COMPARATIVE LEAF ANATOMY OF SIX PECAN CULTIVARS *CARYA ILLINOENSIS* KOCH.

by

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Approved by:

Major Professor

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INTRODUCTION

The anatomical structure of the leaves of plants has been used to a great extent for plant identification, classification, and plant distribution studies. Anatomical measurements of different cultivars within the same species of plant have been used to study the differences in crop yields, water and nutrient absorption, propagation potentials, absorption of chemical through the leaves, plant pest resistance, sugar assimilation efficiency and capacity, and water loss.

Pecan cultivars vary in their productive ability. Some cultivars bear much more precociously bearing than others. There is considerable evidence in the literature that cultural practices as well as genetic factors influence leaf shape and size. Little has been reported on differences that might exist among cultivars of a given species when plants are grown under similar conditions. There is no evidence that a comparative study has been made of the anatomical structure of the leaflet of pecan cultivars. Such a study of the anatomical makeup of the leaves of different pecan cultivars should provide new insight as to why one pecan cultivar is a better producer than another. An increased knowledge of the leaves of pecans might help eventually in enabling horticulturists to regulate the alternate bearing habits of this crop.

Stomata are recognized as playing an important role in the exchange to carbon dioxide and other gases between the air and the leaf as it relates to photosynthate production. Water loss from the plant also occurs through the stomata.

The objects of this research were:

1. To broaden the scope of knowledge concerning the anatomical structure of

leaves of six cultivars of the pecan, Carya illinoensis Koch; Giles, Greenriver, GraTex, Major, Peruque, and Western Schley.

2. To determine if significant differences exist between the different cultivars in regard to:

a. Leaf thickness

b. Stomata density

c. Palisade tissue thickness

d. Palisade cell compactness

e. Midrib diameter

Leaf anatomy, leaf clearing, and leaf stomata imprinting techniques were used in this study.

REVIEW OF LITERATURE

Anatomical comparisons of the leaves of seven different cultivars of apples were made by Pickett (2). Pickett computed the area of the intercellular spaces in different apple cultivars with the use of a planimeter and related in to photosynthetic activities of the leaves. Significant differences in the intercellular space areas were found to exist between cultivars. Pickett (2) also studied the stomata, and he was able to show structural differences among the different cultivars by photographs. He found a correlation between structure and photosynthetic activities of each cultivar.

Simons (8) has studied the anatomy of leaves and shoots of Golden Delicious and Jonared apple trees and correlated anatomical changes with soil moisture supply. In his study, Simons showed that different moisture levels significantly affected the thickness of the leaves. He also was able to determine the width and length of the palisade cells and epidermal cells. Significant differences were found among the cell sizes from plants grown under different moisture levels. The measurements showed that longer palisade cells exist in the Golden Delicious leaves.

Holm (4) describes the structure of Carya alba leaves as having ultimate lateral cell walls but no stomate or hairs in the upper epidermis while lower epidermis has abundant stomata and hairs but no ultimate lateral cell walls. The hairs observed on the leaf were the same as those on the stem. A thick, smooth cuticule covers both sides of the blade. He reports three to five layers of cells containing aggregate crystals in the palisade tissue in the mesophyll. Holm (4) indicated that the rachis, petiole, and midrib all have the same structural appearance.

Fulling (1) has used leaf structural differences as a basis for identifying different cultivars of the fir (Abies). A study of the resin canals in the leaves of different cultivars proved to have taxonomic significance.

Hemtzelman and Howard (5) have used the presence or absence of hairs, the type of hairs, and the types of crystals distributed throughout the leaf in a comparative study of the Icacinaceae. One of the variable features among the species was the pubescence.

Wilkinson (6) used anatomical comparisons in order to study the relative fertility of some species of the tribes Linnaecae and Sambuceae of the Caprifoliaceae.

Wylie (7) has used morophological comparisons to study the leaf structure among the members of a colony of ferns Adiantum pedatum. He found differences in the stomatal intercellular space systems in the leaves of the different ferns. He concluded that these differences were not due to the habitat in which the ferns were growing.

Philpott (10) in his study of the anatomical comparison of the leaf blades of 27 woody species of North Carolina shrub-bog, was able to show some differences in the terminal portions of the leaf veins. He found some differences in the thickness of the leaf blades and also differences in the cuticle thickness. The results of his study showed that thicker leaf blades were correlated with a thicker palisade mesophyll. Philpott (10) stated that "fewer palisade cells are contiguous to an epidermal cell, " according to the ratio of epidermal palisade cells. He found that mountain shrub leaves had fewer differences between palisade and spongy mesophyll than did forest shrub leaves. Philpott (10) found that the leaves of mountain shrubs had numerous veins and that this

vein condition was associated with greater palisade volume. He pointed out that crystals are present in the leaves of mountain shrubs. He has shown that crystals are associated with a particular genus and are not influenced by habitat.

An investigation of the structural modifications of the xeromorphic leaves has been made by Shields (11). He explained that xeromorphic leaves were altered structurally by changes in the environment. Structurally altered leaves had a lower ratio of surface area to leaf volume, reduced external area, decreased cell size, thickened cell walls and a more compact network of veins. The spongy mesophyll was substituted by palisade tissue developed in the leaf. There was also a high stomata frequency.

Turrell (12) has studied the quantitative morphological conditions of large and small leaves of alfalfa. He reported a significant difference in the thickness of the leaflets between the upper and lower epidermis, in the thickness of spongy and palisade mesophyll, and in the diameters of midribs and of major and minor veins. He found that stomata density was lower in the larger leaflets than in the smaller ones. There were also differences between the stomata on the different size leaflets. He concluded there is a positive correlation between leaflet size, internal-external surface ratio, volume of intercellular space, stomata pores, and areole areas.

Wylie (13) in his anatomical survey of 38 species of evergreen dicotyledons from New Zealand found a wide range for both leaf blade and palisade tissue thickness. He reported the mean thickness for leaf blades as 406 U; spongy mesophyll, 193 U; Palisade parenchyma, 124 U; cuticular depth, 93 U; and vein spacing, 199 U. Wylie (13) indicated that large blade thickness is associated

with xeromorphism as related to the evergreen habitat.

In other work by Wylie (14) a relationship was found between leaf internal structure and the distribution of minor veins in the blade. He worked on 66 different herbaceous and woody dicotyledonous species. He reported a significant correlation between the vein distribution and mesophyll organization. The increased amounts of palisade forced the veins closer together while the veins were more widely separated when a large proportion of spongy mesophyll was present.

Wylie (15), working with leaves of 90 dicotyledonous plants from the subtropical regions in Florida, was able to classify leaf cells into two groups. One group of cells (spongy, epidermal layers) was found to be close together and often expanded parallel to the blade plane. The other group of cells were elongated and vertical. The ratio of the volume of these two cell groups in a green leaf was usually related to the vein spacing of the blade.

Morton and Watson (9) have reported on the relationship of physiological growth and efficiency of the leaf. They compared the sugar assimilation of two varieties of sugar beet grown in pot culture. They reported that an increase in the external nitrogen supply increased the rate of production of leaves by the apical meristem. In one variety the number of cells per leaf increased with increase in water and nitrogen supply, but not in other varieties. In the light treatment test, they found that the number of cells per leaf were greatly reduced when initials arose in complete darkness.

Borrill (16) conducted an experiment to test the anatomical relationships between six cultivars of Dactylis. He emphasized the relationship between the leaf and cell size and of the epidermal cell pattern. He found significant differences among the different cultivars.

An extensive work was done by Pyykko (17) in identifying and classifying 284 plants species in Patagonia. The internal leaf structure was used to classify the plants. The main features of structural comparisons were width, shape, thickness of the leaves, thickness of the epidermis, thickness of the cuticule, thickness of the mesophyll, and size of the palisade and spongy mesophyll cells. Pyykko (17) reported a correlation between leaf structure and plant distribution. He found there was less water loss from the leaf where hairs covered the stomata. He concluded that when xeromorphytes receive much light and moisture, they develop strong palisade tissue, but in water-stressed conditions plants develop short, narrow,cubical and closely adherent palisade cells. There was no evidence that environment had an effect on crystal occurrence in the leaf organs. Pyykko concluded that it was due to a genetically controlled.

Dunn et al (18) reported that the imprint of leaves could be useful in determining phylogenetic relationships. By preparing a key through this process might be identified fossils of herbaceous species. Foster (3) used leaf clearings for study of the ontogeny of the terminal sclereid. He was able to show by photomicrographs the lateral veins, and details of differentiating veinlets within the areole. Foster (3) has also used leaf clearing to study sclereid formation and development in different plant species.

Meyer and Anderson (19) have reported that plants grown in different locations may vary in the number of stomata per unit area of the leaf. They have reported on the estimated number of stomata per unit area of the leaf of several different species of plants. The stomata number per square centimeter for lower surface of the apple leaf was 29,400; for black oak, 58,000; for

black walnut, 46,100; and for peach 22,500. In addition they explained that environmental factors have an influence on the stomata numbers. Meyer and Anderson (19) explained that several different plant species have stomata present on the upper and lower surfaces of the leaf, but usually woody plants have stomata only on the lower surface of the leaf with very few on the upper leaf surface.

Miller and McDaniels (20) presented a bibliography on environmental effects on the number of stomata of different species of plant leaves. These workers also reported that in different plant species fewer stomata are produced per unit area of the leaf in a relatively moist condition than in a dryer atmosphere. The highest number of stomata per unit area of leaves of different plants occurred when the plants were grown in sand culture containing the highest per cent moisture. According to Miller et al (20) there was a difference in stomatal density on the different portions of the leaf of citrus crops. The reduction in stomatal number in low light intensity and in the tropical crop growing in nontropical regions have been reported by Miller et al (20).

Pickett (2) has reported that apple trees grown in the greenhouse had fewer stomata than did trees grown in the field.

Gupta (21) has examined the leaves of five different plants of the solanaceae. He reported that the number of stomata per unit area of the leaf had very little value in leaf description since there was a great variation of stomata number from leaf to leaf and from portion to portion of the same leaf. Gupta (21) found a negative correlation between the average number of stomata and the area of the lamina.

MATERIALS AND METHODS

Leaf samples were collected from six pecan cultivars of Carya illinoensis Koch grown in a Bates loam soil (27) near Fredonia, Kansas. The cultivars selected for this study were Major, Greenriver, Peruque, Giles, GraTex, and Western Schley. All trees selected for this study were of varying ages, but as uniform in size as possible. Four trees of each cultivar were used for sampling. Two compound leaves were taken from each of two quadrants per tree. All the trees were bearing. Leaf samples were taken from the tip portions of the branches of the Northeast and Southwest quadrants near the outer edge of the trees about midway up on the side of the tree. From each compound leaf two opposite, middle leaflets were used: one for studying the internal structure of the leaflet, the other for venation. The terminal leaflet (I) on each compound leaf was used to study the frequency of stomata over the leaflet. Figure 1.

Anatomical techniques: In preparing the tissue for sectioning the lower epidermis of Section G, Leaflet 1, (Figure 1), was covered with a thin layer of Elmers Glue-All. After the glue layer was dry, it was peeled off and placed between the microscope slide and cover-glass. The lower surface of the leaflet was studied, and the stomata per 0.156 square millimeter were counted at nine locations on each leaflet. Average counts of nine readings were used for statistical analysis tests. Photomicrographs of the stomata and trichomes were taken at different magnifications.

Section A, which is one centimeter in width, was used for leaf cross-sectional studies.

Section B, one square centimeter area, was used for making abaxial paradermal section of the leaflet.

Section C, D, and E were used for leaf clearing purposes. These sections were of different widths and lengths.

Section D shows the location where studies of the stomata were made on the lower surface of the leaflet.

Sections taken from the terminal leaflets were about two square centimeters.

Section F, Figure 1, was used for cross-sectional studies of the rachis.

Figure 1 Compound leaf of the Pecan showing location of sampling for various studies

Leaflets 1, 2, and 3 were washed with detergent and water and then dried with paper towels by pressing the paper firmly upon the leaflets. Section A, B, and F, (Figure 1,) were cut and placed in FAA solution (23). Leaflet sectioning was done immediately as the leaves were removed from the trees. The procedure suggested by Sass (23) was used for fixing, dehydrating and paraffin embedding of leaf samples. Safranin "O" and fast green staining procedures were used (24, 25, 26).

Measurements of leaflet thicknesses, palisade mesophyll thickness, number of palisade cells per 125 U, and thickness of midribs were taken. Photomicrographs were taken at different magnifications. The mean of ten measurements of the cross-section of the leaflet was used for Least Significant Differences (LSD) statistical analysis tests at the five per cent level.

Leaf clearing: cut Sections C,D,E, (Figure 1,) were placed in jars containing 95% Ethyl alcohol. Fuch's (22) method was used for the leaf clearing process. Permanent slides were prepared and examined.

Stomata imprinting: Leaflet 1 (Figure 1,) was considered for stomatal number determination. The distal leaflets were wetted, covered with wet paper toweling and placed in polyethylene bags. After the samples were brought back to the laboratory, the leaflets were illuminated with a 500 W tungsten bulb. The leaflets were placed in a transparent box in which high humidity was maintained. By providing light, warmth and high humidity, it was expected that the stomata would remain open for the imprinting process.

RESULTS

Aggregates of crystals (druses) were observed primarily in living cells in the cross-sections of the leaflet, midrib, rachis and in adaxial paradermal sections of the leaflets of all cultivars. Druses were present in the pith of the rachis in all cultivars.

There were significant differences between the thickness of the midribs of the leaflets of the different cultivars. The microscopic measurements and statistical analysis tests are shown on Table 3.

Results of the stomata counts on the lower surface of the leaflets are shown in Table 1. There were no stomata present on the upper surface of the leaflets. There were significant differences among the different cultivars in the number of stomata per square millimeter of leaf area at the five per cent level.

Giles, Table 1, had the largest number of stomata, 38460 per square centimeter. Western Schley 35890/cm^2, and GraTex 35250/cm^2, had the second and third largest number of stomata. All cultivars had anomocytic stomata type (28), and the size of the stomata in a given leaflet varied. There was usually one, or occasionally two, large stomata (38U,) per areole. The remaining stomata were small (25U,) and uniform in size. There was considerable variation in shape and size of the stomata within a cultivar and between cultivars. There was no evidence of uniform dispersion of stomata on the leaf surface. Stomata varied in their degree of opening at a given time. On some leaflets stomata were full open, on other half open, even though they were treated the same. There appeared to be no differences as to numbers open or closed between cultivars.

At the five per cent level, as detected by the LSD test, significant differences were found among the leaf thicknesses of the samples from different cultivars. Giles had the thickest leaflets (186U) followed by Western Schley (168U). Greenriver had the thinnest leaflets (146U).

Another feature considered for comparison was palisade tissue thickness. Significant measurements of the palisade tissue (which consists of one or two layers of cells below the upper epidermis down to the spongy mesophyll) are shown in Table 4. Giles had the thickest palisade (860mU). Greenriver and Major had the least extended palisade (640mU).

The results of counts of the palisade cells per 125 microns are shown in Table 5. Significant differences of palisade compactness are present among the different cultivars at the five per cent level. Pictures of abaxial paradermal sections of the leaflets show difference in the size of the spongy mesophyll cells, (plates, VII-XII).

There is an abundance of single hairs present on the surface of the rachis. There are fewer hairs present on the midribs and veinlets in all cultivars. In addition to single hairs, Greenriver and Peruque have a few tufted hairs (28) on the rachis and midribs.

Observations on C, D, and E portions, (Figure 1,) of the cleared leaflets indicated a heavier stomata occured on the middle portions of each leaflet than at the tip or leaflet base. There was a lack of uniformity of areole size and veinlet thicknesses within and between cultivars.

On the lower surface of the leaflets of all cultivars, numerous peltate hairs were found. They varied in diameter, dispersions, and shape between

and within cultivars. They appeared both in the glue imprint and cross-section of the leaflets. There was not a clear opening of the peltate hairs into the internal leaf structure. These hairs nearly always appeared where there was no intercellular opening. In most cases the hairs appeared just below a vein. They stained heavily and appeared dark red. They are marked and shown in (plate I; and plate XIX.).

CODINGS

N = Samples taken from Northeast quadrant of tree

S = Samples taken from Southeast quadrant of tree

G-R = Greenriver

M = Major

P = Peruque

Gx = GraTex

W-S = Western Schley

TABLE 1

Analysis of Stomatal number/0.125 square millimeter leaf area (LSDα test; α=5%)

Variety	Giles		Greenriver		Major		Peruque		GraTex		Western	Schley
Tree Side:	N	S	N	S	N	S	N	S	N	S	N	S
Mean	65	65	49	50	52	45	46	55	55	55	56	53
Σx	518	522	398	400	416	363	378	441	444	440	451	421
Σx^2	1089	544	122	198	280	182	394	601	296	1132	1577	226

LSDα = 9.25 With 84 D. F.

Array Mean:

(S)M	(N)P	(N)G-R	(S)G-R	(N)M	(S)W-S	(S)P(N)GX(S)GX	(N) WS	(N)Giles	(S)Giles
45	46	49	50	52	53	55	56	64	65

*;Means underlined are equal at 5% level

TABLE II

Analysis of measurement of the leaflet thickness ($1x10^{-2}$mm)
LSDα test α=5%

Variety	Giles		Greenriver		Major		GraTex		Peruque		Western Schley	
Tree Side:	N	S	N	S	N	S	N	S	N	S	N	S
Mean	18.6	18.6	15.1	14.1	16.4	16.5	16.3	17.8	16.4	14.4	16.8	16.9
Σx	148.7	149	120.5	112.5	131	132	130.5	142.5	131	115	135	134.8
Σx^2	35	34	24.7	32.2	30	34	49.5	24	12	13,8	47.3	28

LSDα = 2.02 With 84 D.F.

Array Means

(S)GR	(S)P	(N)GR	(N)GX	(N)M&(N)P	(S)M	(S)W-S	(N)W-S	(S)GX	(N)Giles&(S)Giles
14.1	14.4	15.1	16.3	16.4	16.5	16.8	16.9	17.8	18.6

*;Means underlined are equal at 5% level.

TABLE III

Analysis of measurements of midrib diameter in ($1x10^{-2}$mm)
LSDα α=5%

Variety	Giles		Greenriver		GraTex		Major		Peruque		Western Schley	
Tree Side:	N	S	N	S	N	S	N	S	N	S	N	S
Mean	82.1	81	68	73.9	69.9	78.1	79.9	66.2	69.9	81.2	69.1	66.7
Σx	657	648	544	591	559	625	639	530	559	570	553	534
Σx^2	701	1405	276	5838	952.8	396.8	430.8	737.5	210.9	687.5	1322.9	225.5

LSDα = 12.474 With 84 D.F.

Array Means:

(S)M	(S)W-S	(N)GR	(N)W-S	(N)GX&(N)P	(S)GR	(S)GX	(N)M	(S)Giles	(S)P	(N)Giles
66.2	66.7	68	69.1	69.9	73.9	78.1	79.9	81	81.2	82.1

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*;Means underlined are equal at 5% level.

TABLE IV

Analysis of measurements of Palisade tissue thickness
($1x10^{-2}$mm) LSDα α=5%

Variety	Giles		Greenriver		GraTex		Major		Peruque		Western Schley	
Tree Side:	N	S	N	S	N	S	N	S	N	S	N	S
Mean	8.3	8.5	7.1	6.3	7.7	7.9	6.4	6.5	7.4	7.3	7.5	7.6
Ex	66.6	67.9	56.8	50.7	61.3	63.4	51.1	52	59.3	58.6	60.3	61
Ex^2	2.6	5.2	4.5	10.5	22.3	5.3	2.7	5.8	2.6	22.8	17.6	1

LSDα = 1.11 With 84 D. F.

Array Means:

(S)GR	(N)M	(S)M	(N)GR	(S)P	(N)P	(N)W-S	(S)W-S	(N)GX	(S)GX	(N)Giles	(S)Giles
6.3	6.4	6.5	7.1	7.3	7.4	7.5	7.6	7.7	7.9	8.3	8.5

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*;Means underlined are equal at 5% level.

TABLE V

Analysis of measurements of palisade cells compactness
Number of cells counted per 125μ (LSDα α=5%)

Variety	Giles		Greenriver		GraTex		Major		Peruque		Western Schley	
Tree Side:	N	S	N	S	N	S	N	S	N	S	N	S
Mean	27	25	24	20	21	24	22	24	25	22	24	26
Σx	217	204	193	163	171	191	175	190	204	178	196	209
Σx^2	95	40	32.9	5.9	18	34.9	10.9	17.5	15	13.5	91	47.9

LSDα = 2.574, With 84 D.F.

Array Means:

(S)GR	(N)GX	(N)M	(S)P	(S)M;(S)GX;(N)GR;&(N)W-S	(N)P&(S)Giles	(S)W-S	(N)Giles
20	21	22	22	24	25	26	27

*;Means underlined are equal at 5% level.

Plate I -- Cross-section of the leaflet from Giles cultivar, 760X enlarged. Arrow shows the cross-section of peltate hair on the lower epidermis.

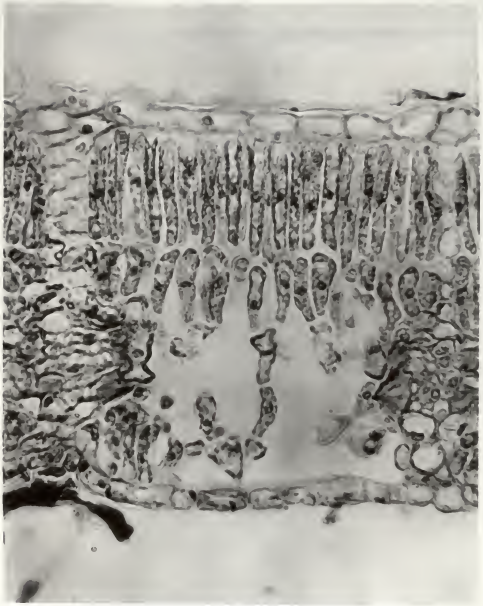

Plate I

Plate II -- Cross-section of the leaflet from Greenriver cultivar, 760X enlarged.

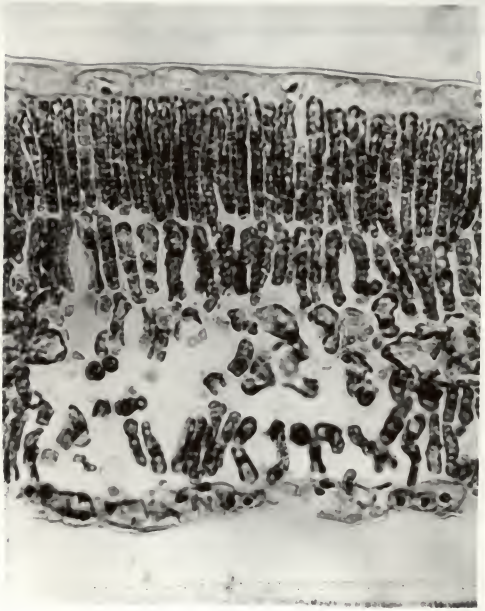

Plate II

Plate III -- Cross-section of the leaflet from GraTex cultivar, 760X enlarged.

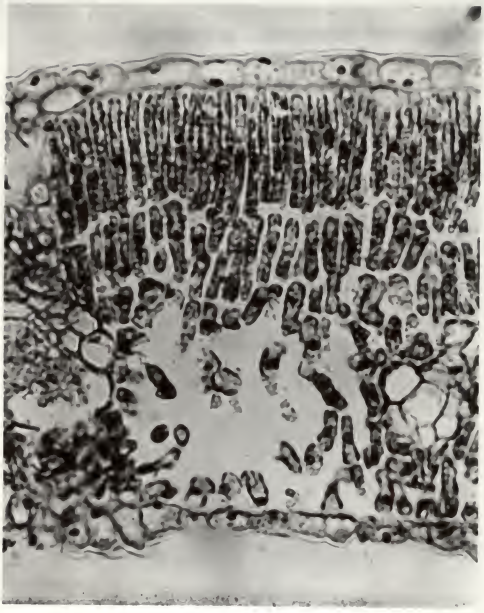

Plate III

Plate IV -- Cross-section of the leaflet from Major cultivar, 760X enlarged.

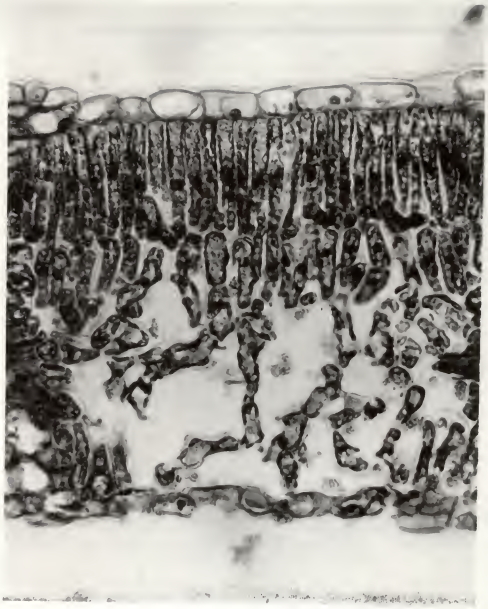

Plate IV

Plate V -- Cross-section of the leaflet from Peruque cultivar, 760X enlarged.

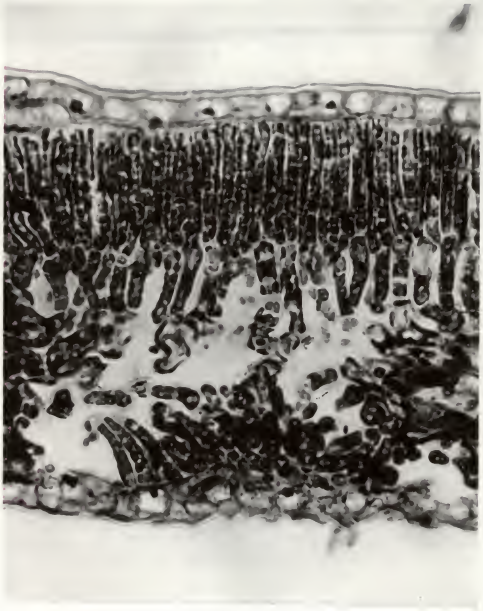

Plate V

Plate VI -- Cross-section of the leaflet from Western Schley cultivar, 760X enlarged. Dark colored curved shape parallel to the lower epidermis shows the cross-section of peltate hair.

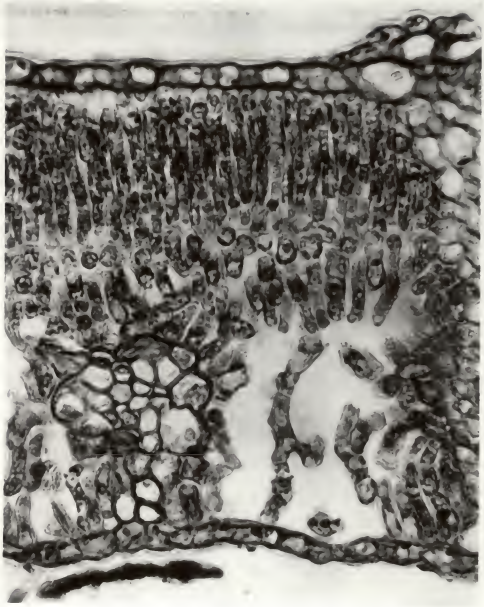

Plate VI

Plate VII -- Tangential section of lower surface of leaflet from Peruque cultivar, 760X enlarged.

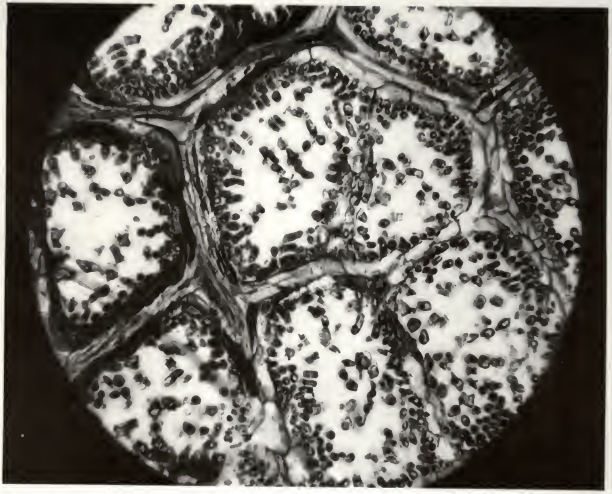

Plate VII

Plate VIII -- Tangential section of lower surface of leaflet from GraTex cultivar, 760X enlarged.

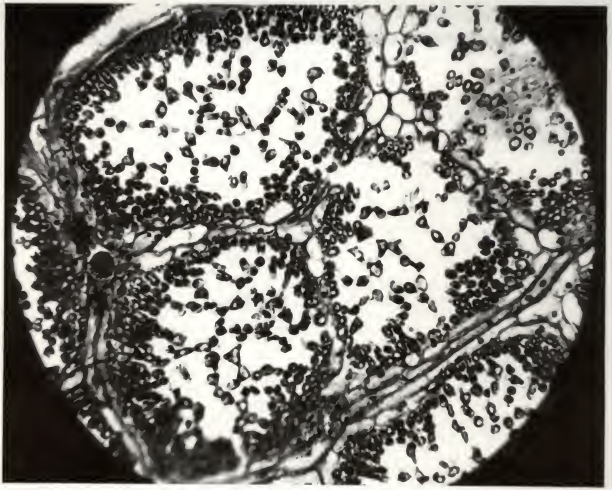

Plate VIII

Plate IX -- Tangential section of lower surface of leaflet from Major cultivar, 760X enlarged.

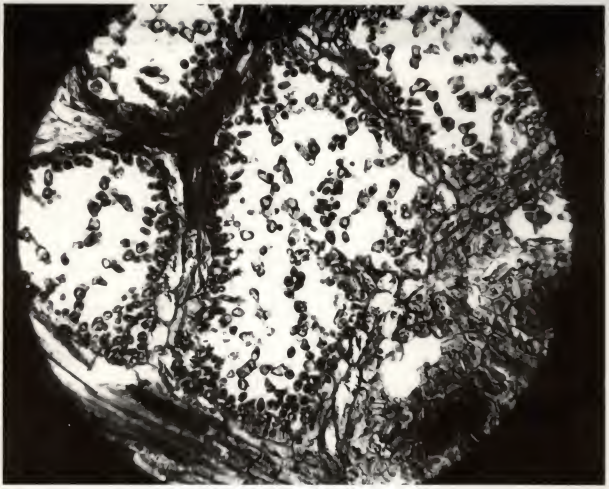

Plate IX

Plate X -- Tangential section of lower surface of leaflet from Giles cultivar, 760X enlarged.

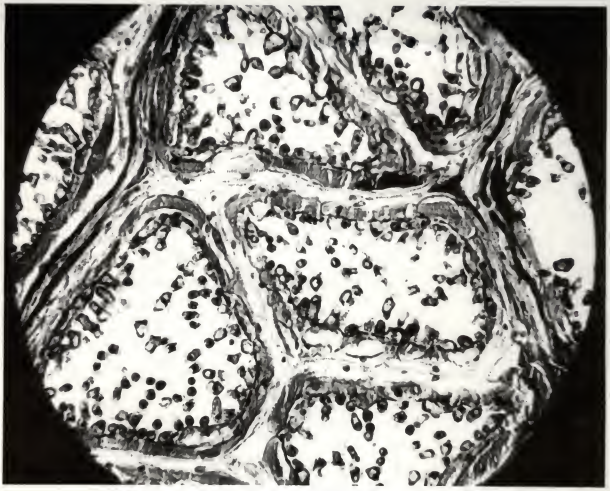

Plate X

Plate XI -- Tangential section of lower surface of leaflet from Western Schley cultivar, 760X enlarged.

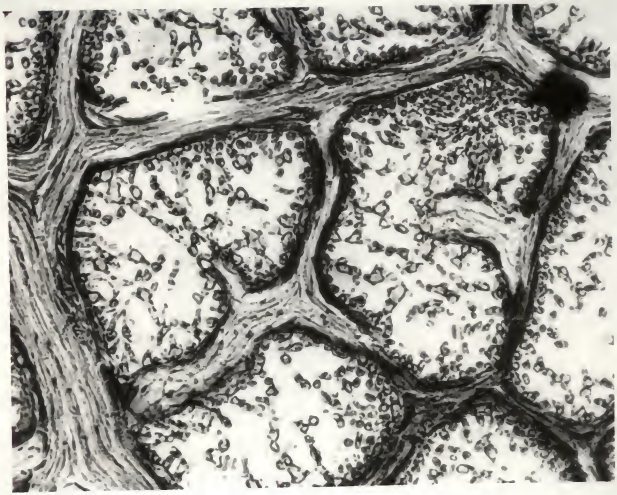

Plate XI

Plate XII -- Tangential section of lower surface of leaflet from Greenriver cultivar, 760X enlarged.

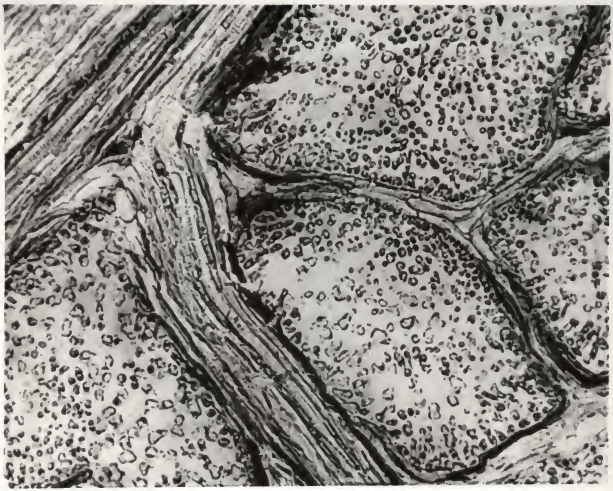

Plate XII

Plate XIII -- Picture of glue imprinted stomata in Giles cultivar, 760X enlarged.

Plate XIII

Plate XIV -- Picture of glue imprinted stomata in Western Schley cultivar, 760X enlarged.

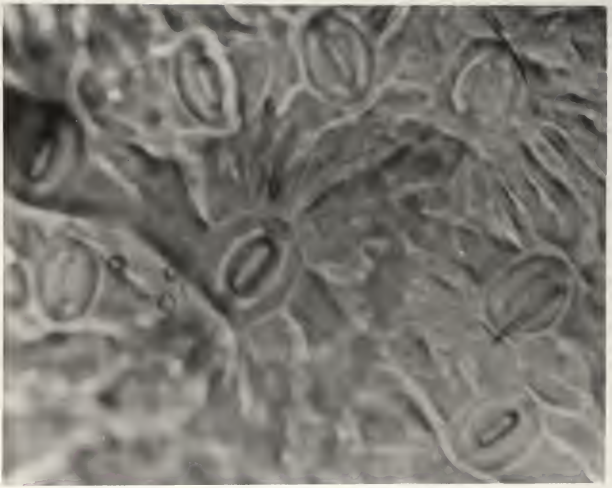

Plate XIV

Plate XV -- Picture of glue imprinted stomata in Major cultivar, 760X enlarged.

Plate XV

Plate XVI -- Picture of glue imprinted stomata in Greenriver cultivar, 760X enlarged.

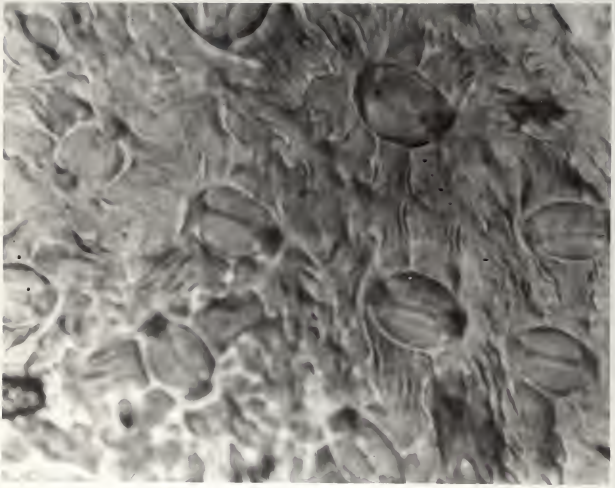

Plate XVI

Plate XVII -- Picture of glue imprinted stomata in GraTex cultivar, 760X enlarged.

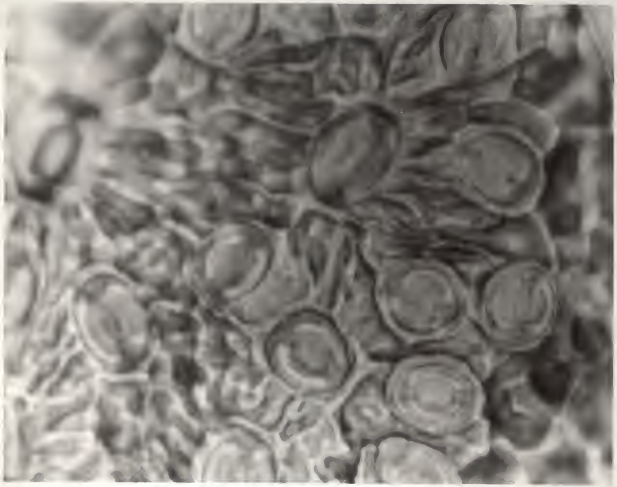

Plate XVII

Plate XVIII -- Picture of glue imprinted stomata in Peruque cultivar, 760X enlarged.

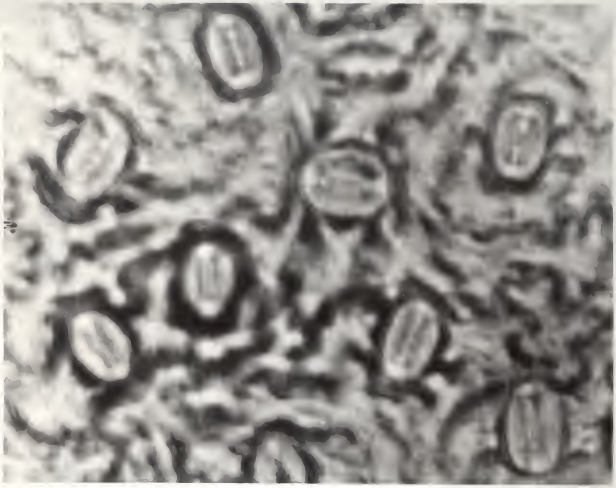

Plate XVIII

Plate XIX -- Lower magnification of stomata glue imprint, showing more detail. Arrow shows the peltate hair in GraTex cultivar, 190X enlarged.

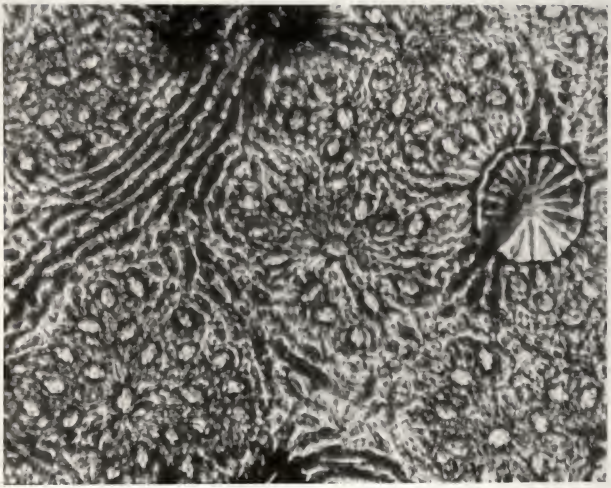

Plate XIX

Plate XX -- Lower magnification of stomata glue imprint showing more detail of leaflet lower surface in Western Schley cultivar, 190X enlarged.

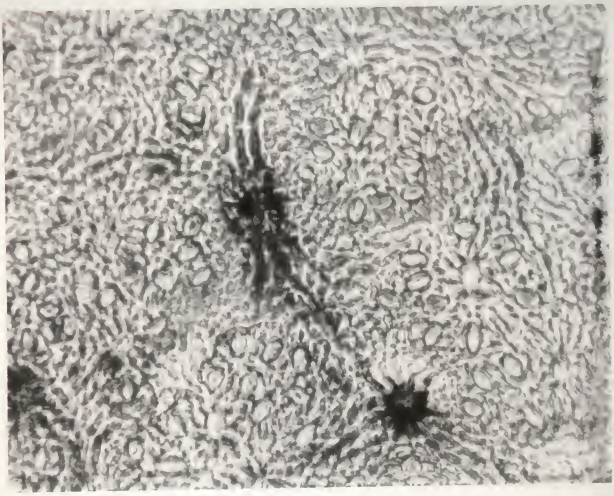

Plate XX

Plate XXI -- Lower magnification of stomata glue imprint showing more detail of leaflet lower surface in Greenriver cultivar, 190X enlarged. Two peltate hairs are shown in the picture.

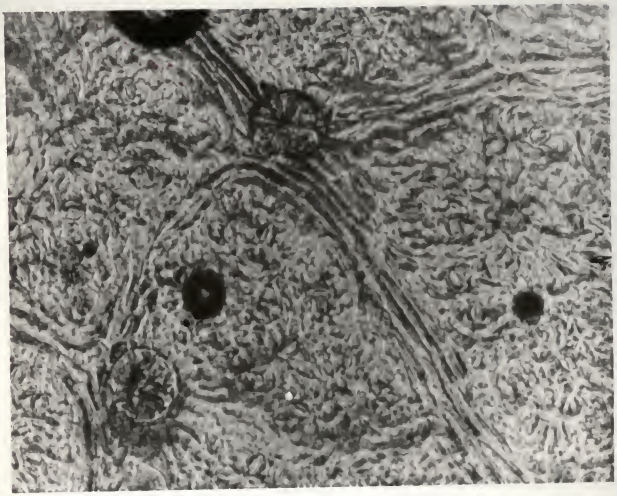

Plate XXI

Plate XXII -- Lower magnification of stomata glue imprint showing more detail of leaflet lower surface in Giles cultivar, 190X enlarged.

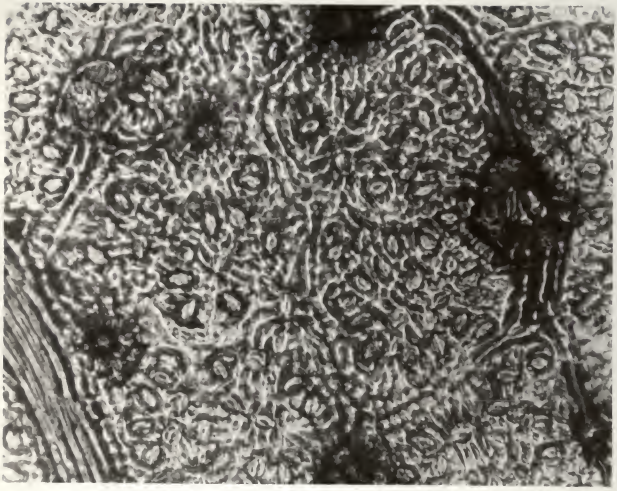

Plate XXII

Plate XXIII -- Lower magnification of stomata glue imprint showing more detail of leaflet lower surface in Peruque cultivar, 190X enlarged.

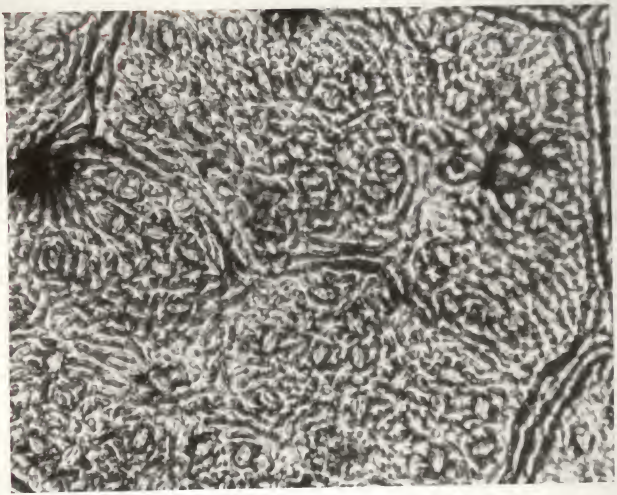

Plate XXIII

Plate XXIV -- Lower magnification of stomata glue imprint, shows more detail of leaflet lower surface in Major cultivar, 190X enlarged.

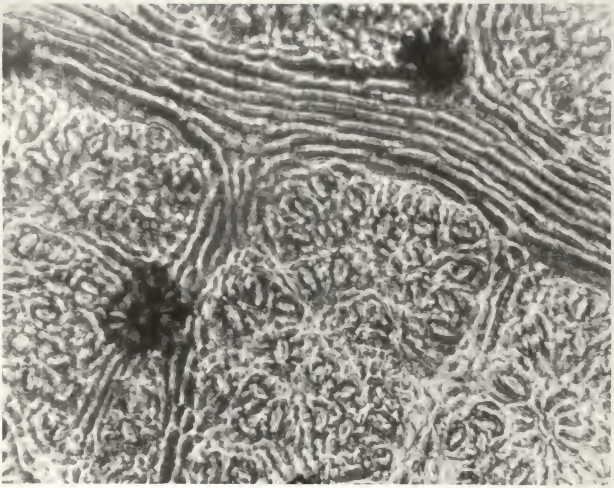

Plate XXIV

DISCUSSION

Significant differences in the thickness of leaflets, and palisade tissue depth and compactness among six different pecan cultivars could indicate that one cultivar is a more efficient manufacturer of photosynthates than another. Such a deduction seems reasonable since the thicker the leaf the more cells present to manufacture food. Philpott (10) noted that when there are large air spaces and thick or numerous palisade layers, the leaves are thicker. The thickness of the palisade tissue is important since the palisade cells contain chlorophyll.

In this study Giles had significantly thicker leaflets from both the north and south sides of the trees than did Greenriver. This was also true of palisade tissue depth.

In this study leaf samples were taken from trees growing under similar environmental conditions; therefore, differences due to external influences would be minimal. The rootstock of the trees, however, are a variable that could conceivably have some effect upon the leaf structure.

Borrill (16) and Pyykko (17) reported that leaf morphological studies would probably enable us to know more about the distribution of plants in different areas and their ability to grow and develop in a certain area.

This conclusion is significant in this study since Greenriver originated in the eastern part of the United States in Henderson County, Kentucky. Giles originated in Cherokee County, Kansas near Chetopa.

In the data shown in this paper, Major, which also originated in Henderson

County, Kentucky, was not significantly different in palisade tissue depth measurements from Greenriver. Means of the measurements show that Major and Greenriver palisade tissue depth are almost identical. The leaflet from the north and south sides of Major trees differed significantly in thickness, but this was not true with Greenriver. Significant differences existed between the thickness of the leaflets of Major trees and the leaflets taken from the south quadrant of Greenriver trees. There was no significant differences between leaflet thickness taken from the north quadrant of Greenriver tree and Major tree.

It is interesting in this study also that Western Schley, which originated in central Texas in San Saba County, is not significantly different in leaf thickness, in palisade tissue depth or palisade cell compactness from Giles. Both Western Schley and Giles, having originated further west, appear to have leaf structures that are significantly different from leaflets taken from Greenriver and Major which originated in the more eastern part of the United States.

There are several environmental factors that have influences on the depth of the palisade layers and intercellular spaces in the leaves of different plant species. Pickett (2) has reported more intercellular space areas in the leaves of apple trees grown in the field than those grown in the greenhouse. He reported that the compactness of the leaf palisade cells varied from year to year in apple trees. Pickett (2) found changes of the intercellular space in different temperatures of the greenhouse-grown apple trees which was reversed in two different cultivars.

The stomata count studies showed Giles leaflets have significantly greater

numbers of stomata per measured area than do leaflets from the other cultivars. Western Schley and GraTex leaflet stomatal densities were more closely allied to Giles than to the other cultivars. Major and Greenriver cultivars had the lowest number of stomata.

Although Miller et al (20) reported that the stomatal numbers on citrus plants were reduced by changing the light intensity, there were no significant differences in stomatal number between north and south exposures within the same pecan cultivar used in this study. This may be explained by the fact that all sides were getting adequate light due to the high light intensities that are common under Kansas conditions.

Gupta (21) found a negative correlation between the average stomatal number and the leaf area. This could be true with the pecan cultivars used in this study, but, leaf area determinations were not included.

It is generally accepted that the plant loses water in vapor form into the atmosphere through the stomata, and the plant will use carbon dioxide of the air which has entered the leaf intercellular spaces through the stomata. Whether stomatal numbers have any relation to the efficiency of a tree to produce photosynthates is not known; however, it seems logical that a plant with a larger number of stomata than another under similar environmental conditions might be a more efficient producer of carbohydrates.

Diameter measurements of the midribs of the leaflets of the six pecan cultivars used in this study failed to produce definitive data; however, significant differences did occur among cultivars. Giles leaflets had the largest midrib diameters while Western Schley and Greenriver had the

smaller midrib measurements. Holm (4) in his anatomical studies of Carya alba, found similarities between midrib, rachis and petiole structure, but such similarities were not observed in this study of Carya illinoensis.

SUMMARY

Anatomical studies were made on leaflet structure from six different pecan cultivars. Comparisons were made between leaflet thickness, depth of palisade tissue, compactness of palisade cells, midrib diameters, and stomatal counts.

Significant differences were found among cultivars in all comparative studies made.

This study indicated that pecan cultivars originating in the same general geographic area of the United States showed similarities in the thickness of the leaflets, stomatal number and palisade tissue measurements. There were significant differences, however, between cultivars that have originated in different geographic areas of the United States. Giles and Western Schley cultivars showed similarites and differed from Greenriver and Major which showed similarities.

The other cultivars studied fell between these two groups. All cultivars showed distinct double layers of palisade tissue. The side of the tree from which the leaf samples were taken had no effect on the anatomical structure of the leaflets.

Single hairs occuring on the midribs and veinlets of all cultivars with tufted and single hairs in Greenriver and Peruque. Peltate hairs were present on the lower surface of the leaflets in all cultivars.

There is lack of uniformity in the thickness of the veinlets and size of the areole in all cultivars.

Further studies to correlate the data from this study with leaf area measurements would be of value. Studies to correlate such information with yield data should also be of great value in determining if one cultivar is better adapted and will produce more fruit in a given area than any other cultivar.

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COMPARATIVE LEAF ANATOMY OF SIX PECAN CULTIVARS

Carya illinoensis Koch.

By

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University Park, New Mexico, 1966

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The purpose of this study was to determine if there are structural differences in the leaves of six pecan cultivars of Carya illinoensis Koch: Giles, Greenriver, GraTex, Major, Peruque, and Western Schley.

Leaf clearing, leaf tissue fixing, dehydrating and embedding and stomata imprinting techniques were employed in this study. Leaf samples were taken from trees growing in Bates loam soil near Fredonia, Kansas. Trees were bearing and samples were taken from four trees of each cultivar. Sass's method was used for leaf tissue fixing (FAA), dehydrating, (TBA), and paraffin embedding. Safranin "O" and fast green were used for staining, basic NaOH Fuchsin for leaf clearing, and Elmer's Glue-All for stomatal imprints.

Least significant difference (LSD) statistical analysis tests at the five percent level were used to compare leaflet thickness, palisade tissue depth, palisade cell compactness, midrib diameter, and stomatal density.

In all comparative studies made, there were significant differences between cultivars. This study indicated that pecan cultivars originating in the same general area of the United States are quite similar in leaf anatomy. However, there were significant differences between cultivars that originated in different geographic areas of the United States. Giles and Western Schley which originated in Kansas and Texas respectively showed structural characteristics which were not common to Greenriver and Major which originated in the state of Kentucky.

No structural differences were observed in the leaves taken from the North and South quadrants of the tree. All leaflets showed distinct double layers of palisade tissue.

There was an abundance of peltate hair on the lower surface of the leaflets of all cultivars. All cultivars had single hairs, Peruque and Greenriver had tufted hairs, also.

Further study would be of value in correlating the information obtained from this experiment with leaf area and crop yield studies.