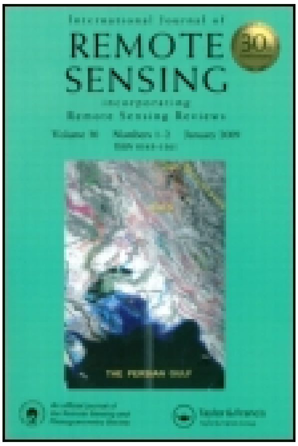


International Journal of Remote Sensing
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tres20

In silico assessment of environmental factors affecting the spectral signature of C_4 plants in the visible domain

Gladimir V. G. Baranoski [a], Bradley Kimmel [a], Tenn F. Chen [a] & Daniel Yim [a]

[a] School of Computer Science, University of Waterloo, Waterloo, ON, Canada, N2L 3G1

To cite this article: Gladimir V. G. Baranoski, Bradley Kimmel, Tenn F. Chen & Daniel Yim (2012): *In silico* assessment of environmental factors affecting the spectral signature of C_4 plants in the visible domain, International Journal of Remote Sensing, 33:4, 1190-1213

To link to this article: http://dx.doi.org/10.1080/01431161.2010.550949

International Journal of Remote Sensing
Vol. 33, No. 4, 20 February 2012, 1190–1213

Taylor & Francis
Taylor & Francis Group

In silico assessment of environmental factors affecting the spectral signature of C_4 plants in the visible domain

GLADIMIR V. G. BARANOSKI*, BRADLEY KIMMEL, TENN F. CHEN
and DANIEL YIM
School of Computer Science, University of Waterloo, Waterloo, ON, Canada, N2L 3G1

(*Received 23 July 2010; in final form 8 December 2010*)

Monocotyledonous (C_4) plants, such as maize and sugarcane, have a central role in the economy and ecology of our planet. In many regions, the main food sources are based on C_4 crops. These crops are also major suppliers of raw materials used in the production of biofuel. Due to their increasing global demand, it becomes essential not only to monitor and analyse the effects of abiotic stress factors, such as limited water and nutrient supplies, on their productivity, but also to determine their ecological impact (e.g. related to their irrigation needs). Computer simulations, or *in silico* experiments, are being routinely employed in remote-sensing investigations aimed at these goals. Besides these applications, *in silico* experiments paired with measured data can also contribute to expand the existing knowledge about the biophysical mechanisms responsible for the remarkable tolerance of C_4 plants to adverse environmental conditions. In this article, we evaluate the applicability of a computer model (ABM-U) to the assessment of biophysical responses of C_4 plants in the visible (photosynthetic) domain when subjected to abiotic stress factors. Initially, we verify the accuracy of model readings obtained in this spectral domain. This verification is performed through quantitative and qualitative comparisons of modelled results with measured data. We then proceed to investigate apparently conflicting reflectance profiles resulting from experiments involving maize specimens under moderate water stress, which is usually associated with unfavourable climate changes. The results of our simulations indicate that ABM-U can reliably predict spectral signature variations caused by abiotic stress factors affecting the photosynthetic apparatus of these plants, which, in turn, have a direct impact on their agricultural yield. Furthermore, our *in silico* experiments suggest that the decrease in the amount of light reflected by (*in vivo*) water-stressed specimens may result from changes in the internal arrangement of the main components of their photosynthetic apparatus, namely the chloroplasts. We close the article with a discussion of putative physiological processes responsible for such changes.

1. Introduction

Plants are primary remote-sensing targets due to their importance in sustaining human and animal life on a global scale. Disruptions to their photosynthetic apparatus have a significant impact not only on food production, but also on increasingly scarce freshwater supplies, which are used predominantly in agriculture (Minorsky

---

*Corresponding author. Email: gvgbaran@curumin.math.uwaterloo.ca

International Journal of Remote Sensing
ISSN 0143-1161 print/ISSN 1366-5901 online © 2012 Taylor & Francis
http://www.tandf.co.uk/journals
http://dx.doi.org/10.1080/01431161.2010.550949

2003, Liu *et al*. 2010). Such disruptions are directly translated to alterations in the spectral signatures of their leaves, whose growth and development are regulated by an array of photobiological processes and environmental factors. For example, the pigments directly involved in the photosynthesis process are contained in organelles (chloroplasts) present in the foliar mesophyll tissue. Variations in the amount of light absorbed by these pigments have a direct influence on the leaves' photosynthetic capacity. These variations may be caused not only by changes in the mesophyll total pigment content, but also by changes in intracellular arrangement of the chloroplasts (Fukshansky 1981, Björn 1992, Vogelmann 1993, Evans *et al*. 2004). The latter may significantly alter the direction of propagation of light travelling in this tissue. This phenomenon, known as the detour effect (Fukshansky 1981, Vogelmann 1993), can result in an increase in the probability of light absorption. It is a well-known fact that certain plants have developed adaptive responses, such as chloroplast displacements, which allow them to adjust light absorption without changing pigment contents (Rabinowitch 1951, Evans *et al*. 2004). However, the underlying mechanisms responsible for these responses and their dependence on environmental factors remain incompletely understood (Wada *et al*. 2003).

Variations in the nutrient and water content of plant leaves can also have a noticeable influence on their photosynthetic capacity (Nátr 1992). For example, a nitrogen deficiency causes a decrease in leaf photosynthetic pigment contents (Zhao *et al*. 2003), that is, the leaf does not have enough nitrogen to produce all the chlorophyll that it needs. This condition, known as chlorosis (Hendry *et al*. 1987) and characterized by leaf colour changes to light green or yellow-green (Lee *et al*. 1999), may also be caused by iron and other mineral deficiencies (Hendry *et al*. 1987). Similarly, severe water stress is also accompanied by an accentuated drop in pigment content (Alberte and Thornber 1977, Hendry *et al*. 1987). The result in both cases is a reduction of the light absorption efficiency, which, in turn, may lead to the plant's death (Lee *et al*. 1999, Loreto *et al*. 2004). According to Loreto *et al.* (2004), when the relative water content (RWC) falls below 70%, it usually results in irreversible or slowly reversible damage of the photosynthetic apparatus. Moderate water stress (RWC >70%), which is observed more often in nature (Loreto *et al*. 2004), may also decrease light absorption due to variations in the foliar optical properties such as those resulting from a reduction of tissue thickness (Woolley 1971). Such a decrease, in turn, can result in changes in the foliar spectral reflectance profiles (Carter 1991). Several studies (Carter and Knapp 2001, Zhao *et al*. 2003) have examined the close relationship between plant physiological parameters and spectral responses (reflectance, transmittance and absorptance). However, despite the spectrophotometric measurements and observational experiments being employed by scientists to study physiological responses of plants under different abiotic stress factors (Carter and McCain 1993, Blackburn 2007), the biophysical and biochemical processes mediating this interdependence at the cellular and tissue levels also remain to be fully unravelled (Evans *et al*. 2004).

Currently, computer simulations, or *in silico* experiments, are being increasingly employed to predict the behaviour of plants under different environmental conditions (Hammer *et al*. 2004). These 'virtual' experiments are usually used to complement traditional 'wet' procedures and are particularly useful when the latter have an invasive nature that may alter the properties of the specimens being investigated. The key element in this context is predictability, that is, a computer model can be used to accelerate the hypothesis generation and validation cycles of research as long as it can provide predictable outputs (Ventura *et al*. 2006). Following this trend, different computer models have been developed to simulate light interactions with plant leaves. Although a review of these models is beyond the scope of this work, the interested reader is referred to the taxonomies presented by Jacquemoud and Ustin (2001) and Baranoski and Rokne (2004). A number of these models have been applied to the study of photosynthesis (Ustin *et al*. 2001) and to the assessment of leaf water content (Ceccato *et al*. 2001). We remark, however, that these studies were primarily directed towards bifacial leaves, which are typical of dicotyledonous plants.

Important monocotyledonous (C_4) plants, notably maize (*Zea mays* L.; corn) and sugarcane (*Saccharum officinarum*), have unifacial leaves. These plants constitute major food supplies for human and animal populations in many regions across the planet. In addition, a substantial amount of raw materials used in biofuel production pipelines comes from these C_4 crops. Due to their increasing worldwide demand, major scientific initiatives are needed to assess and improve their productivity. These initiatives include the acquisition of satellite and ground-based data, which are being widely used in conjunction with modelled data to monitor C_4 crops and their water requirements (Budde *et al*. 2010). Besides their influence on the crops' productivity, these water requirements also have a significant ecological impact. For example, it has been recognized that irrigating C_4 crops for biofuel production on a large scale would have serious environmental implications in certain regions since it would require a large increase in the amount of fresh water withdrawn from aquifers, lakes and rivers (Schneider 2010). For these reasons, a myriad of studies are being conducted to mitigate the effects of adverse environmental conditions on the irrigation needs of C_4 crops and, ultimately, on their yield (Earl and Davis 2003, Chenu *et al*. 2008).

In this context, predictive light transport models can effectively contribute to the early and remote detection of abiotic stress factors that can reduce the photosynthetic efficiency of C_4 plants. Furthermore, the current understanding about their tolerance to less favourable environmental conditions and their capacity to grow in hot climates with sporadic rainfall (Furbank and Taylor 1995) is still limited. Hence, these computational tools can also be used to assist the investigation of open questions involving their physiological responses to changes in water soil levels and the effects of limited water availability on their growing process (Li-Ping *et al*. 2006). From an agricultural and ecological point of view, one of the key benefits of employing such 'virtual' experiments is that they can be performed rapidly to assess the trade-offs *in silico* of different means for increasing water use efficiency of these crops given a set of possible environmental conditions (Minorsky 2003).

The light transport model known as the ABM-U (algorithmic bidirectional scattering distribution function (BDF) for unifacial leaves) was specifically designed to simulate light interactions with unifacial leaves of monocotyledonous plants in the infrared domain (Baranoski 2006). In this article, which is an extended and updated version of a preliminary conference presentation (Baranoski 2009), we investigate its applicability in the visible (photosynthetic) domain, notably with respect to the assessment of spectral variations triggered by abiotic stress factors. As mentioned by Carter and Knapp (2001), this is the region of the light spectrum where leaf reflectance is altered by abiotic stress factors more consistently.

We remark that the effective monitoring of C_4 crops requires the detection of spectral changes under *in vivo* conditions, especially with respect to water requirements (Thenkabail *et al*. 2009). Yet, the spectral data used in studies involving the reduction of foliar water content, particularly in the more conspicuous moderate (RWC $>70\%$) range (Loreto *et al*. 2004), is normally obtained under *in vitro* conditions. In these situations, the specimens (leaves) are usually detached from a living maize plant and air dried (Thomas *et al*. 1971, Carter 1991). Since in both cases the loss of water affects the internal arrangement of the foliar tissues, one might expect that *in vivo* and *in vitro* water reduction procedures would result in similar spectral changes. However, experiments performed by Maracci *et al.* (1991) under *in vivo* conditions (by withholding water from the soil) and later examined by Verdebout *et al.* (1994) suggest otherwise. In order to further investigate these phenomena, we performed simulations to reproduce the results from both sets of conditions. These simulations not only provide further evidence of the predictive capabilities of the ABM-U in the visible domain, but also suggest that the explanation for the apparent spectral discrepancy resulting from *in vivo* and *in vitro* water reduction procedures may lay on light detour effects associated with the adaptive mechanisms of C_4 plants to abiotic stress factors.

2. Methodology

Six sets of *in silico* experiments were carried out during this investigation. In the first set, we used the ABM-U to compute the directional–hemispherical reflectance and transmittance profiles of turgid maize leaves. These profiles were compared to measured data in order to evaluate the model's accuracy in the visible domain. In the second and third sets, we performed simulations to assess the model's predictive capabilities with respect to variations in the illumination geometry and the effects of nutrient deficiency, respectively. We qualitatively compared the results of these simulations to experimental results reported in the literature. The fourth and fifth sets of *in silico* experiments focused on spectral responses triggered by moderate water stress processes in which foliar pigment contents are not affected. More specifically, we used the ABM-U to qualitatively reproduce apparently conflicting spectral responses obtained in experiments involving maize specimens under *in vitro* and *in vivo* moderate water content reduction procedures (Thomas *et al*. 1971, Woolley 1971, Maracci *et al*. 1991, Verdebout *et al*. 1994). The results of these two *in silico* sets of experiments were employed to demonstrate the model's predictive capabilities with respect to spectral changes triggered by water stress. Moreover, these results also suggested that the decrease in the amount of light reflected by the *in vivo* water-stressed specimens may result from an intensification of light detour effects in the mesophyll tissue. In the sixth set of *in silico* experiments, we computed bihemispherical absorptance curves for the specimens under *in vitro* and *in vivo* moderate water content reduction procedures in order to increase our scope of observations.

2.1 *Simulation framework*

The *in silico* experiments described in this article were performed using a light transport model, the ABM-U, whose formulation is based on standard Monte Carlo methods. Although this model has been previously described by Baranoski (2006), we provide a summary of its main characteristics and formulation in this section to facilitate the reproduction of the modelled results presented in this work. In the ABM-U, radiation propagation is simulated as a random walk process whose states correspond to the main tissue interfaces found in unifacial leaves. The transition probabilities of

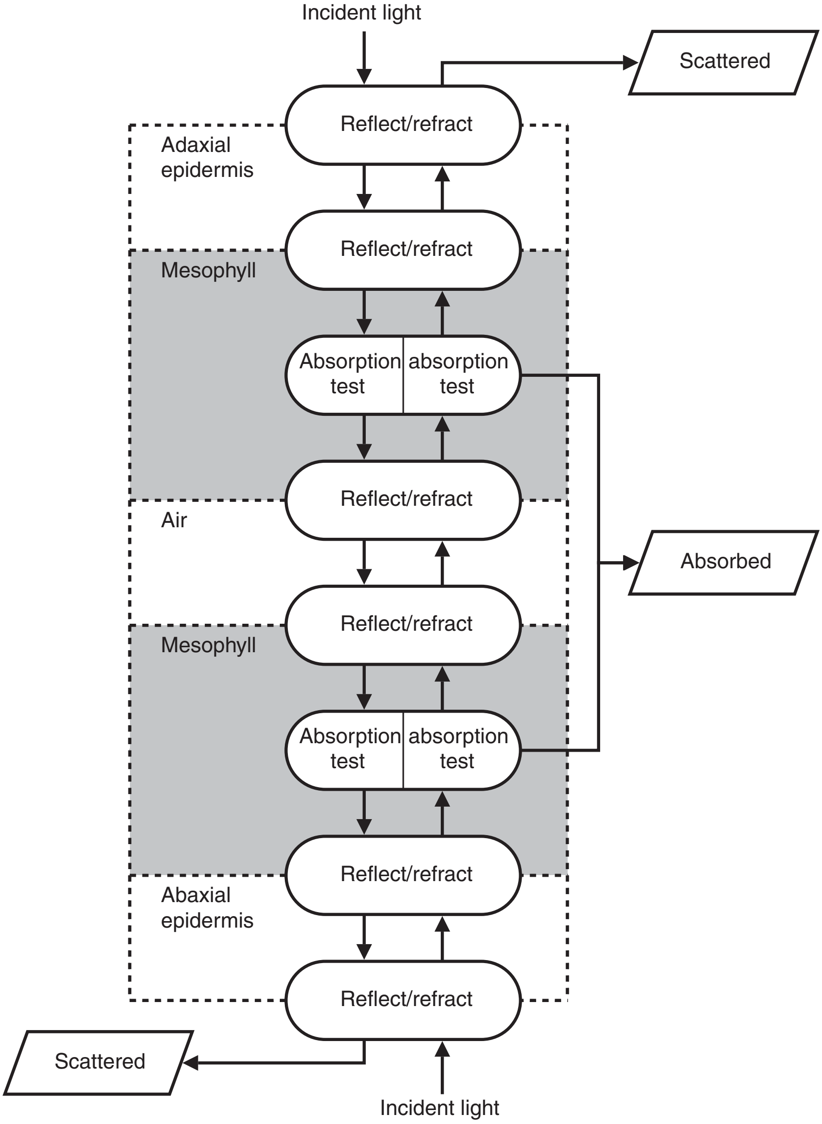


Figure 1. Flowchart depicting the light transport through a unifacial plant leaf as simulated by the ABM-U.

this random walk are associated with the Fresnel coefficients (Meyer-Arendt 1984) computed at each interface, and the termination probabilities are associated with the free path length of the ray travelling in the mesophyll tissue. Figure 1 presents a flowchart depicting the different stages of the random walk process modelled by the ABM-U.

Once a ray enters the mesophyll tissue, it may be propagated or absorbed. The absorption testing performed by the ABM-U is based on the expression used by Allen *et al.* (1969) to compute the transmissivity of a medium according to a variation of Beer's law. The stochastic interpretation of this expression (Prahl 1988) indicates that the probability of absorption of a photon (ray) travelling a distance Δp at a certain wavelength λ in the medium is given by

$$P(\lambda) = 1 - \mathrm{e}^{-\mu_a(\lambda)\Delta p \sec(\theta)}, \tag{1}$$

where $\mu_a(\lambda)$ = effective absorption coefficient of the medium (cm^{-1}), θ = adjusted propagation angle between the ray direction and the medium's normal direction.

The effective absorption coefficient is obtained by adding the absorption coefficients of the medium's constituent materials, which, in turn, are obtained by multiplying the specific absorption coefficient (SAC) of each material (given in $\mathrm{cm}^2\ \mathrm{g}^{-1}$) by its concentration in the medium (given in $\mathrm{g\ cm}^{-3}$). The absorption coefficient of water corresponds simply to its SAC (given in cm^{-1}).

It is important to note that as the angular distribution of light transmitted through plant leaves increases, the probability of light absorption also increases due to the detour effect (Terashima and Saeki 1983, Vogelmann 1993). However, it is also necessary to consider that a non-homogeneous distribution of pigments (under normal conditions, the mesophyll chloroplasts usually remain arrayed along the cell walls (Rabinowitch 1951, Evans *et al*. 2004)) can reduce the probability of light absorption (Björn 1992). In this situation, light can be propagated without encountering the pigment-containing organelles, a phenomenon known as the sieve effect (Fukshansky 1981, Evans *et al*. 2004). Hence, simulations of light transport within foliar tissues need to account for the inverse dependence of detour and sieve effects on the distribution of these absorbers (Vogelmann 1993), and for the correlation of this relationship with respect to the angular deviations of light travelling in the mesophyll tissue (Baranoski and Eng 2007).

When a ray penetrates the mesophyll, its direction of propagation is also probabilistically perturbed to account for tissue structures much larger than the wavelength of light (Baranoski 2006). This perturbation results in an angle of penetration θ_s between the medium's normal direction and the ray's altered trajectory. Note that regardless of the magnitude of θ_s, it is assumed that it is probabilistically possible that the ray goes through the mesophyll tissue without triggering absorption events. This assumption is consistent with the general theory of sieve effect (Fukshansky 1981, 1987). Furthermore, experiments by Seyfried *et al.* (1983) indicated that although the geometrical path length is small for small angles of penetration, the optical differential path length (associated with detour effects) is large. Hence, in order to account for the inverse angular relationship of the sieve and detour effects (Baranoski and Eng 2007), the ABM-U adjusts the ray propagation angle, which is then used to compute the probability of light absorption (equation (1)). More specifically, the adjusted propagation angle θ (given in radians) varies according to the cosine of θ_s, that is, the lower the angle of penetration θ_s, the higher the angle θ, and consequently the higher the probability of absorption (equation (1)). In this case, θ ranges from zero to one radian. This bound was derived from applied optics experiments performed by Okada *et al.* (1996) to determine the optical path length of collimated light interacting with samples whose absorptive and scattering properties are similar to those attributed to plant tissue constituents.

By inverting equation (1), the following expression used to estimate the free path length of a slant ray travelling in the mesophyll tissue is obtained:

$$p(\lambda) = -\frac{1}{\mu_a(\lambda)} \ln(\xi_r)\cos(\theta), \tag{2}$$

where ξ_r = uniformly distributed random number ∈ [0,1].

For each ray incident on an unifacial leaf, we generate a random number ϱ uniformly distributed in the interval [0,1], which we multiply by the thickness of the mesophyll tissue (t_{mu}) to probabilistically determine the location of an internal air space. If the ray is travelling in the portion of the leaf above this internal air space (figure 1), then we compare the $p(\lambda)$ with ϱt_{mu}, otherwise we compare it with $(1 - \varrho)t_{mu}$. If $p(\lambda)$ is greater than the adjusted t_{mu}, then the ray is propagated, otherwise it is absorbed. Based on the morphological characteristics of unifacial leaves (Vogelmann and Martin 1993, Bowes 1996), the parameter t_{mu} is estimated to correspond to approximately 80% of their total thickness.

2.2 *Specimens' characterization data*

The results of the simulations performed to evaluate the predictability of the ABM-U with respect to the interactions of visible light with unifacial leaves were quantitatively compared to measured data available in the LOPEX database (Hosgood *et al.* 1995). The LOPEX project involved leaf optical experiments performed on 120 leaf samples representative of more than 50 species. These experiments included spectral curves of directional–hemispherical reflectance and directional–hemispherical transmittance as well as auxiliary measurements of pigment concentrations, thickness and water content for each specimen.

The LOPEX biochemical data, namely the contents of the main leaf absorbers in the visible range, that characterize the fresh (turgid) specimens used as reference (control) in our simulations are presented in table 1. For each collected sample, the LOPEX database includes spectral measurements and auxiliary measurements performed at different areas on the leaf surface in order to quantify small local variations. We have associated these different groups of measurements to different testing specimens, which are identified by a letter and a subscript. Testing specimens whose characterization and spectral data were obtained from the same LOPEX leaf sample are identified by the same letter (tables 2 and 3).

The auxiliary measurements involving thickness, fresh weight and dry weight were performed for each sample considering an area of 4.1 cm^2. We remark that these

Table 1. Biochemical data measured for plants A (LOPEX sample 12) and B (LOPEX sample 16).

Plant	C_a (mg m^{-1})	C_b (mg m^{-1})	C_c (mg m^{-1})	C_p (%)	C_{cl} (%)
A	2.90	0.80	0.66	26.55	29.63
B	3.16	1.11	0.84	24.09	28.64

Note: Concentration of chlorophyll-*a* (C_a), chlorophyll-*b* (C_b) and carotenoids (C_c) are given as content per fresh weight. Protein (C_p) and cellulose+lignin (C_{cl}) contents are given in terms of percentage of dry weight.
Source: LOPEX (Hosgood *et al.* 1995).

Table 2. Thickness and weight measurements for specimens A1 and A2 (LOPEX sample 12) and specimens B1 and B2 (LOPEX sample 16).

Specimen	Thickness (cm)	Fresh weight (g)	Dry weight (g)
A1	0.0204	0.0668	0.0146
A2	0.0186	0.0688	0.0170
B1	0.0224	0.0796	0.0203
B2	0.0156	0.0595	0.0162

Source: LOPEX (Hosgood *et al*. 1995).

Table 3. Root mean square errors (RMSE) of modelled directional–hemispherical reflectance and directional–hemispherical transmittance curves obtained for specimens A1, A2, B1 and B2.

Specimen	LOPEX spectral file	Spectral quantity	RMSE
A1	0141	Reflectance	0.0080
A1	0142	Transmittance	0.0209
A2	0147	Reflectance	0.0122
A2	0148	Transmittance	0.0112
B1	0141	Reflectance	0.0139
B1	0142	Transmittance	0.0079
B2	0147	Reflectance	0.0103
B2	0148	Transmittance	0.0170

Note: The RMSEs were computed using as reference the directional–hemispherical reflectance and directional–hemispherical transmittance curves (LOPEX spectral files) measured for these specimens.
Source: LOPEX (Hosgood *et al*. 1995).

parameters are used to compute the concentration of the absorbers in terms of g cm^{-3} since in the LOPEX database their contents are given in terms of either *mg* per fresh weight (in the case of chlorophylls and carotenoids) or as percentage of dry weight (in the case of cellulose+lignin and protein).

The upper bound values selected for the cuticle undulations (δ_c), epidermis cell caps (δ_e) and mesophyll cell caps (δ_m) used to perturb the rays interacting with the foliar tissues (Baranoski 2006) were 10, 5 and 5, respectively. These values were derived from data available in the literature (Bone *et al*. 1985, Martin *et al*. 1991, Vogelmann and Martin 1993, Govaerts *et al*. 1996) and also borne out by observations of cross sections of maize leaves (Breece and Holmes 1971). We also note that, to the best of our knowledge, there is no information about actual *in situ* measurements of these parameters available in the literature.

Besides the samples' biochemical and structural parameters, other essential biophysical parameters, such as SACs and refractive indices, were also accounted for in our *in silico* experiments. More specifically, we consider the SAC of the main absorbers present in the foliar tissues, namely pigments (figure 2(*a*)), water (figure 2(*b*)), cellulose+lignin and protein. Although cellulose+lignin and protein have only a minor influence on the absorption of visible light within foliar tissues, we incorporated their SACs (4.38 and 9.96 cm^2 g^{-1}, respectively) in our simulations for completeness. These values were derived from measured data available in the literature (Jacquemoud *et al*. 1996).

The refractive indices used to compute the Fresnel coefficients in our simulations correspond to the main tissue interfaces considered in the ABM-U (figure 1). Usually

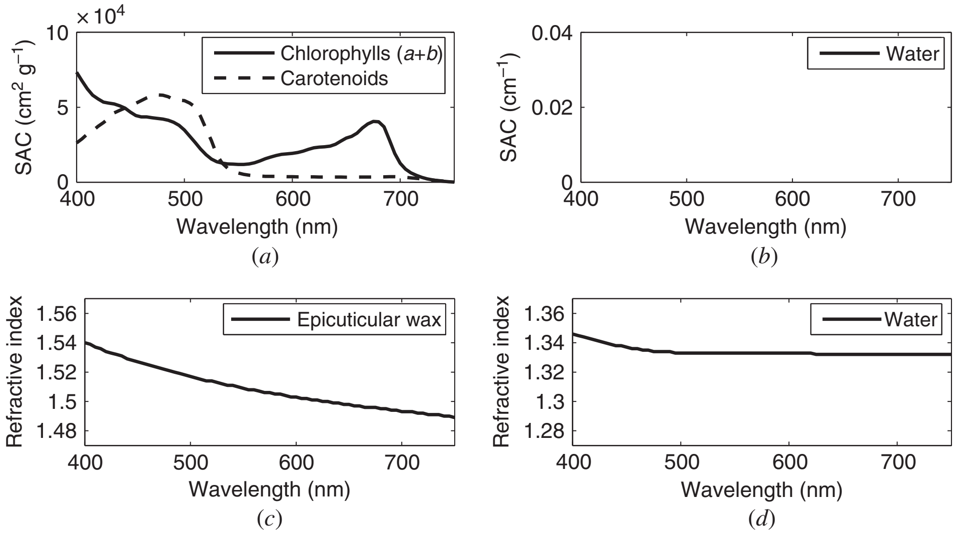


Figure 2. Biophysical data used in the simulations. (*a*) Specific absorption coefficient (SAC) of chlorophylls (*a*+*b*) provided by Jacquemoud *et al.* (1996) and carotenoids (β-carotene) provided by Eng and Baranoski (2007). (*b*) SAC of water provided by Pope and Fry (1997). (*c*) Refractive index of epicuticular wax provided by Vanderbilt and Grant (1985). (*d*) Refractive index of water provided by Palmer and Williams (1974).

the refractive indices available in the literature correspond to either values averaged across the entire electromagnetic spectrum or values measured at a specific wavelength. Despite this practical constraint, we believe that it is worthwhile to make an effort to use spectral refractive indices whenever they are available. For example, the refractive index of the cutinized epidermal cell wall used in our simulations corresponds to the refractive index of the epicuticular wax (figure 2(*c*)). This choice is based on observations that the greatest discontinuity between refractive indices for foliar tissues is across the air–wax boundary (Vanderbilt and Grant 1985, Vanderbilt *et al*. 1991).

According to several studies (Nátr 1992, Zhao *et al*. 2003), all nutrient deficient plants contain less photosynthetic pigments than control plants. Furthermore, the majority of nutrient stress symptoms have a similar effect on foliar spectral responses (Masoni *et al*. 1996, Jørgensen 2002). Hence, for the sake of conciseness, our simulations with respect to nutrient stress involved only nitrogen deficiency. This choice was motivated by two reasons. First, nitrogen is arguably the most important nutrient for growing crops and maintaining a healthy environment (Lee *et al*. 1999). While insufficient nitrogen supply reduces crop yield, excessive application of nitrogen fertilizer usually reduces water quality (Zhao *et al*. 2003). Second, the effects of mineral nutrients on crop yield and their connection with plant water stress have been examined in the literature mainly through studies involving nitrogen-deficient plants (Buckley *et al*. 2002). As stated by Carter and Knapp (2001), this type of stress can be closely simulated by varying the pigment concentration of modelled leaves. Accordingly, we changed the pigment concentrations of our modelled control specimens to obtain the radiometric quantities for the modelled nitrogen-stressed specimens. Since the results of our simulations involving nitrogen deficiency were qualitatively compared with measured data provided by Al-Abbas *et al.* (1974), we applied the same relative change of pigment concentration observed in their experiments, namely a 82% reduction on average in comparison with the control specimen, which was accompanied by negligible variations in the thickness and water content. In order to increase our scope of observations, we also performed simulations considering a 50% nitrogen reduction.

The modelled radiometric quantities for the water-stressed specimens were obtained considering a 25% water reduction and keeping the pigment contents constant. These conditions were selected based on the experimental conditions reported in the experiments by Maracci *et al.* (1991) involving maize leaves under moderate water stress. We also selected this 25% amount to facilitate the comparison of our modelled results with measured data provided by other researchers (Thomas *et al*. 1971, Woolley 1971, Carter 1991). Measurements performed by Woolley (1973) on maize leaves indicate that such a reduction is followed by approximately a 20% reduction in thickness and a 2% reduction in area. We reduced the thickness and area of our turgid (control) specimens accordingly. We also performed a 25% reduction on the fresh weight and a 20% increase in the aspect ratio of its mesophyll cell caps to account for the resulting flattening of these cells (Woolley 1971, Baranoski 2006). Finally, using refractive indices of fully hydrated and dried mesophyll cell walls, 1.415 and 1.53, respectively (Woolley 1975), and the refractive index of water (figure 2(*d*)), we applied the law of Gladstone and Dale (Tuchin 2000, Baranoski 2006) to obtain the spectral refractive indices of mesophyll cell walls before and after the water content reduction.

2.3 *Alignment of measurement and simulation conditions*

All radiometric quantities reported and discussed in this article correspond to readings obtained considering light incident on the adaxial surface of the specimens, and angles of incidence measured with respect to the specimens' normal (zenith). The modelled radiometric quantities were obtained using a virtual spectrophotometer (Baranoski and Rokne 2004).

Although the light transport model used in our simulations provides bidirectional readings (Baranoski 2006), directional–hemispherical quantities can be obtained by integrating the outgoing light (rays) with respect to the outgoing (collection) hemisphere (Nicodemus *et al*. 1992). Similarly, bihemispherical quantities can be calculated by integrating the BDF values with respect to incident and collection hemispheres (Nicodemus *et al*. 1992).

As mentioned earlier, the quantitative comparisons performed to assess the predictability of the ABM-U in the visible domain employed spectral measurements available in the LOPEX database, which were obtained considering an angle of incidence 8°. We used the same angle of incidence to obtain the modelled curves used in these comparisons.

In the case of our simulations involving nutrient (nitrogen) stress effects, we performed qualitative comparisons of modelled results with experimental results provided by Al-Abbas *et al.* (1974). These experimental results were obtained using a Beckman model DK–2A spectrophotometer. In this device, the light beam is incident at an angle close to the specimen's normal (zenith) for most materials (usually about 5° for foliar specimens (Gausman *et al*. 1974)), and the reflected light is scattered into an integrating sphere to provide directional–hemispherical readings. Hence, for consistency with the experimental data used in these comparisons, we considered an angle of 5° to obtain the corresponding modelled curves.

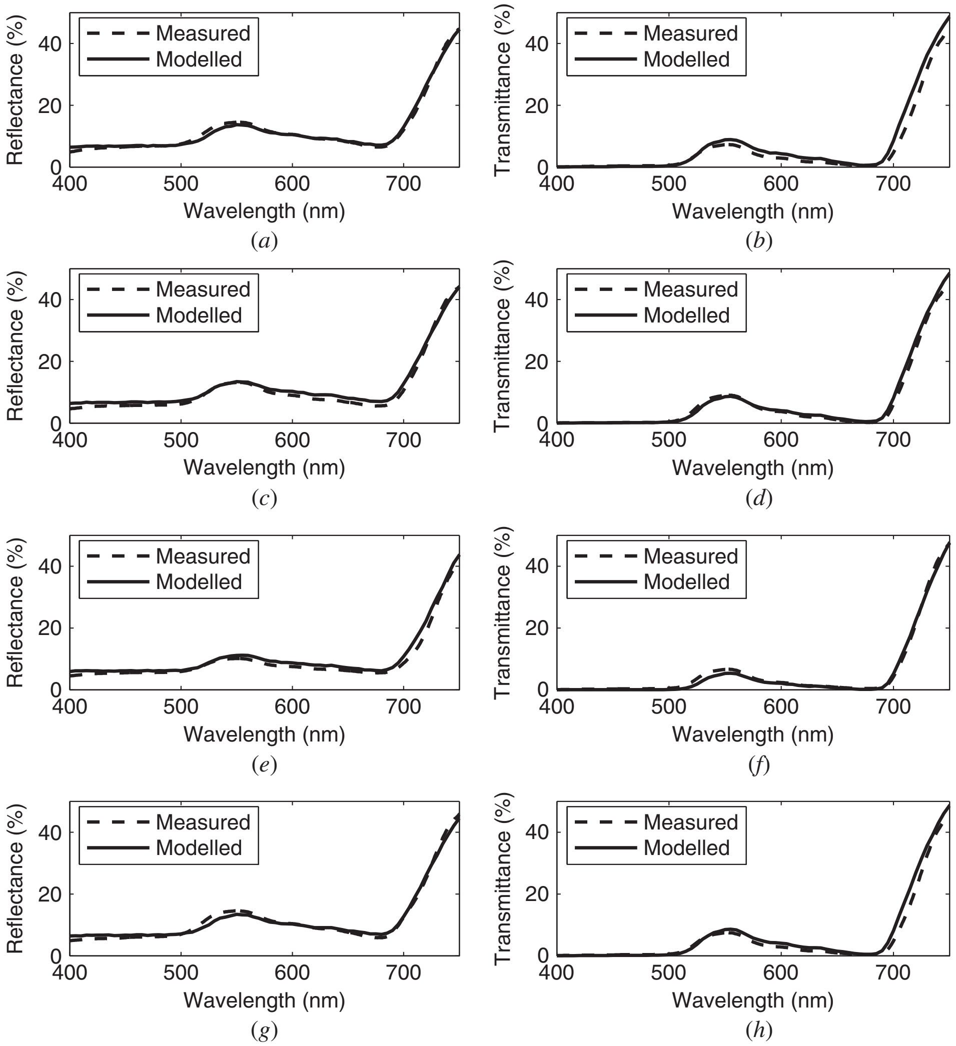
In the case of our simulations involving water stress effects, we qualitatively compared the modelled results with experimental results provided by Woolley (1971), Thomas *et al.* (1971) and Maracci *et al.* (1991). The measured data obtained by Woolley (1971) correspond to directional–hemispherical reflectance values measured considering an angle of incidence of 2.5° from the specimen's normal. Thomas *et al.* (1971) also indicated the use of a Beckman model DK–2A spectrophotometer in their measurements, that is, an angle of incidence around 5°. The measured data provided by Maracci *et al.* (1991) correspond to directional–hemispherical measurements (Hosgood, personal communication, 2008), which were performed considering an angle of incidence around 15°. Since the differences observed among the modelled curves obtained in this angular domain between 2.5° and 15° were negligible, we report the results of our *in silico* experiments involving water-stressed specimens with respect to the same angle of incidence (8°) used to evaluate the accuracy of our simulation results.

3. Results and discussion

In this section, we present the results of the six sets of *in silico* experiments carried out during this investigation. We also examine the practical and theoretical implications of our findings, especially for the detection and study of stress-related changes in the photosynthetic apparatus of C_4 plants.

Initially, modelled directional–hemispherical reflectance and transmittance curves were compared with measured directional–hemispherical reflectance and transmittance curves available in the LOPEX database. The results of these comparisons are presented in figure 3, and they show that the shapes of the modelled curves closely mimic the shapes of their measured counterparts. They also indicate a good quantitative agreement between the modelled and measured curves, specially considering that certain biochemical and biophysical input parameters, such as pigment contents and refractive indices, correspond to averaged data. It is worth noting, however, that the visual proximity between the measured and modelled curves may vary according to the scale of the graphs. For this reason, we also computed the root mean square errors (RMSE) for these curves. The RMSE values obtained for the modelled reflectance and transmittance curves with respect to the measured curves are presented in table 3, and they are all smaller than 0.03, the empirical value usually associated with good spectral reconstruction in the field of remote sensing of vegetation (Jacquemoud *et al*. 1996). According to measured data available in the literature (Walter-Shea *et al*. 1989), the spectral reflectance and transmittance profiles of maize leaves have an inverse dependence on the angle of incidence of the incoming light, that is, an increase on the angle of incidence results in an increase in the reflectance and in a decrease in the transmittance. The modelled spectral curves presented in figure 4, which were obtained in our second set of *in silico* experiments, demonstrate that the ABM-U appropriately simulates this angular (directional) dependency.

The next step of our investigation involved *in silico* experiments to verify the predictability of the proposed simulation framework with respect to changes in the light reflection and transmission profiles of maize leaves due to nitrogen deficiency. In these simulations, we considered nitrogen-deficient and control specimens whose characterization data are provided in §2.2. The simulation results are presented in figure 5, and they clearly depict the changes observed in the actual experiments performed by Al-Abbas *et al.* (1974). More specifically, nitrogen-deficient maize leaves are characterized by higher reflectance and transmittance with respect to control specimens. Furthermore, since this change occurs due to a reduction of photosynthetic pigments,

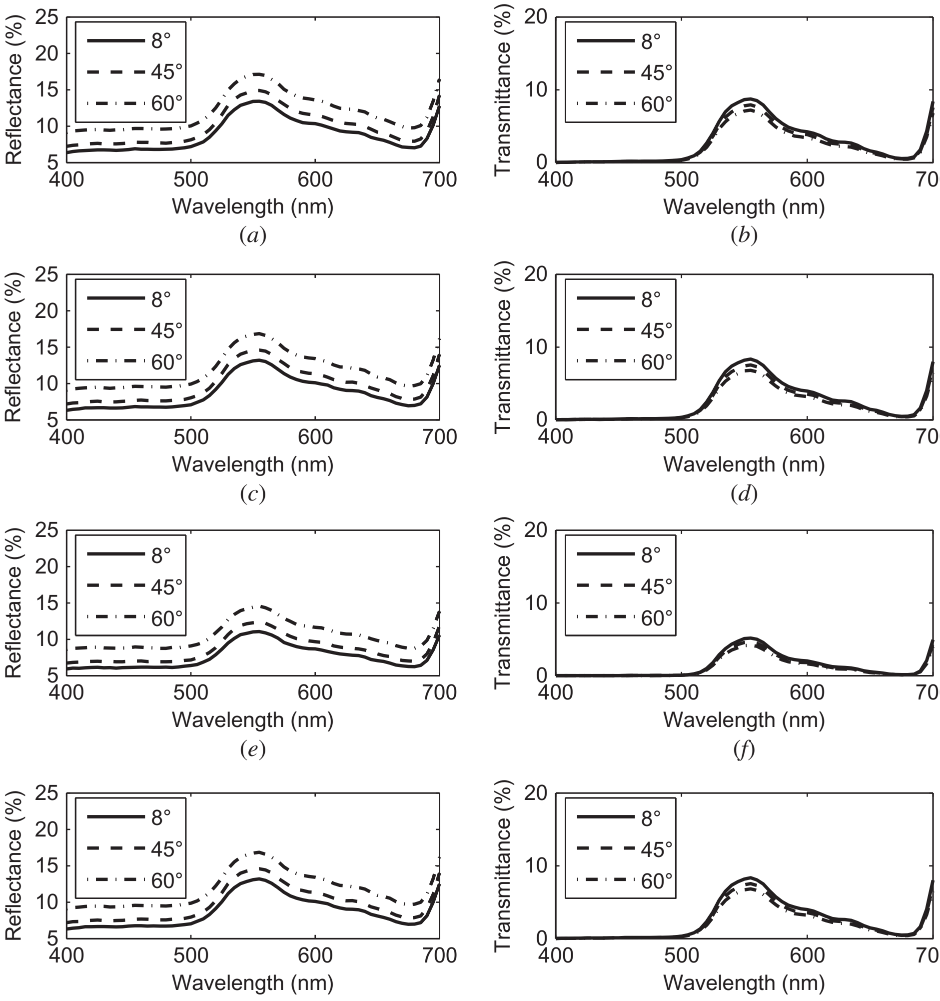


(*a*) (*b*) (*c*) (*d*) (*e*) (*f*) (*g*) (*h*)

Figure 3. Measured and modelled directional–hemispherical reflectance and transmittance curves for maize leaves. The curves were obtained considering an angle of incidence of 8°. (*a*) Reflectance curves for specimen A1. (*b*) Transmittance curves for specimen A1. (*c*) Reflectance curves for specimen A2. (*d*) Transmittance curves for specimen A2. (*e*) Transmittance curves for specimen B1. (*f*) Reflectance curves for specimen B1. (*g*) Reflectance curves for specimen B2. (*h*) Transmittance curves for specimen B2.

it is more pronounced in the region around 550 nm. This behaviour was also observed in more recent experiments by Zhao *et al.* (2003).

Our investigation then proceeded with simulations to qualitatively reproduce reflectance changes observed in specimens under an *in vitro* moderate water content reduction procedure such as air drying (Thomas *et al*. 1971). The results of these simulations, which took into account structural and biophysical changes due to the water content reduction (described in §2.2), are presented in figure 6. The modelled curves show a higher reflectance for the air-dried specimen in comparison with the turgid



(*a*) (*b*) (*c*) (*d*) (*e*) (*f*) (*g*) (*h*)

Figure 4. Modelled directional–hemispherical reflectance and directional–hemispherical transmittance curves for maize leaves illustrating the directional dependency of their spectral profiles. The curves were obtained considering three angles of incidence: 8°, 45° and 60°. (*a*) Reflectance curves for specimen A1. (*b*) Transmittance curves for specimen A1. (*c*) Reflectance curves for specimen A2. (*d*) Transmittance curves for specimen A2. (*e*) Reflectance curves for specimen B1. (*f*) Transmittance curves for specimen B1. (*g*) Reflectance curves for specimen B2. (*h*) Transmittance curves for specimen B2.

(control) specimen. This result is qualitatively consistent with the reflectance increase observed by Thomas *et al.* (1971) on air-dried maize leaves whose water content was reduced by a similar amount (∼25%). Furthermore, it can be observed in the modelled curves depicted in figure 6 that the reflectance increase is more pronounced in the region where the photosynthetic pigments have a lower absorptive capacity (centred at ∼550 nm). This feature is noticeable in the actual measurements performed by Thomas *et al.* (1971). It is also consistent with the measured data obtained by Woolley (1971) for maize leaves and for measured data obtained by Carter (1991) for another

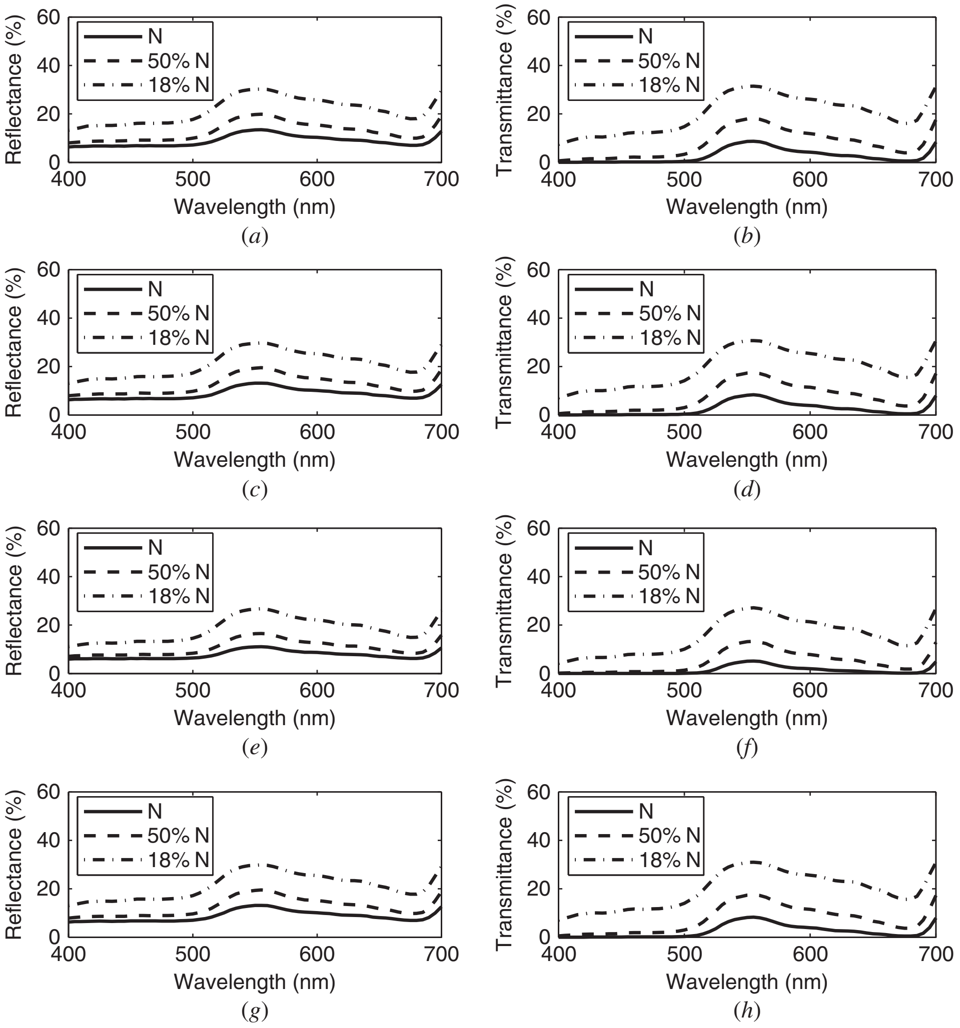
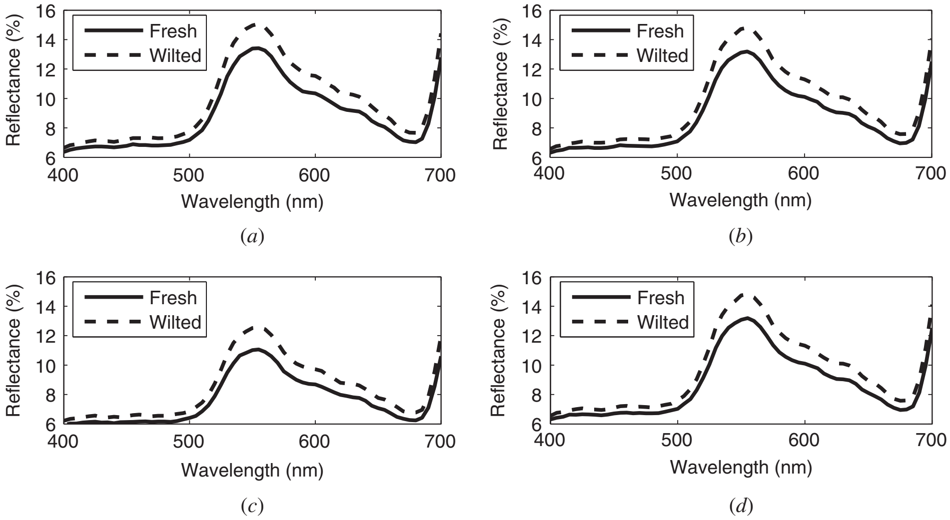


Figure 5. Modelled directional–hemispherical reflectance and directional–hemispherical transmittance curves for maize leaves under different nitrogen treatments: N (normal), 50% N and 18% N. The curves were obtained considering an angle of incidence of 5°. (*a*) Reflectance curves for specimen A1. (*b*) Transmittance curves for specimen A1. (*c*) Reflectance curves for specimen A2. (*d*) Transmittance curves for specimen A2. (*e*) Reflectance curves for specimen B1. (*f*) Transmittance curves for specimen B1. (*g*) Reflectance curves for specimen B2. (*h*) Transmittance curves for specimen B2.

monocotyledonous C_4 species, namely switch cane (*Arundinaria tecta*). In both cases, the leaves' water content was reduced by a similar amount through an *in vitro* water reduction procedure. It is worth noting that under *in vitro* conditions the cell walls may lose their turgor more abruptly resulting in more light diffusion within the foliar tissues, which, in turn, may lead to even higher reflectance values, specially outside the visible domain (Woolley 1971, Carter 1991).

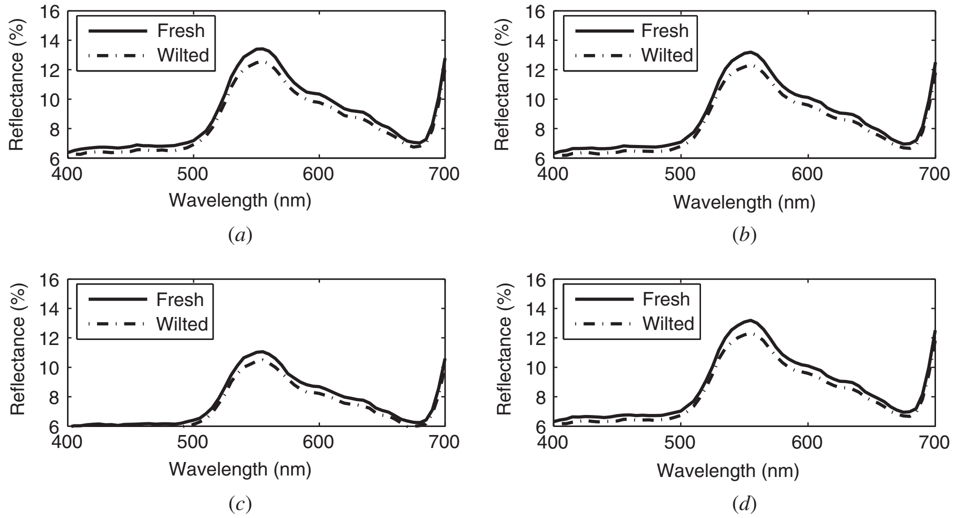
Thomas *et al.* (1971) observed that leaves of plants under water stress may appear darker green than leaves of plants not stressed (control) because they reflect and



(a) (b) (c) (d)

Figure 6. Modelled directional–hemispherical reflectance curves for fresh and wilted maize leaves. The curves for the wilted specimens correspond to leaves whose water content was reduced (25%) using the *in vitro* procedure (through air drying). The curves were obtained considering an angle of incidence of 8°. (*a*) Reflectance curves for specimen A1. (*b*) Reflectance curves for specimen A2. (*c*) Reflectance curves for specimen B1. (*d*) Reflectance curves for specimen B2.

transmit light differently. They also stated that, although the influence of water is often masked by leaf pigment content, water stress may decrease reflectance. This behaviour was observed in the measurements performed by Maracci *et al.* (1991), which were later examined by Verdebout *et al.* (1994). In these experiments, maize leaves whose water content was moderately reduced using an *in vivo* procedure (by withholding water from the soil) showed a reflectance decrease in the visible region even though their pigment content remained constant. Maracci *et al.* (1991) mentioned, however, the need of further experiments to study this tendency. To test the predictive capability of ABM-U with respect to this phenomenon, we performed another set of simulations. More specifically, the goal was to reproduce the reflectance behaviour of the *in vivo* water-stressed specimen considering the same amount of water reduction (25%). Accordingly, it was used with the same data employed in the simulation of the reflectance behaviour of the *in vitro* air-dried specimen, with the exception of the bound for the angular deviations of the propagated light (discussed in §2.1). This bound was removed based on the observation that reflectance of the *in vivo* water-stressed specimen is lower than the reflectance of the turgid specimen even though the pigment contents stayed constant (Maracci *et al*. 1991). This suggests that the water-stressed specimens' absorption efficiency might be affected by changes not present during the *in vitro* water reduction procedure. A possible change could be an intensification of the detour effects within the mesophyll tissue, which, in turn, could be caused by a more homogeneous intracellular distribution of mesophyll chloroplasts. Such a distribution would decrease the sieve effect in this tissue (Fukshansky 1981, Björn 1992). The results of these simulations are presented in figure 7, and they show a lower overall reflectance for the *in vivo* water-stressed specimen in comparison with the turgid (control) specimen. This reflectance decrease is qualitatively consistent with



(*a*) (*b*)

(*c*) (*d*)

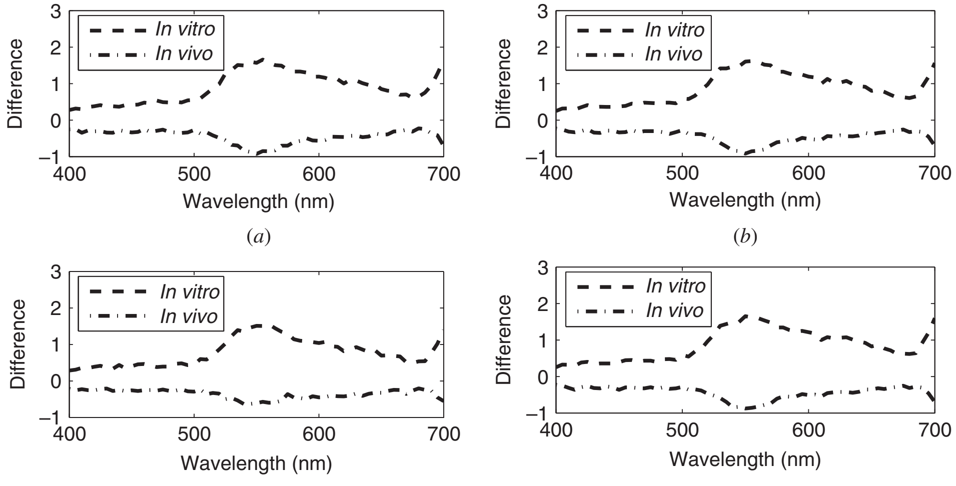
Figure 7. Modelled directional–hemispherical reflectance curves for fresh and wilted maize leaves. The curves for the wilted specimens correspond to leaves whose water content was reduced (25%) using the *in vivo* procedure (by withholding water from the soil). The curves were obtained considering an angle of incidence of 8°. (*a*) Reflectance curves for specimen A1. (*b*) Reflectance curves for specimen A2. (*c*) Reflectance curves for specimen B1. (*d*) Reflectance curves for specimen B2.

the observations made by Maracci *et al.* (1991), and it is more pronounced in the region around 550 nm, a feature also evident in their measurements.

As stated by Carter and Knapp (2001), the differences between the reflectance of stressed and healthy specimens tend to be low in the 400–500 and 670–680 nm regions. As illustrated in figure 8, this trend can be also observed in our simulation results specially with respect to the *in vivo* water-stressed specimens. These small differences are expected since these regions are characterized by strong light absorption by pigments (figure 8). Large amounts of pigment would need to be lost (e.g. following a severe water stress; Alberte and Thornber (1977)) before significant reflectance differences could be detected in these regions (Carter and Knapp 2001). Recall that, in our simulations, we are considering moderate water stress followed by negligible changes in pigment content as observed in the experiments by Maracci *et al.* (1991).

In order to increase our scope of observations, we also compared modelled bihemispherical absorptance curves obtained for the *in vivo* and the *in vitro* water-stressed specimens (figure 9). The former presented a higher magnitude, particularly around 550 nm where the photosynthetic pigments have a lower absorptive capacity. This observation suggests that, if indeed chloroplast displacements occur due to the physiological processes triggered by *in vivo* moderate water stress, they would contribute to an adjustment in the absorption efficiency in this region. This possibility would be consistent with the fact that the detour effects increase absorption and are more pronounced at wavelengths (around 550 nm) where light is weakly absorbed (Garlaschi *et al*. 1989, Vogelmann 1993).

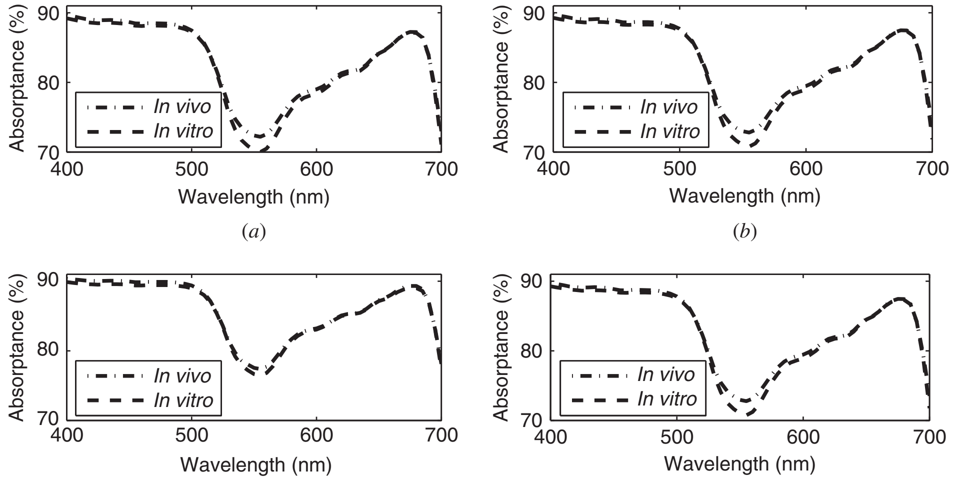
The region of abrupt change in the reflectance spectra of plant leaves between 680 and 800 nm, known as the red edge, results from the combined effects of chlorophyll absorption (low reflectance around 680 nm) and scattering (high reflectance around



(a) (b)

(c) (d)

Figure 8. Differences between the modelled reflectances of *in vitro* and *in vivo* water-stressed maize specimens with respect to the reflectance of the fresh (healthy) specimen. (*a*) Difference curves for specimen A1. (*b*) Difference curves for specimen A2. (*c*) Difference curves for specimen B1. (*d*) Difference curves for specimen B2.

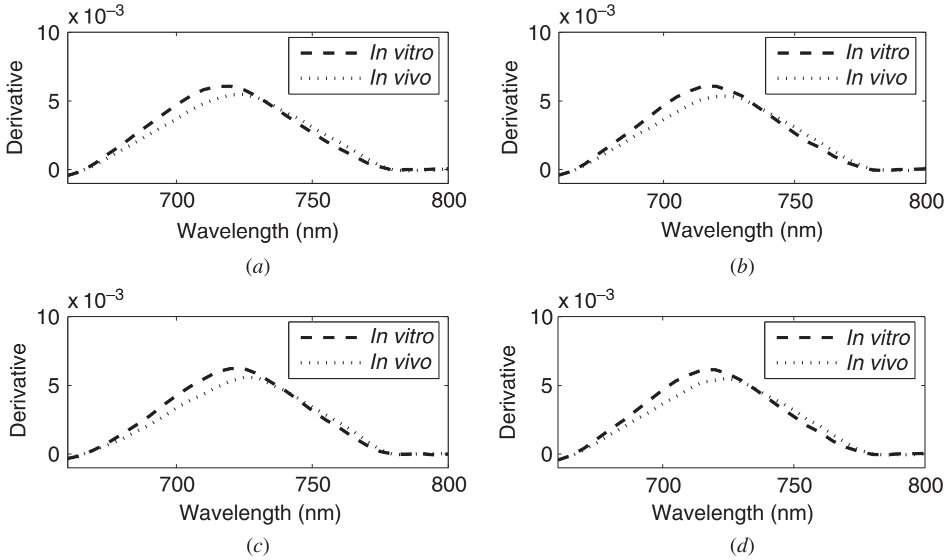


(a) (b)

(c) (d)

Figure 9. Modelled bihemispherical absorptance curves for maize leaves whose water content was reduced (25%) through *in vitro* and *in vivo* procedures. (*a*) Absorptance curves for specimen A1. (*b*) Absorptance curves for specimen A2. (*c*) Absorptance curves for specimen B1. (*d*) Absorptance curves for specimen B2.

800 nm) associated with leaf internal structure and water content (Kumar *et al*. 2001, Baranoski and Rokne 2005). This spectral feature is usually quantified using a parameter that corresponds to the wavelength of the point of maximum slope in the red infrared curve (Kumar *et al*. 2001). The estimation of this parameter, known as the red edge position (REP), can be performed by computing the first derivative of the



(*a*) (*b*)

(*c*) (*d*)

Figure 10. Red edge first derivatives computed for the modelled directional–hemispherical reflectance curves for leaves whose water content was reduced (25%) through *in vitro* and *in vivo* procedures. (*a*) Derivative curves for specimen A1. (*b*) Derivative curves for specimen A2. (*c*) Derivative curves for specimen B1. (*d*) Derivative curves for specimen B2.

leaf reflectance curve (Pu *et al*. 2003). In figure 10, we present the derivatives for the modelled directional–hemispherical reflectance curves obtained in our experiments involving water content reduction procedures. Recall that the chlorophyll contents were kept constant in these experiments. Hence, one would expect only small differences for the REP of the *in vivo* and *in vitro* water-stressed leaves due to their different internal scattering profiles. As expected, such small differences can be observed in the derivative curves presented in figure 10.

The explanation for the apparent spectral discrepancy resulting from moderate *in vivo* (Maracci *et al*. 1991, Verdebout *et al*. 1994) and *in vitro* water reduction procedures (Thomas *et al*. 1971, Woolley 1971, Carter 1991), which was also observed in our *in silico* experiments (figures 6 and 7), is likely to be linked to the adaptive mechanisms of C_4 plants to abiotic stress conditions. Moderate water stress is arguably the most common of these conditions (Loreto *et al*. 2004). Although the decrease in reflectance observed in the *in vivo* water reduction experiments (Maracci *et al*. 1991, Verdebout *et al*. 1994) was relatively small in comparison with the reflectance increase observed in the *in vitro* water reduction experiments (Thomas *et al*. 1971, Woolley 1971, Carter 1991), it is scientifically significant and worth of further investigation due to the economical and ecological importance of C_4 plants and the current scenario of adverse climate changes. According to our simulations, an intensification of light detour effects due to a rearrangement of mesophyll chloroplasts in response to moderate water stress can result in such reflectance decrease. It is important to note that a similar behaviour, that is, a decrease in reflectance in the photosynthetic domain due to *in vivo* water stress, was also reported during reflectance field measurements performed on stressed and unstressed vegetation, namely sunflower (*Helianthus annuus*), by Maracci *et al.* (1991). Maracci *et al.* (1991) also noted that 'the stressed vegetation was still quite green at the time of the measurement although the leaves had wilted'. We remark that Thomas *et al.* (1971) made a similar qualitative observation with respect to water-stressed plants, that is, 'leaves of plants under water stress generally appear darker green than leaves of plants not stressed'. Since both species, maize and sunflower, have the capability of rearranging their chloroplasts in response to certain external stimuli (e.g. light) (Evans *et al*. 2004), it is possible that their adaptive mechanisms may also include chloroplast movements in response to moderate water stress. In the remainder of this section, we concisely examine physiological processes relating this putative rearrangement of chloroplasts to the results of our *in silico* experiments (figures 6 and 7) involving sieve and detour effects caused by the distribution of these organelles.

It has been documented in the literature that the chloroplasts of maize leaves can move in response to light stimuli (Inoue and Shibata 1973, 1974). However, during the *in vivo* water reduction experiments performed by Maracci *et al.* (1991), the illumination conditions were neutral, that is, the control and water-stressed specimens were subjected to the same illumination conditions. Hence, the putative displacement of the maize specimens' chloroplasts cannot be explained by light stimuli. Furthermore, considering that structural changes due to water content reduction were accounted for in both sets of simulations involving the *in vitro* air-dried and the *in vivo* water-stressed specimens, one may assume that they are not responsible for the putatively more homogeneous distribution of the chloroplasts that may occur in *in vivo* water-stressed specimens. Hence, such a distribution might be associated with the *in vivo* nature of the water stress process.

During slowly developing water stress (e.g. when plants sense the reduction of soil water content), water deficit signals are released in the live plant roots and delivered to the leaves (Loreto *et al*. 2004). The main signalling route used in this process is through the abscisic acid (ABA) (Loreto *et al*. 2004, Yokota *et al*. 2006). As ABA is detected, it induces cytosolic Ca^{2+} elevations (Loreto *et al*. 2004). It is important to note that several types of Ca^{2+} channels regulated by membrane-associated and intracellular control mechanisms exist in higher plant cells. The Ca^{2+} channels are essential for the initiation of a large number of other signal transduction processes in these cells (Schroeder *et al*. 2001). For example, experiments by Sato *et al.* (2001) indicate that the influx of external Ca^{2+} through the plasma membrane is required for the early signalling step of chloroplast mechano-relocation movements (but not for photorelocation movements). Considering these observations and the data (measured and modelled) examined in our investigation, we propose the hypothesis that a similar sequence of events may occur in maize leaves under moderate *in vivo* water stress and neutral illumination conditions. More specifically, an elevation of cytosolic Ca^{2+}, induced by water deficit signals, might provide a signalling pathway to trigger the migration of chloroplasts away from the walls of the cells, which, in turn, would result in a more homogeneous distribution of these organelles.

4. Conclusion

We have presented the results of *in silico* experiments involving light interactions with unifacial leaves typical of ubiquitous monocotyledonous (C_4) plants, such as maize and sugarcane, in the visible (photosynthetic) domain. The ray optics based model, ABM-U (Baranoski 2006), used in these experiments employs an algorithmic Monte Carlo formulation which provides a rigorous and yet flexible approach for the simulation of light transport within foliar tissues. The model predictions are consistent with measured data and actual observations reported in the literature, and its parameter space is detailed enough to account for the quantitative differences among the characterization data sets describing different specimens.

When the effects of nutrient and water stress on C_4 crops' physiological parameters are evaluated under *in vivo* conditions, the experimental results may be affected by some unexpected changes in the experimental set up (Masoni *et al*. 1996). For example, as described by Buckley and Farquhar (2004), the foliar transdermal light profile is determined by irradiances at the leaf surface, which can vary independently as the leaf flutters in the wind, as the sun moves through the sky, and as the atmospheric conditions fluctuate. These changes, in turn, may alter the proportions of direct and diffuse light that reach the leaf surface. The proposed *in silico* framework allows the investigation of foliar spectral responses to specific abiotic stress factors while keeping other environmental and experimental conditions, such as illumination and specimen's position, under control.

As stated by Ventura *et al.* (2006), besides allowing the behaviour of a biological system to be simulated under various conditions, a predictive *in silico* experimental framework may also be used to generate new hypotheses, which, in turn, may drive new investigations. This possibility was illustrated by our simulations involving the different effects of *in vitro* and *in vivo* moderate water reduction procedures on the reflectance signature of maize specimens. We proposed the hypothesis that these different effects may be explained by a rearrangement of leaf chloroplasts triggered by water deficit signals. Although an *in situ* investigation of the biochemical mechanisms that might be responsible by this putative rearrangement of chloroplasts is beyond the scope of this work, such an investigation might provide new insights on the adaptive responses of monocotyledonous C_4 plants to unfavourable environmental conditions. Similar studies have been carried out on dicotyledonous plants. For example, Carter and McCain (1993) employed nuclear magnetic resonance microscopy on Norway maple (*Acer platanoides*) leaves under *in vitro* conditions (the leaves were cut from their branches before being dried) to demonstrate the relationship between leaf reflectance and chloroplast water content. However, to the best of our knowledge, no *in situ* verification of these processes has been performed to date on monocotyledonous C_4 plants under *in vivo* conditions.

We remark that traditional intrusive 'wet' laboratory techniques may not be appropriate to monitor the temporal dynamics of the *in vivo* processes mentioned above since these techniques may provoke the mechano-relocation of chloroplasts. Such an *in situ* investigation will likely require a combination of non-invasive time-lapse high resolution imaging methods and *in silico* experiments. We intend to explore this research avenue in our future work.

Acknowledgements

The authors thank the anonymous reviewers for their helpful suggestions, as well as Gregory Carter and Brian Hosgood for providing relevant technical information regarding their own experiments involving the measurement of foliar spectral properties. The authors are also grateful to the Joint Research Centre of the European Commission for granting them access to LOPEX data set. The work presented in this article was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC grant 238337) and the Canada Foundation for Innovation (CFI grant 33418).

References

Al-Abbas, A., Barr, R., Hall, J., Crane, F. and Baumgardner, M., 1974, Spectra of normal and nutrient-deficient maize leaves. *Agronomy Journal*, **66**, pp. 16–20.

Alberte, R. and Thornber, J., 1977, Water stress effects on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplasts of maize. *Plant Physiology*, **59**, pp. 351–353.

Allen, W., Gausman, H., Richardson, A. and Thomas, J., 1969, Interaction of isotropic light with a compact plant leaf. *Journal of the Optical Society of America*, **59**, pp. 1376–1379.

Baranoski, G., 2006, Modeling the interaction of infrared radiation (750 to 2500 nm) with bifacial and unifacial plant leaves. *Remote Sensing of Environment*, **100**, pp. 335–347.

Baranoski, G., 2009, On the predictive modeling of visible light interaction with fresh and environmentally stressed monocotyledonous leaves. In *Proceedings of the International Geoscience and Remote Sensing Symposium – IGARSS'09*, Cape Town, South Africa (New York: Institute of Electrical and Electronic Engineers), pp. 152–155.

Baranoski, G. and Eng, D., 2007, An investigation on sieve and detour effects affecting the interaction of collimated and diffuse infrared radiation (750 to 2500 nm) with plant leaves. *IEEE Transactions on Geoscience and Remote Sensing*, **45**, pp. 2593–2599.

Baranoski, G. and Rokne, J., 2004, *Light Interaction with Plants: A Computer Graphics Perspective* (Chichester: Horwood Publishing).

Baranoski, G. and Rokne, J., 2005, A practical approach for estimating the red edge position of plant leaf reflectance. *International Journal of Remote Sensing*, **26**, pp. 503–521.

Björn, L., 1992, Interception of light by leaves. In *Crop Photosynthesis: Spatial and Temporal Determinants*, N. Baker and H. Thomas (Eds.), pp. 253–276 (Amsterdam: Elsevier Science Publishers B.V.).

Blackburn, G., 2007, Hyperspectral remote sensing of plant pigments. *Journal of Experimental Botany*, **58**, pp. 855–867.

Bone, R., Lee, D. and Norman, J., 1985, Epidermal cells functioning as lenses in leaves of tropical rain-forest shade plants. *Applied Optics*, **24**, pp. 1408–1412.

Bowes, B., 1996, *A Colour Atlas of Plant Structure*, pp. 97–116 (London: Manson Publishing Ltd.).

Breece, H. and Holmes, R., 1971, Bidirectional scattering characteristics of healthy green soybean and corn leaves in vivo. *Applied Optics*, **10**, pp. 119–127.

Buckley, T. and Farquhar, G., 2004, A new analytical model for whole-leaf potential electron transport rate. *Plant, Cell & Environment*, **27**, pp. 1487–1502.

Buckley, T., Miller, J. and Farquhar, G., 2002, The mathematics of linked optimization for water and nitrogen use in canopy. *Silva Fennica*, **36**, pp. 639–689.

Budde, M., Rowland, J. and Funk, C., 2010, Agriculture and food availability remote sensing of agriculture for food security monitoring in the developing world. *IEEE Geoscience and Remote Sensing Newsletter*, **154**, pp. 10–14.

Carter, G., 1991, Primary and secondary effects of water content on the spectral reflectance of leaves. *American Journal of Botany*, **78**, pp. 916–924.

Carter, G. and Knapp, A., 2001, Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *American Journal of Botany*, **88**, pp. 677–684.

Carter, G. and McCain, D., 1993, Relationship of leaf spectral reflectance to chloroplast water content determined using NMR microscopy. *Remote Sensing of Environment*, **46**, pp. 305–310.

Ceccato, P., Flasse, S., Tarantola, S., Jacquemoud, S. and Grégoire, J., 2001, Detecting vegetation leaf water content using reflectance in the optical domain. *Remote Sensing of Environment*, **77**, pp. 22–33.

Chenu, K., Chapman, S., Hammer, G., McLean, G., Salah, H. and Tardieu, F., 2008, Short-term responses of leaf growth rate to water deficit scale up to whole-plant and crop levels: an integrated modelling approach in maize. *Plant, Cell & Environment*, **31**, pp. 378–391.

Earl, H. and Davis, R., 2003, Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. *Agronomy Journal*, **95**, pp. 688–696.

Eng, D. and Baranoski, G., 2007, The application of photoacoustic absorption spectral data to the modeling of leaf optical properties in the visible range. *IEEE Transactions on Geoscience and Remote Sensing*, **45**, pp. 4077–4086.

Evans, J., Vogelmann, T. and Williams, W., 2004, Chloroplast to leaf. In *Photosynthetic Adaptation Chloroplast to Landscape*, W. Smith, T. Vogelmann and C. Critchley (Eds.), *Part 2: Sunlight Capture, Ecological Studies*, vol. 178, pp. 15–41 (New York: Springer).

Fukshansky, L., 1981, Optical properties of plants. In *Proceedings of the Plants and the Daylight Spectrum*, H. Smith (Ed.), January (London: Academic Press), pp. 21–40.

Fukshansky, L., 1987, Absorption statistics in turbid media. *Journal of Quantitative Spectroscopy and Radiative Transfer*, **38**, pp. 389–406.

Furbank, R. and Taylor, W., 1995, Regulation of photosynthesis in C_3 and C_4 plants: a molecular approach. *The Plant Cell*, **7**, pp. 797–807.

Garlaschi, F., Zucchelli, G. and Jennings, R., 1989, Studies on light absorption and photochemical activity changes in chloroplasts suspensions and leaves due to light scattering and light filtration across chloroplast and vegetation layers. *Applied Optics*, **13**, pp. 109–111.

Gausman, H., Allen, W. and Escobar, D., 1974, Refractive index of plant cell walls. *Applied Optics*, **13**, pp. 109–111.

Govaerts, Y., Jacquemoud, S., Verstraete, M. and Ustin, S., 1996, Three-dimensional radiation transfer modeling in a dicotyledon leaf. *Applied Optics*, **35**, pp. 6585–6598.

Hammer, G., Sinclair, T., Chapman, S. and van Oosterom, E., 2004, On systems thinking, systems biology, and the in silico plant. *Plant Physiology*, **134**, pp. 909–911.

Hendry, G., Houghton, J. and Brown, S., 1987, The degradation of chlorophyll – a biological enigma. *New Phytologist*, **107**, pp. 255–302.

Hosgood, B., Jacquemoud, S., Andreoli, G., Verdebout, J., Pedrini, G. and Schmuck, G., 1995, *Leaf Optical Properties Experiment 93 (LOPEX93)*. Technical report EUR 16095 EN, Institute for Remote Sensing Applications, Unit for Advanced Techniques, Ispra, Italy (Brussels, Luxembourg: Office for Official Publications of the European Communities, ECSC-EC-EAEC).

Inoue, Y. and Shibata, K., 1973, Light-induced chloroplast rearrangements and their action spectra as measured by absorption spectrophotometry. *Planta*, **114**, pp. 341–358.

Inoue, Y. and Shibata, K., 1974, Comparative examination of terrestrial plant leaves in terms of light-induced absorption changes due to chloroplast rearrangements. *Plant & Cell Physiology*, **15**, pp. 717–721.

Jacquemoud, S. and Ustin, S., 2001, Leaf optical properties: a state of the art. In *Proceedings of the 8th International Symposium of Physical Measurements & Signatures in Remote Sensing*, January, Aussois, France (Aussois: CNES), pp. 223–332.

Jacquemoud, S., Ustin, S., Verdebout, J., Schmuck, G., Andreoli, G. and Hosgood, B., 1996, Estimating leaf biochemistry using PROSPECT leaf optical properties model. *Remote Sensing of Environment*, **56**, pp. 194–202.

Jørgensen, N., 2002, Study on line imaging spectroscopy as a tool for nitrogen diagnostics in precision farming. PhD thesis, Department of Agricultural Sciences, The Royal Veterinary and Agricultural University, Copenhagem, Denmark.

Kumar, L., Schmidt, K., Dury, S. and Skidmore, A., 2001, Imaging spectrometry and vegetation science. In *Proceedings of the Imaging Spectrometry. Basic Principles and Prospective Applications*, F. van der Meer and S. de Jong (Eds.), pp. 111–155 (Dordrecht: Kluwer Academic Publishers).

Lee, W., Searcy, S. and Kataoka, T., 1999, Assessing nitrogen stress in corn varieties of varying color. In *Proceedings of the ASAE Annual International Meeting*, July, Toronto, Ontario (Toronto, ON: ASAE), Paper 99-3034, pp. 1–24.

Li-Ping, B., Gong, S., Da, G., Hui, S., Yan, L. and Sheng, Z., 2006, Effect of soil drought stress on leaf water stress, membrane permeability and enzymatic antioxidant system of maize. *Pedosphere*, **16**, pp. 326–332.

Liu, L., Wang, J., Huang, W. and Zhao, C., 2010, Detection of leaf and canopy EWT by calculating REWT from reflectance spectra. *International Journal of Remote Sensing*, **31**, pp. 2681–2695.

Loreto, F., Baker, N. and Ort, D., 2004, Chloroplast to leaf. In *Photosynthetic Adaptation Chloroplast to Landscape*, W. Smith, T. Vogelmann and C. Critchley (Eds.), Part 6: Environmental Constraints, Ecological Studies, vol. 178, pp. 231–261 (New York: Springer).

Maracci, G., Schmuck, G., Hosgood, B. and Andreoli, G., 1991, Interpretation of reflectance spectra by plant physiological parameters. In *Proceedings of the International Geoscience and Remote Sensing Symposium – IGARSS'91*, Espoo, Finland (New York, NY: Institute of Electrical and Electronic Engineers), pp. 2303–2306.

Martin, G., Myers, D. and Vogelmann, T., 1991, Characterization of plant epidermal lens effects by a surface replica technique. *Journal of Experimental Botany*, **42**, pp. 581–587.

Masoni, A., Ercoli, L. and Mariotti, M., 1996, Spectral properties of leaves deficient in iron, sulfur, magnesium and manganese. *Agronomy Journal*, **88**, pp. 937–943.

Meyer-Arendt, J., 1984, *Introduction to Modern and Classical Optics* (New Jersey: Prentice-Hall).

Minorsky, P., 2003, Achieving the in silico plant. Systems biology and the future of plant biological research. *Plant Physiology*, **132**, pp. 404–409.

Nátr, L., 1992, Mineral nutrients – a ubiquitous stress factor for photosynthesis. *Photosynthetica*, **27**, pp. 271–294.

Nicodemus, F., Richmond, J., Hsia, J., Ginsberg, I. and Limperis, T., 1992, Geometrical considerations and nomenclature for reflectance. In *Proceedings of the Physics-Based Vision Principles and Practice: Radiometry*, L. Wolff, S. Shafer and G. Healey (Eds.) (Boston: Jones and Bartlett Publishers), pp. 94–145.

Okada, E., Schweiger, M., Arridge, S., Firbank, M. and Delpy, D., 1996, Experimental validation of Monte Carlo and finite-element methods for the estimation of the optical path length in inhomogeneous tissue. *Applied Optics*, **35**, pp. 3362–3371.

Palmer, K. and Williams, D., 1974, Optical Properties of water in the near infrared. *Journal of the Optical Society of America*, **64**, pp. 1107–1110.

Pope, R. and Fry, E., 1997, Absorption spectrum (380–700 nm) of pure water II: integrating cavity measurements. *Applied Optics*, **36**, pp. 8710–8723.

Prahl, S., 1988, Light transport in tissue. PhD thesis, The University of Texas at Austin, TX, USA.

Pu, R., Gong, P., Biging, G. and Larrieu, M., 2003, Extraction of red edge optical parameters from hyperion data for estimation of forest leaf area index. *IEEE Transactions on Geoscience and Remote Sensing*, **41**, pp. 916–921.

Rabinowitch, E., 1951, Light absorption by pigments in the living cell. In *Proceedings of the Photosynthesis and Related Processes*, 2 (New York, NY: Interscience Publishers, Inc.) pp. 672–739.

Sato, Y., Wada, M. and Kadota, A., 2001, External Ca^{2+} is essential for chloroplast movement induced by mechanical stimulation but not by light stimulation. *Plant Physiology*, **127**, pp. 497–504.

Schneider, D., 2010, Biofuel's water problem. *IEEE Spectrum*, **47**, pp. 50–51.

Schroeder, J., Kwak, J. and Allen, G., 2001, Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature*, **410**, pp. 327–330.

Seyfried, M., Fukshansky, L. and Schafer, E., 1983, Correcting remission and transmission spectra of plant tissues measured in glass cuvettes: a technique. *Applied Optics*, **22**, pp. 492–496.

Terashima, I. and Saeki, T., 1983, Light environment within a leaf I. Optical properties of paradermal sections of Camelia leaves with spectral reference to differences in the optical properties of palisade and spongy tissues. *Plant and Cell Physiology*, **24**, pp. 1493–1501.

Thenkabail, P., Turral, H., Biradar, C. and Lyon, J., 2009, *Remote Sensing of Global Croplands for Food Security*, 556 p. (Boca Raton, FL: CRC Press).

Thomas, J., Namkem, L., Oerther, G. and Brown, R., 1971, Estimating leaf water content by reflectance measurements. *Agronomy Journal*, **63**, pp. 845–847.

Tuchin, V., 2000, *Tissue Optics Light Scattering Methods and Instruments for Medical Diagnosis* (Bellingham, WA: The International Society for Optical Engineering).

Ustin, S., Jacquemoud, S. and Govaerts, Y., 2001, Simulation of photon transport in a three-dimensional leaf: implications for photosynthesis. *Plant, Cell & Environment*, **24**, pp. 1095–1103.

Vanderbilt, V. and Grant, L., 1985, Plant canopy specular reflectance model. *IEEE Transactions on Geoscience and Remote Sensing*, **23**, pp. 722–730.

Vanderbilt, V., Grant, L. and Ustin, S., 1991, Polarization of light by vegetation. In *Proceedings of the Photon – Vegetation Interactions – Applications in Optical Remote Sensing and Ecology*, R. Mynemi and J. Ross (Eds.), pp. 191–228 (Berlin: Springer-Verlag).

Ventura, B., Lemerle, C., Michalodimitrakis, K. and Serrano, L., 2006, From *in vivo* to *in silico* biology and back. *Nature*, **443**, pp. 527–533.

Verdebout, J., Jacquemoud, S. and Schmuck, G., 1994, Optical properties of leaves: modeling and experimental studies. In *Proceedings of the Imaging Spectrometry – a Tool for Environmental Observations*, J. Hill and J. Mégier (Eds.), pp. 169–191, Dordrecht: Kluwer Academic Publishers.

Vogelmann, T., 1993, Plant tissue optics. *Annual Review of Plant Physiology and Plant Molecular Biology*, **44**, pp. 231–251.

Vogelmann, T. and Martin, G., 1993, The functional significance of palisade tissue: penetration of directional versus diffuse light. *Plant, Cell and Environment*, **16**, pp. 65–72.

Wada, M., Kagawa, T. and Sato, Y., 2003, Chloroplast movement. *Annual Review of Plant Biology*, **54**, pp. 455–468.

Walter-Shea, E., Norman, J. and Blad, B., 1989, Leaf bidirectional reflectance and transmittance in corn and soybean. *Remote Sensing of Environment*, **29**, pp. 161–174.

Woolley, J., 1971, Reflectance and transmittance of light by leaves. *Plant Physiology*, **47**, pp. 656–662.

Woolley, J., 1973, Change of leaf dimensions and air volume with change in water content. *Plant Physiology*, **41**, pp. 815–816.

Woolley, J., 1975, Refractive index of soybean leaf cell walls. *Plant Physiology*, **55**, pp. 172–174.

Yokota, A., Takahara, K. and Akashi, K., 2006, Water stress. In *Proceedings of the Physiology and Molecular Biology of Stress Tolerance in Plants*, K. Rao, A. Raghavendra and K. Reddy (Eds.), pp.15–39 (Dordrecht: Springer).

Zhao, D., Reddy, K., Kakani, V., Read, J. and Carter, G., 2003, Corn (*Zea mays* L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. *Plant and Soil*, **257**, pp. 205–217.