18 Characteristics of UV-B Radiation Tolerance in Broadleaf Trees in Southern USA

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Abstract Research has indicated that the ozone layer in the earth's stratosphere has decreased significantly in the last two decades. Such a reduction has led to an increase in solar ultraviolet-B (UV-B) radiation (280 nm − 315 nm) striking the earth's surface. Nearly two-thirds of the 400 plant species and cultivars tested to date appear to be UV-B sensitive, one-third of which show certain tolerant characteristics. However, the majority of plants evaluated thus far have been annual agricultural species. Very few studies have been conducted on tree species which account for more than 80% of the global net primary production. Scientists from Southern University, the USDA Forest Service Northeastern Research Station, the USDA UV-B Monitoring and Research Program, and the University of Vermont, have recently completed a five-year program of collaborative research to assess UV-B (280 nm − 315 nm) radiation tolerance characteristics of more than 30 common broadleaf tree species in the southern US. The project has established a database of leaf optical properties, depth of UV-B penetration into leaves, concentration of UV-B absorbing compounds, and leaf anatomy. The study concluded that on a whole leaf basis, the tree leaves absorb 91% − 95%,

reflect 5%－9%, and transmit very little (＜1%) incident UV-B radiation. At the tissue level, the upper leaf epidermis appears to be the main site absorbing the most UV-B radiation. The study has identified 23 broadleaf tree species that possess strong epidermal UV-B screening functions and attenuate 92%－99% of the UV-B through their epidermal layers. These species include Arizona ash, chestnut oak, mocker nut hickory, pecan, American sycamore, bitternut hickory, green ash, sawtooth oak, American elm, blue Japanese oak, cherrybark oak, cottonwood, southern live oak, southern magnolia, shumard oak, sweetgum, American beech, white oak, Chinese tallow, water oak, yellow poplar, Bradford pear, and red maple. The epidermal attenuation is shown to be the dominant UV-B screening characteristic in most of the species studied. Thus, the effectiveness of the epidermal function of UV-B screening underlines the important aspect of the UV-B protection mechanism in the broadleaf trees. Within the species, there are cumulative increases in leaf thickness, leaf total concentration of UV-B absorbing compounds, and leaf chlorophyll content with an increase in UV-B radiation during the period of leaf growth and development (from April to August). The increased concentration in leaf UV-B absorbing compounds may help protect plants against the enhanced UV-B level during the growing season. Comparisons among the species, however, showed that large inter-specific variations exist in the leaf total UV-B absorbing compound concentration, leaf epidermal thickness, leaf total thickness, and depth of UV-B penetration, indicating the individualistic nature of the species. The knowledge of UV-B absorbing compounds and their strategic locations within leaf tissues may increase our current understanding of UV-B tolerance characteristics. Further research is necessary to identify and localize these compounds in leaf tissues. Such information may help us define the biochemical aspects of UV-B protection in broadleaf trees.

Keywords Leaf optical property, UV-B penetration, concentration of total UV-B absorbing compounds, UV-B tolerance, broadleaf tree species

18.1 Introduction

Decreased quantities of total-column ozone have been observed over large parts of the globe, permitting an increased penetration of solar ultraviolet-B (UV-B, 280 nm－315 nm) to the earth's surface (UNEP, 1998). The first reports of potential stratospheric ozone reduction were made more than 30 years ago (Johnston, 1971; Crutzen, 1972). The UV-B radiation on the earth's surface has increased by 6%－14% since the early 1980s (UNEP, 2002). More than 600 papers have been published with much attention directed on the effects of UV-B radiation on higher plants (Caldwell et al., 1998). Studies of the effects of increased solar UV-B at the ecosystem level (Caldwell et al., 1998) have only been undertaken in the past

10 years. Approximately 400 species of plants and cultivars have been screened for sensitivity to UV-B radiation, and of these, about two-thirds were found to be sensitive in some parameter (Sullivan and Rozema, 1999; Sullivan et al., 2003). The balance between damage and protection varies among species, even within varieties of crop species. Many species and varieties can accommodate increased UV-B. Tolerance of elevated UV-B by some species and crop varieties provides opportunities for genetic engineering and breeding to deal with potential crop yield reductions due to elevated UV-B in agricultural systems (UNEP, 1998).

Research indicates that increased UV-B exerts effects more often through altered patterns of gene activity than through damage. These UV-B effects on regulation manifest themselves in many ways, including changes in plant form and production of plant chemicals not directly involved in primary metabolism (UNEP, 1998). Much evidence suggests that plants have evolved two major strategies for resistance to UV-B radiation that involve repair and avoidance mechanisms. The former includes repair of DNA damages by excision repair or by repair of pyrimidine-dimers as photolyase, activated by UV-A and photosynthetically active radiation (PAR) (Taylor et al., 1997). The latter primarily includes epidermal screening of UV-B radiation to protect the mesophyll tissue of a leaf by the accumulation of UV-absorbing compounds in cell vacuoles and/or cell walls of the epidermis (Caldwell et al., 1983; Hutzler et al., 1998). Research has shown that flavonoids and related phenolics play an important role in plant defense against the UV-B radiation (Caldwell et al., 1983; Tevini et al., 1991; Day et al., 1992; Day, 1993; Li et al., 1993; Beggs and Wellmann, 1994; Day et al., 1994; Karabourniotis and Fasseas, 1996; Reuber et al., 1996; Bornman et al., 1997; Hutzler et al., 1998; Karabourniotis et al., 1998; Laakso et al., 2000; Sullivan et al., 2005).

Structural and biochemical changes induced by enhanced levels of UV-B radiation ultimately modify the penetration of UV radiation into plants. In order for UV radiation to be effective in plants, it must effectively penetrate into the tissues and be absorbed. The ability to predict the consequences of enhanced ambient UV-B levels on plants depends in part on our understanding of how much of this radiation reaches the chromophores within the mesophyll (Day, 1993). Ultraviolet penetration varies with plant species. Penetration of UV-B was found to be the greatest in herbaceous dicotyledons (broad-leafed plants) and was progressively less in woody dicotyledons, grasses and conifers (Day et al., 1992). The UV penetration also changes with leaf age; younger leaves attenuate UV-B radiation less than do the more mature leaves in some conifers (DeLucia et al., 1991; DeLucia et al., 1992).

Although some 400 plant species and cultivars have been studied, the vast majority tested have been herbaceous, annual agricultural species grown in laboratory or glasshouse conditions. Fewer than 5% of the studies have been conducted under field conditions. Relatively little information exists on the effects of UV-B radiation on forest tree species (Caldwell et al., 1998), which account for more than 80% of global net primary production (Whittaker, 1975; Barnes et

al., 1998). Tropical forests have received very little attention with respect to the ozone reduction problem (Searles et al., 1995; Caldwell et al., 1998) even though they represent nearly one half of global productivity and much of the total tree species diversity. Our knowledge is far from complete in regard to the effects of enhanced UV-B on trees and forest communities in various landscapes. The diverse range of UV-B radiation responses observed within annual plant species suggests that direct extrapolations from these species to long-lived woody plants many not be feasible. Therefore, it would be useful to know the differences in UV-B screening effectiveness in various tree species and to determine what general leaf properties are responsible for these differences. This paper is a result of a USDA-funded collaborative research project involving the scientists from Southern University, the USDA-FS Northeast Research Station, the USDA UV-B Monitoring and Research Program (UVMRP), and the University of Vermont. The research was the first in the southern US that focused on assessing UV-B radiation tolerance characteristics in diverse southern broadleaf tree species (Table 18.1, column 1). The specific objectives were to: (1) measure leaf reflectance, transmittance, and absorption of UV (280 nm − 400 nm) and visible (400 nm − 760 nm) radiation spectrums on a whole-leaf basis, (2) measure the light distribution and depth of UV-B light penetration (310 nm) into the leaves, (3) investigate leaf surface morphology and leaf anatomy, and (4) measure the total concentration of UV-B-absorbing compounds in the leaves throughout the growing season.

Table 18.1 Leaf reflectance (R_λ), transmittance (T_λ), and absorbance ($A_{\lambda)}$ to 300 nm UV-B radiation on a whole leaf basis, measured from the mature leaves of 35 southern tree species, grown in Baton Rouge, Louisiana, USA

Scientific name-common name	Whole leaf reflectance to 300nm (%) $R_{\lambda=300nm}$	Whole leaf transmittance to 300nm (%) $T_{\lambda=300nm}$	Whole leaf absorbance to 300nm (%) $A_{\lambda=300nm}$
Broadleaf evergreen trees			
Magnolia grandiflora - Southern magnolia	6.051	0.079	93.870
Magnolia virginiana - Sweet bay magnolia	6.004	0.003	93.993
Quercus glauca - Blue Japanese evergreen oak	6.756	0.005	93.239
Quercus virginiana - Southern live oak	8.475	0.000	91.525
Deciduous trees			
Acer rubrum - Red maple	6.017	0.003	93.979
Betula nigra - River birch	6.866	0.004	93.130
Carya cordiformis - Bitternut hickory	6.044	0.001	93.957
Carya illinoinensis - Pecan	8.576	0.010	91.415
Carya tomentosa - Mockernut hickory	6.223	0.002	93.775
Castanea dentata - American chestnut	7.852	0.000	92.148
Celis laevigata - Sugar hackberry	5.388	0.005	94.607

(Continued)

Scientific name-common name	Whole leaf reflectance to 300nm (%) $R_{\lambda=300nm}$	Whole leaf transmittance to 300nm (%) $T_{\lambda=300nm}$	Whole leaf absorbance to 300nm (%) $A_{\lambda=300nm}$
Cercis canadensis - Red bud	4.766	0.000	95.234
Cornus florida - Dogwood	8.610	0.048	91.342
Fagus grandifolia - American beech	6.970	0.003	93.027
Fraxinus pennsylvanica - Green ash	5.467	0.003	94.530
Fraxinus velutina - Arizona ash	6.570	0.003	93.427
Liquidamber styraciflua – Sweetgum	7.464	0.001	92.536
Liriodendron tulipifera - Yellow poplar	4.807	0.002	95.191
Morus rubra - Red mulberry	7.440	0.023	92.537
Platanus occidentails – Sycamore	6.824	0.012	93.164
Populus deltoides - Cottonwood	7.337	0.001	92.662
Pyrus calletyana "Bradford" - Bradford pear	6.088	0.002	93.910
Quercus acutissima - Sawtooth oak	6.634	0.167	93.199
Quercus alba - White oak	7.073	0.005	92.922
Quercus michauxii - Swamp chestnut oak	5.637	0.004	94.359
Quercus nigra - Water oak	7.186	0.001	92.813
Quercus palustris - Pin oak	5.843	0.005	94.152
Quercus phellos - Willow oak	5.503	0.008	94.490
Quercus shumardii- Shumard oak	8.144	0.005	91.851
Quercus stellata - Post oak	6.922	0.001	93.077
Quercus falcata - Southern red oak	5.974	0.004	94.022
Quercus falcata var. pagodifolia - Cherrybark oak	5.700	0.008	94.291
Sapium sebufrum - Chinese tallow	5.998	0.002	94.000
Ulmus americans - American elm	5.758	0.002	94.240
Ulmus parvifolia - Chinese elm	6.387	0.004	93.608
Mean	6.553	0.012	93.435
Max	8.610	0.167	95.234
Min	4.766	0.000	91.342
SD	1.005	0.031	1.008

The project has established a database of leaf optical properties (reflectance, transmittance, and absorbance to UV-B, UV-A, and visible light), leaf UV-B absorbing compound profiles, depth of UV-B penetration into leaves, and leaf anatomical characteristics. It is expected that through the comparative analyses of these properties across the species, we may have a better understanding of the biophysical and biochemical aspects of UV-B tolerance characteristics in diverse broadleaf trees in the South.

18.2 Methodology

18.2.1 Plant Materials

Leaves of the selected southern broadleaf tree species (Table 18.1, column 1) were collected from individual trees growing in Baton Rouge, Louisiana, USA, during two growing seasons from April to October in 2000 and in 2001. Leaf samples were collected each month from the sun portions of four unshaded individuals per species at the terminal 20 cm – 50 cm of a branch or stem, and were placed in humidified plastic bags in an insulated box for transporting to the laboratory. The experiment was a randomized complete block design with seven blocks arranged across time in a growing season. Each month was a block in which three trees per species were randomly selected for sampling. Species order was randomized. All species within a block were sampled in the shortest time possible during the first week of the month to minimize the environmental variation within the block. Samples were taken at approximately the same time each day between 8:00 a.m. – 10:00 a.m. Analyses were finished within the six hours of the sample collection. A total of 980 samples (35 species×4 samples/species/month×7 months) were collected in the field during the 2000 growing season to monitor the leaf optical properties, leaf UV-B absorbing compounds and chlorophyll concentration, and leaf thickness. The light penetration and spectral distribution within leaves and the anatomical research were conducted during the 2001 growing season.

18.2.2 Measuring Leaf Optical Properties

Leaf reflectance and transmittance were measured with an integrating sphere (Optronic IS-1000) following the procedures 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 (Optronic OL Series 752-10) and a high accuracy UV/Visible spectroradiometer (OL 754) connected to a computer. Spectral scanning was performed from 250 nm – 800 nm at 5 nm intervals to produce a spectral distribution of the reflectance and transmittance on a whole-leaf basis for each species. Leaf adaxial/upper side was illuminated using a 200-W tungsten coiled-coil filament lamp with a 1.3 cm×7 cm quartz envelope. The spectral irradiance values were provided within the wavelength ranging from 250 nm to 800 nm and were based on the National Institute of Standard and Technology (NIST) 1973 scale of spectral irradiance (Saunders and Shumaker, 1977). Measurements of both spectral transmittance and reflectance were based on the procedure of direct substitution

(Optronic Laboratory, 1997). The spectral absorbance, A_λ, of a leaf sample, was computed from the knowledge of its spectral transmittance, T_λ, and spectral reflectance, R_λ, as follows:

$$A_\lambda = 1 - T_\lambda - R_\lambda$$

18.2.3 Measuring the Light Penetration and Distribution within Leaf Tissues

The depth of light penetration into leaves and spectral distribution within leaf tissues were measured using a fiber optic microprobe system (Fig. 18.1) (Qi et al., 2003a), which was modified based on Vogelmann and Bjorn (1984) and Vogelmann et al. (1991). The microprobes were fabricated in Dr. T. C. Vogelmann's lab, using 150 μm diameter (OD) multimode step-index fibers made of fused silica (Polymicro Technologies, Phoenix, AZ, USA). The fibers were heated and drawn to a tip diameter of around 10 μm. The tapered regions of the probes were then coated with evaporated chromium and truncated with a diamond knife. Thus, light entry was confined to the tip of the probes that had near-perfect Gaussian acceptance angles (50% acceptance half width) of 27° to 34°. To operate the microprobe, the tip was threaded through a needle eye and firmly glued onto it (Fig. 18.2(a)); the other end of the probe was connected to a spectroradiometer (Model 754, Optronic Laboratory, Florida, USA) (Fig. 18.2(b)). The needle carrying the probe tip was firmly mounted to a Stepper Mike (Model 18515, Oriel, Stratford, CT, USA) on an XYZ translator (Fig. 18.2(c)). The leaf sample was mounted between two aligned Plexiglas slides perforated with a 5 mm diameter opening in the center of both slides to allow the light to illuminate and the fiber to penetrate. The leaf sample was placed between the slides, with the abaxial side facing the fiber probe and the adaxial side facing the radiation beam from a 75-W Xenon-arc lamp (Hanovia 901C-1), which generated 4 $W/m^2/nm$ UV-B flux at 310 nm, or 0.23 W/m^2 erythemal UV-B at the leaf surface level. This amount of UV-B was comparable to the ambient level of the UV-B in Baton Rouge, LA, USA, measured by the USDA UVMRP. The erythemal UV-B radiation at the Baton Rouge Station peaked in June at 0.3 W/m^2 UV-B with the maximum daily sum of 8.2 kJ/m^2.

The measurements of light penetration were made at zero orientation at which the largest portion of light flux moves through the leaf. Photons captured by the microprobe were measured with the calibrated spectroradiometer. The light penetration was measured at four wavelengths including UV-B at 310 nm, UV-A at 360 nm, blue at 430 nm, and red at 680 nm. The spectroradiometer was preset for the wavelength selected before each measurement. The microprobe was auto-advanced at 4 μm/s through a vein-free region from the abaxial side toward the irradiated surface. Light transmitted to the probe was calculated as the relative amount of light, expressed as the ratio of light measured by the probe inside the

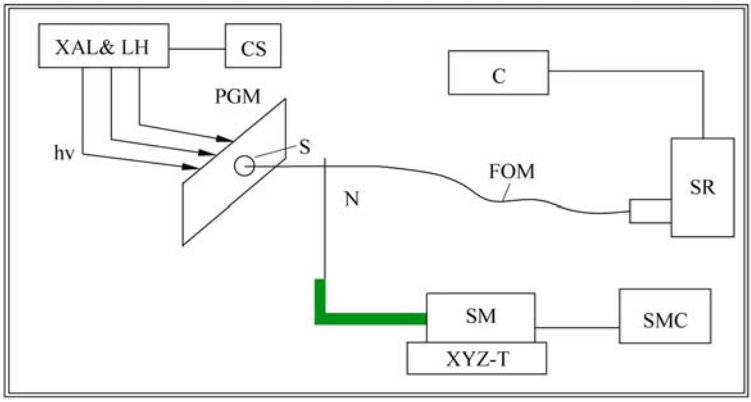

Figure 18.1 Diagram of the fiber optical microprobe system for measurement of light penetration, modified based on Vogelmann and Bjorn (1984). C = Computer, FOM = Fiber Optic Microprobe, S = Leaf Sample, N = Needle, SR = Spectroradiometer, SM = Stepper Motor, XYZ-T = XYZ Translator, SMC = Stepping Motor Controller, PGM = Plexiglas Mount, XAL&LH = Xenon-arc Lamp and Lamp House, CS = Current Source, hv = high voltage (Qi et al., 2003a)

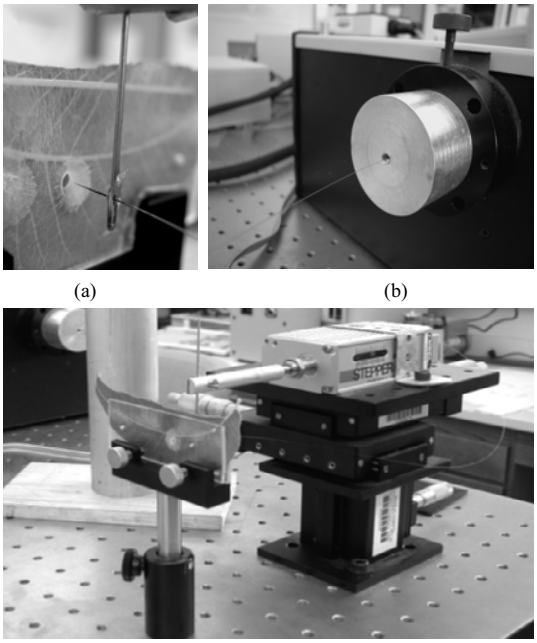

Figure 18.2 The fiber optic microprobe tip is threaded through the eye of a needle and firmly glued on to it (a), and the other end of the microprobe is connected to the OL754 Spectroradiometer (b). A leaf sample is mounted between two aligned Plexiglas slides perforated with a 5mm diameter opening in the center of each slide to allow the light to illuminate from the leaf adaxial surface and the microprobe to penetrate from the leaf abaxial surface (c)

tissue to light measured by the probe without the tissue (probe normal to incident light, the control). Fifteen mature leaves per species were measured for each of the four wavelengths selected.

18.2.4 Scanning Electron Microscopy and Light Microscopy of Leaves

Mature leaves were dissected, fixed in FAA (ethanol, glacial acetic acid, and formaldehyde), dehydrated in ethanol series, and dried in carbon dioxide using a Denton DCP-1 critical point drying apparatus. Leaves were mounted on stubs, coated with 25 nm gold palladium using a Hummer Ⅱ Sputter Coater, and examined using a Cambridge S-260 scanning electron microscope. Thicknesses of total leaf, upper and lower epidermis, mesophyll, and sponge tissues were measured with a light microscope equipped with a micrometer. The leaf thicknesses were monitored from April to October 2000 for all 35 species.

18.2.5 Measurements of UV-B Absorbing Compounds and Chlorophyll Concentrations

Extraction of leaf UV-B absorbing-compounds, mainly flavonoids and related phenolic compounds, was based on the method by Gorton and Vogelmann (1996). 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 powder in liquid nitrogen using a Teflon pestle. One mL of acidified methanol (methanol:H_2O:HCl 79:20:1 v/v) was added to the microfuge tube and homogenized well. The extracts were stored frozen ($-80^{\circ}C$) for up to one week, then clarified by centrifugation (Micro 12, National Labnet Company, Inc.). Forty μl of the supernatant were transferred to a quartz cuvette and diluted in 3 mL of extraction medium (resulting in a volume dilution factor of 76). Spectra of the extracts were obtained from 200 nm－820 nm with a computer-controlled, split-beam, dual-detector UV/Visible spectrophotometer (Genesis 2 Spectronic, Inc.) at 1 nm increments. The absorbance spectra of the extract were then standardized for the leaf area and volume to obtain the absorbance values on a leaf area basis (A/cm^2). In order to calculate the UV-B absorbing compound concentration for each sample, we decided to include the entire UV-B wavelength region by calculating the cumulative absorbance values from 280 nm－320 nm for each sample. This avoided the arbitrary selection of the absorbance at a single wavelength, e.g., at 300 nm, 310 nm, or 330 nm, which has been used in numerous studies. Our recent report also indicates that the values of UV-B absorbing compounds measured at 280 nm, 300 nm, and 310 nm were not always in agreement (R^2 ranged from 0.76－0.93) due to their wavelength specific nature (Qi et al., 2002). Therefore, the cumulative absorbance from

280 nm – 320 nm at 1 nm intervals was used, then standardized for leaf area and volume, and expressed as total absorbance ($A_{280\ nm-320\ nm}/cm^2$) to represent the total UV-B absorbing compound content or concentrations per unit of leaf area. Leaf total chlorophyll content was measured with a Minolta Chlorophyll Meter (Spad-502, Spectrum Technologies Inc, IL). The values were then converted to the true chlorophyll content based on the method developed by Yadava (1986).

18.2.6 Statistical Analysis

Data were analyzed using ANOVA ($p = 0.05$). Duncan's multiple range tests were used to compare means ($p = 0.05$). Regression analyses were used to assess the correlation and predictive power among the selected variables.

18.3 Results and Discussion

18.3.1 Leaf Optical Properties

Changes in leaf spectral reflectance, transmittance, and absorbance to UV/visible light of pecan leaves throughout a growing season are illustrated in Fig. 18.3. The changes in leaf optical properties were relatively small in UV-B (280 nm – 320 nm) and UV-A (320 nm – 400 nm) spectral regions compared to that of the visible spectral region (400 nm – 760 nm). Throughout the growing season, pecan leaves reflected 4% – 8%, transmitted 0% – 1%, and absorbed up to 96% of UV radiation (280 nm – 400 nm). In the visible light region, leaf absorbance to green light (at 555 nm) and to red light (at 680 nm) increased dramatically during leaf development from April to July. This trend seems to hold true for all other species studied. Since the leaf optical properties remained relatively steady within the UV region, this indicated we could make the species comparison at a single wavelength. Since our focus was primarily on UV-B, we chose 300 nm for the comparison of the leaf optical properties amongst the 35 species (Table 18.1). The species were ranked from the highest to the lowest based on their reflectance (Fig. 18.4). As shown in Table 18.1 and Fig. 18.4, leaf reflectance to the 300 nm UV-B was generally low across the species, ranging between 4.77% – 8.61% with a mean value of 6.55%, ± 1% of standard deviation. The leaf transmittance to the 300 nm was rather low for all the species, ranging between 0% – 0.17%. The leaf absorbance to the 300 nm UV-B was above 90% across the species, ranging between 91.34% – 95.23% with a mean value of 93.44%, ± 1% of standard deviation. These findings are generally in agreement with the results reported by Gausman et al. (1975), Robberecht et al. (1980), Cen and Bornman (1993), and Yang et al. (1995). It is generally agreed that leaves absorb over 90% of incident UV-B; leaf surface

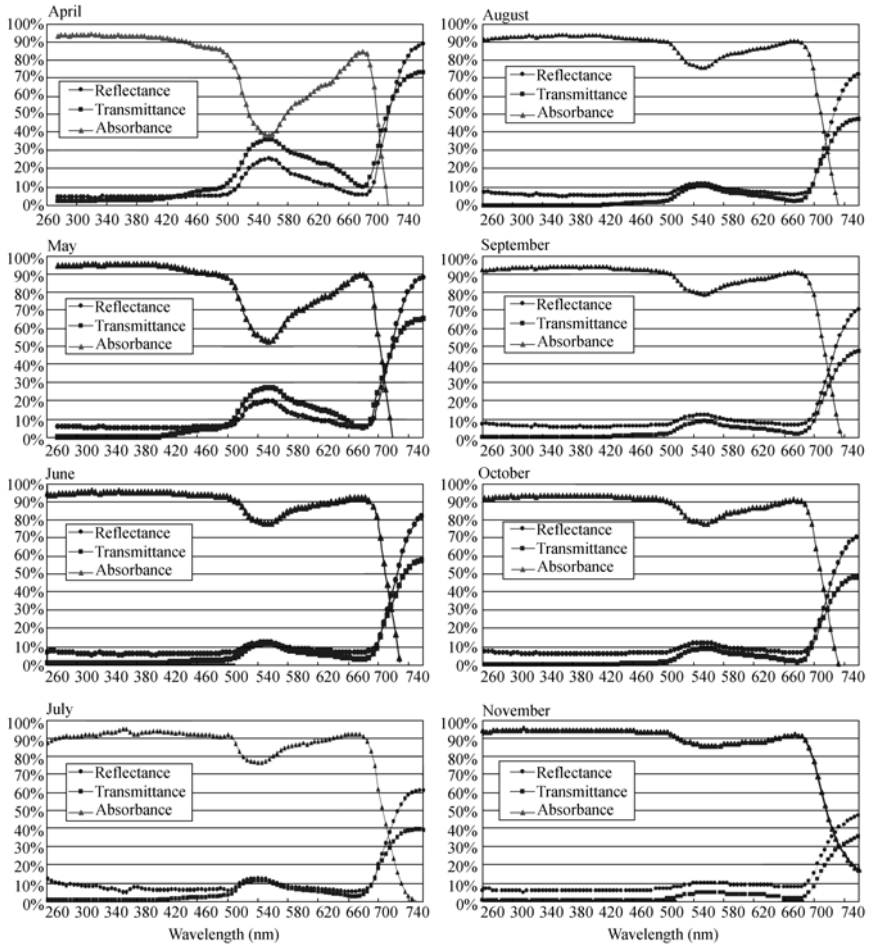

Figure 18.3 Leaf spectral reflectance, transmittance, and absorbance to UV/visible light during a growing season in leaves of pecan (Qi et al., 2003b)

reflection, as a first line of defense against UV-B, is less than 10% in most species, and there is negligible transmission of UV-B through leaves.

18.3.2 Depth of Light Penetration into Leaf Tissues

Light attenuation in pecan leaves at four wavelengths including UV-B, UV-A, blue light, and red light into leaf tissues is illustrated in Fig. 18.5. The depths of the light penetration into the leaf tissues are presented in Fig. 18.6. Pecan leaf epidermis strongly attenuated 310 nm UV-B, 98% of which was absorbed within the first 10 μm of the 15 μm-thick upper epidermal tissue (Figs. 18.5 and 18.6). High UV-B attenuation by the epidermal layer means low epidermal transmittance to UV-B.

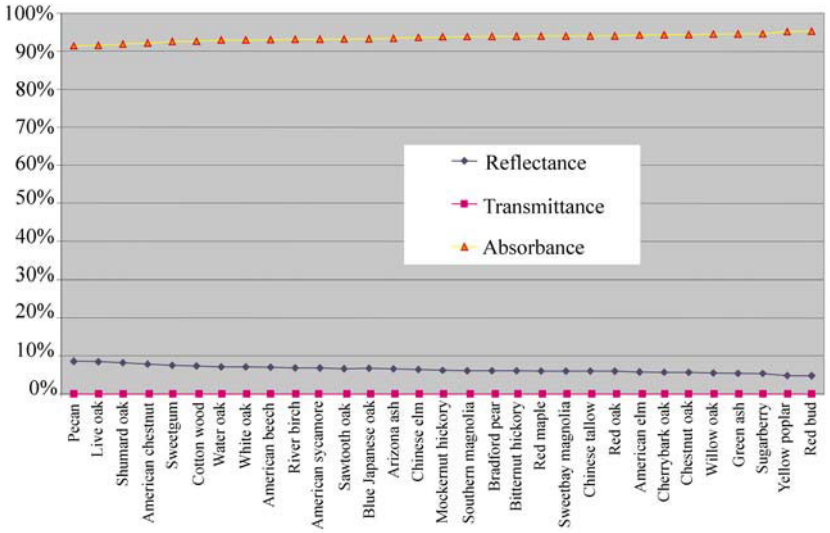

Figure 18.4 Leaf reflectance, transmittance, and absorbance to 300 nm UV-B radiation on a whole leaf basis in the selected broadleaf tree species. The measurements were made on the mature leaves collected in August in Baton Rouge, LA. The species were ranked based on the reflectance from the highest to the lowest

Since the epidermis of the pecan leaves allowed very little UV-B (< 2%) transmittance into the mesophyll tissues, the photosynthetic apparatus were essentially protected from the UV-B damage. Thus, such an effective epidermal function of the UV-B screening characterizes the UV-B tolerance mechanism in pecan. In addition to the UV-B attenuation, pecan leaf epidermis attenuated 96% of the UV-A (360 nm), 83% of the blue light (430 nm), and 58% of the red light (680 nm). However, the blue and the red light penetrated much deeper into the mesophyll tissues. The 430 nm light was mainly attenuated within the first 100 μm thickness of the leaf, including the upper epidermis and the palisade mesophyll tissue. The 680 nm penetrated even deeper into the sponge mesophyll and was mainly attenuated within the first 160 μm thickness of the leaf. Overall, mesophyll tissues alone attenuated 17% of the blue light and 42% of the red light that were available for photosynthesis (Figs. 18.5 and 18.6).

Comparisons among the species are presented in Fig. 18.7, which contains 31 species, excluding dogwood, red mulberry, pin oak, and post oak, for which the data were not available. The 31 species were ranked from the lowest to the highest based on their epidermal UV-B transmittance. The differences in the depth of the UV-B penetration, epidermal transmittance to UV-B, and upper epidermal thickness are clearly considerable amongst the species (Fig. 18.7). The first 23 species in Fig. 18.7, including Arizona ash, chestnut oak, mocker nut hickory, pecan, American sycamore, bitternut hickory, green ash, sawtooth oak, American elm, blue Japanese oak, cherrybark oak, cottonwood, southern live oak, southern magnolia, shumard oak,

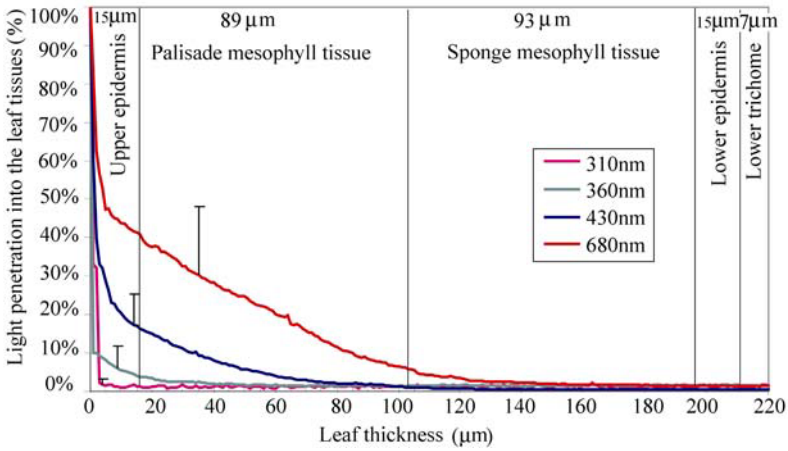

Figure 18.5 Light penetration into leaf tissues from the adaxial surfaces in pecan by four different wavelengths, the error bar represents +1SD. The measurements were made on 15 mature leaves sampled in August

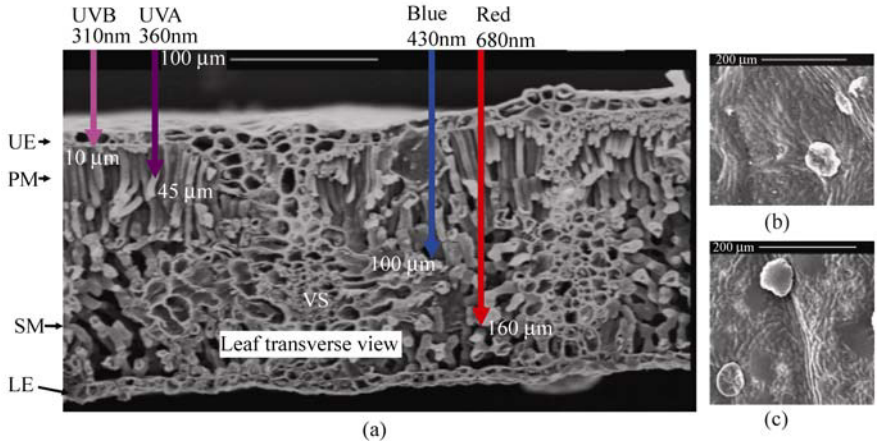

Figure 18.6 Visual illustration of the light penetration into leaf tissues in pecan. (a) Shows a pecan leaf cross-section. UE = upper epidermis, PM = palisade mesophyll, SM = sponge mesophyll, LE = lower epidermis, VS = vascular system. The downward arrows show the relative positions of depths of light penetration at different wavelengths; (b) shows the upper leaf surface and trichome; and (c) shows the lower leaf surface and trichome

sweetgum, American beech, white oak, Chinese tallow, water oak, yellow poplar, Bradford pear, and red maple, had relatively low epidermal transmittance, ranging from 1% – 8%. This means the leaf epidermises of these species were capable of attenuating 92% – 99% of the UV-B absorbed. Such a strong epidermal function of the UV-B screening underlies the fundamental UV-B protection mechanism in these species. This finding also concurs with the research by Caldwell et al.

(1983) and Day (1993), asserting that the epidermal attenuation appears to be the dominant UV-B screening mechanism in the majority of plants. On the other hand, the last five species in Fig. 18.7, including river birch, American chestnut, red bud, Chinese elm, and sugarberry, exhibited high epidermal transmittance (ranging from 27% − 49%). These species may be less UV-B tolerant. They may also experience up to 50% UV-B penetration into their mesophyll tissues, which could result in damage to their photosynthetic apparatus, unless there are additional UV-B absorbing compounds present in their mesophyll tissues. In addition, the three species in Fig. 18.7, including sweet bay magnolia, red oak, and willow oak, are intermediate regarding their epidermal transmittance (13% − 17%), which falls between the low values (under 8%) of the first 23 species and the high values (27% − 49%) shown by the last 5 species.

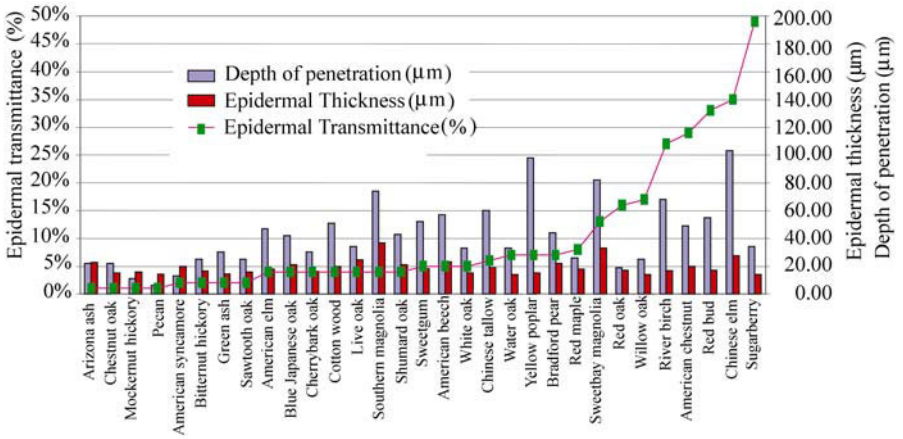

Figure 18.7 Comparisons of upper epidermal transmittance to 310 nm UV-B radiation, depth of 310 nm penetration into leaves, and epidermal thickness among the selected broadleaf tree species. Note: The species were ranked based on the epidermal transmittance from the lowest to the highest. In all cases, mature leaves were used and light was illuminated toward the upper leaf surfaces in the light penetration study

18.3.3 The Concentration of Leaf UV-B Absorbing Compounds

The inter-specific comparison of the total UV-B absorbing compound concentration per unit of leaf area ($A_{280\ nm-320\ nm}/cm^2$, assessed by integrating the absorbance values from 280 nm − 320 nm at 1 nm intervals) in the mature leaves is presented in Fig. 18.8. Large variations exist among the species. These UV-B absorbing compounds possess strong absorbance to the UV radiation (Qi et al., 2002; Qi et al., 2003b, c). It is possible that those species with the strong epidermal UV-B screening abilities, such as the first 23 species in Fig. 18.7, may have accumulated

more UV-B absorbing compounds in their epidermal layers than the rest of the species. Further research is necessary to localize the UV-B absorbing compounds in leaves. The accumulation of flavonoids in the epidermis has been shown to reduce epidermal transmittance of UV-B radiation (Robberecht and Caldwell, 1978; Tevini et al., 1991). Research by Karabourniotis and Fasseas (1996) and Karabourniotis et al. (1998) suggests that these flavonoids, and possibly other phenolics, are present throughout the leaf, but accumulate significantly in leaf trichomes and epidermal cells. Research has also demonstrated that the *Arabidopsis* mutants' lack of UV-B absorbing compounds (e.g., cinnnamic acid precursors and flavonoids) were hypersensitive to UV-B radiation (Li et al., 1993; Reuber et al., 1996). Those species with weak epidermal screening abilities, such as river birch, American chestnut, red bud, Chinese elm, and sugarberry (see Fig. 18.7), may be able to accumulate UV-B absorbing compounds throughout the leaf cross-sections in order to provide additional UV-B defense. Ultraviolet-absorbing compounds and specific leaf anatomical features are important in determining leaf screening efficiency (Bornman, 1999). The identifications and locations of flavonoids and related phenolics in leaf tissues have been advanced by many studies (Schnitzler et al., 1996; Hutzler et al., 1998; Laakso et al., 2000; Semerdjieva et al., 2003; Sullivan et al., 2005). Using fluorescence microscopy, Semerdjieva et al. (2003) discovered *Vaccinium myrtillus* contained the highest concentration of methanol-extractable UV-B absorbing compounds which were distributed throughout the leaf, and were particularly concentrated in chlorophyll-containing cells, while in *Vaccinium vitisidaea*, most phenolic compounds were cell wall-bound and concentrated in the walls of the epidermis. These two plants represent extreme forms of two divergent strategies for UV-B screening.

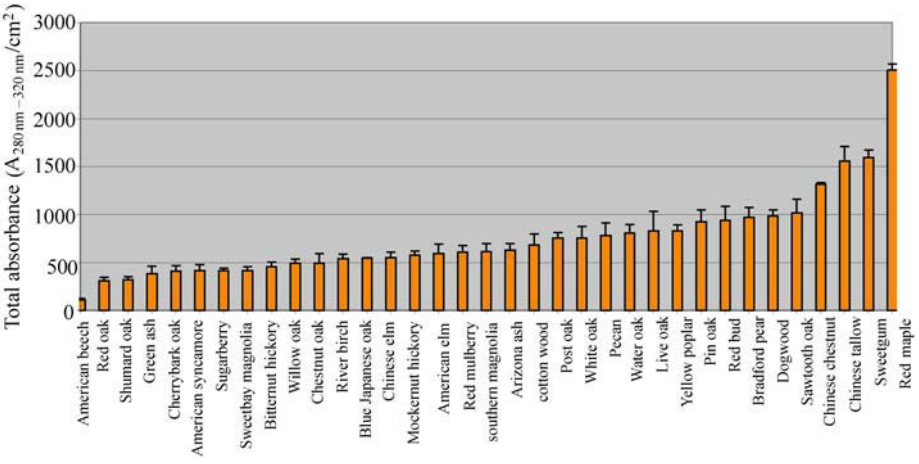

Figure 18.8 Comparisons of UV-B absorbing-compound concentrations in mature leaves among the selected broadleaf tree species grown in Baton Rouge, LA. The value for each species was the mean over four month measurements from July to Oct with four samples per month from sun-exposed leaves. The error bar indicates + 1SE

18.3.4 Correlations among the UV-B Related Variables within and among the Species

This study has shown that with increased UV-B radiation during the growing season (Fig. 18.9(a)), there are cumulative increases in leaf concentration of UV-B absorbing compounds (Fig. 18.9(b)), leaf total thickness (Fig. 18.9(c)), and leaf chlorophyll content (Fig. 18.9(d)), in all the 35 species combined. However, these parameters are all delayed responses from increasing solar UV levels. Although the leaves from each species were sampled in the first week of each month to represent that month, they perhaps better represent the previous month in relation to their responses to the UV-B radiation. Even so, there are still delays in the responses in UV-B absorbing compounds and other parameters as shown in Fig. 18.9. Perhaps a cumulative response might be in effect. The increased UV-B absorbing compound content over the growing season helps enhance the plants defense against the enhanced UV-B level. Correlation analyses of these variables (Table 18.2) indicated that during the growing season, there is a good correlation between total leaf thickness and total concentration of leaf UV-B absorbing compounds ($r = 0.9$). Also, considering that chlorophyll synthesis is very dynamic throughout the season, while UV-B absorbing compounds are probably only produced at very specific developmental stages it would make sense that chlorophyll concentrations would correlate much better with solar irradiance over the long haul than would UV-B absorbing compounds, which may be locked in at the time of synthesis and are not continuously being recycled. Thus, it is not surprising that the ambient UV-B radiation was correlated better to leaf chlorophyll content ($r = 0.94$) than to the concentration of UV-B absorbing compounds ($r = 0.45$). This may also be attributed to the fact that the UV-B radiation follows the trend of the total solar radiation, within which the PAR is well-correlated to chlorophyll development during the growing season. The chlorophyll content declined significantly in October (Fig. 18.9(d)) while the UV-B absorbing compound concentration remained at a steady state during the latter part of the growing season from August to October (Fig. 18.9(b)).

As we have demonstrated in Figs. 18.7 and 18.8, considerable inter-specific variations exist in the leaf total UV-B absorbing compound concentration, leaf epidermal thickness, and depth of UV-B penetration, as well as the epidermal transmittance among the species investigated. In order to reveal if there is any inter-specific correlation between these variables, we also performed the correlation analyses (Table 18.3). The results show there is a good inter-specific correlation between leaf total thickness and leaf epidermal thickness ($r = 0.84$), meaning that the species with thicker leaves may have thicker epidermis. Some positive inter-specific relationships seem to exist between the leaf total thickness and its chlorophyll content ($r = 0.66$), and between the epidermal thickness and the depth of UV-B penetration into leaves ($r = 0.53$). The correlation coefficients between the epidermal transmittance and depth of UV-B penetration ($r = 0.36$) and between

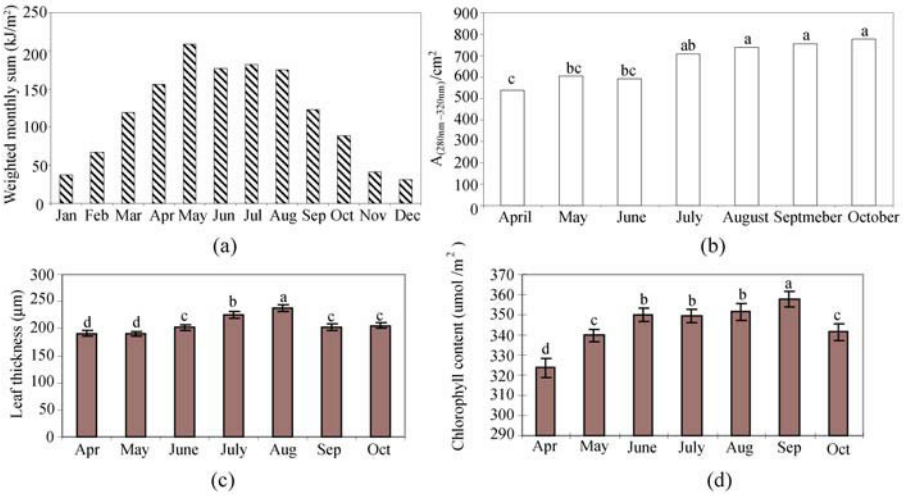

Figure 18.9 Monthly summation of ambient UV-B radiation in Baton Rouge, LA in year 2000 provided by the USDA-UV-B Monitoring Program Baton Rouge Station (a); and seasonal trends in leaf UV-B absorbing-compound concentration (b); leaf thickness (c); and leaf chlorophyll content (d); of the 35 species combined. Within each chart, means with unlike letters differ significantly according to Duncan's multiple range tests, $p \leqslant 0.05$. The error bars represent ± 1SE

Table 18.2 Correlation coefficients during leaf growth and development in all the species combined

Correlation coefficient	UV-B radiation (kJ/m^2/month)	Leaf thickness (μm)	Leaf chlorophyll (μmol/m^2)	Leaf UV-B absorbing compounds ($A_{280\ nm-320\ nm}/cm^2$)
UV-B radiation (kJ/m^2/month)	1			
Leaf thickness (μm)	0.48	1		
Leaf chlorophyll (μmol/m^2)	0.94	0.66	1	
Leaf UV-B absorbing-compounds $A_{280\ nm-320\ nm}/cm^2$	0.45	0.90	0.72	1

leaf total thickness and the depth of the penetration ($r = 0.36$) were too low to depict any inter-specific relationships. No inter-specific relationships were discovered between the leaf total UV-B absorbing compound concentration and any other variables, including leaf total thickness, epidermal thickness, epidermal transmittance, and depth of UV-B penetration (r values ranged from -0.02 to 0.06, as shown in Table 18.3). This may be attributed to the individualistic nature (randomness) of the species, or is perhaps due to the fact that the trees selected in this study,

Table 18.3 Correlation between the selected UV-B related properties in mature leaves across all the species studied

Correlation coefficient	Leaf total thickness (μm)	Leaf upper epidermal thickness (μm)	Leaf upper epidermal transmittance to 310 nm UVB (%)	Depth of 310 nm UVB penetration into leaves (μm)	Leaf concentration of total UV-B absorbing compounds ($A_{280\ nm\ -\ 320\ nm}/cm^2$)	Leaf chlorophyll content ($\mu mol/m^2$)
Leaf total thickness (μm)	1.00					
Leaf upper epidermal thickness (μm)	0.84	1.00				
Leaf upper epidermal transmittance to 310nm UVB (%)	−0.23	0.02	1.00			
Depth of 310 nm UVB penetration into leaves (μm)	0.36	0.53	0.36	1.00		
Leaf concentration of total UV-B absorbing compounds ($A_{280\ nm\ -\ 320\ nm}/cm^2$)	0.04	0.06	−0.02	0.02	1.00	
Chlorophyll content ($\mu mol/m^2$)	0.66	0.52	−0.17	0.10	−0.08	1.00

including 4 evergreen and 31 deciduous broadleaf species, generally belong to two life forms (evergreen angiosperms and deciduous dicotyledon trees) that may not be able to warrant for any significant correlations. A study involving species from 6 plant life forms (evergreen gymnosperms, evergreen angiosperms, deciduous dicotyledon trees, deciduous dicotyledon shrubs/vines, herbaceous dicotyledon, and grasses) did reveal better correlations between some of the UV-B related variables across the species (Day, 1993).

18.4 Conclusions

This study investigated the UV-B related biophysical, biochemical, and anatomical characteristics of more than 30 southern broadleaf tree species. It is concluded that leaves of the broadleaf trees generally reflect 4%−9%, transmit 0%−0.2%, and absorb 91%−95% of the incident UV-B radiation. The main site of UV-B attenuation takes place within the upper leaf epidermis. The project has identified 23 broadleaf tree species (Arizona ash, chestnut oak, mocker nut hickory, pecan, American sycamore, bitternut hickory, green ash, sawtooth oak, American elm, blue Japanese oak, cherrybark oak, cottonwood, southern live oak, southern magnolia, shumard oak, sweetgum, American beech, white oak, Chinese tallow, water oak, yellow poplar, Bradford pear, and red maple) possessing a strong epidermal UV-B screening functions (attenuating over 90% UV-B). The effectiveness of the epidermal function of the UV-B screening underlines the major UV-B protection mechanism in most of the trees studied. Three species (sweet bay magnolia, red oak, and willow oak) possessed intermediate epidermal screening function, and five other species (river birch, American chestnut, red bud, Chinese elm, and sugarberry) showed somewhat weak epidermal screening functions. Considerable inter-specific variations exist in leaf UV-B absorbing compound concentrations, depth of UV-B penetration, and leaf anatomical features. In addition to the epidermal screening function, the leaf UV-B absorbing compounds may play an important role in providing additional defense against UV-B radiation penetration into mesophyll tissues. Further studies are necessary to focus on localization and identification of UV-B absorbing compounds in leaf tissues, to promote a better understanding of the UV-B tolerance mechanisms in trees.

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