ON THE PREDICTIVE MODELING OF VISIBLE LIGHT INTERACTION WITH FRESH AND ENVIRONMENTALLY STRESSED MONOCOTYLEDONOUS LEAVES

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ABSTRACT

The author has recently proposed a model to simulate light interactions with monocotyledonous (unifacial) leaves in the infrared domain. In this paper, we evaluate the applicability of this model to simulations performed in the visible (photosynthetic) domain, and aimed at the investigation of biophysical responses triggered by nutrient and water stress. The model's fidelity and predictability in this spectral domain are assessed through quantitative and qualitative comparisons of modeled results with measured data obtained for maize specimens. Its predictive capabilities are further demonstrated through the simulation of reflectance profiles resulting from experiments involving maize leaves under different water reduction procedures.

***Index Terms*—** Stress, reflectance, transmittance, absorption, chloroplast, simulation.

1. INTRODUCTION

Due their increasing global demand (used not only for food, but also for fuel production), monocotyledonous C_4 plants, such as maize (*Zea mays* L.; corn) and sugarcane (*Saccharum officinarum*), are becoming the focal point of studies involving the impact of abiotic stress factors on crop photosynthetic efficiency. Clearly, the availability of simulation tools specifically designed for these plants can contribute to expand the current understanding about their tolerance to less favorable environmental conditions. Furthermore, these tools can be used to assist the investigation of open questions involving their physiological responses to changes in water soil levels and the effects of limited nutrient and water supplies on their growing process. From an agricultural and ecological point of view, one of the key benefits of performing *in silico* experiments using these tools is that such "virtual" experiments can provide quick feedback with respect to different strategies for increasing nutrient and water use efficiency of these crops given a set of possible environmental conditions [1].

Recently, a light transport model, namely the ABM-U (algorithmic BDF (bidirectional scattering distribution function) model for unifacial leaves), was specifically designed to simulate light interactions with monocotyledonous (unifacial) leaves [2, 3]. Its results showed good qualitative and quantitative agreement with measured data with respect to the infrared domain. In this paper, its predictability in the visible (photosynthetic) domain is evaluated along with its applicability to the study of processes relating nutrient and water stress to light absorption in this domain. As mentioned by Carter and Knapp [4], this is the region of the light spectrum where foliar spectral signatures are altered by abiotic stress factors more consistently.

2. MATERIALS AND METHODS

2.1. Evaluation and Characterization Data Sets

The simulations performed to evaluate the predictability of the ABM-U with respect to the interactions of visible light with monocotyledonous (unifacial) plant leaves were quantitatively compared to measured data available in the LOPEX [5] spectral files 147 (directional-hemispherical reflectance) and 148 (directional-hemispherical transmittance), which correspond to a maize specimen whose measured structural and biophysical attributes are also available in LOPEX. This set of attributes was used to characterize the fresh (turgid) specimen used as the reference (control) in this work, and it includes: area (4.1 cm^2), fresh weight (0.0688 g), dry weight (0.0171 g), thickness (0.0186 cm) and the main absorber contents, namely chlorophyll a (0.2004 mg), chlorophyll b (0.0571 mg), carotenoids (0.0454 mg), cellulose (0.0037 g), lignin (0.00037 g) and protein (0.0043 g). It is worth mention that the protein content used in the simulations corresponds to the percentage of nitrogen per dry weight of the sample provided in the LOPEX database. As stated by Jacquemoud et al. [6], there is a strong relationship between total nitrogen (N) and protein content. Such a relationship is taken into account in the simulations. Besides these species dependent parameters, other essential biophysical parameters, such as the refractive indices of the main foliar materials and the specific absorption coefficients of chlorophyll a, chlorophyll b, carotenoids, cellulose+lignin, protein and water [2, 7, 6], were also accounted for in our *in silico* experiments.

In the simulations involving changes in the foliar water content, we consider a 25% water loss. This figure was selected to facilitate the comparison of modeled results with measured data available in the literature [8, 9, 10]. According to measurements performed by Wooley [11] on a maize leaf with thickness (0.0191 cm) and density thickness (17.78 $mg\ cm^{-2}$) similar to the thickness (0.0186 cm) and density thickness (16.78 $mg\ cm^{-2}$) of the LOPEX maize specimen described above (which was selected among other LOPEX maize specimens for this reason), such a reduction is followed by approximately a 20% reduction in the leaf thickness and a 2% reduction in leaf area. The thickness and area of the fresh (control) specimen were reduced accordingly, along with a 25% fresh weight reduction and a 20% increase in the aspect ratio of its mesophyll cell caps to account for the resulting flattening of these cells [2, 9]. Finally, using refractive indices of fully hydrated and dried mesophyll cell walls, 1.415 and 1.53 respectively [12], the law of Gladstone and Dale [2] was applied to obtain the refractive index of mesophyll cell walls (1.44375) after the water content reduction.

2.2. Simulation Issues

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 changes in the direction of propagation of light traveling in the mesophyll, a phenomenon known as the detour effect [13]. Since the distribution of absorbers within the mesophyll cells is not complete homogeneous (under normal conditions, the mesophyll chloroplasts usually remain arrayed along the cell walls [14]), the angular deviations due to light interactions with these absorbers are not completely random either. In addition, it is necessary to consider that any heterogeneity in a pigment distribution can only reduce the probability of light absorption (in comparison with a homogeneous distribution) since light can be propagated without encountering the pigment-containing organelles, a phenomenon known as the sieve effect [13]. 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, and for the correlation of this relationship with respect to the angular deviations of light traveling in the mesophyll tissue. Accordingly, the ABM-U incorporates a bound for the angular deviations caused by the heterogeneous distribution of chloroplasts under normal conditions. This bound was derived from applied optics experiments performed on samples with absorptive and scattering properties similar to those attributed to plant tissue constituents [3]. In the ABM-U, this bound is incorporated into the probabilistic algorithm used to guide the propagation of the light rays within the foliar tissues.

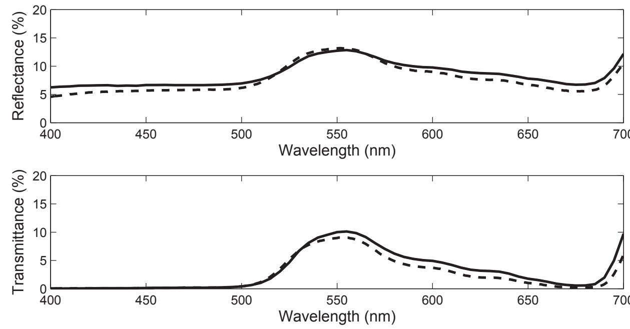

Fig. 1. Measured (dashed line) and modeled (solid line) directional-hemispherical reflectance (top) and directional-hemispherical transmittance (bottom) curves for a fresh maize leaf. The curves were obtained considering an angle of incidence of 8°.

3. RESULTS AND DISCUSSION

Initially, modeled directional-hemispherical reflectance and transmittance spectral 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 1, and they show that the shapes of the modeled curves closely mimic the shapes of their measured counterparts. They also indicate an excellent quantitative agreement, 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 modeled 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 modeled reflectance and transmittance curves with respect to the measured curves were 0.0096 and 0.0093 respectively, which are smaller than 0.03, the empirical value usually associated to good spectral reconstruction in the field of remote sensing of vegetation [6].

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, which can be closely simulated by varying the pigment concentration of modeled leaves [4]. In these simulations, we considered the same pigment reduction (82%) employed in the experiments performed by Al-Abbas et al. [15]. The simulation results are presented in Figure 2, and they clearly depict the changes observed in the actual experiments performed by Al-Abbas et al. [15]. First, nitrogen-deficient maize leaves are characterized by higher reflectance and transmittance with respect to control specimens. This change occurs due to a reduction of photosynthetic pigments, and, for this reason, it is more pronounced in the region around

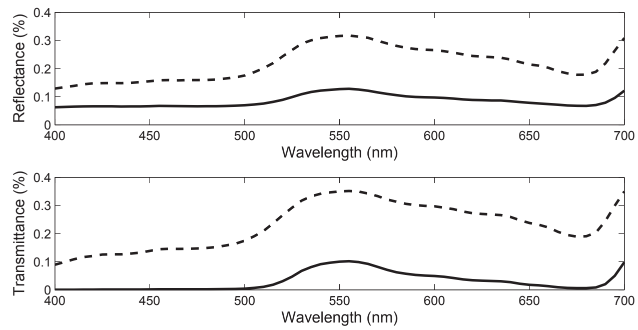

Fig. 2. Modeled directional-hemispherical reflectance (top) and directional-hemispherical transmittance (bottom) curves for fresh (solid line) and nitrogen stressed (dashed line) maize leaves. The curves were obtained considering an angle of incidence of $5°$.

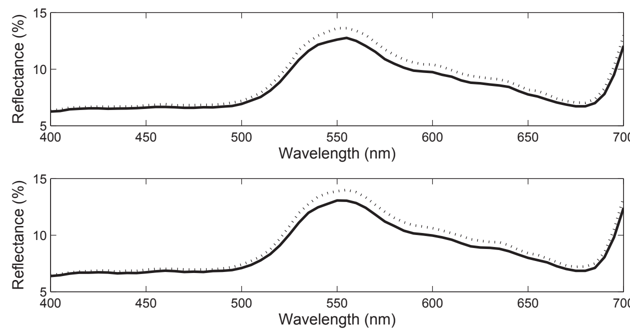

Fig. 3. Modeled directional-hemispherical reflectance curves of fresh (solid line) and wilted (dashed line) maize leaves. The curves were obtained considering two angles of incidence, $2.5°$ (top) and $15°$ (bottom), to avoid a directional bias in the qualitative comparisons with measured data.

$550nm$ [15]. Second, the increase in transmittance is larger than the increase in the reflectance for angles of incidence close to the zenith.

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 [8]. The results of these simulations, which took into account the structural and refractive index changes caused by a 25% water loss (Section 2.1), are presented in Figure 3. The modeled curves show a higher reflectance for the air-dried specimen in comparison with the fresh (turgid) specimen. This result is qualitatively consistent with the reflectance increase observed by Thomas *et al.* [8] in air-dried maize leaves whose water content was reduced by a similar amount (approximately 25%). Furthermore, it can be observed in the modeled curves depicted in Figure 3 that the reflectance increase is more pronounced in the region where the photosynthetic pigments have a lower absorptive capacity (centered at approximately 550 nm). This feature is also noticeable in the actual measurements performed by Thomas *et al.* [8], and it is consistent with the measured data obtained by Wooley [9] for maize leaves whose water content was reduced by a similar amount through an *in vitro* water reduction procedure.

Thomas *et al.* [8] observed that leaves of plants under water stress may appear darker green than leaves of plants not stressed (control) because they reflect and transmit light differently. They also stated that, although the influence of water is often masked by leaf pigment content, water stress may, under certain conditions, decrease reflectance. This behavior was observed in measurements performed by Maracci *et al.* [10]. In their 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.* [10] mentioned, however, the need of further experiments to study this tendency.

Another set of simulations was then carried out to reproduce this reflectance behavior. All characterization parameters were kept the same (including the amount of water loss, 25%). The bound for the angular deviations of the propagated light, however, was removed to account for a less heterogeneous distribution of the chloroplasts, and consequently to allow a diffuse distribution of light traveling in the mesophyll [3]. Its removal was based on the observation that the reflectance of the *in vivo* water stressed specimen decreased even though the pigment contents stayed constant [10]. This suggests that its absorption efficiency might be affected by factors not present during an *in vitro* water reduction procedure. A possible factor could be an intensification of the detour effects within the mesophyll tissue, which could be caused by a more homogeneous intracellular distribution of mesophyll chloroplasts, possibly triggered by water deficit signals [16]. Such a distribution would decrease the sieve effect in this tissue [13]. The results of these simulations are presented in Figure 4, and they show a lower overall reflectance for the *in vivo* water stressed specimen in comparison with the fresh (control) specimen. This reflectance decrease is qualitatively consistent with the observations made by Marracci *et al.* [10], and it is more pronounced in the region around 550 nm, a feature also evident in their measurements.

4. CONCLUSION

Besides allowing the behavior of a biological system to be simulated under various conditions, a predictive *in silico* experimental framework may also be used to drive new investigations. In line with these guidelines, the proposed framework may open new opportunities to the study of physiological perturbations caused by abiotic stress factors. Such an opportunity was illustrated in previous section by the simu-

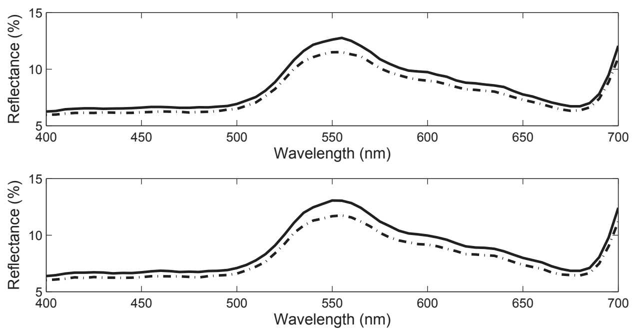

Fig. 4. Modeled directional-hemispherical reflectance curves of fresh (solid) and water stressed (dash-dotted) maize leaves. The curves were obtained considering two angles of incidence, $2.5°$ (top) and $15°$ (bottom), to avoid a directional bias in the qualitative comparisons with measured data.

lations involving the apparent discrepancy in the reflectance profiles of specimens under *in vitro* and *in vivo* moderate water reduction procedures. Considering that identical structural and refractive index changes due to water loss were accounted for in both sets of simulations, one may assume that these changes are not responsible for a less heterogeneous distribution of the chloroplasts attributed to the *in vivo* water stressed specimen. Hence, such a distribution would likely be associated with the *in vivo* nature of the water stress process. 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, our simulations suggest that such an investigation might provide new insights on the adaptive responses of monocotyledonous C_4 plants to unfavorable environmental conditions.

5. REFERENCES

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

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

[3] G.V.G. Baranoski and D. Eng, "An investigation on sieve and detour effects affecting the interaction of collimated and diffuse infrared radiation (750 to 250 nm) with plant leaves," *IEEE Transactions on Geoscience and Remote Sensing*, vol. 45, pp. 2593–2599, 2007.

[4] G.A. Carter and A.K. Knapp, "Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration," *American Journal of Botany*, vol. 88, no. 4, pp. 677–684, 2001.

[5] B. Hosgood, S. Jacquemoud, G. Andreoli, J. Verdebout, G. Pedrini, and G. Schmuck, "Leaf Optical Properties Experiment 93 (LOPEX93]," Tech. Rep. EUR 16095 EN, Institute for Remote Sensing Applications, Unit for Advanced Techniques, Ispra, Italy, 1995.

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

[7] D. Eng and G.V.G. Baranoski, "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*, vol. 45, pp. 4077–4086, 2007.

[8] J.R. Thomas, L.N. Namkem, G.F. Oerther, and R.G. Brown, "Estimating leaf water content by reflectance measurements," *Agronomy Journal*, vol. 63, pp. 845–847, 1971.

[9] J.T. Woolley, "Reflectance and transmittance of light by leaves," *Plant Physiology*, vol. 47, pp. 656–662, 1971.

[10] G. Maracci, G. Schmuck, B. Hosgood, and G. Andreoli, "Interpretation of reflectance spectra by plant physiological parameters," in *International Geoscience and Remote Sensing Symposium - IGARSS'91*, Espoo, Finland, 1991, pp. 2303–2306.

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

[12] J.T. Woolley, "Refractive index of soybean leaf cell walls," *Plant Physiology*, vol. 55, pp. 172–174, 1975.

[13] L. Fukshansky, "Optical properties of plants," in *Plants and the Daylight Spectrum*, H. Smith, Ed., London, January 1981, pp. 21–40, Academic Press.

[14] E.I. Rabinowitch, "Light absorption by pigments in the living cell," in *Photosynthesis and Related Processes*. 1951, vol. 2, pp. 672–739, Interscience Publishers, Inc.

[15] A.H. Al-Abbas, R. Barr, J.D. Hall, F.L. Crane, and M.F. Baumgardner, "Spectra of normal and nutrient-deficient maize leaves," *Agronomy Journal*, vol. 66, pp. 16–20, 1974.

[16] F. Loreto, N.P. Baker, and D.R. Ort, "Chloroplast to leaf," in *Photosynthetic Adaptation Chloroplast to Landscape*, W.K. Smith, T.C. Vogelmann, and C. Critchley, Eds., chapter 9, pp. 231–261. Springer, NY, USA, 2004, Part 6: Environmental Constraints, Ecological Studies, Vol. 178.