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Changes in ultraviolet-B and visible optical properties and absorbing pigment concentrations in pecan leaves during a growing season

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Abstract

UV-B (280–320 nm) and visible (400–760 nm) spectral reflectance, transmittance, and absorptance; chlorophyll content; UV-B absorbing compound concentration; and leaf thickness were measured for pecan (*Carya illinoensis*) leaves over a growing season (April–October). Leaf samples were collected monthly from a pecan plantation located on the Southern University Horticultural Farm, Baton Rouge, Louisiana. The leaf spectral reflectance and transmittance were measured from 280 to 760 nm using a high-accuracy UV-Vis spectroradiometer with an integrating sphere. The spectral absorptance was calculated based on the reflectance and transmittance percentages. Total UV-B absorbing compounds concentration ($A_{280\text{–}320\,\mathrm{nm}}$ (cm^{-2})), chlorophyll content, and leaf thickness were related to the leaf optical properties. The patterns of the spectral distributions of optical properties over the growing season, effects of leaf age on the measured parameters, and their relationships were determined. Leaf optical properties were significantly affected by leaf age and the effects were more dramatic in the visible spectral region than in the UV-B region. Within the UV-B spectral region, leaf reflectance ranged between 4.27% (April) and 8.29% (July), transmittance between 0.01% (June) and 0.05% (April), and absorptance between 91.70% (July) and 95.68% (April). Within the visible spectral region, leaf optical properties changed significantly with the wavelength and leaf development. The visible spectral reflectance and transmittance had maxima at 555 nm and minima at 680 nm, and increased sharply afterwards. The highest visible spectral reflectance and transmittance occurred in the newly developed leaves, whereas the visible spectral absorptance increased with leaf maturation. Leaf chlorophyll content, thickness, and UV-B absorbing compound concentration significantly increased during leaf development (April–July). Correlation and regression analyses indicated that leaf chlorophyll content was a strong predictor of the green light reflectance, transmittance, and absorptance. The strongest predictors of UV-B reflectance were leaf thickness and leaf UV-B absorbing compound concentrations.

Keywords: UV-B/visible radiation; Spectral absorptance/reflectance/transmittance; UV-B absorbing compound concentration; Chlorophyll; Leaf thickness; Pecan (*Carya illinoensis*)

1. Introduction

Decreased quantities of total-column ozone are now observed over large parts of the globe, permitting increased penetration of solar UV-B radiation (280–320 nm) to the Earth's surface (UNEP, 1998). Plants have developed different defense mechanisms against

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UV-B radiation, such as thicker and smaller leaves (Bornman and Vogelmann, 1991; Sullivan and Teramura, 1988), increased production of UV-absorbing compounds such as flavonoids (Caldwell et al., 1983; Tevini et al., 1991), and higher amounts of reflective waxes (Barnes and Cardoso-Vilhena, 1996; Dubé and Bornman, 1992; Rozema et al., 1997). Flavonoids have been considered a major protective mechanism from UV-B radiation because of their strong absorbance in this wavelength region and highly increased concentration in epidermal layers due to UV-radiation (Beggs and Wellmann, 1994; Bornman et al., 1997; Caldwell et al., 1983; Robberecht and Caldwell, 1978, 1983; Tevini et al., 1991). The accumulation of flavonoids in the epidermis has been shown to reduce epidermal transmittance of UV-B radiation and thus may provide some protection (Robberecht and Caldwell, 1978; Tevini et al., 1991). At the structural level, increased leaf thickness is often induced by UV-B radiation, reducing UV-B penetration to internal leaf tissues (Bornman and Vogelmann, 1991). Penetration of UV-B radiation also changes with leaf age, younger leaves attenuating UV-B radiation less than the more mature leaves, as was shown in some conifers (DeLucia et al., 1991, 1992). Leaf age may be an important factor when assessing the influence of UV-B on plant performance (Teramura and Caldwell, 1981). During leaf ontogeny, chlorophyll accumulates, generally reaching a maximum concentration (per unit area) when the leaf is fully expanded (Sestak, 1985).

It is generally agreed that leaves absorb over 90% of incident UV-B; leaf surface reflection in this wavelength range is generally below 10% and there is negligible transmission of UV-B through leaves (Cen and Bornman, 1993; Gao et al., 1996; Gausman et al., 1975; Qi et al., 2002; Robberecht et al., 1980; Yang et al., 1995). Though leaves of all species measured to date have low reflectivity in the UV range, on a percentage basis, reflectivity could differ significantly inter- and intra-specifically.

Review of the leaf optical properties bibliography containing more than 600 publications compiled by Jacquemoud (1996) as well as recent publications, suggests that most plant species tested to date have been annual agricultural species, which account for less than 10% of global net primary production. Very few studies have been conducted on tree species, which account for more than 80% of global net primary production. In addition, the studies dealing with leaf optical properties in trees have focused more on the visible and infrared spectral regions than the UV or UV-B regions, and mature leaf samples were used in most cases. Little information exists on systematically and simultaneously monitoring leaf optical properties in UV and visible spectral regions and their spectral distributions spanning a growing season in pecan (*Carya illinoensis*) except our initial report (Qi et al., 2002). Such information along with biochemical changes (such as formation of flavonoids and chlorophylls) can be useful for updating existing UV-B models and developing new models to predict UV-B screening abilities of plants.

Pecan is an important agronomic crop tree species in North America. The current paper reports the UV-B and visible spectral distributions in leaves of pecan over a growing season (April–October); age effects of leaves on optical properties, UV-B absorbing compound concentration, chlorophyll content, and leaf thickness; and the relationships among these parameters.

2. Materials and methods

2.1. Materials

Leaf samples were collected monthly from April (the month of leaf emergence) to October (the month before leaf senescence) from a 40-year-old pecan plantation located on the Southern University Horticultural Farm. April through October represents the period of the long growing season for pecan in Southern Louisiana. In the first week of each month, sun-exposed leaves were collected from four unshaded individual trees from terminal 20–50 cm of branches, and were placed in a humidified plastic bag in an insulated box for transporting to the laboratory, about 200 m from the plantation. Since pecan has compound leaves with 9–17 leaflets, the middle pair of the leaflets from leaf samples of each tree was then selected randomly for the study. The measurements of leaf optical properties, chlorophyll content, and leaf thickness were completed the same day of leaf collection, while samples for measuring UV-B absorbing compounds concentration were prepared the same day and the analyses were completed a week later. The experiment was a completely randomized design with months as treatments representing the various ages of the leaves in a growing season.

2.2. Measurements of leaf optical properties

Leaf reflectance and transmittance were measured with an integrating sphere (IS-1000, Optronic, FL) from 280 to 760 nm as described by Optronic Laboratories Inc. (1997). The integrating sphere, powered by a constant power-output supply (OL 65 Programmable Current Source, Optronic, FL), was used in combination with an irradiance standard (OL Series 752-10, Optronic, FL) and a high-accuracy UV-Vis spectroradiometer (OL 754, Optronic, FL) that was connected to a computer and printer. Spectral scanning was performed between 280 and 760 nm at 5-nm intervals to produce spectral distributions of the reflectance and transmittance. The leaf samples were illuminated with a 200 W tungsten coiled-coil filament lamp with a small 1.3 cm × 7 cm quartz envelope. The spectral irradiance values were provided within the wavelength range of 250–800 nm based on the National Institute of Standards and Technology (NIST) 1973 scale of spectral irradiance. The calibrated OL 754 measured photons captured at each wavelength. The same location on the adaxial surface of the leaf sample was irradiated for both reflectance and transmittance scans. Measurements of both spectral transmittance and reflectance were based on the direct substitution method (Optronic Laboratories, 1997). Measuring spectral transmittance involved two scans; a calibration (or 100%) scan and a test scan. The system automatically calculated the transmittance as the ratio of the test sample signal to the 100% signal at each wavelength. Measuring spectral reflectance also involved two scans; a calibration (or 100%) scan and a test scan. The system automatically calculated the reflectance as a ratio of the test signal to the 100% scan at each wavelength. The test sample's reflectance was calculated by multiplying the calibrated reference standard's reflectance value. The spectral absorptance (A_λ) of a leaf sample was computed from its spectral transmittance (T_λ) and spectral reflectance (R_λ) as: $A_\lambda = l - T_\lambda - R_\lambda$. The leaf optical properties were measured for pecan leaves from April to October with at least four replicates each month.

2.3. UV-B absorbing compounds

Extraction of leaf UV-B absorbing compounds, mainly flavonoids and related phenolic compounds, was performed following Gorton and Vogelmann (1996) that was consistent with Markham (1982). Two 10-mm-diameter leaf discs (the total leaf area was 1.57 cm^2) were placed in a 1.5-ml microfuge tube and ground to a fine power in liquid nitrogen with a Teflon pestle. One milliliter of acidified methanol (methanol:H_2O:HCl 79:20:1, v/v/v) was added to the microfuge tube and homogenized well. The extracts were stored frozen (−80 °C) for up to 1 week, then clarified by centrifugation (Micro 12, National Labnet Company, Inc.). Forty microliters of the supernatant were transferred to a quartz cuvette and diluted in 3 ml of extraction medium (resulting in a dilution factor of 76) and scanned from 200 to 820 nm with a computer-controlled, split-beam, dual-detector UV-Vis spectrophotometer (Genesis 2 Spectronic Inc.) at 1 nm increments. The spectra of the extract were then divided by the sample leaf area (1.57 cm^2 of two leaf discs) to obtain the absorbance values on a leaf area basis (A (cm^{-2})). It should be noted that the absorbance values on a leaf area basis, used further in the results and discussion, were not standardized for volume, because the absorbance values would then have been enhanced by 76 times based on the dilution factor. This method kept the absorbance scale relative to the normal reading scale of the spectrophotometer. The results of the statistical comparison among the months were not affected by the lack of standardization because the absorbance reading was relative and the same dilution factor was used for each sample extraction. The leaf UV-B absorbing compounds were monitored monthly from April to October with at least four replicates per month.

2.4. Chlorophyll concentration

Leaf total chlorophyll content was measured with a Minolta chlorophyll meter (Spad-502, Spectrum Technologies Inc., IL). The values measured by the chlorophyll meter corresponded to the amount of chlorophyll present in the plant leaf. The values were calculated based on the amount of light transmitted by the leaf in two wavelength regions, a red

LED (peak wavelength: approximately 650 nm) and an infrared LED (peak wavelength: approximately 940 nm). During calibration, the two LEDs emitted light sequentially without any sample in the sample slot. The received light was converted into electrical signals and the ratio of their intensities was calculated. After a sample was inserted in the measuring head, the two LEDS emitted light again. The light transmitted by the leaf stroked the receptor and was converted into electrical signals. Then the ratio of the intensities of the transmitted light was calculated. The content of the chlorophyll present in the sample leaf was then calculated based on the calibration and sample intensity ratios and presented in percentage (%). The procedure was fairly quick and nondestructive. The chlorophyll content was measured monthly from April to October with at least ten replicates each month.

2.5. Total leaf thickness

A thin leaf transverse section was obtained by holding two double-edged razor blades tightly together and cutting through a leaf sample. New razor blades were used for each sample to ensure good sectioning. The leaf sections were used for anatomical examination. This procedure is fairly easy to operate with a little practice, and suitable for observing a large amount of fresh leaves in a short period of time so that leaf samples could be kept at or close to their original moisture condition. The leaf sections obtained by this procedure were thin enough to show various types of tissues under a light microscope. Based on this procedure, total leaf thickness (μm) was measured with a light microscope equipped with a micrometer. The leaf thickness was measured monthly from April to October 2000 with at least ten replicates per month.

2.6. Data analyses

The spectral distributions of leaf reflectance, transmittance, and absorptance from 280 to 760 nm were plotted from April to October based on the monthly averages. Three representative wavelength regions; UV-B (280–320 nm), green light (555 nm), and red light (680 nm); were chosen for the purpose of characterization of the spectral changes during the growing season. In the UV-B region, the simple averages from 280 to 320 nm were determined monthly for each optical property. Effects of leaf age (represented by the months during the growing season from April through October) on optical properties, UV-B absorbing pigment concentration, chlorophyll content, and leaf thickness were analyzed using the ANOVA procedure (SAS, version 8). If a significant difference ($P \leq 0.05$) was found in the above variables, the means were separated using Tukey's studentized range (HSD) test. Further, correlation analyses were performed to obtain a correlation matrix, showing the coefficients of correlation for all pairs among the variables. Linear regressions were also performed to determine the levels of significance of the correlations within the matrix.

3. Results

3.1. General trends of the UV-B and visible spectral distributions over a growing season

Leaf spectral distributions from 280 to 760 nm from April to October in pecan are presented in Fig. 1. In the UV-B (280–320 nm) and UV-A (320–400 nm) spectral regions the pattern of the leaf spectral distributions changed very little with wavelength over the growing season. In general across the UV (280–400) spectral region, leaf reflectance was less than 10%; transmittance was close to zero; and absorptance was above 90% (Fig. 1).

In contrast, the leaf spectral distribution within the visible (400–760 nm) spectral region changed dramatically with both the wavelength and the growing season (Fig. 1). Leaf reflectance and transmittance increased from 400 to 555 nm, reached maxima at 555 nm (green wavelength), then decreased from 555 to 680 nm and reached minima at 680 nm (red wavelength), and thereafter increased sharply from 680 to 760 nm. The reverse occurred for visible spectral absorptance. Although the pattern of the visible spectral distribution remained the same from April to October, the magnitude of the pattern was greatly affected by the leaf age, especially during the leaf growth and development (April–June). Both reflectance and transmittance of visible light were the highest in the newly developed leaves (April), and decreased as

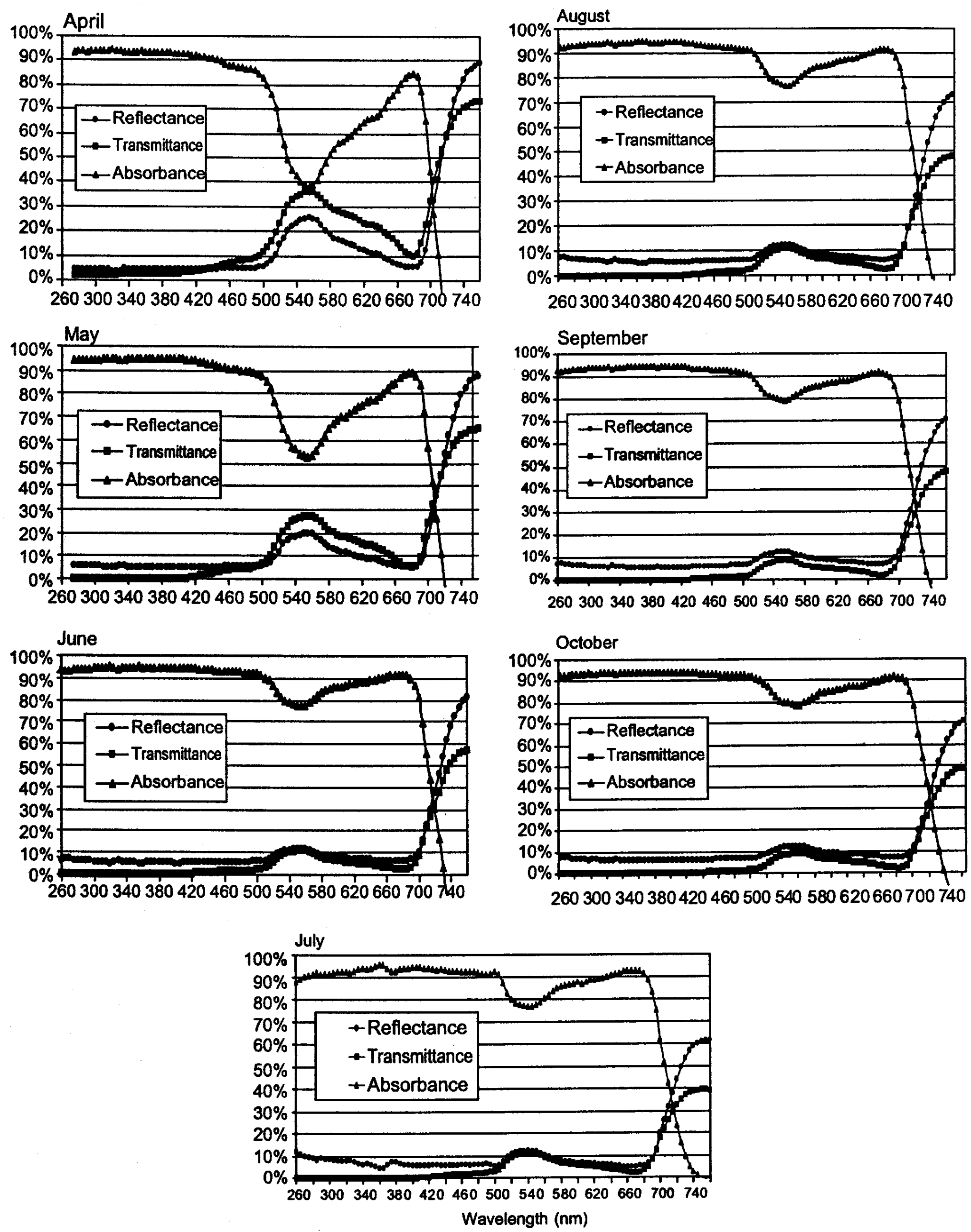

Fig. 1. Leaf spectral distributions from 280 to 760 nm in pecan during a growing season from April to October.

Table 1
Changes in reflectance (R_λ), transmittance (T_λ), and absorptance (A_λ) of pecan leaves during a growing season at three wavelength regions, UV-B (280–320 nm), green (555 nm), and red (680 nm) wavelengths

Months	UV-B (280–320 nm)			Green (555 nm)			Red (680 nm)		
	R_λ (%)	T_λ (%)	A_λ (%)	R_λ (%)	T_λ (%)	A_λ (%)	R_λ (%)	T_λ (%)	A_λ (%)
April	4.27 d	0.05 a	95.68 a	27.35 a	39.21 a	33.44 c	5.50 b	9.64 a	84.86 d
May	5.41 c	0.02 ab	94.58 b	19.96 b	27.39 b	52.75 b	5.73 b	5.35 c	88.91 b
June	5.69 c	0.01 ab	94.30 b	11.59 c	11.07 c	77.34 a	6.05 ab	2.19 d	91.77 a
July	8.29 a	0.01 b	91.70 d	11.05 c	10.28 dc	78.67 a	5.51b	6.20 bc	86.62 bc
August	6.26 b	0.01 b	93.73 c	12.23 c	10.93 dc	76.83 a	6.14 ab	6.95 b	84.38 c
September	6.36 b	0.01 b	93.64 c	12.14 c	8.59 d	79.28 a	6.72 a	5.61 bc	85.24 bc
October	6.43 b	0.01 b	93.57 c	12.31 c	9.21 dc	78.49 a	6.75 a	6.16 bc	84.58 c
P^*	<0.0001	0.0150	<0.0001	<0.0001	<0.0001	<0.0001	0.0223	<0.0001	<0.0001

Within each column, means with the same letter are not significantly different at alpha = 0.05, according to Tukey's studentized range (HSD) test.

* P is the probability of one-way ANOVA, indicating the level of significance among the months for each variable.

leaves grew and matured (May–July); whereas the absorptance of the visible light increased during this period. The spectral pattern maintained relatively unchanged from leaf maturation to the end of the growing season (July–October).

3.2. *Age effects of leaves on optical properties at three wavelength regions*

Based on the spectral distribution patterns over the growing season (Fig. 1), three characteristic wavelength regions, UV-B (280–320 nm), green wavelength peak at 555 nm, and red wavelength peak at 680 nm, were chosen. Because the spectral distributions were relatively constant across the UV-B wavelength region, the simple averages from 280 to 320 nm determined monthly for each optical properties were used for comparisons. Effects of leaf age on reflectance, transmittance, and absorptance in these three wavelength regions were analyzed individually and the results are shown in Table 1.

3.2.1. *280–320 nm*

Although the spectral distributions in the UV-B region appeared to be quite stable throughout the growing season (Fig. 1), statistical analyses by ANOVA revealed subtle but significant changes in UV-B optical properties with leaf age (Table 1). There was a significant increase in UV-B reflectance from April (4.27%) to July (8.29%) and no significant changes were detected from August to October. Young leaves (in April) seemed to have higher transmittance than mature leaves (July–October), but the values were extremely low, ranging from 0.01 to 0.05%. The absorbance was significantly higher in young leaves than in mature leaves, ranging from 91.70 to 95.68% (Table 1).

3.2.2. *555 nm*

Leaf age had significant effects on reflectance, transmittance, and absorptance at 555 nm ($P < 0.0001$ for all cases). There were significant decreases in leaf reflectance and transmittance, and a significant increase in leaf absorptance from leaf emergence (April) to leaf maturation (July) (Table 1). Reflectance and transmittance maxima at 555 nm occurred in April with 27.35 and 39.21%, respectively, both then decreased significantly through June to 11.59 and 11.07%, respectively, and were relatively stable for the rest of the season. Leaf absorptance at 555 nm increased significantly from 39.21% in April to 77.34% in June and thereafter kept relatively steady through October.

3.2.3. *680 nm*

At 680 nm reflectance was moderately affected by leaf age, showing a small increase from April (5.50%) to September (6.72%); while transmittance appeared decreasing during leaf development from April (9.64%) to June (2.19%), averaging 6.01% over the growing season. The absorptance at 680 nm also significantly increased from April (84.86%) to June

(91.77%) with a growing season average of 87.92% (Table 1).

3.3. Age effects of leaves on UV-B absorbing compound concentration, chlorophyll content, and leaf thickness

3.3.1. UV-B absorbing compounds

The absorbance spectra of leaf UV-B absorbing compounds from 280 to 320 nm from April to October are presented in Fig. 2. The absorbance spectra increased remarkably during leaf development and peaked in July at the full leaf expansion. Despite the magnitude differences among the months, the absorbance spectra followed a similar pattern, showing a decline from 280 to 320 nm except May, for which the spectrum was somehow inconsistent and this could relate to the sampling variation and needs to be future investigated. The declining trend also indicated that the UV-B absorbing compounds were strongly wavelength dependent. To facilitate the statistical comparison among the months, and to avoid the arbitrary selection of a single wavelength for such a comparison, we decided to include the entire UV-B wavelength region by calculating the cumulative absorbance values from 280 to 320 nm for each sample, and there were four samples each month. This cumulative absorbance in our opinion would be the best criterion to represent the total UV-B absorbing compounds concentration. Our recent report on 35 tree species (Qi et al., 2002) indicated that the absorbance of UV-B absorbing compounds measured at 280, 300, and 310 nm were not always in agreement with each other although fairly good correlations existed among these wavelengths (R^2 range: 0.76–0.93) and this was mainly attributed to the wavelength-specific nature of the UV-B absorbing compounds. As such, instead of conducting a comparison at any selected wavelengths, the cumulative absorbance from 280 to 320 nm at a 1-nm interval for each sample, standardized for leaf area and expressed as $A_{280\text{–}320\,nm}$ (cm^{-2}), were analyzed statistically to assess the age effects, and the results are presented in Table 2. $A_{280\text{–}320\,nm}$ (cm^{-2}) was significantly affected by leaf age ($P < 0.0001$) with

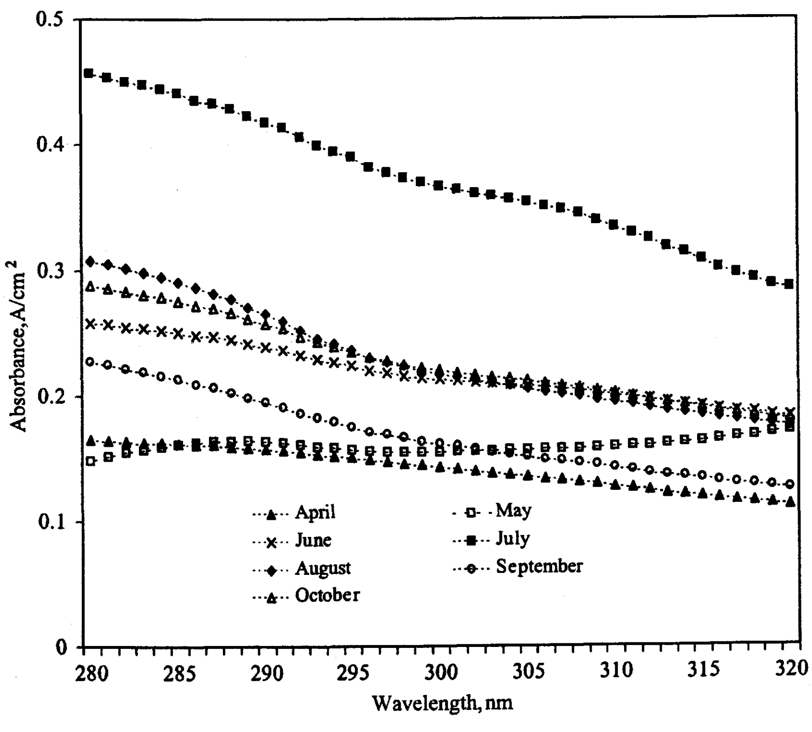

Fig. 2. Absorbance spectra of UV-B absorbing compounds in pecan leaves during a growing season from April to October.

Table 2
Changes in total absorbance of UV-B absorbing compounds, chlorophyll content, and thickness in pecan leaves during a growing season

Months	Cumulative absorbance of UV-B absorbing compounds ($A_{280-320\,nm}$) (cm^{-2})	Chlorophyll content (%)	Total thickness (μm)
April	5.82 c	29.70 d	160 e
May	6.54 c	36.11 c	185 d
June	8.96 b	38.76 b	199 dc
July	15.28 a	40.59 ab	233 a
August	9.43 b	38.58 b	220 ab
September	6.92 c	40.88 a	206 bc
October	9.38 b	39.95 ab	189 dc
P[a]	<0.0001	<0.0001	<0.0001

Within each column, means with the same letter are not significantly different at alpha = 0.05, according to Tukey's studentized range (HSD) test.

[a] P is the probability of one-way ANOVA, indicating the level of significance among the months for each variable.

the lowest absorbance in the newly grown leaves in April and the highest in mature leaves in July; thereafter, the values generally declined or fluctuated for the remaining growing season (August–October). This decline indicated that the degradation of certain UV-B absorbing compounds took place as leaves aged.

3.3.2. Chlorophyll content

Leaf chlorophyll content was significantly affected by leaf age ($P < 0.0001$, Table 2). A significant increase was shown during leaf growth and development from April to July, indicating that the time for full leaf expansion and further leaf maturity occurred in July. Thereafter, leaf chlorophyll level remained relatively steady until October and showed no significant changes compared to July (Table 2). It was not surprising that little decline was shown in chlorophyll content in October because of the warm subtropical weather in Southern Louisiana associated with a long growing season where pecan leaves typically do not begin their senescence process until late November. In fact, in our study, the leaves throughout the tree crowns in October were still dark green showing no visible difference from September.

3.3.3. Leaf thickness

Leaf thickness was also significantly influenced by leaf age ($P < 0.0001$, Table 2). It increased significantly from April (160 μm) to July (233 μm), and slowly declined toward the end of the growing season due to aging effects. The increase in leaf thickness through July coincided with the trend of chlorophyll development, supporting that July was the time for full leaf expansion.

3.4. Relationships between leaf optical properties and absorbing pigment concentrations and leaf thickness

The correlation matrix for the eight variables measured in pecan leaves during the growing season is presented in Table 3. The matrix shows the coefficients of correlation for all pairs of dependent and independent variables. The levels of the significance of the correlations were assessed based on linear regression analyses. This information is useful in determining the intercorrelations among the variables and in selecting the final independent variables to be included in models. The leaf chlorophyll content was related negatively to the light reflectance ($r = -0.965$, $P < 0.001$) and transmittance ($r = -0.964$, $P < 0.001$), and positively to absorbance at 555 nm ($r = 0.967$, $P < 0.001$). There was no significant relationship between the chlorophyll content and the UV-B absorbing compound concentrations in pecan ($r = 0.567$, $P = 0.184$). The strongest predictors of UV-B reflectance were leaf thickness ($r = 0.890$, $P < 0.01$) and leaf UV-B absorbing compound concentration ($r = 0.900$, $P < 0.01$). The leaf thickness also appeared to be a fair predictor of the total UV-B absorbing compound concentration ($r = 0.791$, $P < 0.05$). Significant intercorrelations existed between the leaf optical properties at 555 nm ($r > 0.99$, $P < 0.0001$ for all cases) (Table 3).

Table 3
Correlation matrix for selected variables in pecan leaves during a growing season

	Correlation coefficients (r)							
	Chlorophyll (%)	Thickness (μm)	Absorbance of UV-B absorbing compounds ($A_{280-320\,nm}$) (cm^{-2})	UV-B reflectance ($R_{280-320\,nm}$) (%)	Green reflectance ($R_{555\,nm}$) (%)	Green transmittance ($T_{555\,nm}$) (%)	Green absorptance ($A_{555\,nm}$) (%)	Red absorptance ($A_{680\,nm}$) (%)
Chlorophyll (%)	1.000							
Thickness (μm)	0.801*	1.000						
Absorbance of UV-B absorbing compounds ($A_{280-320\,nm}$) (cm^{-2})	0.567	0.791*	1.000					
UV-B reflectance ($R_{280-320\,nm}$) (%)	0.812*	0.890**	0.900**	1.000				
Green reflectance ($R_{555\,nm}$) (%)	−0.965***	−0.832*	−0.618	−0.782*	1.000			
Green transmittance ($T_{555\,nm}$) (%)	−0.964***	−0.789*	−0.560	−0.753*	0.991***	1.000		
Green absorptance ($A_{555\,nm}$) (%)	0.967***	0.805*	0.580	0.765*	−0.996***	−0.999***	1.000	
Red absorptance ($A_{680\,nm}$) (%)	0.081	0.003	0.014	−0.091	−0.124	−0.028	0.062	1.000

* The correlation is significant at $P < 0.05$.
** The correlation is significant at $P < 0.01$.
*** The correlation is significant at $P < 0.001$.

4. Discussion and conclusions

Based on our study, pecan leaves during the growing season proved to have a great impact on UV-B interception, reflecting 4–8%, transmitting near 0%, and absorbing 92–96% of UV-B radiation. This finding agrees with the previous reports (Cen and Bornman, 1993; Gao et al., 1996; Gausman et al., 1975; Robberecht et al., 1980; Yang et al., 1995). Such effectiveness of leaves and of tree canopies collectively in blocking harmful UV-B radiation is a significant contribution of trees to the environments where people live. Planting more trees and proper management of urban and community forests will significantly reduce the harmful UV-B radiation. In pedestrian locations where trees obscure both the sun and most of the sky from view, there are large reductions in UV-B irradiance (Grant et al., 2002; Heisler and Grant, 2000). People who are active outdoors should utilize the tree canopy as a natural UV-B protective measure. The fact that leaf absorptance of UV-B radiation was consistently high (over 90%) from April to October (Fig. 1), suggests that young leaves were just as effective as mature leaves in blocking UV-B radiation.

Measurements of leaf reflectance are useful in determining tree influences on exposure to UV radiation of other life forms in the vicinity of urban trees (Grant and Heisler, 1996; Grant et al., 2002; Heisler and Grant, 2000). The significant increases in leaf UV-B reflectance, leaf thickness, chlorophyll content and UV-B absorbing compounds all occurred from April to July, indicating that pecan leaves had reached their full expansion by July (Tables 1 and 2). There were fairly good correlations existing among leaf UV-B reflectance, leaf thickness, and UV-B absorbing compounds (Table 3). It is interesting that in the UV-B range, leaf absorbance was at a minimum in July, while the averaged pools of UV-B absorbing compounds were at their maximum and so was the reflectance. Since many researchers would believe in the dogma that such compounds "absorb" the UV-B radiation, rather than reflect UV-B. A question stemming from these results is whether the "UV-B absorbing compounds" could also be "reflecting compounds"? It is not clear to us, and this question deserves a further investigation. However, one may argue that although leaf UV-B absorbing compounds play an important role in UV-B absorption, they may not be the only factor for such determination. Obviously, the change in leaf structure and surface morphology would affect leaf reflectance and, in turn, it would affect the absorbance. Studies have shown increases in leaf surface reflective wax and cuticle thickness may increase UV-B reflectance (Barnes and Cardoso-Vilhena, 1996; Dubé and Bornman, 1992; Rozema et al., 1997). It was observed that the adaxial surfaces of pecan leaves sampled in June had already developed trichomes and ridging waxy layers (Qi, unpublished data). Increase in waxy content on leaf surfaces during leaf development has also been reported in other trees such as yellow poplar, red maple, and red oak (Brakke et al., 1993). In addition, with regard to the UV-B absorbing compounds, a recent report by Warren et al. (2002) indicated that leaf age affected both the accumulation of phenolic compounds and the flavonoids composition in field grown *Pinus ponderosa*, *Quercus rubra*, and *Pseudotsuga menziesii*. It was also pointed out that crude tissue MeOH extracts and spectrophotometric measurements can provide a measure of total UV absorption, but provide little information concerning the specific compounds affecting the absorption (Warren et al., 2002). Therefore, a further investigation should be devoted to isolation, identification, and quantification of specific absorption profiles of the UV-B absorbing compounds so that it may provide the insight for better understanding of the chemistry of UV-B absorption in pecan.

The fact that leaves absorb more than 90% of UV-B radiation has prompted us to investigate where in the leaves the UV-B is absorbed. Qi et al. (2002) studied the UV and visible spectral distributions within mature leaves of four tree species including pecan using a fiber-optic microprobe system. It was found that in all cases, little or no UV-B light is transmitted to leaf palisade and sponge mesophyll tissues. Light within palisade and sponge mesophyll was mainly in the visible wavelength region. This suggests that the upper epidermal layer is primarily responsible for absorbing the 90% of UV-B radiation. Therefore, the mesophyll tissues beneath the epidermis are protected from UV-B damage. An unavoidable consequence of the exposure of plants to natural sunlight is the exposure to UV-radiation, which is cumulative through the growing season. Under such conditions, pecan would likely be UV-B resistant by absorbing over 90% of UV-B in the leaf epidermis, which accumulates UV-B absorbing compounds as leaves develop. However, this may differ under higher levels of UV-B exposure. Future study should explore the effects of different levels of enhanced UV-B radiation on leaf optical properties, and related biochemical and morphological changes.

The results of the present study suggest that there was no significant correlation between the UV-B absorbing compound concentration and chlorophyll content (Table 3). This finding represents the intraspecific situation. In addition, the same result held true interspecifically when we related the UV-B absorbing compound concentrations to the chlorophyll contents measured in the mature leaves of 35 southern tree species ($r^2 = 0.02$) (Qi et al., 2002). This is reasonable because they have little in common. The UV-B absorbing flavonoids, the secondary metabolites, are mainly accumulated in the epidermis, where they strongly absorb UV-B radiation and transmit visible or photosynthetically active radiation (PAR) (Hahlbrock and Grisebach, 1979; Jordan, 1996; Krauss et al., 1997; Robberecht and Caldwell, 1986). In contrast, chlorophylls, the primary metabolites, are situated in the leaf mesophyll tissues and strongly absorb PAR.

The spectral absorptance pattern in the visible wavelength region in pecan (Fig. 1) is in agreement with the previous studies for other species by Gates (1965), Gates et al. (1965), Gausman et al. (1976), Gupta and Woolley (1971), and Vogelmann and Moss (1993). Mature leaves absorbed more visible light than young leaves. Within the visible region, leaves absorbed more blue and red than green wavelengths and this is consistent with the absorption spectra of chlorophylls a and b (Buchanan et al., 2000). Within the leaves of pecan, palisade tissues absorbed more light than sponge mesophyll (Qi et al., 2002). The seasonal spectral reflectance pattern in the visible region in pecan agrees with Gates (1980) who reported the seasonal reflectance changes in a white oak (*Quercus alba*) based on chronological age of the leaves.

In conclusion, leaf optical properties of pecan were compared between UV and visible spectral regions. Changes in leaf optical properties were associated with leaf growth and development and were more remarkable in the visible than in the UV region. Pecan leaves absorbed an average of 94% of UV-B radiation. UV-B reflectance could be predicted by leaf UV-B absorbing compound concentrations and thickness. The information presented here is useful in many applications including determining vegetation characteristics using remote sensing, predicting UV-B resistance features, and establishing models to assess the impact of tree canopy on UV exposure in the environment. The information helps us understand how plants interact with solar UV and visible radiation.

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