

On the detection and monitoring of reduced water content in plants using spectral responses in the visible domain

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ABSTRACT

The water status of cultivated plants can have a significant impact not only on food production, but also on the appropriate usage of increasingly scarce freshwater supplies. Accordingly, the cost-effective detection and monitoring of changes in their water content are longstanding remote sensing goals. Existing procedures employed to achieve these goals are largely based on the spectral responses of plant leaves in the infrared domain where the light absorption within the foliar tissues is dominated by water. Recently, it has been suggested that such procedures could be implemented using spectral responses, more specifically spectral subsurface reflectance to transmittance ratios, obtained in the visible domain. The basis for this proposition resides on the premise that a reduced water content (RWC) can result in histological changes whose effects on the foliar optical properties may not be limited to the infrared domain. However, the experiments leading to this proposition were performed on detached leaves, which were not influenced by the whole plant's adaptation mechanisms to water stress. In this work, we investigate whether the spectral responses of living plant leaves in the visible domain can lead to reliable RWC estimations. We employ measured biophysical data and predictive light transport simulations in order to extend qualitatively and quantitatively the scope of previous studies in this area. Our findings indicate that the living specimens' physiological responses to water stress should be taken into account in the design of new procedures for the cost-effective RWC estimation using visible subsurface reflectance to transmittance ratios.

Keywords: foliar optical properties, water stress, chloroplast relocation, simulation.

1. INTRODUCTION

In recent years, we have been observing an accentuation of adverse climatic conditions leading to extensive drought periods in certain regions of the planet. At the same time, the demand for increasing agricultural yields for food and biofuel production continues to grow steadily. The combination of these factors has become a catalyst for research efforts toward the effective detection and monitoring of changes in crops' water status. These procedures are essential not only from an economical, but also from an ecological point of view. An underestimation of crops' water requirements may lead to reduced yields, while an overestimation may result in detrimental effects to the environment and human health such as the contamination (*e.g.*, due to the excessive use of fertilizers) and even depletion of limited freshwater supplies¹.

Remote sensing initiatives combined with the acquisition and analysis of ground-based spectral data for plants have a central role in this scenario. They can provide valuable support for the development of accurate drought stress detecting procedures and sensors aimed at limiting irrigation water overuse and preventing long-term production losses. Since monocotyledonous C_4 species characterized by unifacial leaves, such as maize (*Zea mays L.*; corn) and sugarcane (*Saccharum officinarum*), are not only large-scale providers of raw materials for food and biofuel production, but also endowed with advanced mechanisms for adaptation to adverse environmental conditions, they have been object of a wide range of experimental investigations in this area²⁻⁷. Despite these efforts, however, the current understanding about these species' capacity to grow in hot climates with sporadic rainfall is still relatively limited. These aspects have motivated us to further examine the variations in these plants' spectral signatures in response to water stress.

The assessment of a plant's water status is associated with the measurement of its leaves' relative water content (RWC). According to Loreto *et al.*⁸, when the RWC falls below 70%, it can result in an irreversible damage of

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the foliar photosynthetic apparatus. More specifically, the RWC reduction is accompanied by an accentuated drop in pigment content^{9,10}. This reduces light absorption efficiency, which may result in the plant's death^{8,11}. Moderate water stress (RWC > 70%), which is observed more often in nature⁸, may also prompt structural variations, such as tissue thickness reductions¹², that can affect foliar optical properties and, consequently, the leaves' spectral signatures.

Although the effective monitoring of C_4 crops' water status requires the detection of changes in foliar spectral responses under *in vivo* conditions, the spectral data used in studies involving the reduction of foliar water content, particularly in the more conspicuous moderate range, is normally obtained under *in vitro* conditions. In these situations, in which the specimens (leaves) are usually detached from a living plant and air dried, an increase in the visible reflectance is observed^{2,13}. One might expect that *in vivo* and *in vitro* water reduction procedures would result in similar spectral changes since in both cases the loss of water affects the internal structure of the foliar tissues. However, experiments performed by Maracci *et al.*³ under *in vivo* conditions (by withholding water from the soil), which were also examined by Verdebout *et al.*⁴, suggested that this may not be the case for certain species. More specifically, in these experiments, maize leaves whose water content was moderately reduced using an *in vivo* procedure showed a decrease in the visible reflectance even though their pigment content remained relatively constant³.

According to Loretto *et al.*⁸, 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. The main signalling route used in this process is mediated by abscisic acid (ABA)¹⁴. It is worth noting that, as ABA is detected, it induces cytosolic Ca^{2+} elevations⁸. Also, Ca^{2+} channels are essential for the initiation of a large number of signal transduction processes in higher plant cells¹⁵. For example, experiments by Sato *et al.*¹⁶ indicate that the influx of external Ca^{2+} through the plasma membrane is required for the early signalling step of chloroplast mechano-relocation movements. Accordingly, it has been proposed that a similar sequence of events may occur in maize leaves under moderate *in vivo* water stress and neutral illumination conditions⁷. 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 chlorophyll-containing organelles. Such a distribution pattern would increase the probability of light detour effects, and consequently, increase light absorption in the visible domain^{17,18}. This would explain the reflectance decrease observed during moderate *in vivo* water reduction procedures, and it would also provide further evidence about the adaptive mechanisms of these plants to adverse environmental conditions^{5,6}.

Estimations of plant's water content usually involve spectral measurements in the infrared domain^{19,20}. However, different studies have addressed the possibility of using foliar spectral signatures in the visible domain to assist in the assessment of plant's water status^{13,21,22}. Recently, Vanderbilt *et al.*²³ have performed experiments in which optical polarization techniques have been employed to remove the surface (specular) component of green healthy maize leaves' reflectance in order to demonstrate that its subsurface (diffuse) component has a direct correlation with the leaves' relative water content (RWC). This correlation was based on the premise that RWC-linked changes in the visible reflectance are associated with structural alterations in the internal arrangement of the foliar tissues, and are not attributable to molecular configuration changes in cellular pigments, provided that the leaves' RWC is above 65%. As result, Vanderbilt *et al.*²³ indicated that the subsurface reflectance to transmittance ratio (R_d/T) can potentially be used to estimate the RWC of wilted specimens, notably for RWC values between 85% and 65%. It is important to note, however, that these experiments were performed on leaves detached from the living plant, *i.e.*, under *in vitro* conditions.

Computer simulations, or *in silico* experiments, are routinely being employed to accelerate the different cycles of research involving optical processes that cannot be fully studied through traditional laboratory procedures due to technical limitations. Among these limitations, one can highlight the difficulties of performing *in situ* measurements requiring the control of a large number of biophysical and experimental variables. In the case of plants, it has been demonstrated that computer simulations paired with measured data can be effectively employed in the investigation of open questions involving their physiological responses to changes in water soil levels⁷ as well as the effects of limited water availability on their growing process²⁴. From agricultural and environmental perspectives, one of the key benefits of employing such "virtual" experiments is that they can

enable rapid assessments of different approaches for increasing water use efficiency of crops given a set of possible environmental conditions²⁵.

In this paper, we employ computer simulations to assess whether variations on the visible spectral signatures of C_4 plants characterized by unifacial leaves can be effectively employed in the detection and monitoring of RWC changes under both *in vitro* and *in vivo* conditions. We selected maize as the focal point of our investigation for two main reasons. First, for consistency with the related work outlined above. Second, for the relatively larger amount of biophysical data available for this plant in the scientific literature in comparison with other C_4 plants like sugarcane.

Our controlled *in silico* experiments are performed using the predictive light transport model known as ABM-U (algorithmic BDF (bidirectional scattering distribution function) model for unifacial leaves)^{26,27}. Besides allowing to overcome the technical limitations associated with actual experiments, the use of such a model allow us to obtain the subsurface component of foliar reflectance in a straightforward manner. Our findings confirm the need to account for the living specimens' physiological responses to water stress when designing new procedures for the cost-effective RWC estimation using visible spectral reflectance to transmittance ratios. They also suggest possible indicators of moderate water stress in unifacial C_4 specimens under *in vivo* conditions, which can potentially contribute to more reliable RWC estimations.

2. MATERIALS AND METHODS

2.1 Simulation Framework Overview

The ray-optics based model, ABM-U^{26,27}, used in this investigation employs an algorithmic Monte Carlo formulation that provides a rigorous and yet flexible approach for the simulation of light transport within foliar tissues. Its detailed parameter space enables specific biophysical characteristics of different specimens to be appropriately taken into account during the simulations.

Within the ABM-U formulation, a ray interacting with a given leaf specimen can be associated with any selected wavelength within the spectral regions of interest. Hence, ABM-U can provide reflectance and transmittance quantities with different spectral resolutions. For consistency, however, we considered a spectral resolution of $5nm$ in all modeled curves depicted in this work. In terms of illumination and collection geometries, ABM-U can provide bidirectional reflectance and transmittance values by recording the direction of the outgoing rays using a virtual gonireflectometer^{28,29}. In addition, one can obtain directional-hemispherical reflectance and transmittance values by integrating the outgoing rays with respect to the collection hemisphere using a virtual spectrophotometer^{29,30}. The spectral curves presented in this investigation correspond to directional-hemispherical readings obtained considering an angle of incidence equal to 8° (for consistency with experiments aimed at remote sensing observations of plants³¹) and 10^6 sample rays per wavelength.

To enable the full reproduction of our investigation results, we made ABM-U available online³² via a model distribution system³³ along with the supporting biophysical data (*e.g.*, refractive indices and extinction coefficients) used in our *in silico* experiments³⁴. This system enables researchers to specify experimental conditions (*e.g.*, angle of incidence and spectral range) and specimen characterization parameters (*e.g.*, pigments and water content) using a web interface (Figure 1), and receive customized simulation results.

2.2 Specimens' Characterization Data

Three groups of maize specimens were considered in this investigation, namely fresh (turgid, baseline), *in vitro* wilted and *in vivo* wilted. Moreover, within each group, we also considered exemplars, henceforth referred to as A and B, with relatively distinct biophysical characteristics. We used measured datasets, which are available in the LOPEX database³¹, in the characterization of the fresh A and B specimens, and modified versions of these datasets in the characterization of the corresponding wilted specimens. These datasets and their modifications are reported in the remainder of this section.

The LOPEX project³¹ involved experiments performed on 120 leaf samples representative of more than 50 species. These experiments included directional-hemispherical reflectance and transmittance measurements as well as auxiliary measurements of pigment concentrations, thickness and water content for each specimen. Incidentally, the modeled (using the ABM-U) reflectance and transmittance curves obtained for the baseline

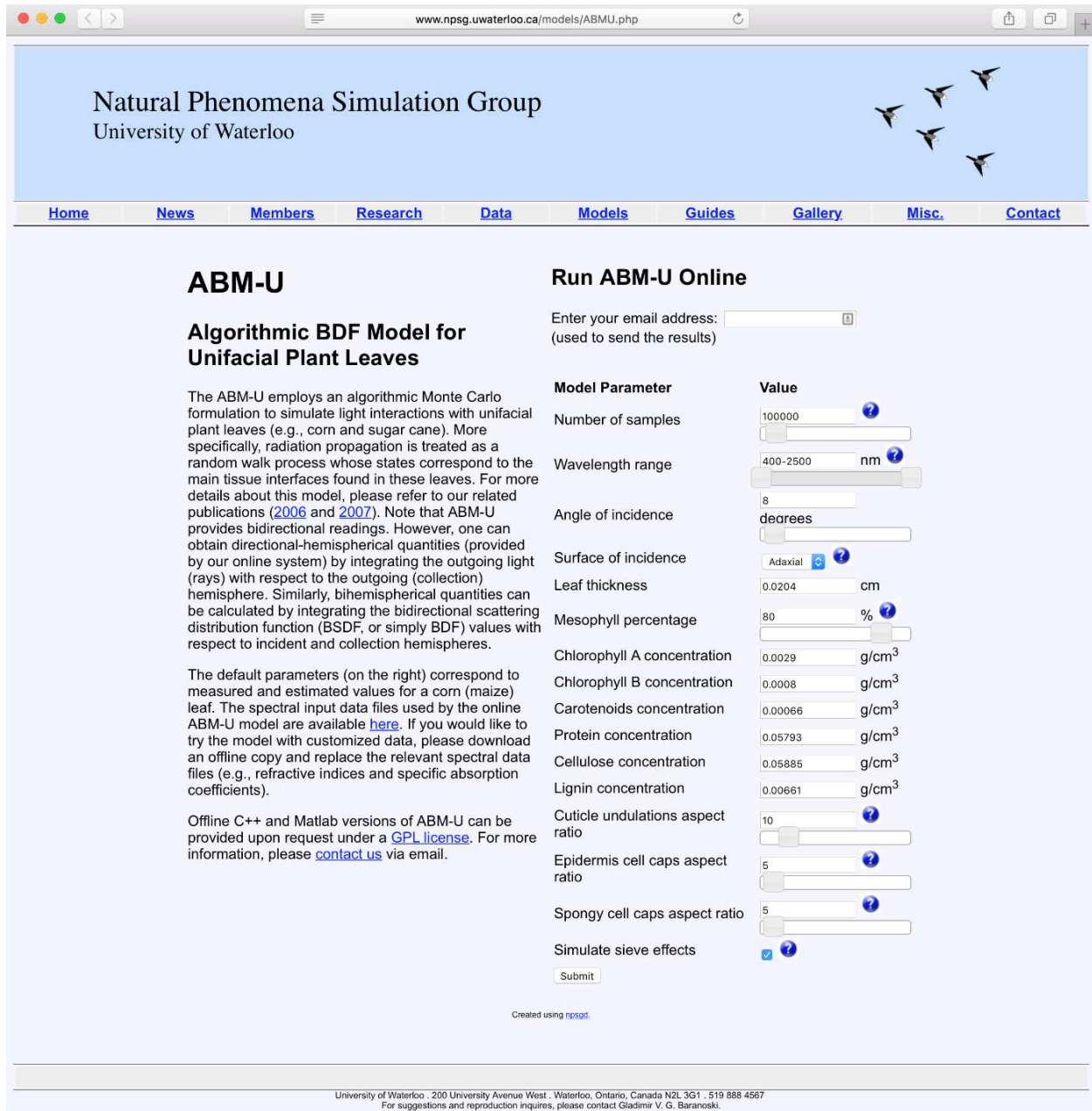


Figure 1: The web interface for the ABM-U model³² available through the Natural Phenomena Simulation Group Distributed (NPSGD) system³³. Through this interface, researchers can configure biophysical parameters and execute light transport simulations involving C_4 plants characterized by unifacial plant leaves.

specimens considered in this investigation closely approximate their measured counterparts as depicted in previous works^{7,26}.

The LOPEX biochemical data, namely the contents of the main leaf absorbers in the visible range, used to characterize the baseline (fresh) specimens are presented in Table 1. The remaining LOPEX data employed in the characterization of these specimens, namely thickness, fresh weight and dry weight, is presented in Table 2. The values for these parameters correspond to measurements performed for each specimen during the LOPEX project considering a foliar area of 4.1 cm^2 . These parameters are used to compute the concentration of the absorbers in terms of g/cm^3 since their contents are given either in terms of mg per fresh weight (in the case of

specimen	C_a ($mg\ g^{-1}$)	C_b ($mg\ g^{-1}$)	C_c ($mg\ g^{-1}$)	C_p (%)	C_{cl} (%)	C_l (%)
A	2.90	0.80	0.66	26.55	26.60	3.03
B	3.16	1.11	0.84	24.09	25.89	2.75

Table 1: Biochemical data for specimens A (LOPEX sample 12) and B (LOPEX sample 16). The concentration of chlorophyll a (C_a), chlorophyll b (C_b) and carotenoids (C_c) are given as content per fresh weight. Protein (C_p), cellulose (C_{cl}) and lignin (C_l) contents are given in terms of percentage of dry weight.

specimen	thickness (cm)	fresh weight (g)	dry weight (g)
A	0.0204	0.0668	0.0146
B	0.0156	0.0595	0.0162

Table 2: Thickness and weight values for specimens A (LOPEX sample 12) and B (LOPEX sample 16). These values correspond to the biophysical characteristics of the actual specimens used to obtain the LOPEX spectral measurements 141 and 537 respectively.

chlorophylls and carotenoids) or as percentage of dry weight (in the case of cellulose, lignin and protein) in the LOPEX database. The volume considered in these concentration computations corresponds to the sampled foliar area multiplied by the thickness of the mesophyll tissue, which we estimated to be approximately 80% of the leaves' total thickness^{35,36}. The upper bound values selected for the cuticle undulations, epidermis cell caps and mesophyll (spongy) cell caps used to perturb the light rays interacting with the foliar tissues²⁶ were 10, 5 and 5, respectively. These values were derived from data available in the literature and also borne out by observations of cross sections of maize leaves⁷.

The modeled radiometric quantities for the wilted specimens were obtained considering a 25% water reduction accompanied by minor changes in the pigment contents as reported in the experiments by Maracci *et al.* (1991) involving maize leaves under moderate water stress. Measurements performed by Wolley³⁷ on maize leaves indicate that such a water content reduction is followed by approximately a 20% reduction in thickness and a 2% reduction in area. We reduced the thickness and area of our baseline specimens accordingly, and we also performed a 25% reduction on their fresh weights. A summary of ABM-U parameter values employed in this investigation is provided in Table 3. Note that, as expected,³ the performed morphological changes resulted in the pigment concentrations remaining relatively unchanged. Finally, we applied a 20% increase in the aspect ratio of the mesophyll (spongy) cell caps to account for their resulting flattening^{12,26}.

Parameter	A (fresh)	A (wilted)	B (fresh)	B (wilted)
Thickness (cm)	0.02040	0.01632	0.02240	0.01792
Mesophyll percentage (%)	80	80	80	80
Chlorophyll A concentration (g/cm^3)	0.00290	0.00277	0.00342	0.003227
Chlorophyll B concentration (g/cm^3)	0.00080	0.00076	0.00120	0.00115
Carotenoids concentration (g/cm^3)	0.00066	0.00063	0.00091	0.00087
Protein concentration (g/cm^3)	0.05793	0.07389	0.06656	0.08490
Cellulose concentration (g/cm^3)	0.05804	0.07403	0.07152	0.09124
Lignin concentration (g/cm^3)	0.00661	0.00844	0.00760	0.00969
Cuticle undulations aspect ratio	10	10	10	10
Epidermal cell caps aspect ratio	5	5	5	5
Spongy cell caps aspect ration	5	6	5	6

Table 3: Summary of ABM-U parameters employed in the characterization of the maize specimens considered in this investigation.

We remark that, as the angular distribution of light transmitted through plant leaves increases, the probability of light absorption also increases due to the detour effect¹⁸. 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^{38,39}) can reduce the probability of light absorption⁴⁰. In this situation, light can be propagated without encountering these organelles, a phenomenon known as the sieve effect^{39,41}. 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 absorbers¹⁸ and on the angular deviations of light travelling in the mesophyll tissue²⁷.

In order to account for the inverse angular relationship of the sieve and detour effects, ABM-U adjusts the ray propagation angle using a bound derived from applied optics experiments²⁷. Accordingly, we kept this bound in place (see sieve effects checkbox in Figure 1) during the experiments involving fresh specimens. For the experiments involving the corresponding *in vitro* and *in vivo* wilted specimens, we employ the same modified biophysical datasets (Table 3) and simulation parameters for both, with the exception of this bound, which we removed for the latter. The removal of this bound resulted in sieve effects not being considered for these specimens. This choice was based on the putative intensification of the detour effects caused by a more homogeneous intracellular distribution of mesophyll chloroplasts in response to water stress⁷.

Finally, we applied the law of Gladstone and Dale⁷ to obtain the spectral refractive index of mesophyll cell walls after the water content reduction. Although this adjusted index of refraction was considered in the simulations presented in this paper for completeness, we note that its impact in the modeled results was minor.

3. RESULTS AND DISCUSSION

Initially, we computed the subsurface reflectance and transmittance for the three groups of specimens. As it can be observed in Figure 2, while the subsurface reflectance of the *in vitro* wilted specimens has increased in comparison with the subsurface reflectance of the fresh specimens, a behaviour consistent with the *in vitro* experiments performed by Vanderbilt *et al.*²³, the subsurface reflectance of the *in vivo* wilted specimens has decreased, a behaviour consistent with the *in vivo* experiments performed by Maracci *et al.*³. On the other hand, as it can be observed in Figure 3, the transmittance of both groups of wilted specimens, *in vitro* and *in vivo*, has increased in comparison with the transmittances computed for the fresh specimens. These observations indicate that subsurface reflectance alone cannot be used in RWC estimations since some species, like maize, may adapt to moderate water stress conditions. Such an adaptation, in turn, may result in a decrease in the amount of reflected light instead of an increase. These different reflectance variation patterns may render remote RWC estimations unreliable. Hence, in order to detect and monitor moderate water stress conditions before the damage to the plants' photosynthetic apparatus becomes irreversible, it may be necessary to acquire supporting ground-based information, including both reflectance and transmittance data.

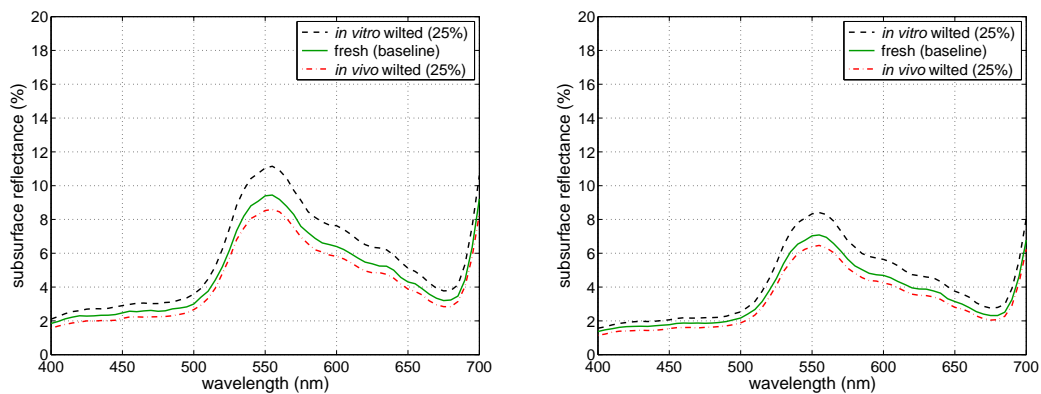


Figure 2: Subsurface reflectance spectra computed for the maize leaf specimens A (left) and B (right) considering them in fresh and wilted states.

At a first glance, it might seem that the use of transmittance readings could be sufficient for obtaining reliable RWC estimations since this spectral quantity has increased for both groups of wilted specimens. However, it is

important to note that significant quantitative transmittance variations can be observed even among different fresh specimens belonging to the same plant species as illustrated by the spectral curves presented in Figure 3. Ideally, one should employ as an indicator of moderate water stress a quantity whose interpretation would not be significantly affected by intra-species biophysical variations. Accordingly, we proceeded to explore the use of subsurface reflectance (R_d) to transmittance (T) ratios.

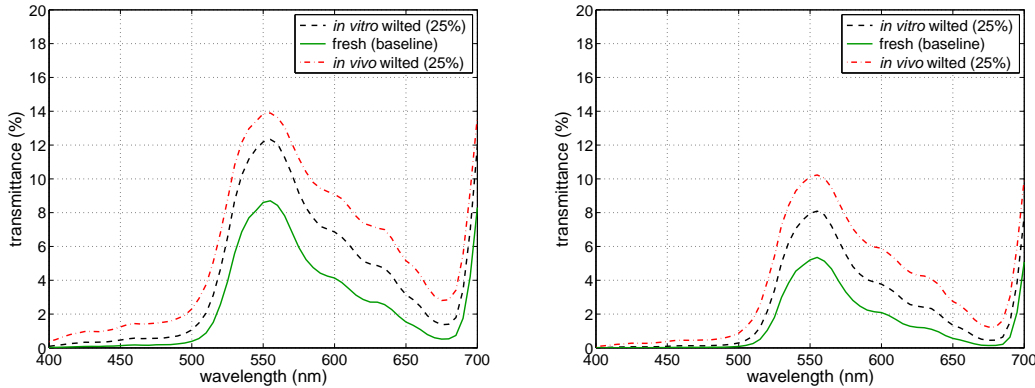


Figure 3: Transmittance spectra computed for the maize leaf specimens A (left) and B (right) considering them in fresh and wilted states.

The R_d/T curves computed for the different groups of specimens are depicted in Figure 4. As expected, the same qualitative trends can be observed in the curves computed for the two different groups of maize leaf specimens (A and B). However, one can also observe significant quantitative differences, notably in regions characterized by low transmittance values. These quantitative inconsistencies make R_d/T values computed for these regions poor indicators of moderate water stress. In addition, it is important to consider that small errors that may occur during the acquisition of transmittance data may lead to larger errors in the computation of the R_d/T values for these regions.

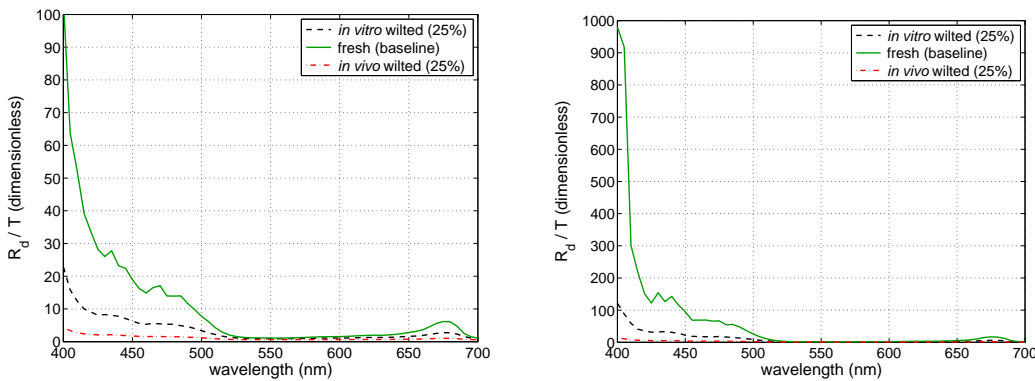


Figure 4: Ratios of subsurface reflectance (R_d) to transmittance (T) computed for the maize leaf specimens A (left) and B (right) considering them in fresh and wilted states.

Possible errors during the acquisition of transmittance data are less likely to affect the computation of the R_d/T values around $555nm$ since this region is characterized by relatively large subsurface reflectance and transmittance values. In addition, a closer examination of the R_d/T values within this region reveals a consistent trend for both groups of specimens. More specifically, at $555nm$, the R_d/T ratio approaches 0.6 for the two distinct *in vivo* wilted specimens as depicted in Figure 5. This suggests that the R_d/T ratio at $555nm$ can be employed as an indicator of moderate water stress in maize, and possibly in other C_4 species with similar characteristics like sugarcane, under *in vivo* conditions. The use of such indicators, in turn, may contribute to the development of new procedures for the reliable estimation of plants' RWC.

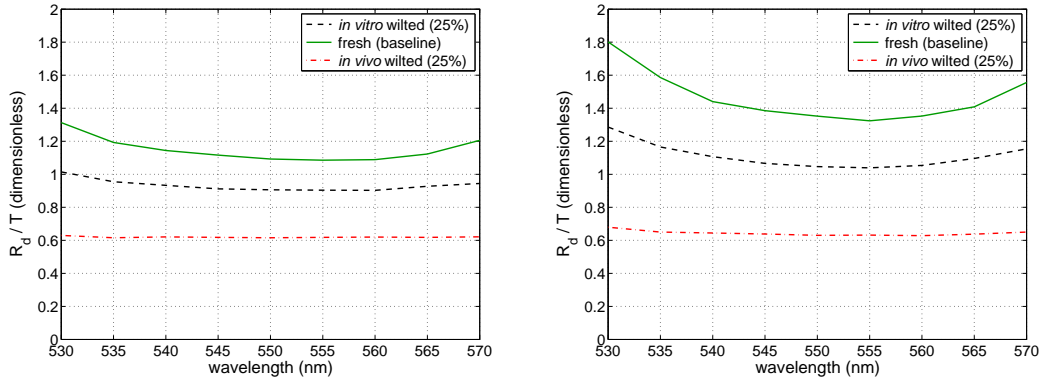


Figure 5: Zoom-in of the spectral R_d/T ratios provided in Figure 4 for the maize leaf specimens A (left) and B (right).

Clearly, the use of a R_d/T value computed at 555nm as a water stress indicator, and possibly in RWC estimations, needs to be further investigated through more comprehensive experiments involving more representative specimens and a wider range of experimental variables. In addition, it is also necessary to examine practical aspects involving the acquisition of subsurface reflectance and transmittance data in field campaigns. For example, since actual subsurface reflectance measurements require the use of optical polarization techniques and hardware²³, one might wonder whether this requirement might render these measurements impractical outside laboratory settings.

It is worth noting that measured foliar spectral data used in remote sensing investigations is usually obtained at low angles of incidence, with values between 2.5° to 15° being commonly found in the literature^{2, 3, 7, 12, 31, 42}. It has been reported that surface reflectance contributions are minor for low angles of incidence close to zero.¹² Moreover, these contributions result from light interactions at the interface between the air and the cuticle wax layer⁴³, whose optical properties are likely to remain unchanged as a result of moderate water stress. The net effect of these aspects is that, for practical purposes, the relatively small surface contributions can be treated as a constant under these conditions. Hence, it may be possible to use total reflectance (R) in order to obtain a reliable moderate water stress estimator using radiometric quantities computed at a low angle of incidence. This would effectively eliminate the need for separating surface and subsurface reflectance components through additional optical equipment. Accordingly, we computed R/T curves in the $530\text{-}570\text{nm}$ range for the different groups of specimens. As it can be observed in the plots presented in Figure 6, at 555nm , this ratio is close to 1.0 for the two distinct *in vivo* wilted specimens.

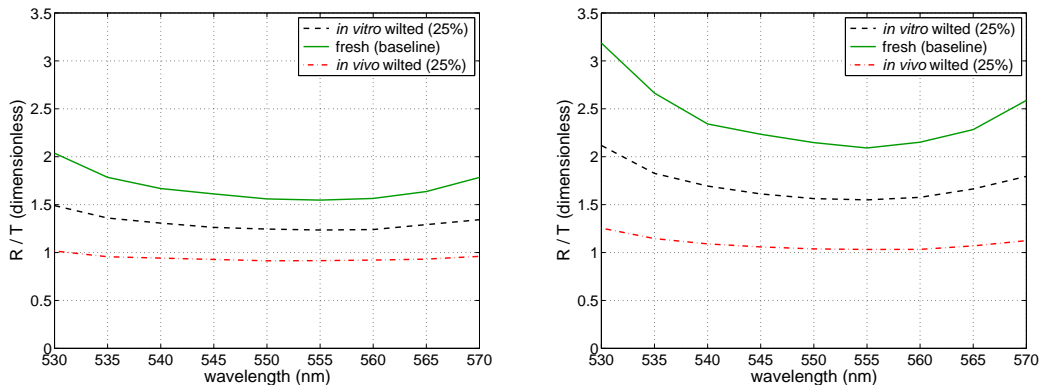


Figure 6: Ratios of total reflectance (R) to transmittance (T), in the $530\text{-}570\text{ nm}$ range, computed for the maize leaf specimens A (left) and B (right) considering them in fresh and wilted states.

In summary, our *silico* experiments indicate that unreliable assessments of water status may result from overlooking the adaptation mechanisms developed by certain plants, notably C_4 species like maize, to cope with adverse environmental conditions. This is particularly relevant if one aims to prevent an irreversible damage to their photosynthetic apparatus, which requires taking action before the plants' water losses exceed the moderate to severe threshold. We remark that these adaptation mechanisms, developed to increase a plant's chance of survival, can affect its spectral signature.

4. CONCLUSION AND FUTURE WORK

In this work, we have revisited the visible spectral responses of maize specimens to moderate water stress, under both *in vitro* and *in vivo* conditions, in order to explore alternatives for the early detection and monitoring of situations affecting their RWC. The results of our *in silico* experiments suggest that the subsurface reflectance to transmittance ratio and the total reflectance to transmittance ratio, both measured at 555nm , may serve as indicators of moderate water stress in C_4 plants under *in vivo* conditions and contribute to the development of reliable and cost-effective procedures for RWC estimation. Although further research is necessary to assess the full potential of using leaf spectral responses in the visible domain to detect and monitor water stress conditions, this possibility is tangible and it may be less constrained by practical issues, such as the use of polarization optics, than it was originally expected.

As future work, we plan to extend our *in silico* experiments to a wider range of specimens in order to assess the generality of the proposed water stress indicator. We also intend to investigate whether the polarization optics requirement can be effectively relaxed by considering the relative impact of increasing angles of incidence to the consistency of the proposed water stress indicators.

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REFERENCES

- [1] Gutiérrez, R., "System biology for enhanced plant nitrogen nutrition," *Science* **336**, 1673–1675 (2012).
- [2] Thomas, J., Namkem, L., Oerther, G., and Brown, R., "Estimating leaf water content by reflectance measurements," *Agronomy Journal* **63**, 845–847 (1971).
- [3] Maracci, G., Schmuck, G., Hosgood, B., and Andreoli, G., "Interpretation of reflectance spectra by plant physiological parameters," in [*International Geoscience and Remote Sensing Symposium - IGARSS'91*], 2303–2306 (1991).
- [4] Verdebout, J., Jacquemoud, S., and Schmuck, G., "Optical properties of leaves: modeling and experimental studies," in [*Imaging Spectrometry - a Tool for Environmental Observations*], Hill, J. and Mégier, J., eds., 169–191, Kluwer Academic Publishers, Dordrecht, The Netherlands (1994).
- [5] Earl, H. and Davis, R., "Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize," *Agronomy Journal* **95**, 688–696 (2003).
- [6] Chenu, K., Chapman, S., Hammer, G., Mclean, G., Salah, H., and Tardieu, F., "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**, 378–391 (2008).
- [7] Baranoski, G., Kimmel, B., Chen, T., and Yim, D., "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 (2012).
- [8] Loreto, F., Baker, N., and Ort, D., "Chloroplast to leaf," in [*Photosynthetic Adaptation Chloroplast to Landscape*], Smith, W., Vogelmann, T., and Critchley, C., eds., ch. 9, 231–261, Springer, NY, USA (2004). Part 6: Environmental Constraints, Ecological Studies, Vol. 178.
- [9] Alberte, R. and Thornber, J., "Water stress effects on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplasts of maize," *Plant Physiology* **59**, 351–353 (1977).

- [10] Hendry, G., Houghton, J., and Brown, S., "The degradation of chlorophyll - a biological enigma," *New Phytologist* **107**, 255–302 (1987).
- [11] Lee, W., Searcy, S., and Kataoka, T., "Assessing nitrogen stress in corn varieties of varying color," in [*ASAE Annual International Meeting*], 1–24, ASAE, Toronto, Ontario, Canada (July 1999). Paper 99-3034.
- [12] Woolley, J., "Reflectance and transmittance of light by leaves," *Plant Physiology* **47**, 656–662 (1971).
- [13] Carter, G., "Primary and secondary effects of water content on the spectral reflectance of leaves," *American Journal of Botany* **78**, 916–924 (1991).
- [14] Yokota, A., Takahara, K., and Akashi, K., "Water stress," in [*Physiology and Molecular Biology of Stress Tolerance in Plants*], Rao, K., Raghavendra, A., and Reddy, K., eds., 15–39, Springer, Dordrecht, The Netherlands (2006). chapter 2.
- [15] Schlerf, M. and Atzberger, C., "Imaging spectrometry and vegetation science," in [*Imaging Spectrometry. Basic Principles and Prospective Applications*], van der Meer, F. and de Jong, S., eds., 111–155, Kluwer Academic Publishers, Dordrecht (2001).
- [16] Sato, Y., Wada, M., and Kadota, A., "External Ca^{2+} is essential for chloroplast movement induced by mechanical stimulation but not by light stimulation," *Plant Physiology* **127**, 497–504 (2001).
- [17] Terashima, I. and Saeki, T., "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**, 1493–1501 (1983).
- [18] Vogelmann, T., "Plant tissue optics," *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 231–251 (1993).
- [19] Govender, M., Dye, P., Weiersbye, I., Witkowski, E., and Ahmed, F., "Review of commonly used remote sensing and ground-based technologies to measure plant water stress," *Water SA* **35**(5), 741–752 (2009).
- [20] Liu, L., Wang, J., Huang, W., and Zhao, C., "Detection of leaf and canopy EWT by calculating REWT from reflectance spectra," *ISPRS Journal of Photogrammetry and Remote Sensing* **31**(10), 2681–2695 (2010).
- [21] Dobrowski, S., Pushnik, J., Zarco-Tejada, P., and Ustin, S., "Simple reflectance indices track heat and water stress-induced changes in steady-state chlorophyll fluorescence at the canopy scale," *Remote Sensing of Environment* **97**, 403–414 (2005).
- [22] Baranoski, G., Chen, T., Kimmel, B., Miranda, E., and Yim, D., "On the high-fidelity monitoring of c3 and c4 crops under nutrient and water stress," in [*Asia-Pacific Remote Sensing Conference, Proc. of SPIE, Vol. 8524, Land Surface Remote Sensing*], Honda, D. E. Y., Sawada, H., and Shi, J., eds., 85240W–1–9 (2012).
- [23] Vanderbilt, V., Daughtry, C., and Dahlgren, R., "Relative water content, bidirectional reflectance and bidirectional transmittance of the interior of detached leaves during dry down," in [*IEEE International Geoscience & Remote Sensing Symposium - IGARSS*], (2015). Abstract.
- [24] Li-Ping, B., Gong, S., Da, G., Hui, S., Yan, L., and Sheng, Z., "Effect of soil drought stress on leaf water stress, membrane permeability and enzymatic antioxidant system of maize," *Pedosphere* **16**(3), 326–332 (2006).
- [25] Minorsky, P., "Achieving the *in silico* plant. Systems biology and the future of plant biological research," *Plant Physiology* **132**, 404–409 (2003).
- [26] Baranoski, G., "Modeling the interaction of infrared radiation (750 to 2500 nm) with bifacial and unifacial plant leaves," *Remote Sensing of Environment* **100**, 335–347 (2006).
- [27] Baranoski, G. and Eng, D., "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**, 2593–2599 (2007).
- [28] Krishnaswamy, A. and Baranoski, G., "A biophysically-based spectral model of light interaction with human skin," *Computer Graphics Forum* **23**(3), 331–340 (2004).
- [29] Baranoski, G. and Rokne, J., [*Light Interaction with Plants: A Computer Graphics Perspective*], Horwood Publishing, Chichester, UK (2004).
- [30] Baranoski, G., Rokne, J., and Xu, G., "Virtual spectrophotometric measurements for biologically and physically-based rendering," *The Visual Computer* **17**(8), 506–518 (2001).
- [31] Hosgood, B., Jacquemoud, S., Andreoli, G., Verdebout, J., Pedrini, G., and Schmuck, G., "Leaf optical properties experiment 93," Tech. Rep. Report EUR 16095 EN, Joint Research Center, European Commission, Institute for Remote Sensing Applications (1995).

- [32] NPSG, *Run ABM-U Online*. Natural Phenomena Simulation Group, University of Waterloo, Canada (2011). <http://www.npsg.uwaterloo.ca/models/ABUB.php>.
- [33] Baranoski, G., Dimson, T., Chen, T., Kimmel, B., Yim, D., and Miranda, E., “Rapid dissemination of light transport models on the web,” *IEEE Computer Graphics & Applications* **32**(3), 10–15 (2012).
- [34] NPSG, *Plant Leaf Data*. Natural Phenomena Simulation Group, University of Waterloo, Canada (2011). <http://www.npsg.uwaterloo.ca/data/leaves.php>.
- [35] Vogelmann, T. and Martin, G., “The functional significance of palisade tissue: penetration of directional versus diffuse light,” *Plant, Cell and Environment* **16**, 65–72 (1993).
- [36] Bowes, B., [*A Colour Atlas of Plant Structure*], Manson Publishing Ltda (1996). pages 97-116.
- [37] Woolley, J., “Change of leaf dimensions and air volume with change in water content,” *Plant Physiology* **41**, 815–816 (1973).
- [38] Rabinowitch, E., “Light absorption by pigments in the living cell,” in [*Photosynthesis and Related Processes*], **2**, 672–739, Interscience Publishers, Inc. (1951). Part 1.
- [39] Evans, J., Vogelmann, T., and Williams, W., “Chloroplast to leaf,” in [*Photosynthetic Adaptation Chloroplast to Landscape*], Smith, W., Vogelmann, T., and Critchley, C., eds., ch. 2, 15–41, Springer, NY, USA (2004). Part 2: Sunlight Capture, Ecological Studies, Vol. 178.
- [40] Björn, L., “Interception of light by leaves,” in [*Crop Photosynthesis: Spatial and Temporal Determinants*], Baker, N. and Thomas, H., eds., ch. 11, 253–276, Elsevier Science Publishers B. V. (1992).
- [41] Fukshansky, L., “Optical properties of plants,” in [*Plants and the Daylight Spectrum*], Smith, H., ed., 21–40, Academic Press, London (January 1981).
- [42] Al-Abbas, A., Barr, R., Hall, J., Crane, F., and Baumgardner, M., “Spectra of normal and nutrient-deficient maize leaves,” *Agronomy Journal* **66**, 16–20 (1974).
- [43] Vanderbilt, V., Grant, L., and Ustin, S., “Polarization of light by vegetation,” in [*Photon-vegetation interactions: Applications in optical remote sensing and ecology*], Nynemi, R. and Ross, J., eds., 191–228, Springer Verlag, Berlin, Germany (1991).