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

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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

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In 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 C₄ 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 $\Delta p$ at a certain wavelength $\lambda$ in the medium is given by

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

(1)

where $\mu_a(\lambda)$ = effective absorption coefficient of the medium (cm$^{-1}$), $\theta$ = 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 cm$^2$ g$^{-1}$) by its concentration in the medium (given in 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 $\theta_s$ between the medium’s normal direction and the ray’s altered trajectory. Note that regardless of the magnitude of $\theta_s$, 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 $\theta$ (given in radians) varies according to the cosine of $\theta_s$, that is, the lower the angle of penetration $\theta_s$, the higher the angle $\theta$, and consequently the higher the probability of absorption (equation (1)). In this case, $\theta$ 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:

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

where \( \xi_r \) = uniformly distributed random number \( \in [0,1] \).

For each ray incident on an unifacial leaf, we generate a random number \( \varrho \) 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 \( \varrho t_{mu} \), 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 cm². We remark that these

<table>
<thead>
<tr>
<th>Plant</th>
<th>( C_a ) (mg m⁻¹)</th>
<th>( C_b ) (mg m⁻¹)</th>
<th>( C_c ) (mg m⁻¹)</th>
<th>( C_p ) (%)</th>
<th>( C_{cl} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.90</td>
<td>0.80</td>
<td>0.66</td>
<td>26.55</td>
<td>29.63</td>
</tr>
<tr>
<td>B</td>
<td>3.16</td>
<td>1.11</td>
<td>0.84</td>
<td>24.09</td>
<td>28.64</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Thickness (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.0204</td>
<td>0.0668</td>
<td>0.0146</td>
</tr>
<tr>
<td>A2</td>
<td>0.0186</td>
<td>0.0688</td>
<td>0.0170</td>
</tr>
<tr>
<td>B1</td>
<td>0.0224</td>
<td>0.0796</td>
<td>0.0203</td>
</tr>
<tr>
<td>B2</td>
<td>0.0156</td>
<td>0.0595</td>
<td>0.0162</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>LOPEX spectral file</th>
<th>Spectral quantity</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0141</td>
<td>Reflectance</td>
<td>0.0080</td>
</tr>
<tr>
<td>A1</td>
<td>0142</td>
<td>Transmittance</td>
<td>0.0209</td>
</tr>
<tr>
<td>A2</td>
<td>0147</td>
<td>Reflectance</td>
<td>0.0122</td>
</tr>
<tr>
<td>A2</td>
<td>0148</td>
<td>Transmittance</td>
<td>0.0112</td>
</tr>
<tr>
<td>B1</td>
<td>0141</td>
<td>Reflectance</td>
<td>0.0139</td>
</tr>
<tr>
<td>B1</td>
<td>0142</td>
<td>Transmittance</td>
<td>0.0079</td>
</tr>
<tr>
<td>B2</td>
<td>0147</td>
<td>Reflectance</td>
<td>0.0103</td>
</tr>
<tr>
<td>B2</td>
<td>0148</td>
<td>Transmittance</td>
<td>0.0170</td>
</tr>
</tbody>
</table>

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 (\(\delta_c\)), epidermis cell caps (\(\delta_e\)) and mesophyll cell caps (\(\delta_m\)) 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
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 C₄ 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,
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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...
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.

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
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monocotyledonous C₄ 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
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
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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.

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...
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.

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
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 C₄ 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 C₄ 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 (C₄) 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.

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