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Ultraviolet leaf reflectance of common urban trees and the prediction of reflectance from leaf surface characteristics

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Abstract

The spectral reflectance and transmittance over the wavelength range of 250–700 nm were evaluated for leaves of 20 deciduous tree species and leaf sheaths of five isogenic wax variants of *Sorghum bicolor* differing in visible reflectance due to cuticular waxes. Using the sorghum sheath reflectance and cuticle surface characteristics as a model, it was concluded that tree leaf reflectance above 0.06 was likely due to the presence of variously-shaped fine epicuticular wax structures on the leaf surface. Increasing the density of sub-micron wax structures corresponded to an enhanced ultraviolet (UV) reflectance over the PAR reflectance of a given leaf surface—either *S. bicolor* sheath or tree leaf. Amorphous globular epicuticular wax structures did not appear to scatter UV as well as wax filaments or vertical plates in varying patterns even when the dimensions of the structures were similar. Further work is needed to clarify this relationship and the influence of cellular pigments on subsurface contributions to the reflectance.
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Keywords: UV; Sorghum; Reflectivity; Deciduous forest; Radiation

1. Introduction

The reduction of stratospheric ozone currently occurring over many parts of the world results in increased ultraviolet-B (UV-B) radiation at the earth's surface. This increased irradiance may impact the productivity of agriculture across the globe, as irradiance in the UV-B wavelengths (280–320 nm) has been shown to affect plant growth and development (Caldwell, 1971; Caldwell et al., 1998). To estimate the effects of UV-B on plants we need to determine the spectral distribution of UV-B radiation received on leaf surfaces within the plant canopy as well as penetrating the leaf surfaces and affecting the physiology of the plant. Modeling the distribution of UV-B radiation on leaf surfaces requires information on the spectral leaf reflectance and transmittance, reflectance of soil under the canopy, the canopy structure, and the spectral distribution of UV-B radiation reaching the top of the canopy.

Due to the extensive efforts in the past and present to model photosynthetic activity in canopies, extensive information on the spectral leaf transmittance and reflectance exists for wavelengths from 400 nm on up to around 1500 nm. However, similar modeling efforts to assess UV receipt and resulting plant response are quite limited. Consequently, little information on the spectral transmittance and reflectance of leaves exists

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for wavelengths less than 400 nm and thus many assumptions have to be made to model the UV radiation in the canopy. The first extensive effort to measure the UV optical properties of tree leaves was made by Yang et al. (1995), although the non-standard methodology used in the study limits confidence in the measurements. Measurements of the spectral leaf reflectance have been made for tomato, corn, and soybean leaves in the UV wavelength bands (Brabham and Briggs, 1975), but no corresponding spectral leaf transmittances measurements were made. Typically, plants appear to have leaf UV reflectance less than 0.10 and essentially no leaf transmittance in UV wavelengths (Gates et al., 1965).

UV leaf reflectance is not likely to be similar to that of the oft-measured reflectance of visible radiation. The scattering and transmission of radiation in the leaf is strongly influenced by the relatively large chromatophores and vacuoles, the internal scattering at the intercellular air spaces, and internal scattering associated with the palisade cell shapes (Wooley, 1971; Fukshansky, 1991; Cui et al., 1991; DeLucia et al., 1996). While leaf reflection in the visible wavelengths appears to be a result of internal scattering and absorption processes deep within the leaf, UV reflection appears to occur from the cuticle or first layer of epidermal cells (Grant, 1987).

Initial absorption of radiation is in the leaf cuticle. Krauss et al. (1997) studied the spectral transmittance of cuticles of a number of plant species and found that UV-B attenuation within the cuticle was nearly identical for a wide range of species. Similar results were found by Gausman et al. (1975) with a range of plant species having UV absorption by leaf epidermal cuticles of between 0.91 and 0.96 and UV reflectance varying between 0.058 and 0.091 (Gausman et al., 1975). In contrast, other studies have shown wide variability in the UV-B transmittance of the epidermal layer for trees and shrubs (Day et al., 1994). Krauss et al. (1997) concluded that the UV-B attenuation in the cuticles of the species they studied was largely a result of cuticular pigments.

Penetration of radiation further into the leaf varies with wavelength. In UV-B and UV-A, flavonoids and other phenylpropanoids that strongly absorb in the UV are most certainly under the cuticle and apical cell walls of the epidermis (Gausman et al., 1975) as well as in the vacuole and on the walls of the epidermal cells (Day et al., 1993; Kootstra, 1994). The location of absorbing pigments for a given wavelength of radiation varies by species. In *Picea pungens* leaves, UV-B radiation penetrated only to a depth of 0.02 mm, not even penetrating the epidermis layer (Day et al., 1993). In other species such as *Chenopodium album* and *Smilacina stellata*, the UV-B penetrated through spongy mesophyll layers to a depth of 0.16–0.15 mm (Day et al., 1993). In contrast to the absorption of UV radiation, these phenylpropanoid compounds that absorb in the UV-B transmit in the PAR (van de Staaij et al., 1995), causing the epidermal layer of cells to be nearly transparent to the PAR.

Leaf reflectance is partly due to roughness features on the surface with varying density, dimension and refractive indices, and partly due to scattering off cell wall surfaces (Grant, 1987). Tucker and Garratt (1977) and others have modeled the scattering by leaves based on refractive differences in the leaf, with spectral variation in part due to particle scattering theory. PAR leaf reflection is largely due to scattering off internal cell walls of the leaf (Tucker and Garratt, 1977). In contrast, UV-B leaf reflectance must occur at or near the leaf surface due to the intense UV absorption in the cuticle, cell walls, and cell chromatophores (Day et al., 1993; Kootstra, 1994; van de Staaij et al., 1995; Gausman et al., 1975). Rod-like epicuticular wax structures on the surface of *Picea pungens* Engelm. and *Picea pungens* Engelm. Var. hoopsi leaves were shown by Clark and Lister (1975) and Reicosky and Hanover (1978) to contribute more to the UV reflectance than the visible wavelengths. Clark and Lister (1975) attributed the enhanced reflectance of UV over visible radiation to Rayleigh-sized wax particles of varying length.

Can leaf UV-B reflectance be predicted on the basis of leaf surface properties such as the dimensions and density of structures on a leaf surface? This paper describes the spectral scattering properties of leaves from 20 species or varieties of common deciduous urban trees and relates the surface structure of the tree leaves to the UV spectral reflectance. To help understand the interplay of surface structure dimension and density on reflectance, the study used a series of single gene epicuticular wax mutants of *S. bicolor* (Peters, 1993) that exhibited a wide range of sheath wax densities and structures (Jenks et al., 1992, 2000). It is proposed that the scattering from leaves and sheaths

in the UV wavelengths are largely influenced by the density and dimension of the epicuticular structures on the leaf or sheath surface.

2. Materials and methods

2.1. Sample collection and optical measurements

The spectral leaf transmittance and the spectral adaxial and abaxial leaf reflectance of white *oak* (*Quercus alba* L.), red oak (*Quercus rubra* L.), pin oak (*Quercus palustris* Muench.), apple (*Malus* × *domestica* Borkh.), Callery pear (*Pyrus calleryana* Dcne.), hackberry (*Celtis occidentalis* L.), sweetgum (*Liquidambar styraciflua* L.), white ash (*Fraxinus americana* L.), green ash (*Fraxinus pennsylvanica* Marsh.), crabapple (*Malus floribunda* Sieb. ex Van Houtte), little leaf linden (*Tilia cordata* Mill.), saucer magnolia (*Magnolia* × *soulangiana* Soulange-Bodin), river birch (*Betula nigra* L.), sycamore (*Platinus occidentalis* L.), tulip poplar (*Liriodendron tulipifera* L.), redbud (*Cercis canadensis* L.), red maple (*Acer rubrum* L.), silver maple (*Acer saccharinum* L.), sugar maple (*Acer saccharum* Marsh), and Norway maple (*Acer platanoides* L.) were measured on whole leaves collected during the summer of 1994 from trees growing in the West Lafayette, IN, area. The spectral abaxial leaf transmittance and reflectance of wild-type *Sorghum bicolor* (L.) Moench. and six isogenic epicuticular wax mutants were measured from sheaths removed from field-grown plants during the summer of 1998.

Three healthy-appearing mature sunlit leaves from each of the species were collected from the field before sunrise. Leaves were stored on wet paper in plastic bags in a way that avoided contact that might disrupt the wax crystallization patterns, and kept cool until optical properties were measured later that day. Spectral leaf reflectance and transmittance were measured using a Perkin-Elmer Lambda 19 spectrophotometer fitted with an integrating sphere. The spectrophotometer light source was a collimated beam with an 8° incidence angle to the measured leaf surface within a Spectralon® (Labsphere, Inc.) integrating sphere. Measurements at 5 nm steps between 700 and 250 nm were made using a Spectralon® reference standard (Labsphere, Inc.). Leaf midribs were avoided. The mean and standard error of the mean (SE) of the leaf blade and leaf sheath reflectance and transmittance across the wavelength bands of PAR (400–700 nm), UV-A (320–400 nm), and UV-B (280–320 nm) were calculated.

2.2. Leaf surface morphology measurements

Leaf surface morphology was determined using a JEOL JSM-840 scanning electron microscope (SEM). Samples were prepared for microscopy at 6 kV using techniques reported in Jenks (1993). In short, air-dried leaf tissue samples were mounted on aluminum stubs and sputter-coated with gold–palladium. Although air drying of some tissues caused collapse (wrinkling) of the epidermal cells, noncollapsed areas for wax structural analysis were present on all samples. Leaf surface morphology was evaluated by qualitative and quantitative image analysis of 1500× and 250× scanning electron micrographs of the leaf surface. Image analysis was only performed on images with excellent resolution and contrast, and showed no evidence of collapsed tissue due to SEM sample preparation. As the wrinkled epidermal-surface regions of our air-dried samples showed the typical downward collapse of apical cell walls between anticlinal walls that does not occur in quench-frozen samples viewed using low temperature SEM (Jenks et al., 1992), these leaf surfaces were presumed to have been smooth before the air-drying process. Quantitative dimensional assessment of leaf surface features was made manually by measuring 10–15 features in 5000× to 10,000× SEM photomicrographs of each leaf surface.

The coverage of scattering features on the leaf, termed the scattering cross-section, was defined as the ratio of brightly lit regions of the leaf surface (produced by scattering the electron beam of the SEM in the direction of the photomicrograph negative) to total leaf surface area from 1500× SEM photomicrographs. The mean scattering cross-sections on the tree leaf surfaces were determined by three replicates of counts of bright intersections on an 18 × 40 grid. More conclusive statements concerning the features influencing the tree leaf reflectance were however not possible because we did not have replicate SEM photomicrographs of the same leaf surfaces for which reflectance was measured, and shadows created on the surfaces by the SEM precluded accurate surface

feature measurements. No SEM photomicrographs were made for the specific sorghum mutant plants in which sheath reflectance is reported here. Since the measured sheath reflectance in the PAR (R_{PAR}) was higher for the field-grown sheath samples used in this study than those reported for greenhouse-grown plants in Jenks (1993), the scattering cross-sections for the sorghum mutants used in this study were approximated using a logarithmic function relating the measured R_{PAR} values and filament density ($r^2 = 0.998$) reported in Grant et al. (1995) (Table 2).

2.3. Application of scattering theory to leaf surfaces

Walter-Shea and Norman (1991) and others have translated particle scattering theory to scattering by surface roughness features. It has been surmised that scattering efficiency of the surface (K) follows the Mie or Rayleigh theory based on the differences between the dimension of the surface feature facets and the radiation wavelength (n). Then the fraction of incident radiation scattered off a surface (R) depends on K and the scattering cross-section or effective surface area (s) for scattering objects of dimension (r) according to (modified from Kyle, 1991)

$$R(n) = \int_0^{\infty} s(r)K(n,r)\frac{\mathrm{d}n}{\mathrm{d}r}\,\mathrm{d}r \qquad (1)$$

where the kernel of the integral is a peaked function, with the dominant peaks associated with high K and high s and the tails associated with either high s and low K of relatively large objects (compared to the wavelength of radiation) or low s and high K for relatively small objects with low frequencies of occurrence on the surface. K depends on the dimensions of the scattering surface roughness relative to the wavelength of incident radiation (Grant, 1987). Scattering in any given direction depends on the shape, density and positioning (orientation) of scattering objects on the rough surface. Measurements of the leaf and sheath scattering were made in an integrating sphere and therefore include scattering from all directions.

Information concerning the shape of $K(n, r)$ in the UV can be derived from a ratio of reflectance at two wavelengths (say 260 and 370 nm: termed R_{260}/R_{370} ratio) if we assume that s and the refractive index (that influences K) are the same across the range of wavelengths. This R_{260}/R_{370} ratio was used to represent wavelength-dependent scattering from the leaf and sheath surfaces. It is proposed in this study that the scattering objects of interest for the UV wavelengths are the epicuticular wax structures on the leaf surface.

An approximation of the magnitude of s for all tree leaf surfaces with distinct scattering objects on the surface was based on the measured scattering cross-section of the objects from the SEM photomicrographs of the surface. The impact of wax structures on the sheath spectral reflectance was evaluated by subtracting the sheath spectral reflectance of mutant *bm6* that exhibited no wax structures from the spectral reflectance of the other mutants and the wild-type.

The mean radius r of the epicuticular objects on the leaf and sheath surfaces was determined from 10 measurements of the objects visible on the 1500× photomicrographs. The mean radius was a mean minimum radius, as measurements were always made perpendicular to the edge of the object.

The effect of similar surface structures, and hence similar values of $K(n, r)$, but differing s from Eq. (1) on $R(n)$ was studied using the abaxial leaf sheaths of a number of chemically induced wax mutants of *Sorghum bicolor* that are morphologically identical except for the epicuticular wax; wild type P954035 and isogenic epicuticular wax mutants *h7*, *h10* (previously *bm28*), *bm2* (previously *bm22*), and *bm11* (previously *bm38*) (Peters, 1993). These isolines exhibit a range in filamentous and globular epicuticular wax structures on the sheaths (Jenks et al., 1992).

3. Results and discussion

3.1. Leaf transmittance

In general, leaf transmittance in the UV was immeasurably small. The lack of UV leaf transmittance is in agreement with Brabham and Briggs (1975) and Yang et al. (1995). Penetration through the leaf requires penetration through the (adaxial) cuticle, epidermis, mesophyll, a second (abaxial) epidermis, and a second cuticle. In a study by Krauss et al. (1997), transmittance through the epidermis and cuticle varied among species from 0.004 to 0.50. *Malus domestica* had an epidermal transmittance of 0.50 in the UV-B

(Krauss et al., 1997), while the whole leaf transmittance was measured in this study at less than 0.006. *Quercus velutina* had an epidermal transmittance in the UV-B of 0.13 (Day et al., 1994), while the whole leaf transmittance was also measured in this study at less than 0.006. Thus it appears that the transmission of UV-B through the leaf is negligible, and much of the absorption of the UV-B is in the cuticle. The penetration of radiation into leaves generally increases with increasing wavelength (Cui et al., 1991; Krauss et al., 1997; and Day et al., 1994). In this study, the PAR leaf transmittance varied from 0.05 to 0.24 (Table 1).

3.2. Broadband reflectance

The UV-B and UV-A leaf reflectance for both adaxial and abaxial sides of the majority of tree species evaluated had values of approximately 0.05 (Table 1), with standard deviations of less than 0.01. The PAR leaf reflectance was always greater than that in the UV-B (Table 1), with standard deviations of less than 0.02. Across the range of tree species and leaf surfaces evaluated, the leaf reflectance in the PAR waveband varied more than that in the UV-B waveband (Table 1). The UV-B and UV-A reflectances for the sorghum mutants were similar to those of the tree species; however, the UV-B and UV-A reflectance of the sorghum wild-type was nearly twice that for all other surfaces (Tables 1 and 2). The leaf reflectances in the PAR and UV-B were similar to reflectances in many other species reported by Caldwell (1971) and Gates et al. (1965).

While all leaves with UV-B leaf reflectance above 0.07 had PAR leaf reflectance above 0.16, several species had leaf reflectance in the PAR above 0.16 but leaf reflectance in the UV-B less than 0.07 (Fig. 1). The values for UV reflectance were very similar to previous studies of leaf epidermis reflectance (Brabham and Briggs, 1975; Gausman et al., 1975). Of particular note is the similarity in the rate of change in reflectance between 250 and 370 nm for all tree species, with both UV-A and UV-B reflectance of less than 0.07 (Fig. 2a). What characteristic(s) of a leaf surface causes the UV-B leaf reflectance to be high in some species and low in others even while the PAR leaf reflectance is high? If the surfaces on the leaf that scatter the PAR and UV radiation are similar, then the PAR and UV reflectance should be monotonically (but not linearly) related. However, since the PAR penetrates the leaf to a greater depth than the UV and is absorbed by different chromatophores than the UV, the effective surfaces scattering the PAR differ from the UV. Therefore, we would not expect a good correlation between the PAR and UV reflectance of a leaf, nor might we expect a straightforward means to estimate the commonly unmeasured UV reflectance from the commonly measured PAR reflectance. Our results showed a poor linear correlation between the PAR reflectance and UV-B reflectance ($r = 0.65$) but a good correlation between PAR reflectance and UV-A reflectance ($r = 0.97$) for the combined set of abaxial and adaxial leaf surfaces of the 20 species studied. This may be expected as UV-A and PAR radiation are both utilized in plant development (photosynthesis, UV-B repair processes) while UV-B appears to be damaging to plant development (Grant, 1997). Thus, plants may have adapted mechanisms for utilizing UV-A but minimizing absorption of UV-B.

3.3. Trichomes versus broadband reflectance

Trichomes were evident on the abaxial leaf surface of five tree species and on both abaxial and adaxial surfaces of one species (Table 1). Trichomes influence the scattering of all wavelengths of light, partly by redirecting the radiation at oblique angles from the radiation source. Many studies have found that the presence of trichomes increases leaf reflectance in visible and near-infrared wavelengths (Grant, 1987). In this study, the presence of trichomes commonly corresponded with greater density of epicuticular structures—resulting in a confounding of the scattering influence of trichomes. For species with no trichomes and smooth leaf surfaces, we found the mean difference between the adaxial and abaxial UV-B reflectance ($n = 9$; mean difference, 0.0056; S.D., 0.0044) was greater than the mean difference for species with smooth surfaces and trichomes present ($n = 3$; mean difference, 0.0047; S.D., 0.0064). In the PAR, the difference in abaxial and adaxial reflectance of species with smooth surfaces and no trichomes ($n = 9$; mean difference, 0.056; S.D., 0.021) was not significantly different from the species with smooth surfaces and trichomes ($n = 3$; mean difference, 0.060; S.D., 0.003). This suggests that trichomes do not affect PAR leaf reflectance in the species studied,

Table 1
Leaf surface characteristics of tree species

Code	Species	Adaxial leaf surface					Abaxial leaf surface					Trans-mittance
		Reflectance			Wax crystal structure		Reflectance			Wax crystal structure		PAR
		UV-B	UV-A	PAR	Shape	Mean radius (nm)	UV-B	UV-A	PAR	Shape	Mean radius (nm)	
Trichomes present on both surfaces												
Co	*C. occidentalis*	0.064	0.057	0.090	Smooth	NA	0.088	0.082	0.159	(Smooth)	NA	0.082
Trichomes present on abaxial surface												
Asc	*A. saccharinum*	0.058	0.052	0.088	(Smooth)[a]	NA	0.138	0.155	0.241	Horizontal plates	200	0.038
Cc	*C. canadensis*	0.056	0.051	0.082	Stellate plates	150	0.049	0.057	0.144	Stellate vertical plates	200	0.057
Fp	*F. pennsylvanica*	0.060	0.052	0.068	Smooth	NA	0.048	0.049	0.125	(Smooth)	NA	0.037
Ms	*M. soulangiana*	0.053	0.046	0.069	Smooth	NA	0.055	0.062	0.132	Smooth	NA	0.056
M	*M. domestica*	0.059	0.051	0.075	Smooth	NA	0.059	0.054	0.135	Smooth	NA	0.034
Qa	*Q. alba*	0.061	0.053	0.067	Vertical plates	–	0.127	0.114	0.165	Vertical plates	100	0.049
No trichomes present												
Ap	*A. platinoides*	0.052	0.047	0.057	(Smooth)	NA	0.060	0.068	0.110	Smooth	NA	PAR
Ar	*A. rubrum*	0.057	0.049	0.076	(Smooth)	NA	0.129	0.130	0.223	Filaments	350	0.025
As	*A. saccharum*	0.052	0.050	0.068	(Smooth)	NA	0.066	0.068	0.157	Vertical plates	400	0.036
Bn	*B. nigra*	0.054	0.046	0.082	Globules	400	0.064	0.057	0.187	Stellate vertical plates	150	0.057
Fa	*F. americana*	0.060	0.052	0.082	Smooth	NA	0.053	0.050	0.168	(Smooth)	NA	0.029
Ls	*L. styraciflua*	0.061	0.056	0.078	Smooth	NA	0.057	0.053	0.131	Smooth	NA	0.055
Lt	*L. tulipifera*	0.056	0.048	0.082	Globules	200	0.075	0.060	0.191	Globules	200	0.048
Mr	*M. floribunda*	0.060	0.052	0.067	Smooth	NA	0.053	0.055	0.158	Smooth	NA	0.032
Po	*P. calleryana*	0.045	0.040	0.072	(Smooth)	NA	0.057	0.053	0.146	(Smooth)	NA	0.028
Pc	*P. occidentalis*	0.055	0.048	0.078	(Smooth)	NA	0.054	0.055	0.137	Smooth	NA	0.053
Qp	*Q. palustris*	0.057	0.050	0.112	Smooth	NA	0.056	0.051	0.175	Smooth	NA	0.022
Qr	*Q. rubra*	0.052	0.045	0.071	Smooth	NA	0.061	0.054	0.118	Smooth	NA	0.075
Tc	*T. cordata*	0.056	0.051	0.065	(Smooth)	NA	0.061	0.065	0.139	Smooth	NA	0.058

NA: not applicable.

[a] Parenthesis means smooth is assumed: the SEM photomicrograph showed either significant collapse of tissues or breakage of the epicuticular surface.

Table 2
Sorghum bicolor sheath characteristics

Species, mutant	Code	Surface reflectance			Epicuticular structure	
		UV-B	UV-A	PAR	Density ($\mu m^2\ \mu m^{-2}$)[a]	Morphology[b]
S. bicolor, wild-type	wild	0.245	0.233	0.299	0.63	Dense long filaments
S. bicolor, h7	*h7*	0.143	0.134	0.206	0.44	Sparse long filaments
S. bicolor, h10	*h10*	0.059	0.059	0.161	0.31	Sparse short filaments
S. bicolor, bm11	*bm11*	0.051	0.052	0.135	0.22	Sparse crystals
S. bicolor, bm2	*bm2*	0.061	0.071	0.166	0.32	None

[a] Calculated on the basis of PAR reflectance and epicuticular structure density in Grant et al. (1995).
[b] From Jenks et al., 1992.

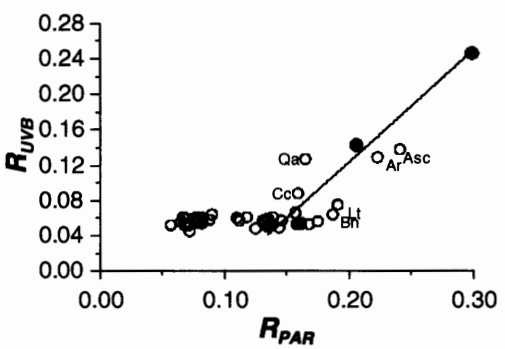

Fig. 1. Comparative UV-B and PAR reflectance of leaves. The UV-B and PAR reflectance for tree species are indicated by the open circles, while those for the sorghum mutants are indicated by the closed circles. A linear regression of the sorghum mutants is indicated (solid line). The labeled outlier is abaxial surface of *Q. alba*.

in contrast with prior studies of other species (Grant, 1987; Holmes and Keiller, 2002). Holmes and Keiller (2002), however, do point out that the effect of trichomes is not consistent across all species.

3.4. Spectral reflectance

The decrease in reflectance with increasing wavelength ($-\partial R/\partial n$) is smooth and monotonic. Since the UV reflectance is a broadband value, high levels of reflectance can occur either by increasing the reflectance in all UV wavelengths or by increasing $-\partial R/\partial n$ in the UV. For tree species with UV-B reflectance above 0.07, the greater reflectance in the UV-B was due in some cases to an increase in $-\partial R/\partial n$ (*L. tulipifera*, Lt; *Q. alba*, Qa; and *C. Canadensis*, Cc) and in some cases to an overall increase in $R(n)$ (offset) (*A. rubrum*, Ar; *A. saccharinum*, Asc; and *B. nigra*, Bn) (Fig. 2b). Differences in UV-B reflectance between the sorghum wild-type and *h7* mutant were due to an overall increase in $R(n)$, while that between the *h10* and *h7* mutants was due to both an overall increase in $R(n)$ and an increase in $-\partial R/\partial n$ (Fig. 2b).

3.5. Epicuticular surface structures versus reflectance

For the tree species, three types of epicuticular structures were evident: filamentous, plate, and globular structures (Table 1; Figs. 3 and 4). For the sorghum wild-type and mutants with similar filamentous epicuticular wax structures, those with greater density of filaments had greater reflectance (Table 2; Fig. 5). Most leaves of tree species with distinct epicuticular wax structures (*Q. alba*, *C. canadensis*, *L. tulipifera*, *A. rubrum*, *A. saccharinum*, and *B. nigra*) had relatively high leaf reflectance in the PAR and UV-B (Qa, Cc, Lt, Ar, Asc, and Bn respectively in Fig. 1). For the sorghum mutants, those with smoother surfaces (*bm2* and *bm11*) showed less UV-B, UV-A, and PAR reflectance than those with a dense layer of long filamentous structures (wild type and *h7*) (Table 2). Leaves from many of the tree species with fine (sub-micron) wax structures (*L. tulipifera*, *Q. alba*, and *C. canadensis*; Table 1) appeared to have the greatest $-\partial R/\partial n$ of species evaluated (Fig. 2b). However, *A. rubrum* and *B. nigra* with fine wax structures on the leaves had relatively low $-\partial R/\partial n$ (Fig. 2b). A better understanding of the effect of surface wax structures on scattering can be gained by comparing

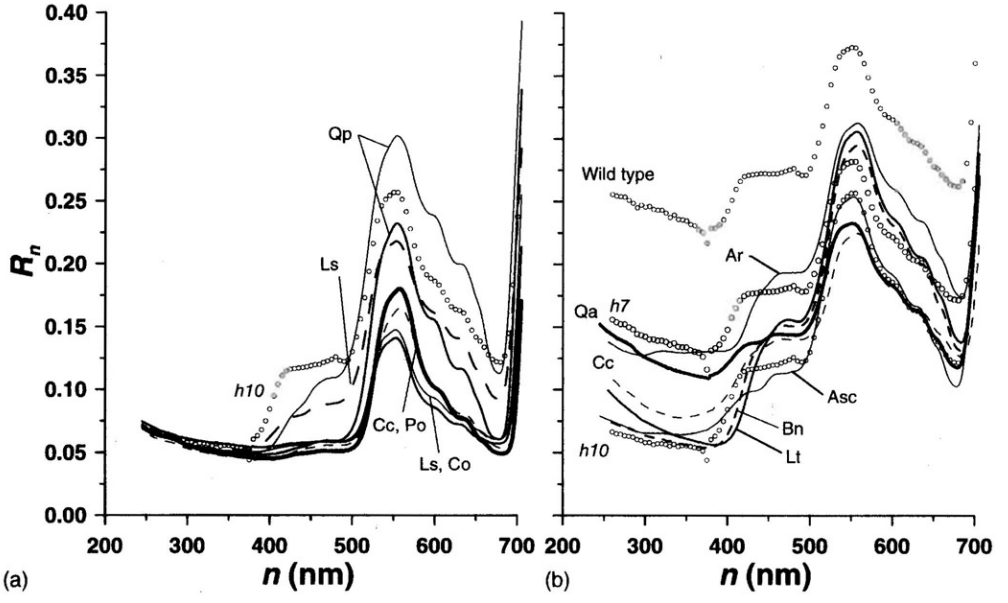

Fig. 2. Spectral reflectance of leaf surfaces. The spectral reflectance of representative adaxial and abaxial tree leaves are indicated by lines while those of the sorghum wild-type and mutant sheaths are indicated with open circles. Reflectance of tree species with UV reflectance <0.07 are plotted in panel (a) while those >0.07 are plotted in panel (b). Abbreviations are defined in Tables 1 and 2.

the spectral reflectance of the tree leaves and sorghum sheaths through an analysis of the terms in Eq. (1).

3.5.1. Dimension of scattering elements

Scattering efficiency K, which the R_{260}/R_{370} ratio represents, is dependent on n and r (Eq. (1)). The dimension of the epicuticular wax structures on the sorghum mutants, as determined by Jenks (1993), were similar with the filaments of the wild-type, *h7* and *h10* mutants of *Sorghum bicolor* with rectangular cross-sections of approximately 1000 nm × 500 nm (Fig. 5), while the sparse crystalline waxes on the *bm11* mutant had radii of 200 ± 100 nm. The measured radii of the epicuticular structures on the tree leaf surfaces varied from 150 to 400 nm (Table 1). The similar radii of the wax structures of *A. saccharinum*, *A. rubrum*, and *Q. alba* abaxial leaf surface (Fig. 4) resulted in similar UV-B but differing PAR

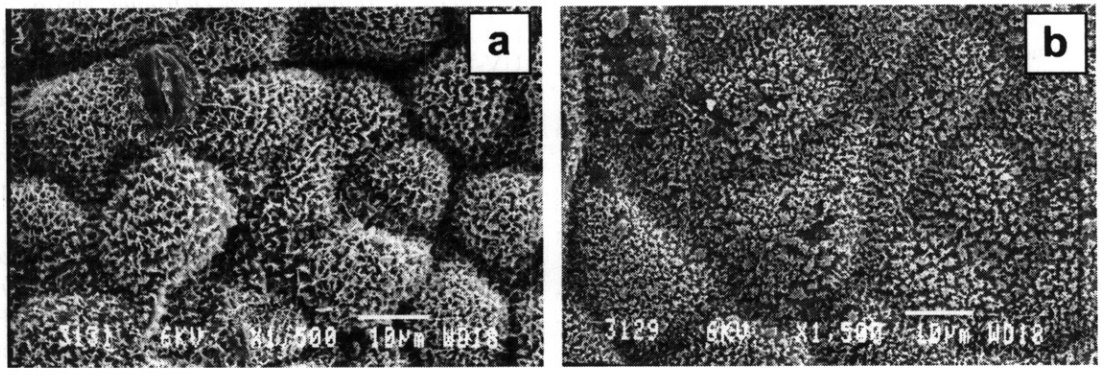

Fig. 3. Leaf surface morphology for *C. canadensis*. SEM images of the (a) abaxial and (b) adaxial surfaces are illustrated. The surfaces have similar $R_{UV\text{-}B}$ while R_{PAR}(abaxial) > R_{PAR}(adaxial).

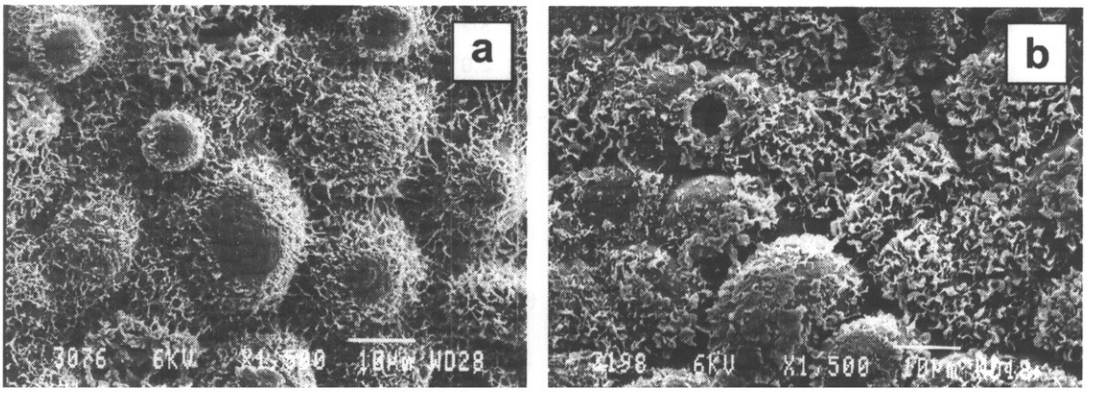

Fig. 4. Leaf surface morphology for species with UV-B reflectance >0.08. SEM images of the abaxial leaf surfaces of (a) *Q. alba* and (b) *A. saccharum* are illustrated. $R_{UV\text{-}B}$ of *Q. alba* is two times that of *A. saccharum*. In panel (a), wax has been rubbed off several cells, resulting in an artifact-smoothed surface.

reflectances (Table 1). The stellate plates of the abaxial surface of *B. nigra* leaves had a similar thickness as the structures of the *A. saccharinum*, *A. rubrum*, and *Q. alba* abaxial leaf surfaces but lower UV reflectance (Table 1). The sub-micron size of the adaxial-surface amorphous globules or the abaxial-surface stellate plates of *B. nigra* did not correspond to high UV reflectance (Table 1).

3.5.2. Scattering efficiency

The R_{260}/R_{370} ratio gives some insight into the efficiency of scattering (K) from the leaf blade or leaf

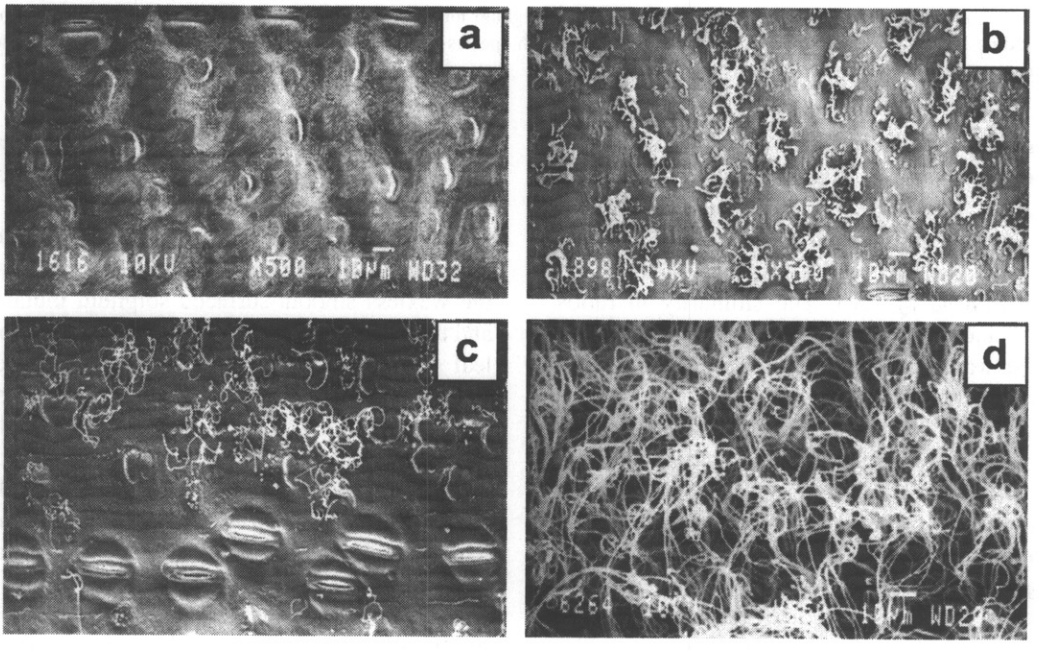

Fig. 5. Sheath surface morphology of *Sorghum* mutants. SEM images of the sheath surfaces of (a) *bm11*, (b) *h10*, (c) *h7*, and the (d) wild-type sheaths are illustrated.

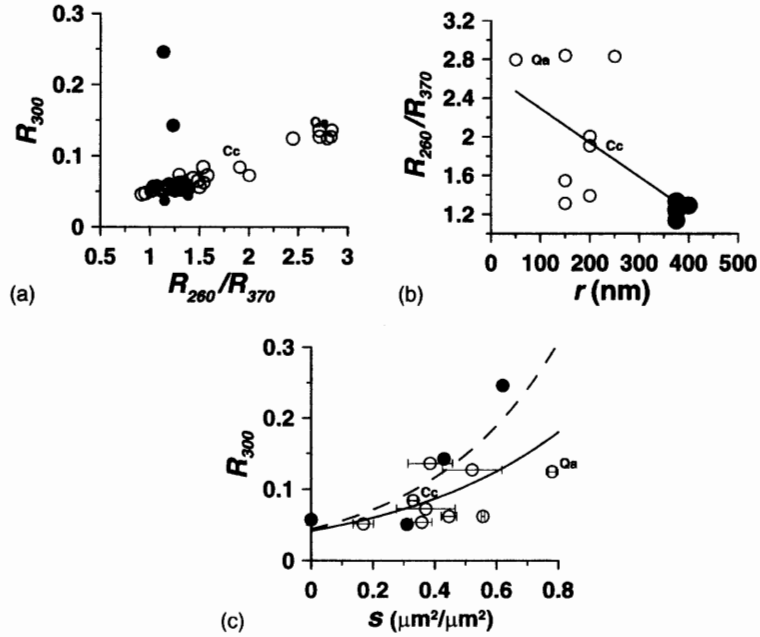

Fig. 6. Analysis of processes involved in spectral reflectance. The reflectance at 300 nm is related to the ratio of reflectance in the 260 and 370 nm wavelengths for all leaf and sheath surfaces (panel (a)) and the scattering cross-section for only surfaces with distinct epicuticular structures (panel (c)). The R_{260}/R_{370} ratio is compared to the radius of the distinct epicuticular structures in panel (b). Values for the abaxial and/or adaxial leaf surfaces of the tree species are indicated by the open circles, while those for the sorghum mutants are indicated by the closed circles. Regression line in panel (b) represents the linear fit to both sorghum mutants and tree species combined. Dashed and solid regression lines in panel (c) are exponential fits for the sorghum mutants only ($r^2 = 0.70$) and the combined sorghum and tree species excluding the labeled data ($r^2 = 0.36$). Labeled values (with species label from Table 1) refer to surfaces with trichomes. Bars represent one standard deviation.

sheath surface. Sorghum sheath wax mutants with varying amounts of wax filaments of similar width (*h7*, *h10*, and wild type) all had similar R_{260}/R_{370} ratios of between 1.15 and 1.30 (Fig. 6a). However, smooth sheath surface of the sorghum mutant *bm2* had the R_{260}/R_{370} of 1.15. The similarity in ratios suggests that differences in the length and type of epicuticular wax structures between mutants did not influence the efficiency of scattering.

A comparison of the spectral reflectance of the sorghum wild-type and mutants to the smooth-surfaced *bm2* mutant spectral reflectance further shows only minor differences in efficiency of scattering. The spectral reflectance of the *h7* mutant and wild-type with long filaments increased with decreasing wavelength below 500 nm. This indicates that there is some wavelength-dependent scattering of long filaments on the sheath surface. The spectral reflectance of the *h10* with few short, thick filaments (Fig. 7) showed less wavelength-dependent scattering than the mutants with denser filament coverage and longer filaments.

The R_{260}/R_{370} ratio of the leaf surfaces varied from less than 1.0 to almost 3.0 (Fig. 6a). As found for the waxless smooth-surfaced sorghum mutant (*bm2*) and the nearly-smooth mutant (*bm11*), the smooth tree leaf surfaces with R_{260}/R_{370} values of approximately 1.0 were among the lowest-reflecting leaf surfaces (Table 1). The structures evident on the other leaf surfaces (epicuticular wax filaments, plates, and amorphous globs) tended to result in greater leaf reflectance in the UV than the surfaces without epicuticular wax structures. In contrast with the sorghum mutants, increases in R_{300} of the tree species leaf surfaces were linearly related to the R_{260}/R_{370} ratio ($r^2 = 0.95$, Fig. 6a). This linear relationship suggests that the reflectance over the range of 260–370 nm is from similar surfaces.

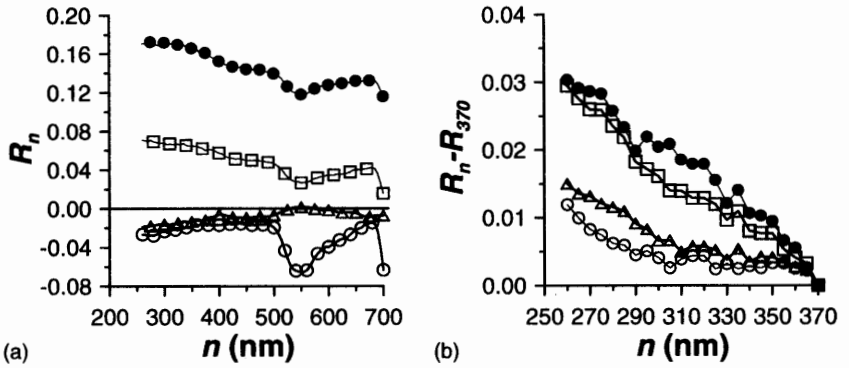

Fig. 7. Spectral reflectance relative to filamentous surface waxes. The difference in reflectance of wild type–*bm2* (closed circle), *h7–bm2* (open square), *bm11–bm2* (open triangle), and *h10–bm2* (open circle) are indicated in panel (a). The reflectance normalized to that at 370 nm of wild type–*bm2* (closed circle), *h7–bm2* (open square), *bm11–bm2* (open triangle), and *h10–bm2* (open circle) is indicated in panel (b).

In general, the R_{260}/R_{370} ratio was inversely related to the radius of epicuticular structure, with leaf surfaces that had structures with an r less than the wavelengths of interest (260 and 370 nm) having a greater ratio than those with r greater than the wavelengths of interest (Fig. 6b). A linear regression relating the R_{260}/R_{370} ratio to r had an r^2 of 0.42. Results were however inconsistent with three surfaces inexplicably having small r but R_{260}/R_{370} ratios similar to the sorghum surfaces. The two surfaces with distinct epicuticular wax structures and trichomes (Cc and Qa in Fig. 6a and b) did not deviate from the trend of the overall population, suggesting negligible effect of the trichomes on the scattering efficiency. Overall, it appears that the monotonic increase in reflectance with decreasing wavelength through the UV-B is probably not a simple function of the dimension of the scattering elements.

3.5.3. Scattering cross-section

Theoretically the scattering cross-section for the leaf surface is in part a function of the density of the wax structures on the leaf surface If the scattering efficiency is similar across the sorghum mutants with similar R_{260}/R_{370} ratios, differences in sorghum mutant sheath reflectances must be due to differences in the scattering cross-sections. The estimated density of sorghum sheath filaments did appear to influence R_{300} (Fig. 6c).

The density of filaments on the sheaths (defining the scattering cross-section) of the sorghum mutants reported in Grant et al. (1995) were generally small (less than 0.15 $\mu m^2\ \mu m^{-2}$) except for the wild-type (0.80 $\mu m^2\ \mu m^{-2}$). After applying the empirical function relating wax density and PAR reflectance, the density varied from 0 to 0.6 $\mu m^2\ \mu m^{-2}$. An exponential function of the scattering cross-section approximated the R_{300} (Fig. 6c). The scattering cross-section s (Eq. (1)) for the leaf surfaces with distinct epicuticular structures ranged form 0.17 to 0.8 $\mu m^2\ \mu m^{-2}$, but only weakly corresponded with the R_{300} of the surface (Fig. 6c). However, the scattering cross-sections for the tree leaf surfaces with epicuticular structures generally followed the relationship indicated by the sorghum mutants (Fig. 6c). The relationship between R_{300} and s appeared to be exponential, with an r^2 of 0.70 for the sorghum surfaces with similar epicuticular structures and an r^2 of 0.36 for the combined sorghum and tree leaf surfaces with a wide range of epicuticular structures (Fig. 6c). The values labeled 'Qa' and 'Cc' in Fig. 6c corresponded to leaf surfaces with trichomes that may have reduced the R_{300} by absorbing the UV radiation in accordance with Karabourniotis et al. (1992) and Holmes and Keiller (2002). The overall relationship suggests that the measured cross-section does influence R_{300}, with the more distinct relationship evident in the sorghum mutant sheath surfaces than the tree leaf surfaces due to lesser variation in the epicuticular structures (Table 2) and hence scattering efficiency (represented by the R_{260}/R_{370} ratio; Fig. 6a).

4. Conclusions

In general, leaf reflectance in the UV wavelength bands is approximately 0.05 for the plants studied. Differences between the leaf abaxial and adaxial surface reflectance were smaller for the UV-B and UV-A wavelengths than for the PAR wavelengths. The UV reflectance of leaf surfaces with filament or plate wax structures were generally higher than those with smooth surfaces.

Results suggest that the magnitude of the UV reflectance was largely a function of the shape, diameter, and distribution of epicuticular wax leaf surface structures. This was evident in the many tree species evaluated as well as the *Sorghum bicolor* sheaths. Amorphous globular wax structures did not appear to scatter UV as well as filaments or vertical plates in varying patterns. Increasing density of sub-micron wax structures corresponded to an enhanced UV reflectance of the *S. bicolor* mutants due to the similarity in the dimensions of the epicuticular structures. The smaller epicuticular wax structures on most of the tree leaf surfaces appeared to contribute to greater scattering efficiency. Further work needs to be done to substantiate that the UV reflectance is largely from the leaf surface, how the density of fine structures on the surface influences the leaf or sheath reflectance, and what shapes and dimensions of epicuticular wax structures are better at scattering UV radiation. More precise measurements of K and s for various leaf surfaces are needed to determine if the combined effects do indeed account for the variation in R_{300}.

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