (Fig. Aa). Upon further breakdown, provenance 8 and 18 clustered together and separated from the other provenances in the group (Fig. Ab). When the provenances were grouped into four clusters, 8 and 18 grouped together and 1, 6, 15 and 16 still showed similarities by

grouping together as a cluster (Fig. Ac). However, provenance 7 separated into a different cluster while the other provenances formed two separate clusters (Fig. Ac).

In a plotted pie graph generated by a hierarchical cluster analysis (5 clusters) based on leaflet number, leaf area and leaf fresh weight, provenances 8 and 18 clustered together (Fig. Ad). Provenances 10, 12 and 5 also formed a cluster, while it is interesting to see the branching of provenance 13 and 17 from the same location into two different clusters (Fig. Ad). It is also useful to note that provenances 6 from Illinois and 16 from Central Mexico were still grouped together along with provenance 1 and 15 from Northern Mexico (Fig. Ad). Provenance 13 was closely related to 2, 3, 4, 9, 11, 14, and 19 while provenance 17 was related to 5, 10, and 12 (Fig. B). In the plotted pie graph generated by a hierarchical cluster analysis based on mean leaflet fresh weight and mean leaflet area provenance 1 and 7 formed a cluster while all the other provenances were grouped together as one cluster (Fig. Ca). Analysis of three clusters (Fig. Cb) revealed that provenance 8 and 18 from the west coast grouped together while provenances 13 and 17 from the same location branched into two different clusters. Upon examination of the clusters as four groups, provenance 7 and 1 were still grouped together and the cluster formed by 8 and 18 was still intact (Fig. Cc). The close relationship between provenance 8 and 18 was exhibited as they still remained clustered even upon dividing the provenances into five clusters (Fig. Cd). Provenance 17 was related to 8 and 18, while 13 was closely related to 2, 3, 4, 9, 11, 16, and 19 (Fig. D).

In the plotted pie graph generated by a hierarchical cluster analysis based on specific leaf area and fresh/dry weight, division into two groups revealed that

provenances 1, 7, 11, 12, and 14 were grouped together while all the other provenances formed one cluster (Fig. Ea). Upon dividing the provenances into three clusters, 8 and 18 diverged into different clusters (Fig. Eb). Provenances 3, 6, 10, 13, 15, 17, and 18 grouped together into a cluster while provenances 2, 4, 5, 8, 9, 16, and 19 formed a separate cluster (Fig. Eb). Further division into four clusters led to the branching of provenances 13 and 17 from the same location into different clusters (Fig. Ec). The plotted pie with five clusters showed that provenance 18 is more closely related to provenances 15 (Fig. Ed) and 13 (Fig. F).

A plotted pie graph generated by a hierarchical cluster analysis based on net CO_2 assimilation, stomatal conductance and transpiration measured in June showed that provenances 8 and 18 were clustered with 1, 2, 3, 5, 7, 9, 10, 14, and 16, while provenances 13 and 17 from the same location diverged into two different clusters (Fig. Ga). Provenance 19 from the west coast was clustered with 4, 6, 12, and 15 (Fig. Ga). Upon further scrutiny, provenances 8 and 18 separated into different clusters resulting in a grouping of provenances 1, 3, 5, 7, 9, 14, 16, and 18 into a cluster (Fig. Gb) and provenances 2, 8, and 10 into a separate cluster (Fig. Gc). Further division into four clusters indicated that provenance 8 was more closely related to 2, 10, and 17 (Fig. Gc). The plotted pie graph with five clusters (Fig. Gd) indicated that provenance 17 was closely related to 2, 8, and 10.There was also an indication that provenance 13 was closely related to 19 (Fig. H).

A plotted pie graph generated by a hierarchical cluster analysis based on net CO_2 assimilation, stomatal conductance and transpiration measured in July indicated a similar

pattern to the June data with provenances 13 and 17 from the same location in Mexico diverging into different clusters (Fig. Ia). Provenances 6, 7, 8, 9, 12, and 14, were clustered together while provenances 1, 2, 3, 4, 10, 11, 15, 16, 18, and 19 formed one cluster (Fig. Ia). Division into three clusters indicated that provenance 17 was closely related to 6, 7, 8, 9, 12, and 14 (Fig. Ib). Upon closer observation, division of provenances into four clusters indicated that provenance 17 was more closely related to provenance 6 than to the other provenances as observed earlier (Fig. Ic). Provenances 7, 8, 9, 12, and 14 were clustered together, whereas 2 and 19 formed a cluster together (Fig. Id).There was also an indication that provenance 17 was more closely related to 8 and 12 in a manner similar to the pattern in June (Fig. J Fig.). In June there was an indication that provenance 13 was closely related to provenance 19 (Fig. H), but in July provenances 13 and 18 were related (Fig. JFig.).

A plotted pie graph generated by a hierarchical cluster analysis based on photosynthetic efficiency and photosynthetic yield (July 2006) shows that provenances 2. 12, 16, and 19 were closely related to each other (Fig. Ka). The plotted pie with three clusters (Fig. Kb) indicated that provenances 13 and 17 from the same location in El Carmen, Mexico, were not very closely related to each other. When clustering was done taking fluorescence and gas exchange parameters into consideration, the dendrogram showed that provenance 13 was more closely related to provenances 3, 4, and 5 than to 17 (Fig. L). Provenance 17, however, grouped together with provenances 1, 7, 8, 9, 10, 11, 14, 15, and 18 (Fig. Kb). Provenances 2, 12, 16, and 19 were closely related while 7 and 9 formed a cluster together (Fig. Kc). Division into five clusters indicates that

provenances 19 and 16, respectively from the west and east coast, were closely related (Fig. Kd). Provenances 6, 7, 10, 15, 16, and 17 were also closely related (Fig. Ma) while provenance 13 was closely related to provenances 1, 8, and 19 (Fig. N and Fig. Mb). Scrutiny after division into further clusters indicates that provenance 9 was closely related to 12 (Fig. Mc) and provenance 8 to 19 (Fig. Md). However, a plotted pie graph generated by a hierarchical cluster analysis based on photosynthetic efficiency and photosynthetic yield (August 2006) shows that provenances 8 and 19 were closely related to each other (Fig. Md).

Discussion

The exploratory approach using cluster analysis with Ward's method in SPSS (SPSS, Inc., Chicago, Ill.) provides information complimenting the results obtained from the analysis of variance (ANOVA) using SAS (SAS, Inc., Cary, N.C.).

Provenances 6 and 16 were closely related when leaflet number, leaf area, and leaf fresh weight were taken into consideration for clustering. Morphologically, provenances 6 and 16 seemed to be closely related to each other but, when the physiological responses were examined, they grouped into different clusters. However, when clustering was done on the basis of specific leaf area and fresh/dry weight, provenances 6 and 16 seemed unrelated and appeared in two different clusters. Provenance 6 was closely related to 18 while provenance 16 was related to 8. The other important relationships that emerged from cluster analysis and ANOVA are that provenances 8 and 18 were very closely related to each other when morphological

characteristics are studied on the whole leaf basis (Table 3) as well as on a leaflet basis (Table 4). On the other hand, even though provenances 8 and 18 seemed to be related initially, when specific leaf area and fresh/dry weight were taken into account it was revealed they were not closely related. Provenances 13 and 17 obtained from El Carmen were very distinct from each other, possibly because of the different sources used in obtaining seed. This also informs us that a greater number of representatives from a provenance need to be studied to obtain a true picture of the behavior of the individuals in that location.

When morphology was studied on a leaflet basis and on the criteria of specific leaf area and fresh/dry weight, provenances 16 from the eastern coast and 19 from the western coast clustered together. Thus, the results from the cluster analysis corroborated with the results obtained from the ANOVA (Table 3 and Table 4) for the above parameters and indicated that a definite geographic pattern did not exist for these morphological traits.

When clustering was done on the basis of gas exchange data, an important feature was the divergence of provenances 13 and 17 into different clusters. However, provenance 13 seemed to be closely related to 4, 5, 6, 12, and 19 while provenance 17 was closely related to provenance 2, 8, 10, 16, and 18 in June. In July, provenance 13 was related to 18 while 17 was related to 8. It is to be noted that provenances 16 and 19 were members of different clusters in June but belonged to the same cluster in July. This indicates a phenotypic plasticity already exhibited by the ANOVA (Table 5 and 6).

The fluorescence parameters (photosynthetic efficiency and yield) once again indicated that provenances 13 and 17 are not very closely related to each other. In July, provenance 17 belonged to the cluster that consisted of both provenances 8 and 18 while provenance 13 was related to provenances 3, 4 and 5. In July, provenance 19 was closely related to provenance 2, 12, and 16. In August, provenance 19 was closely related to provenances 1, 8, and 13 while provenance 17 was related to the cluster that consisted of 6, 7, 10, 15, and 16.

Overall, there was a clear indication that different sources from the same location can exhibit different characteristics and there is great plasticity associated with physiological traits. There is also a great possibility that there exists a founding effect due to the source of the seed material as it was obtained from a nut Conference held at Piedras Negras, Coahuila, Mexico, in November of 1994. Nevertheless, the cluster analysis pointed towards the need to include multiple individuals and multiple seed sources from the same location in the analysis. Also, pecan is an open-pollinated species and therefore, there is a definite need to include multiple seed sources to represent populations and provenances. Future research and analysis could benefit from using the results from the current exploratory analysis and use greater number of individuals in a multivariate approach.

CHAPTER VI

CONCLUSIONS AND FUTURE PROSPECTS

Carya illinoinensis (Wangenh.) C. Koch is a species distributed over an area of varied geographic and climatic variation. The species is also distributed in the form of sporadic populations and regenerating stands throughout north-central Mexico (Fig. 1) and as far south as the state of Oaxaca (Grauke, 1990; Thompson and Grauke, 1991a). Such a wide distribution produces exposure to varied environmental conditions providing a potential for anatomical and physiological adaptation within the cultivars and provenances (i.e., the area of origin of seed).

Conclusions

In the present study pecan provenances were differentiated based on morphological and anatomical traits to a greater extent as compared to the physiological traits. Intraspecific variation in pecan provenances has been exhibited for the morphological and anatomical traits along the east-west gradient. The physiological parameters (Net CO_2 assimilation rate, stomatal conductance, transpiration, WUE, photosynthetic efficiency and yield, photochemical quenching and non-quenching) measured were similar for most of the provenances. These results could be caused by the plasticity of most ecophysiological traits as described by Ackerly et al., (2000). In tandem with the present study, photosynthetic acclimation to drought was not apparent in a study conducted on loblolly pine to study the genetic differences in morphology and physiology (John and Johnson, 1988).

All provenances studied in the present study were grown in the same location in Somerville, Texas. Hence, physiological traits that could be very plastic may have responded to the same environmental conditions using similar acclimatization strategies, thus resulting in a lack of differences. Woodword and Kelly (1995) found that changes in SD were generally greater in samples from amphistomatous species than those from hypostomatous species, such as pecan. It is possible that being a hypostomatous species, the anatomical traits in pecan may not be as plastic as the physiological traits. This indicates that certain species may not show plasticity to environmental changes in a single generation for some ecogeographical traits. There is also the possibility that leaf anatomical traits, such as stomatal density (SD), are genetically controlled to a great extent in pecan. The result of the study conducted on three pecan cultivars suggests that SD, epidermal density (ED) and stomatal index (SI) are stable within a pecan cultivar despite ecogeographical differences of the growing sites. Poole et al. (1996) stated that leaf SD is not affected by leaf expansion in relation to abiotic factors in the surrounding environment but that instead it is influenced by local differences in stomatal differentiation. Such patterns of local differentiation can be attributed to genetic control, for traits with such differentiation patterns can be found in nearly all plant species, as most of them are subject to restricted gene flow in their native distribution (Bradshaw, 1959). As a result, SD may be linked to the long-term climatic conditions of the location where the species (or cultivar) developed. The stability of certain leaf anatomical characteristics, such as SD and ED, for pecan cultivars grown at different locations

confirms that these traits can be used for screening ecotypes and provenances for breeding and cultivar development.

The types of trichomes and patterns of trichome density (TD) observed in the pecan cultivar study (Chapter II) varied at the three locations and were different between cultivars. However, a dissimilar pattern was observed in the provenance study. There was a major overlap between the provenances for all the three types of trichomes on either side of the tree. It has been suggested that the glandularity of trichomes may be a result of long-term predator pressure (Levin, 1973). If the above proposal is taken into consideration, it is imperative to note that there is a major difference between the pecan cultivar and provenance studies. The leaf material used in the cultivar study was obtained from genetically identical material grown at different locations and the types and patterns of trichomes varied at the three locations. In the provenance study, even though the trees used in the study were genetically different, they were all grown at the same orchard in Somerville, Texas. So, it is possible that the predator pressure varied with the location in the first study, but it was similar in the second study. These tentative conclusions also indicate that unlike SD, TD may not be genetically controlled but rather it could be an effect of the environmental (biotic and abiotic) factors.

It is also interesting to note that western provenances of *C. illinoinensis* displayed the least SD while an eastern provenance showed the greatest SD. This trend can be attributed to the gradient in moisture availability from the wetter conditions in the east to the arid conditions in the west in North America. This may have an implication for water use by the provenances when exposed to drought conditions. A positive but weak

correlation between Δ and SD was observed in ponderosa pine (Cregg et al., 2000). Masle et al., (2005) have further identified the specific role of a gene 'ERECTA' in instantaneous WUE, SD, ED expansion and cell to cell functions. The ERECTA mutants exhibited increased SD and reduction in epidermal cell size. The mutants had smaller epidermal cells but had to compensate with an increase in their number in order to keep the stomatal index constant. The wild type had fewer stomata per unit area and larger but fewer epidermal cells associated with lower stomatal conductance and therefore, higher WUE than the mutants (Masle et al., 2005). Based upon the SD trend, it can be proposed that the western provenance with least SD may be associated with a conservative water use strategy during water deficit while the eastern provenances may have a less conservative water use strategy. A negative correlation was reported between provenance mean driest quarter rainfall and root mass/foliar area ratio, foliar area/stem cross sectional area, WUE, and ^{13}C (Li et al., 2000). WUE was correlated with gas exchange variables and growth traits among five *E. microtheca* populations studied (Li, 1998; Li, 2000). The relationship between plant growth and WUE may provide a basis for genotype selection for drought adaptation and improved biomass productivity at locations with deficit water conditions (Li, 2000). However, the relationship between water use strategies and climate remain controversial. Lloyd and Farquhar (1994) compiled a global survey which showed that plants from habitats with a high evaporative demand have greater water use. In other words, these plants transpire more even under well-watered conditions. There is a possibility that *C. illinoinensis* being a riparian species, thrives along river banks in its natural habitat even in arid environments. This

could be a probable reason for the low WUE exhibited in the current and earlier studies for the species. However, Zhang and Marshall (1995) and Palmroth (1999) did not find any trend in water use among provenances of *Pinus ponderosa* Dougl. *ex* Laws. or *Pinus sylvestris* L., respectively. A similar lack of trend without any differences between provenances for WUE has been reported in the present study.

It is clear that any conclusions about the adaptive significance of differences that are found between populations and provenances can only be tentative at this stage of the study. The behavior of different provenances in the study on provenances of *E. microtheca* suggests that the changes in plant morphological and physiological characteristics are closely related to the climate and habitat that the plants are adapted to (Li et al., 2000). Of the climatic variables that were investigated, rainfall during the driest months had the highest correlation with plant characteristics (Li et al., 2000). Even though, the literature emphasized that it is important to include climatic factors into consideration while studying trends along latitudes and longitudes. Unfortunately, climatic data corresponding to the populations under scrutiny in the present studies could not be obtained. Limited data available pertaining to elevation and precipitation was collected to provide an approximate picture of the distribution of populations along the gradient (Fig. O and Fig. P). There is a vague indication available from the above figures that there is a possibility that provenances from areas of similar precipitation and elevation could be showing similar behavioral patterns.

Future Prospects

The current study was tailored to provide a broad picture of the variation patterns across a wide geographical area. However, it was not possible to incorporate specific details of climatic differences between the areas of origin of the pecan provenances under consideration. Thus, as a result the study was able to provide an outline of the morphological and anatomical adaptations of the provenances in relation to their locations of origin but could not explain the reasons behind such behavioral patterns. Further detailed research in the direction of evaluation of variation between specific provenances of interest is required to be able to draw concrete conclusions about the morphology and anatomy across geographical gradients. It is also important to include a greater number of seed sources from a provenance to have a representative sample with reference to the location of the origin of the seed. This strategy could facilitate the development of a more powerful study and help in the identification of desirable morphological and anatomical traits suitable for breeding purposes in the future. It would also be of interest to include climatological parameters like altitude and precipitation gradients and patterns into the scope of the study. This would help in providing an understanding of the variation as well as the non variation patterns between provenances for morphological and anatomical traits.

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APPENDIX

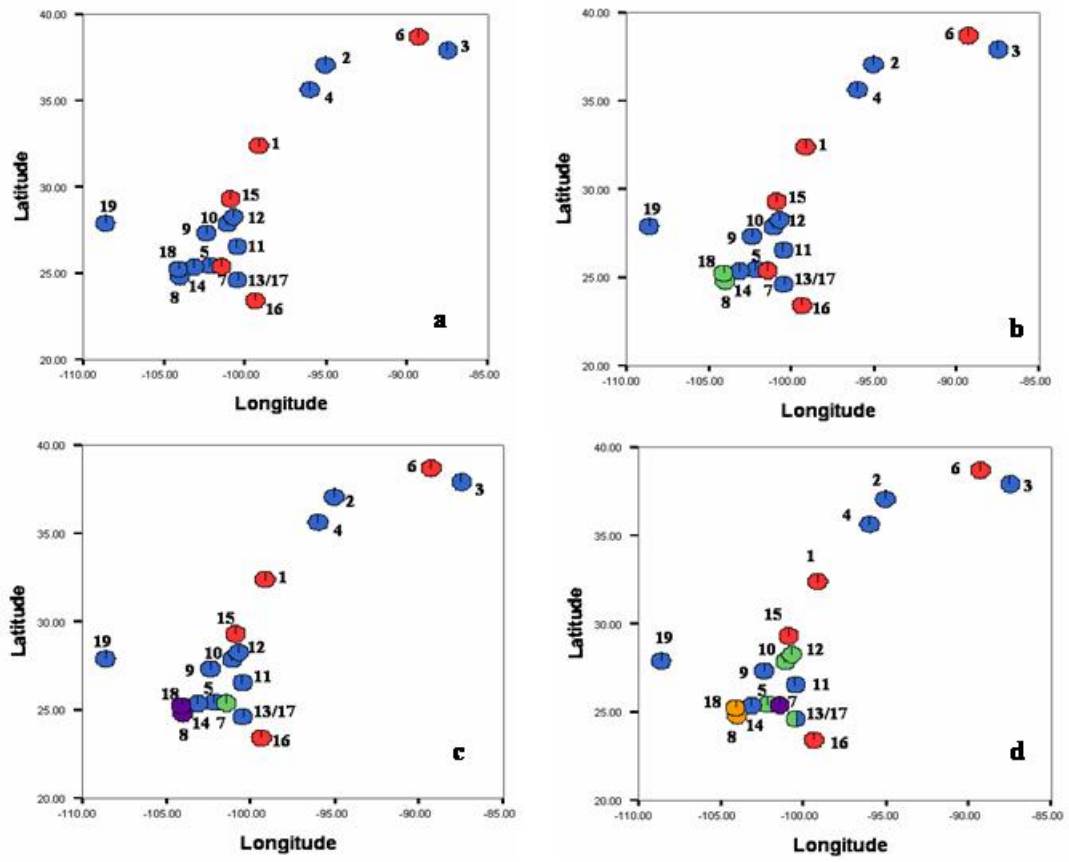

Fig. A. Plotted pie graph generated by a hierarchical cluster analysis based on leaflet number, leaf area and leaf fresh weight showing the distribution of 19 open-pollinated pecan provenances from 18 locations in a. two b. three c. four d. five clusters. Populations grouped together as a cluster are indicated by the same color and the numbers denote the provenances specified in Table 3.

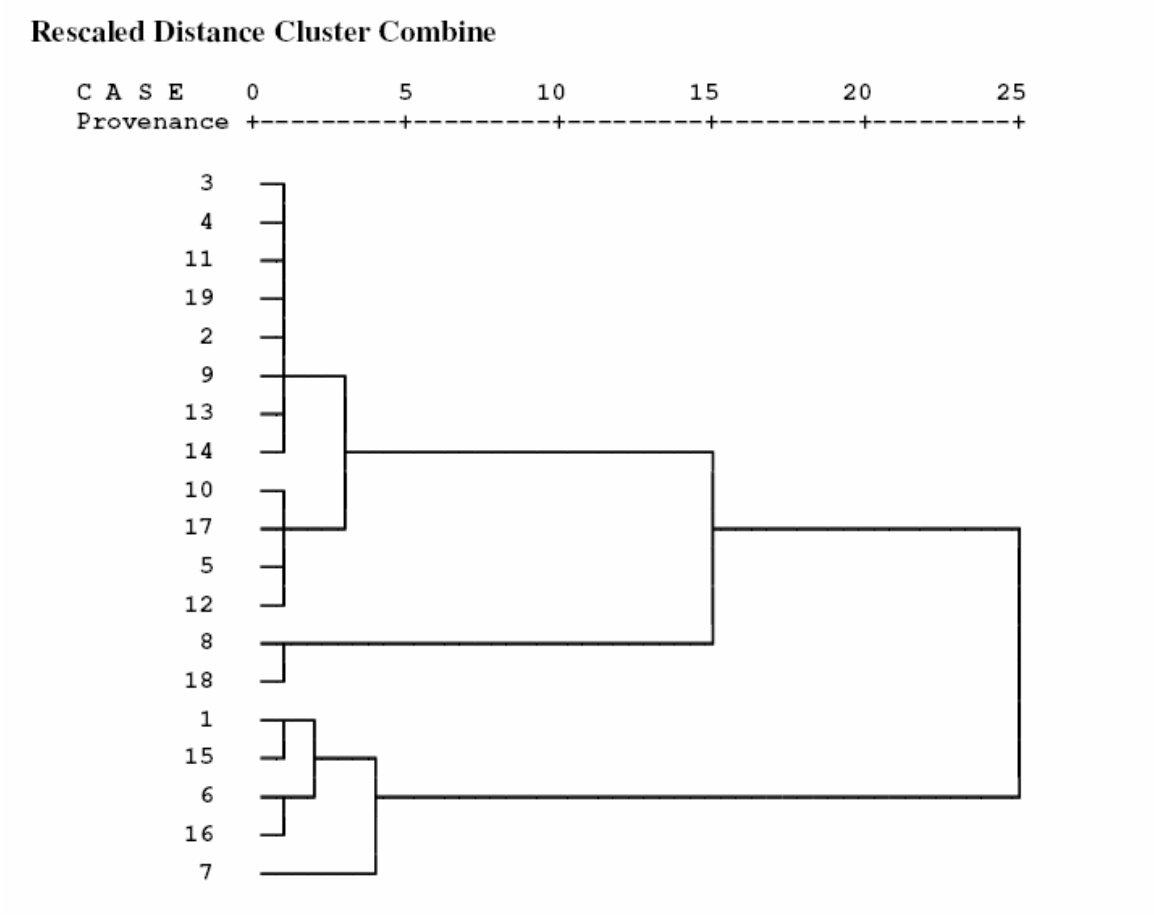

Fig. B. Dendrogram generated by a hierarchical cluster analysis using Ward's method based on leaflet number, leaf area and leaf fresh weight showing the relationship among 19 open-pollinated pecan provenances.

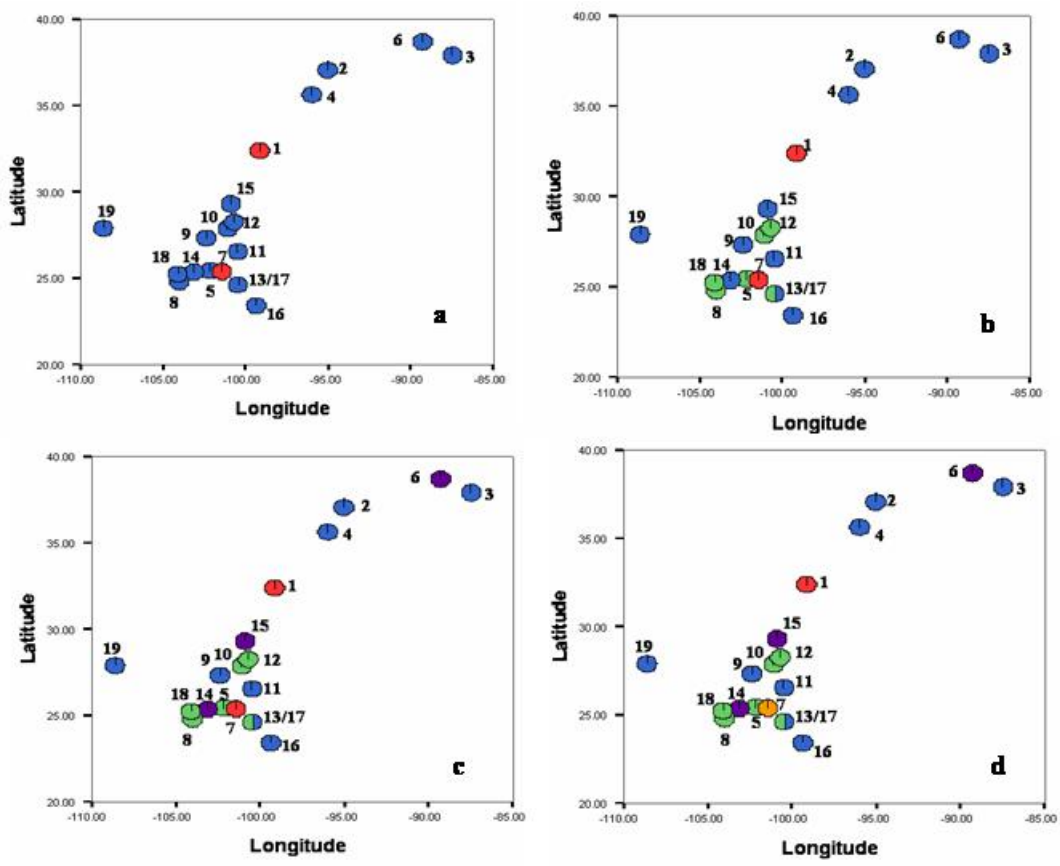

Fig. C. Plotted pie graph generated by a hierarchical cluster analysis based on mean leaflet fresh weight and mean leaflet area showing the distribution of 19 open-pollinated pecan provenances from 18 locations in a. two b. three c. four d. five clusters. Populations grouped together as a cluster are indicated by the same color and the numbers denote the provenances specified in Table 3.

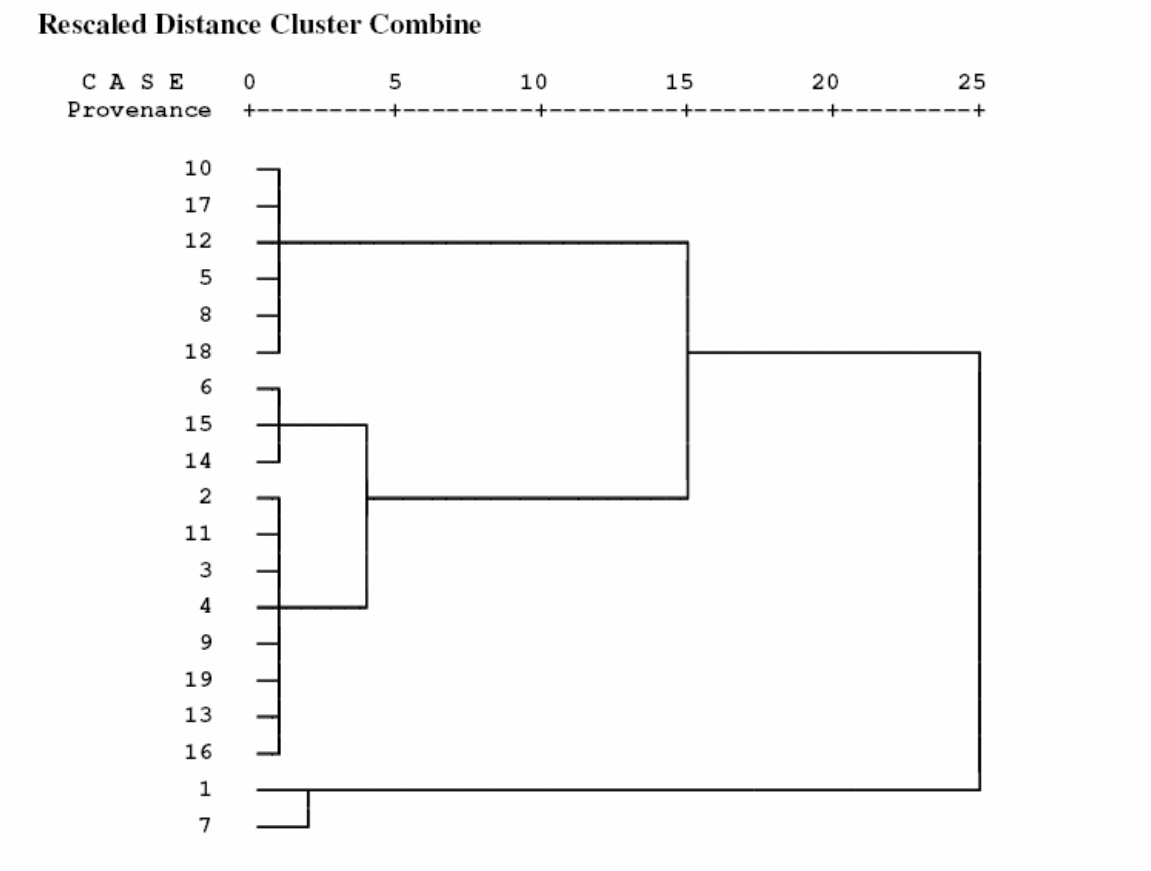

Fig.D. Dendrogram generated by a hierarchical cluster analysis using Ward's method based on leaflet fresh weight and mean leaflet area showing the relationship among 19 open-pollinated pecan provenances.

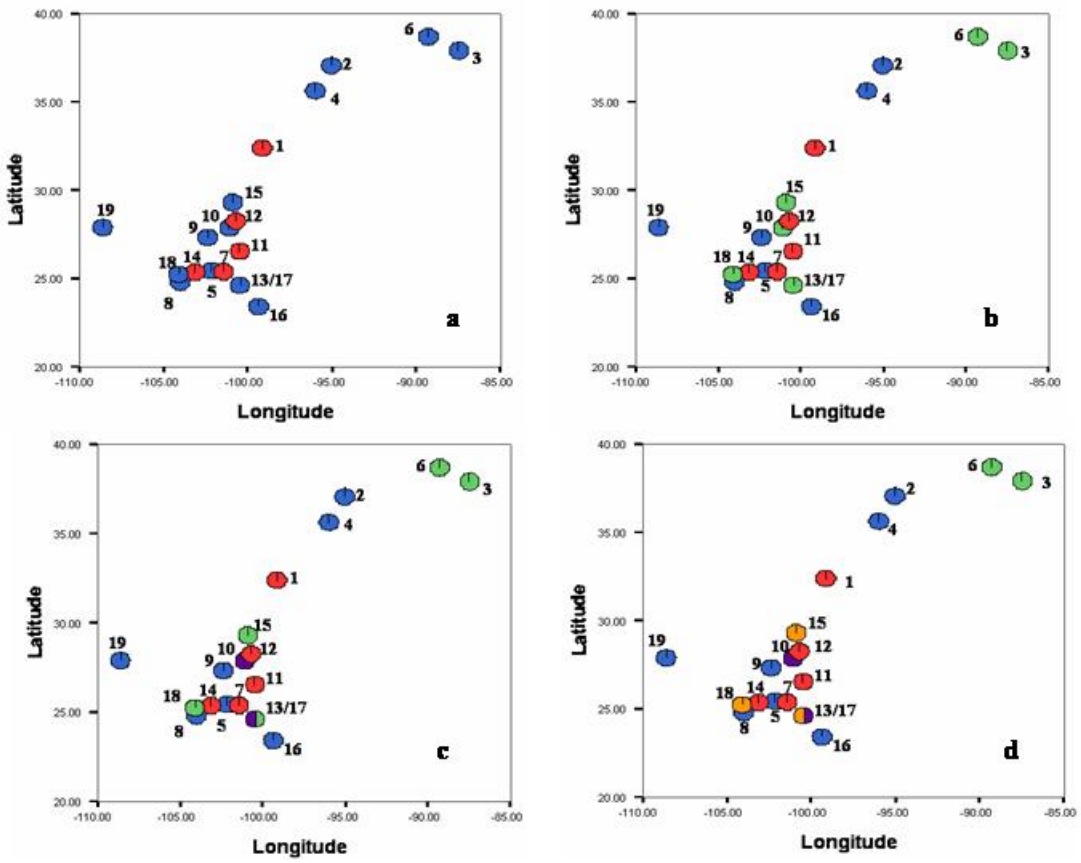

Fig. E. Plotted pie graph generated by a hierarchical cluster analysis based on specific leaf area and fresh/ dry weight showing the distribution of 19 open-pollinated pecan provenances from 18 locations in a. two b. three c. four d. five clusters. Populations grouped together as a cluster are indicated by the same color and the numbers denote the provenances specified in Table 3.

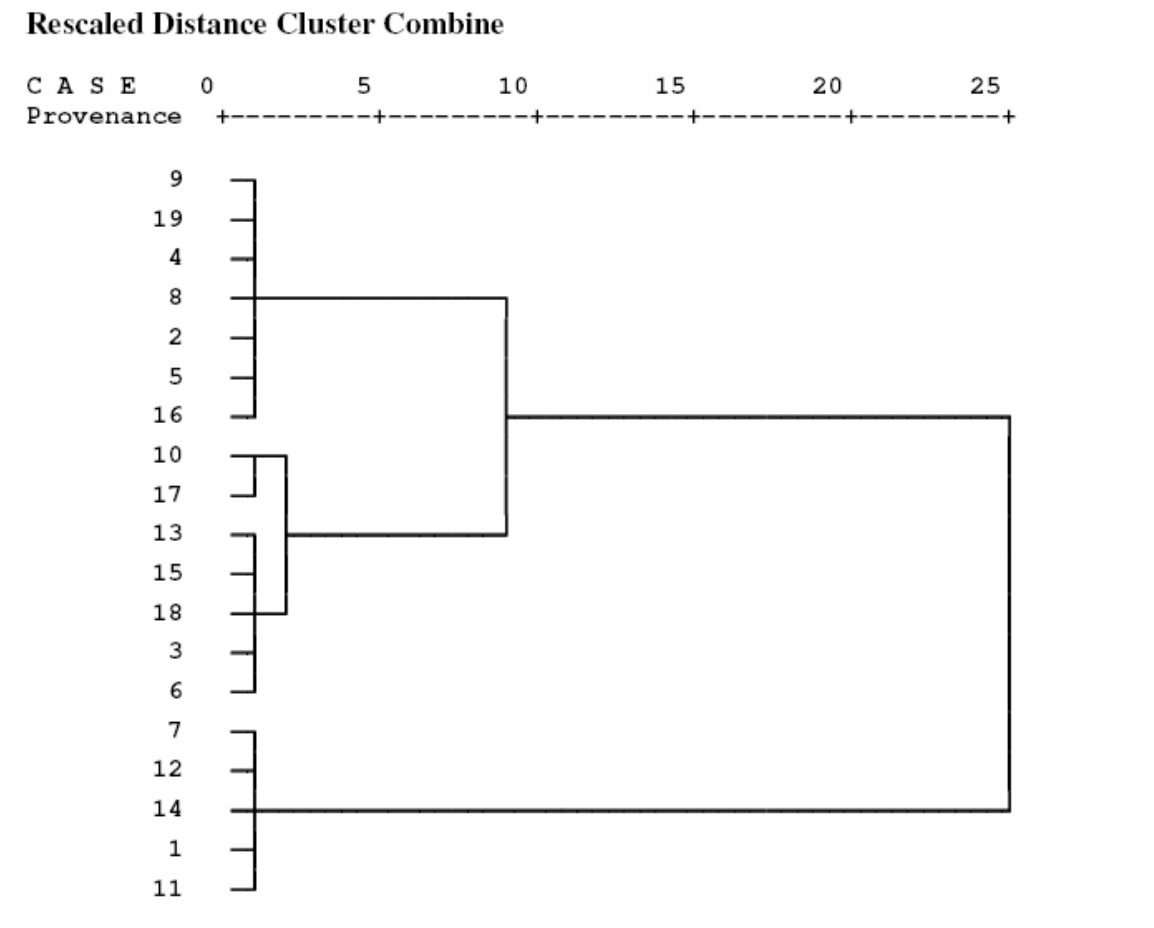

Fig. F. Dendrogram generated by a hierarchical cluster analysis using Ward's method based on specific leaf area and fresh/dry weight showing the relationship among 19 open-pollinated pecan provenances.

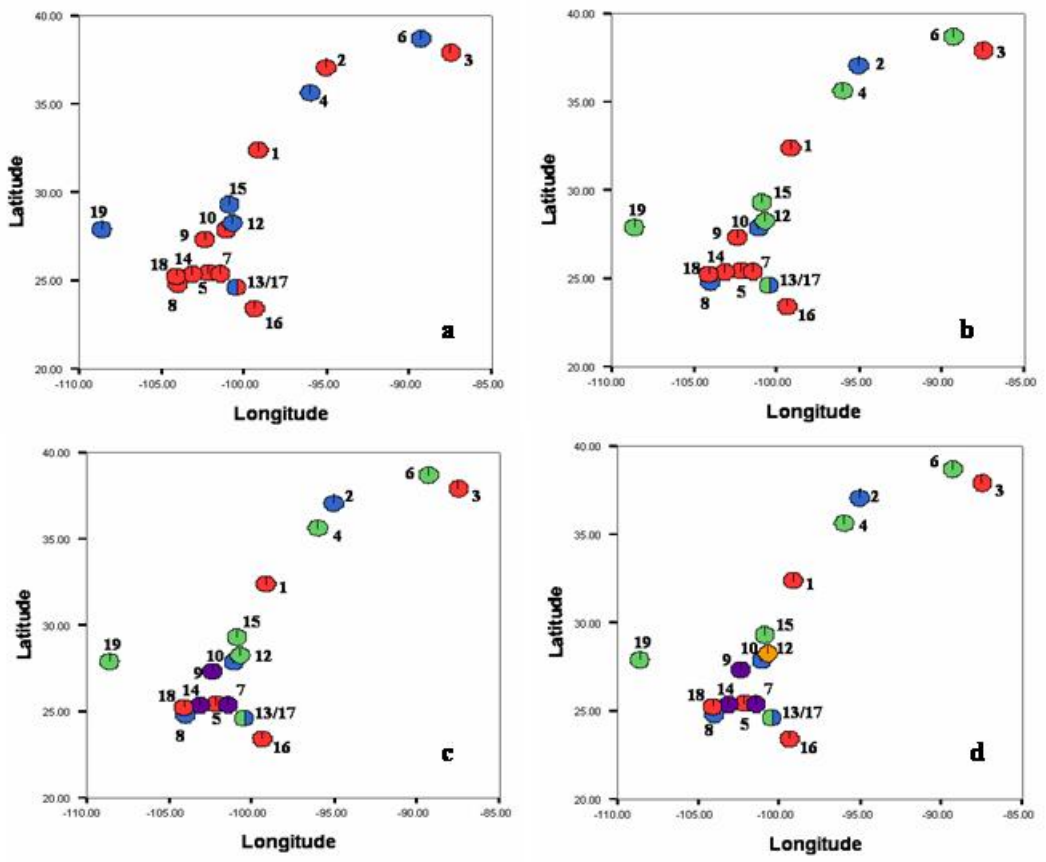

Fig. G. Plotted pie graph generated by a hierarchical cluster analysis based on net CO_2 assimilation, stomatal conductance and transpiration (June 2006) showing the distribution of 19 open-pollinated pecan provenances from 18 locations in a. two b. three c. four d. five clusters. Populations grouped together as a cluster are indicated by the same color and the numbers denote the provenances specified in Table 3.

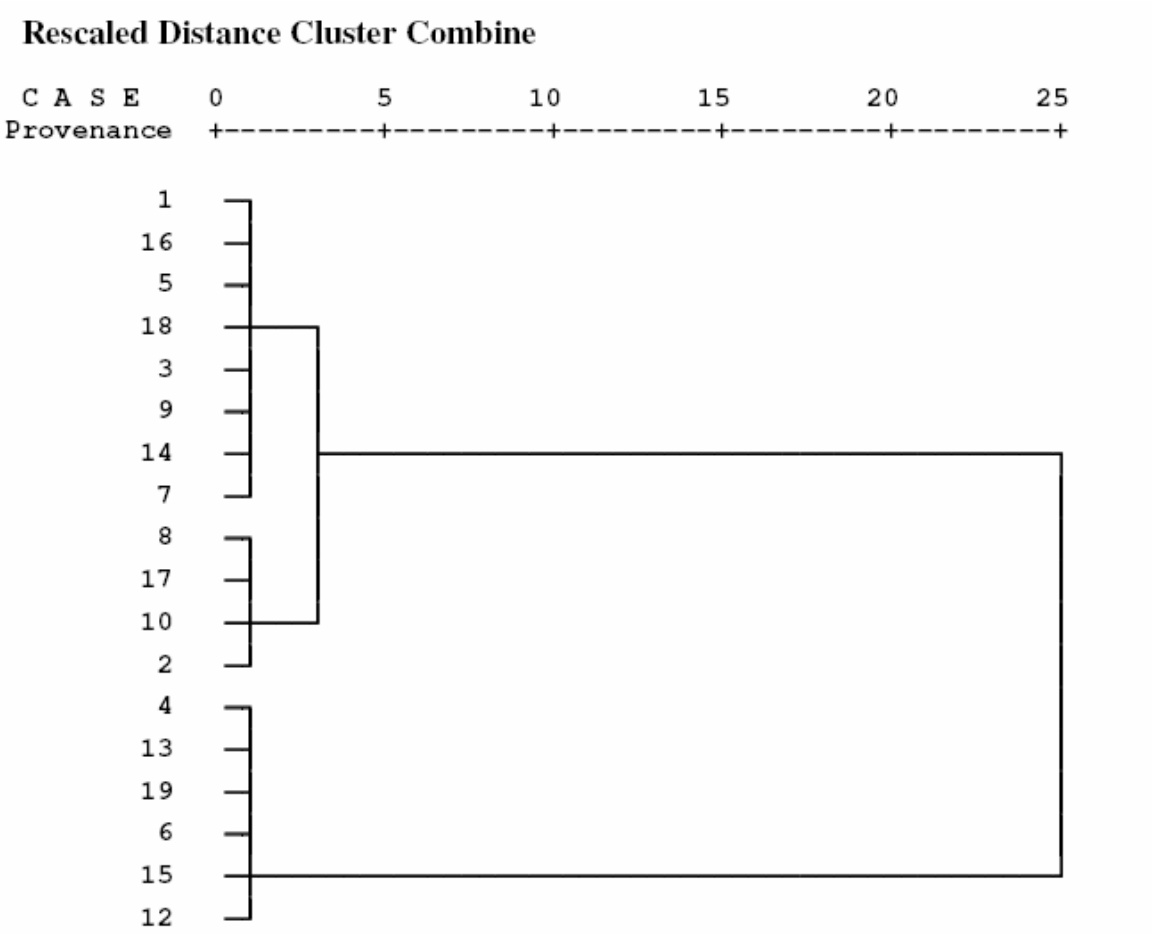

Fig. H. Dendrogram generated by a hierarchical cluster analysis using Ward's method based on net CO_2 assimilation, stomatal conductance and transpiration (June 2006) showing the relationship among nineteen 19 open-pollinated pecan provenances.

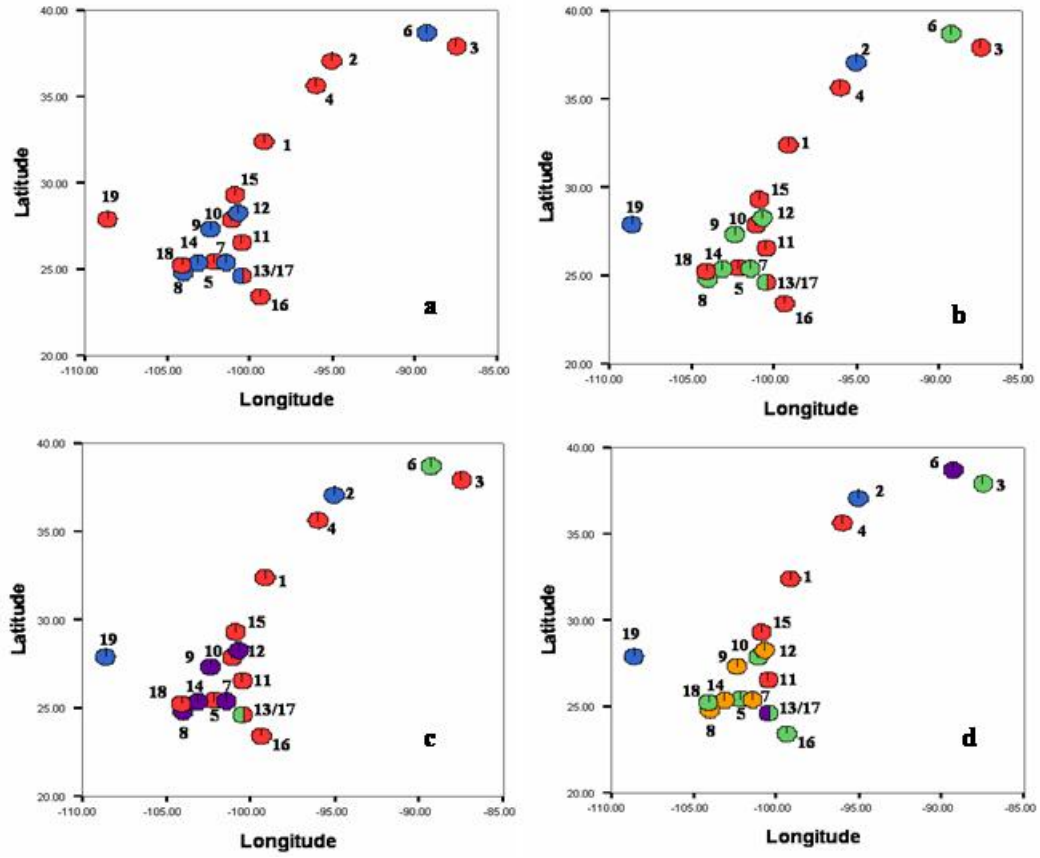

Fig. I. Plotted pie graph generated by a hierarchical cluster analysis based on net CO_2 assimilation, stomatal conductance and transpiration (July 2006) showing the distribution of 19 open-pollinated pecan provenances from 18 locations in a. two b. three c. four d. five clusters. Populations grouped together as a cluster are indicated by the same color and the numbers denote the provenances specified in Table 3.

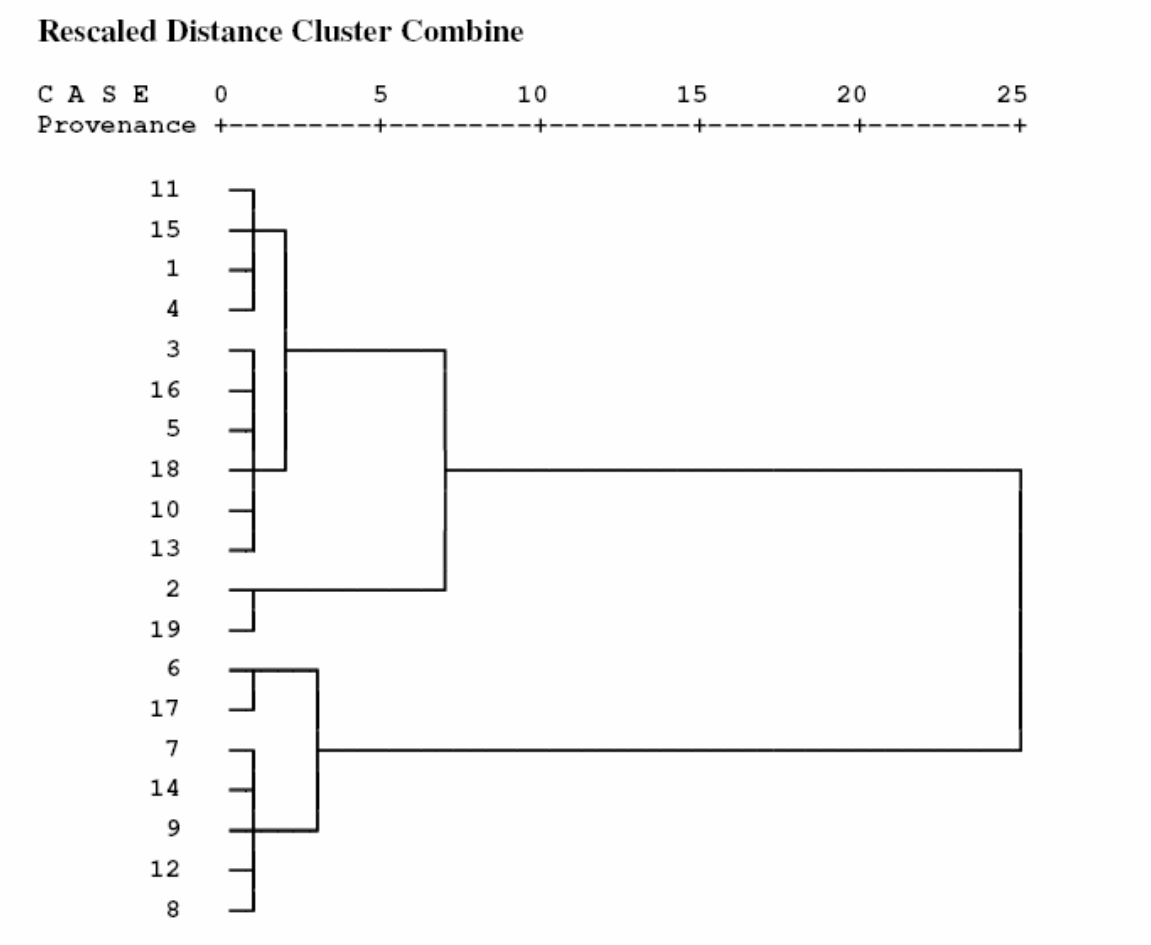

Fig. J. Dendrogram generated by a hierarchical cluster analysis using Ward's method based on net CO_2 assimilation, stomatal conductance and transpiration (July 2006) showing the relationship among 19 open-pollinated pecan provenances.

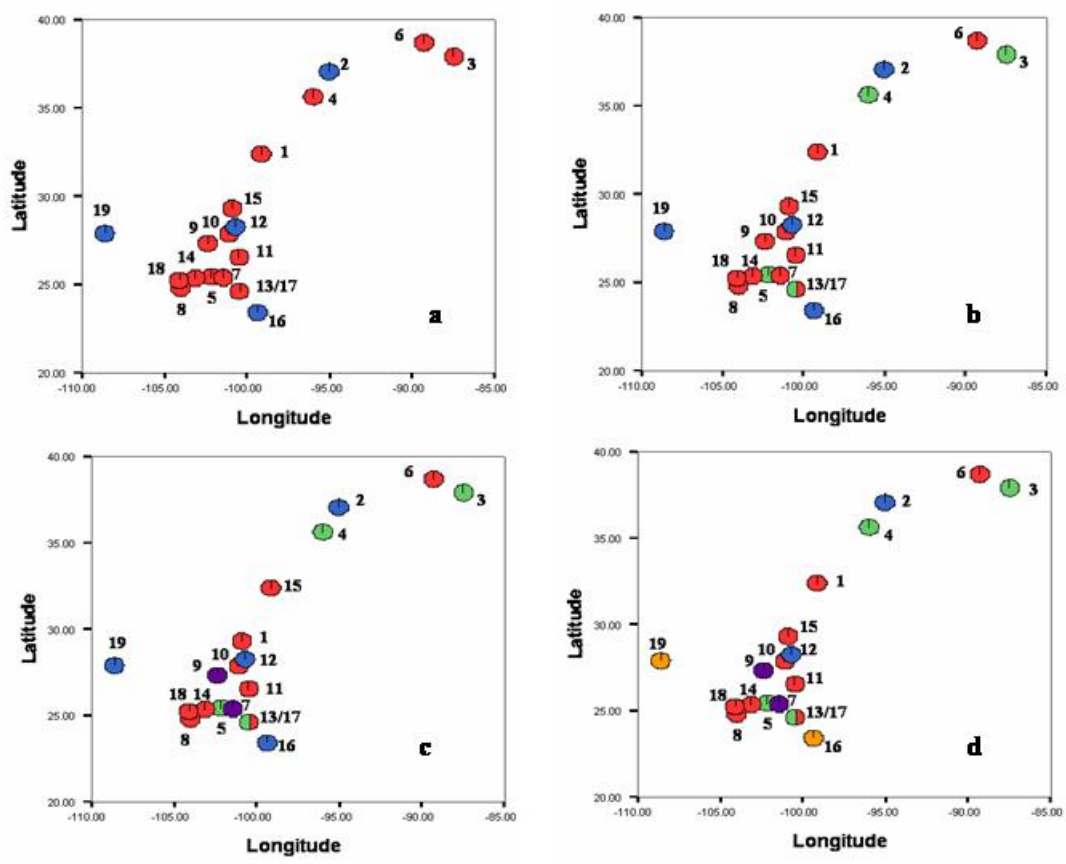

Fig. K. Plotted pie graph generated by a hierarchical cluster analysis based on photosynthetic efficiency and photosynthetic yield (July 2006) showing the distribution of 19 open-pollinated pecan provenances from 18 locations in a. two b. three c. four d. five clusters. Populations grouped together as a cluster are indicated by the same color and the numbers denote the provenances specified in Table 3.

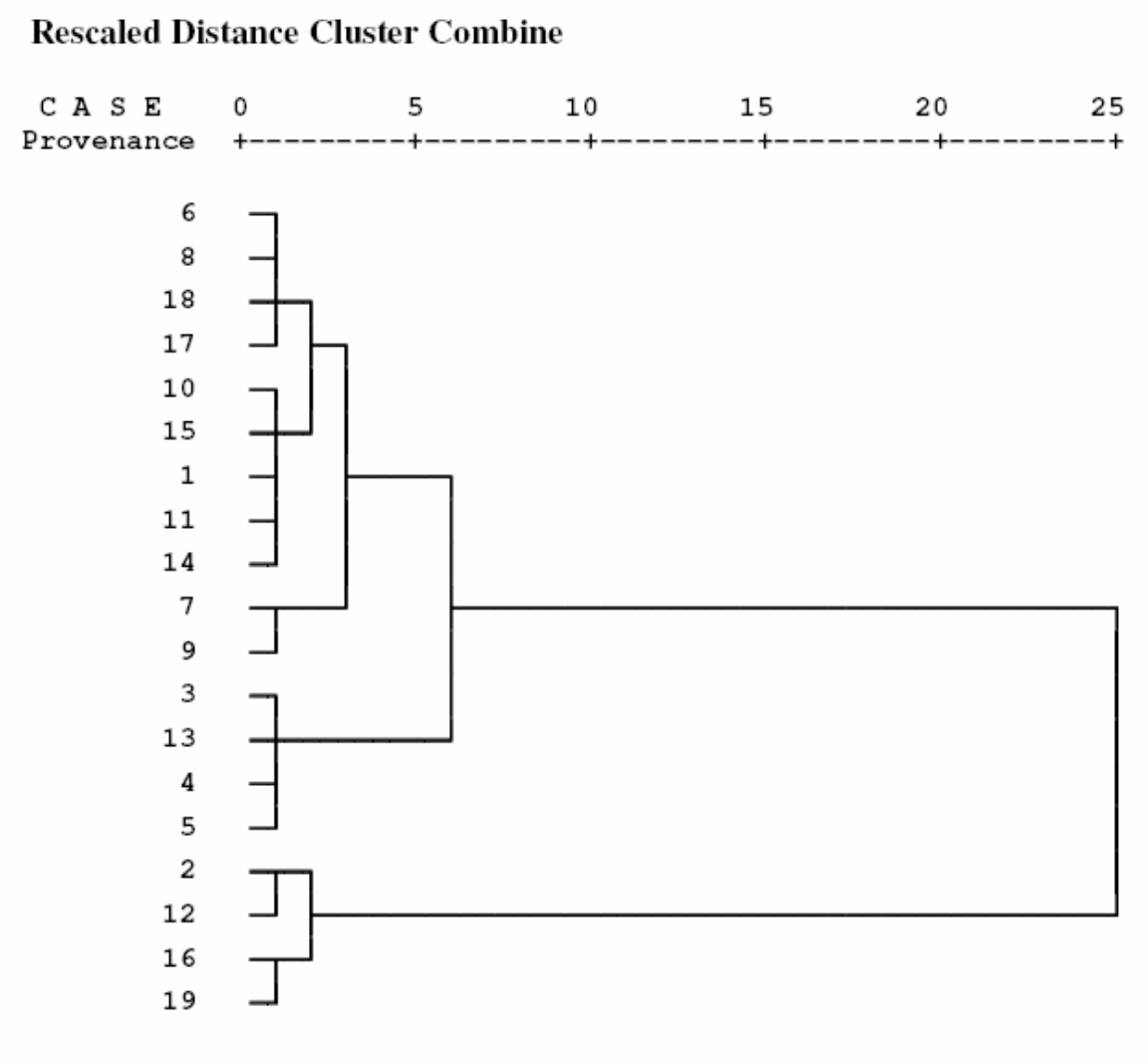

Fig. L. Dendrogram generated by a hierarchical cluster analysis using Ward's method based on photosynthetic efficiency and photosynthetic yield (July 2006) showing the relationship among 19 open-pollinated pecan provenances.

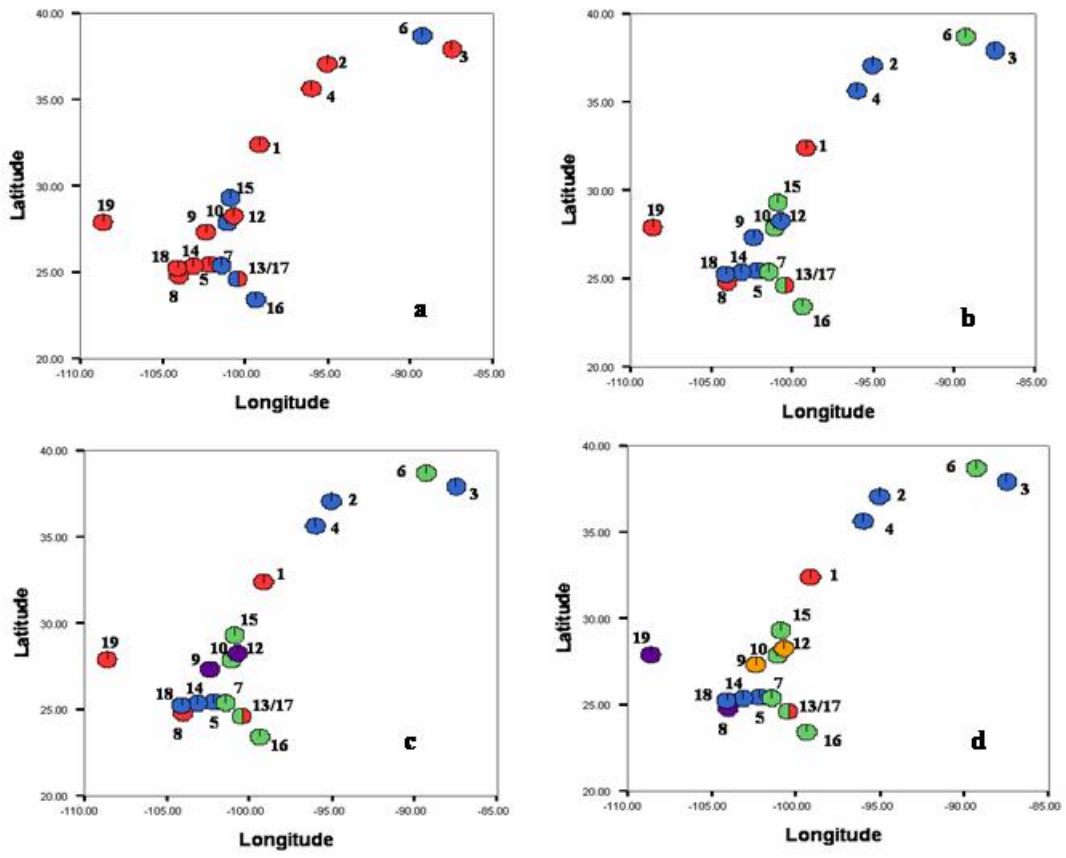

Fig. M. Plotted pie graph generated by a hierarchical cluster analysis based on photosynthetic efficiency and photosynthetic yield (August 2006) showing the distribution of 19 open-pollinated pecan provenances from 18 locations in a. two b. three c. four d. five clusters. Populations grouped together as a cluster are indicated by the same color and the numbers denote the provenances specified in Table 3.

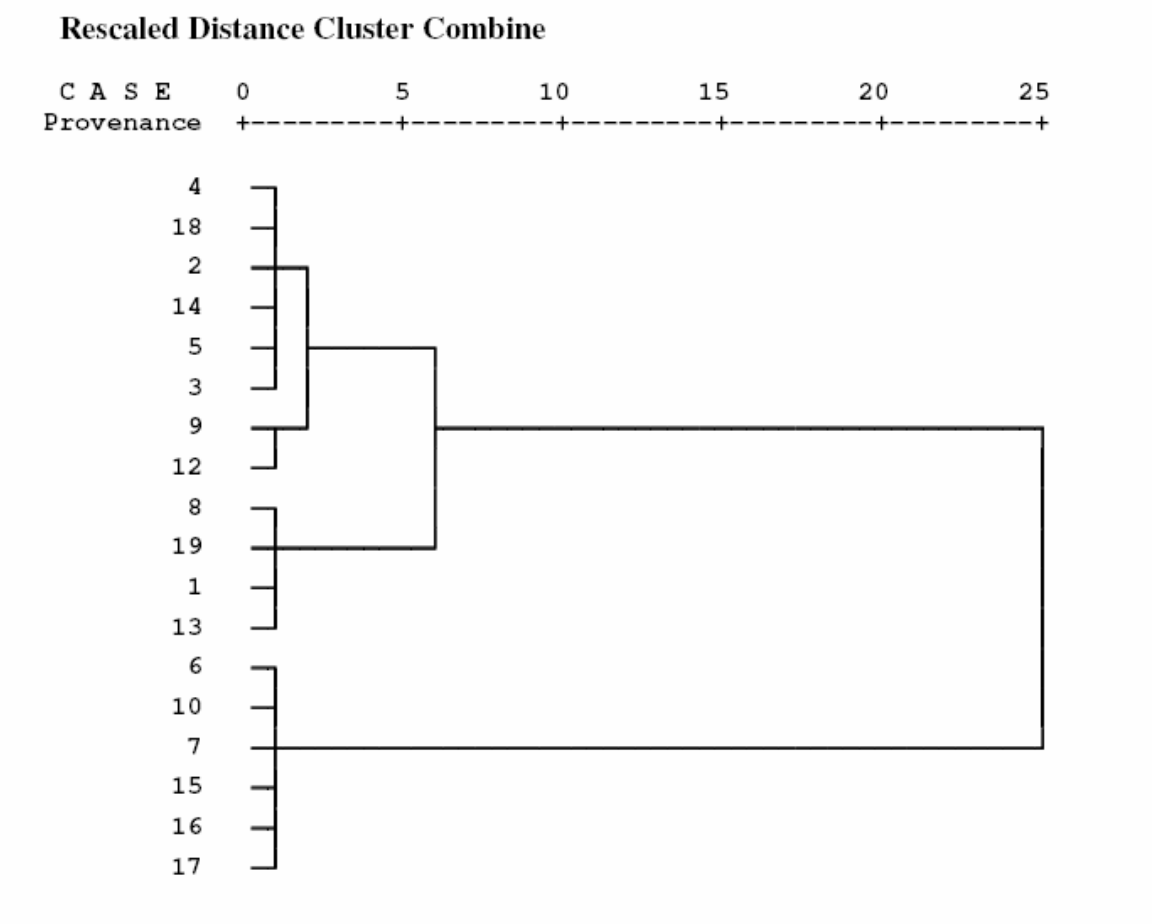

Fig. N. Dendrogram generated by a hierarchical cluster analysis using Ward's method based on photosynthetic efficiency and photosynthetic yield (August 2006) showing the relationship among 19 open-pollinated pecan provenances.

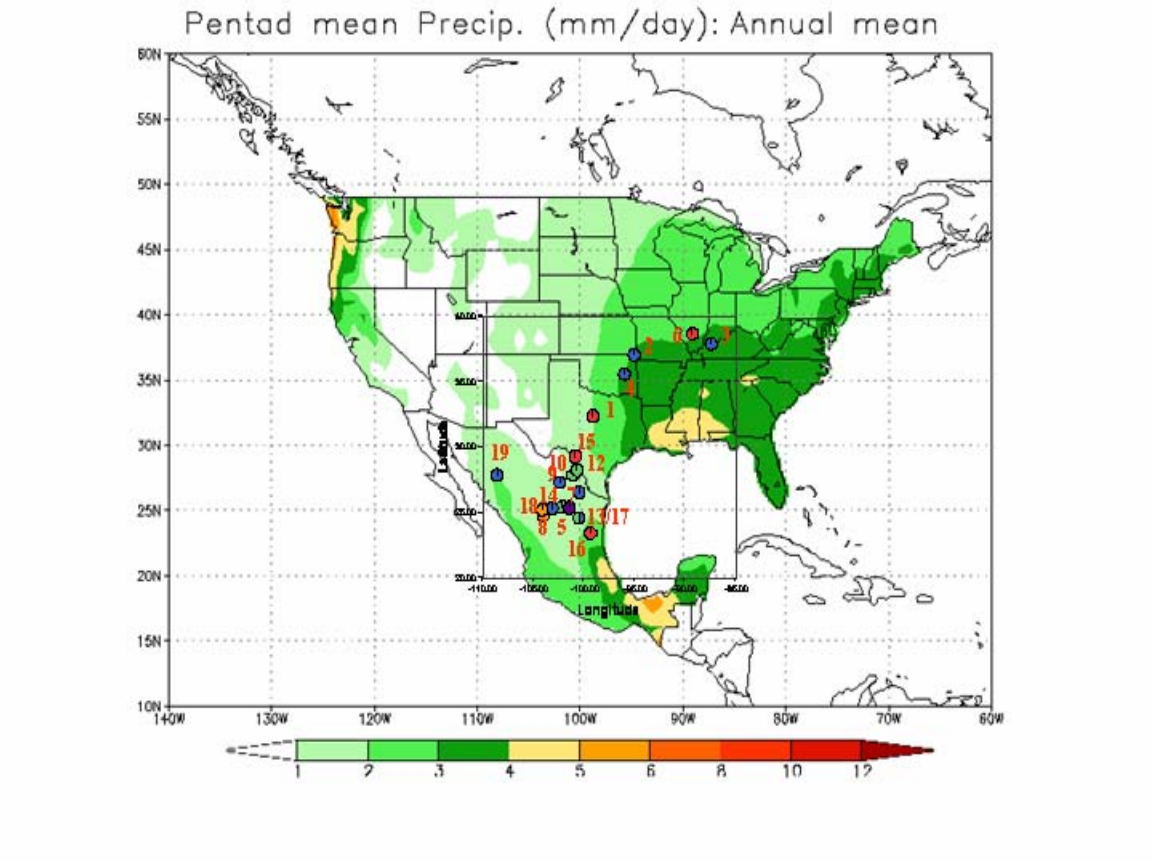

Fig. O. Distribution of the 19 pecan provenances utilized in the present study across United States and Mexico over a precipitation gradient. Map obtained from NOAA (2002).

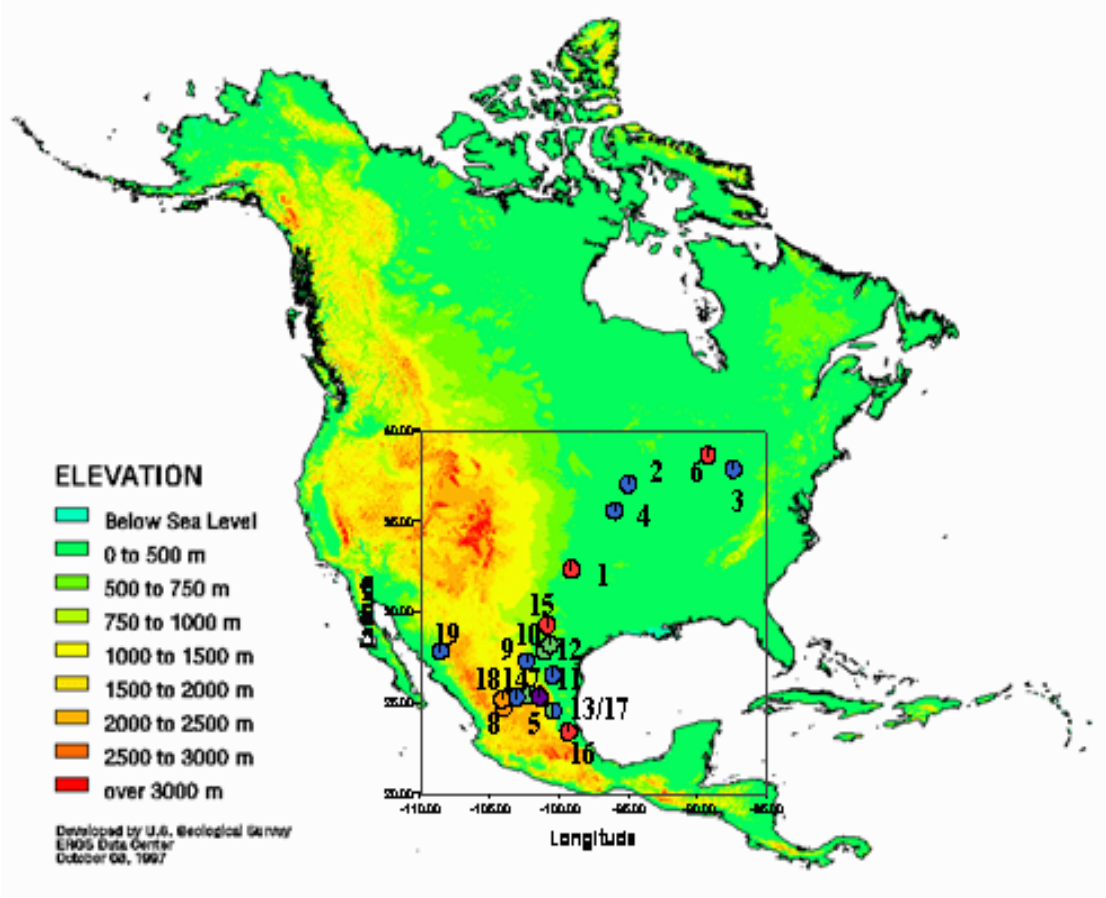

Fig. P. Distribution of the 19 pecan provenances utilized in the present study across United States and Mexico over an elevation gradient. Map obtained from the USGS (1997).

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