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Non-destructive detection of water stress and estimation of relative water content in maize

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[1] Non-destructive estimation of leaf water content provides vital information about vegetation productivity. We report here on controlled seven day experiments using greenhouse-grown maize. Fifty plants were randomly assigned to two equal groups: water stressed and well watered. Spectroscopic, relative water content (RWC), and chlorophyll concentration measurements were made daily. Because water molecules absorb radiation in near- and middle-infrared, most efforts to sense water deficit remotely utilize infrared wavelengths. In these experiments, we identified a strong, systematic, and repeatable relationship between photosynthetically active radiation (PAR, 400–700 nm) albedo and leaf RWC. We show that visible spectrum reflectance provides a means to detect early stages of plant stress and estimate leaf RWC. **Citation:** Zygielbaum, A. I., A. A. Gitelson, T. J. Arkebauer, and D. C. Rundquist (2009), Non-destructive detection of water stress and estimation of relative water content in maize, *Geophys. Res. Lett.*, 36, L12403, doi:10.1029/2009GL038906.

1. Introduction

[2] Virtually all life on Earth depends upon photosynthesis. Absorbed photons provide energy and water facilitates electron transport reactions for photosynthesis. Water enables the incorporation of CO₂ carbon into carbohydrates. It also provides the hydraulic pressure required for opening stomata and maintaining structural integrity. Thus, estimating leaf water content is important in determining the health and productivity of vegetation. This information is vital for climate studies, carbon cycle research, and maximizing agricultural irrigation efficiency.

[3] Water molecules absorb radiation in near- and mid-infrared (NIR and MIR) wavelengths near 970, 1240, 1400, and 1900 nm. Because the amount of absorption is related to total water content, these wavelengths are traditionally used in non-destructive and remote estimation of vegetation water content [e.g., *Ceccato et al.*, 2001; *Hunt and Rock*, 1989]. However, infrared reflected radiation is affected greatly by plant architecture, vegetation density and leaf structure, thus increasing estimation uncertainty [*Elachi*, 1987].

[4] Several authors have documented an increase in reflectance in the visible spectrum when plants become

stressed [*Carter*, 1991, 1993; *Yu et al.*, 2000; *Ceccato et al.*, 2001; *Aldakheel and Danson*, 1997]. However, this effect has never been quantified and employed to retrieve vegetation water content.

[5] In this paper, we report the results of experiments designed to (a) study the optical properties of water-stressed and non-stressed maize leaves using visible, NIR and MIR reflectance spectroscopy, (b) establish a relationship between relative water content (RWC) in leaves and visible spectrum reflectance, and (c) develop a non-destructive technique using visible spectrum reflectance to accurately estimate leaf RWC.

2. Methods

[6] For each of the two experiments, fifty maize plants (DeKalb DKC 63–46) were grown in a greenhouse. No artificial illumination was used. Seeds were planted in a mixture of 1/3 peat moss, 1/3 greenhouse soil (silty clay loam) and 1/3 perlite by volume in single pots. The pot size was 7.6 liters (approx. 0.22 m diameter by 0.20 m height). Fertilizer was applied to ensure nutrient sufficiency. Plants emerged approximately eight weeks prior to initiation of the experiments. Phenologically, the plants were V18 to VT [*Ritchie et al.*, 1997] during both experiments. The first experiment was conducted in late April/early May 2008, the second in mid-February 2009. Ancillary data, including outside and inside greenhouse temperature and humidity and outside visible and NIR downwelling irradiance, were recorded.

[7] Half of the plants were randomly selected for treatment. Treated plants were not watered during the course of the seven days. The remaining plants, the untreated (control) plants, were watered daily after reflectance measurements. Sufficient water was applied to assure the soil was at field capacity. Several plants were randomly selected as buffer plants, placed at the end of each row of pots, and not used for measurement.

[8] Reflectance measurements using a hyperspectral ASD FieldSpec Pro radiometer (350 to 2500 nm) and a self-illuminated leaf probe were performed each afternoon at 3 pm. Data were interpolated to 1 nm spectral resolution. Calibration was performed using a 99% reflective Spectralon reference panel. Adaxial leaf reflectance measurements were made approximately 2 cm from the plant stem. Optically absorbing foam (spectrally flat 4% reflectance) was placed behind the leaf. The three leaves measured per plant included the middle leaf (“mid” – most likely to become the ear leaf), the leaf positioned two above the middle leaf (“top”), and the leaf positioned two below the middle leaf (“bottom”).

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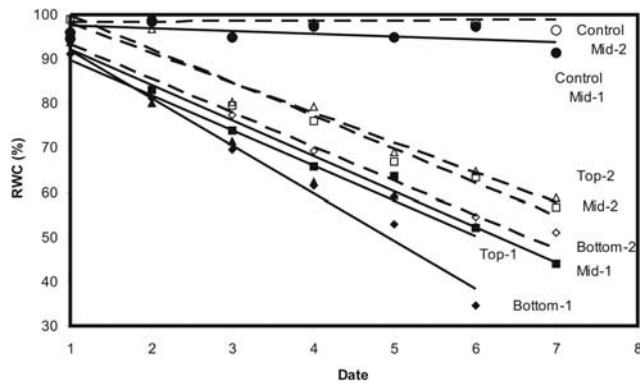


Figure 1. Relative water content (RWC) plotted versus date: bottom (diamonds), middle (squares), and top (triangles) leaves of two treated plants, and middle leaf (circle) of two control plants. Closed symbols denote first experiment; open symbols denote second experiment. Lines are the best fit functions. The graph represents 28 separate plants for each experiment.

[9] Following the reflectance measurements, two treated and two control plants were randomly selected for gravimetric determination of RWC. Ten 1.0 cm leaf punch samples were taken from each of the leaves (cited above) on each plant. Punches were quickly sealed into pre-weighed vials. Differencing the filled vial weights from the empty weights provided the fresh weight (FW). The vials were filled with distilled water and refrigerated in darkness at 5 °C for 15 hours to allow sample rehydration. The punches were then removed from the vials, patted surface dry, and weighed, providing full turgor weight (TW). Next, the punches were placed into open vials and heated in an oven at 105 °C for 24 hours. Upon removal, the vials were immediately sealed and weighed. The contents were discarded and the empty vials weighed. The difference between weights provided the dry weight (DW). RWC (%) was calculated using the formula: $RWC = 100[(FW-DW)/(TW-DW)]$. This was used as a proxy for the RWC in all of the plants at each temporal point in the experiment. Due to destructive procedures, sampled plants were discarded.

[10] In the second experiment, additional punches were taken from one treated and one control RWC sample plant to determine chlorophyll content analytically [Lichtenthaler, 1987].

3. Results and Discussion

[11] During both experiments, RWC in control plant leaves remained above 90%; mean RWC was 95.8% and 98.7% with coefficients of variation (CV) of 2.9 and 3.3% for experiments 1 and 2, respectively. Leaves from the treated plants showed linearly decreasing RWC during the treatment period (Figure 1). Coefficients of determination, R^2 , were higher than 0.96 for all leaves but the top leaf in the second experiment ($R^2 > 0.9$). During the first experiment, RWC decreased from more than 90% to below 50%. In the second, the minimum RWC was 50% in the bottom leaf, and above 60% in the top and middle leaves. Differing ranges of RWC in the two experiments can be attributed to variable environmental conditions. While greenhouse temperature averaged near 28°C for both periods, the humidity and downwelling irradiance were, appropriate to the season, different. During the first experiment, downwelling irradiance averaged 670 Wm^{-2} , and greenhouse relative humidity averaged 27%. For the second experiment, the average irradiance was 463 Wm^{-2} and the average relative humidity 42%.

[12] The spectra for the eight treated and eight control plants in the first experiment were separately averaged. The mean reflectance of the treated leaves (Figure 2a) increased nearly monotonically throughout the visible (400–750 nm) and MIR (1400–2500 nm) spectrum. The coefficient of variation had four pronounced peaks: in the visible spectrum (reaching 30%), near 1450 nm (>25%), near 2000 nm (>35%) and at 2500 nm (>35%). CV was minimal in the NIR (750 to 1300 nm), near 1650 nm, and around 2200 nm (Figure 2a). The reflectances of control plant leaves were virtually invariant with maximal CV below 5% (Figure 2b for middle leaves). The outcome of the second experiment was consistent with these results.

[13] PAR albedo in all treated plant leaves increased significantly during both experiments. Mid leaf albedo increased from 6.5% to 10.9% during experiment 1 and

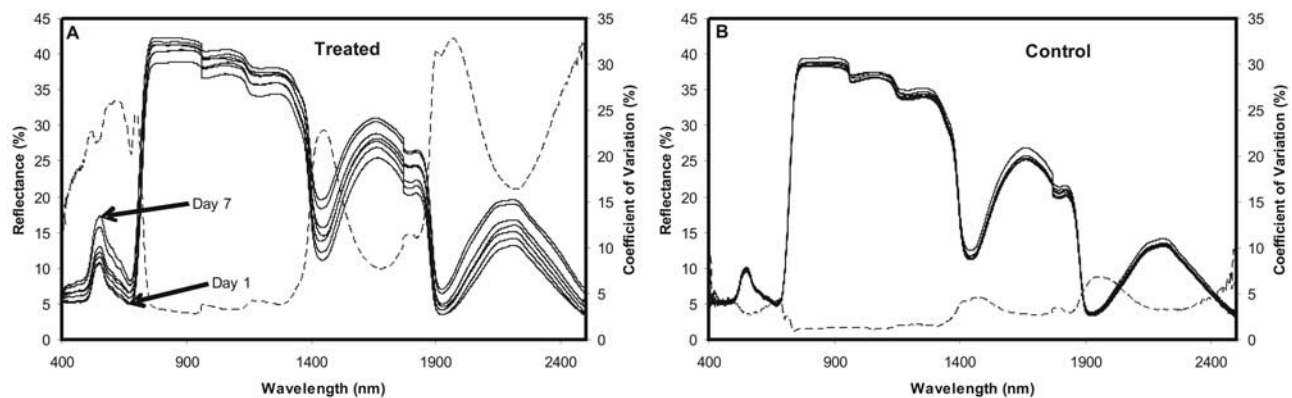


Figure 2. Reflectance spectra for (a) treated and (b) control leaves. The spectra (solid lines) represent the averages of reflectances of eight middle leaves from the first experiment. Treated plants were not watered during the seven days and exhibited increasing reflectance from day 1 through day 7. Control plants were watered each afternoon. Coefficients of variation (dashed lines) are also presented.

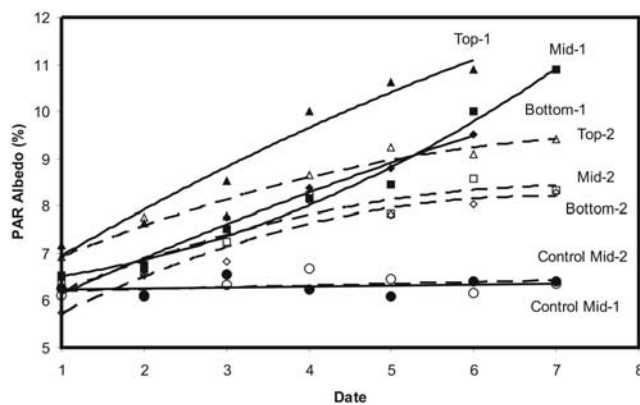


Figure 3. Average PAR albedo of leaves from treated and control plants plotted versus time. The bottom (diamonds), middle (squares), and top (triangles) leaves of two treated plants are shown along with the middle (circles) leaves of two control plants. Closed symbols denote first experiment; open symbols denote second experiment. The points represent the daily average of PAR albedo for eight separate treated plants not watered during each seven-day experiment and eight separate control plants watered daily.

from 6.3% to 8.3% in experiment 2. The daily albedo CV among the eight leaves measured in each experiment was below 10% and varied non-systematically over the experiment period (Figure 3).

[14] In both experiments, total chlorophyll content (sum of chlorophyll *a* and *b*) remained virtually unchanged in control plant leaves and, during days 1 through 4, in treated plant leaves (CV < 5%). However, during days 4 through 7, chlorophyll in treated plant top and bottom leaves decreased 7–9% and in middle leaves decreased about 15%. This decrease affected reflectance only in the green (around 550 nm) and in the red edge, around 700 nm [Gitelson *et al.*, 2003]. Analysis of the relationship between reflectance and chlorophyll content in maize leaves [Gitelson *et al.*, 2003; Ciganda *et al.*, 2009] indicated that none of the observed increase in albedo between days 1 and 4 and less than half of the observed increase in albedo between days 4 and 7 can be attributed to a change in chlorophyll content.

[15] Thus, PAR albedo is a sensitive indicator of water stress in maize. We also established a quantitative relationship between PAR albedo and leaf RWC (Figure 4). PAR albedo can, therefore, be used as an accurate proxy for RWC in maize leaves.

[16] Reflectance systematically increases with decreasing RWC at all PAR wavelengths (λ). However, the increase is not uniform (note CV spectrum in Figure 2a). Several approaches can be used to exploit this characteristic to detect water stress and estimate RWC. First, one can use the reflectance in specific spectral regions in blue (400 to 500 nm) and red (650–700 nm), where pigments absorb maximally. Reflectance in these spectral regions correlates highly ($R^2 > 0.95$) with RWC.

[17] Second, a model for RWC retrieval can be developed using a normalized difference of the form $[\rho(\lambda_1) - \rho(\lambda_2)] / [\rho(\lambda_1) + \rho(\lambda_2)]$, where λ_1 and λ_2 are in the visible spectrum. The accuracy of the RWC estimation is higher when λ_1 is located closely to λ_2 . For example, for $\lambda_2 = 570$ nm, as in the Photochemical Reflectance Index (PRI) [Gamon *et al.*,

1992], when the position of λ_1 is moved from 500 to 568 nm or from 660 to 575 nm, maximal $R^2 > 0.95$ were observed for λ_1 at 565 and 575 nm. No spectral feature gives an advantage to $\lambda_1 = 530$ nm as in PRI. Thus, setting $\lambda_1 = 540$ and $\lambda_2 = 560$ nm (both with 10 nm bandwidth), for example, results in a model for estimating RWC that is insensitive to chlorophyll content.

[18] Third, differences of reciprocal reflectances, $\rho^{-1}(\lambda_1) - \rho^{-1}(\lambda_2)$ [Gitelson *et al.*, 2003], relate very closely to RWC and can be used as proxies. As above, R^2 was higher with λ_1 located closely to λ_2 .

[19] Fourth and finally, a model can be based on the non-uniform behavior of reflectance in the visible range. An iterative search algorithm to identify models with minimal RMSE, was applied to the reflectance spectra in the PAR region and RWC data for the middle leaves in the first experiment. Several accurate models were found to estimate RWC. As an example, in Figure 5, the ratio of average reflectance in the range 515 to 525 nm and 715 to 725 nm was plotted versus RWC. The linear regression equation parameters were used to validate the R_{520}/R_{720} model by comparing measured and predicted RWC for all remaining leaves in both experiments. The standard error of RWC prediction was less than 8% despite differing environmental conditions in the two experiments, and differing RWC and albedo characteristics among the leaves. This model is insensitive to chlorophyll content above 200 mg m⁻² (in green to dark-green leaves).

[20] The spectral signatures upon which these findings are based result from changes in leaf anatomy and/or physiology driven by water deficit. Photoprotection mechanisms invoked to prevent damage to photosynthetic processes and structures as well as photodamage itself are likely causes [Kasahara *et al.*, 2002; Björkman and Powles, 1984; Long *et al.*, 1994; Ristic and Cass, 1992; Utrillas and Alegre, 1997]. Changes in indexes of refraction in vacuoles due to increasing solute concentration and surface changes in cellular structural elements may also

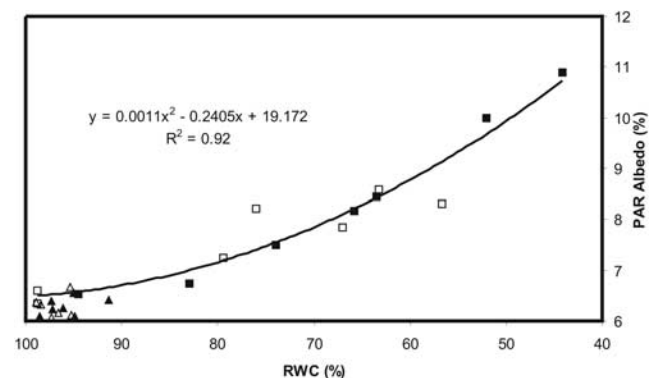


Figure 4. PAR albedo plotted versus relative water content (RWC). Square symbols represent the average PAR albedo from the middle leaf of treated plants not watered during the seven-day experiments. Triangle symbols represent the average PAR albedo from the middle leaf of control plants watered daily. Closed symbols indicate first experiment, open symbols indicate second experiment. The solid line is the quadratic best fit function for all treated plant leaves.

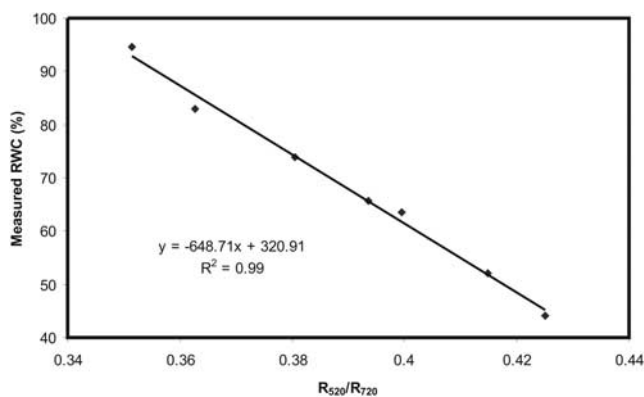


Figure 5. Measured RWC in treated middle leaves (first experiment) plotted versus the ratio of reflectances at 520 and 720 nm with 10 nm bandwidth.

play a role. These hypotheses should be investigated during future experiments.

4. Conclusions

[21] We studied the optical properties of water-stressed and non-stressed maize leaves using reflectance spectroscopy in two independent controlled tests. An immediate, consistent and statistically significant increase in visible spectrum reflectance of stressed leaves was detected and documented. We found a strong and repeatable relationship between PAR albedo and leaf RWC. Our data indicated a 70% increase in the amount of reflected PAR light as maize plants became increasingly water stressed (50% RWC). PAR albedo was shown sensitive to very early stages of plant water stress. To the best of our knowledge, this is the first report identifying the systematic nature of the quantitative relationship between PAR albedo and RWC. Further, we have demonstrated that RWC can be retrieved using a model based on non-destructive reflectance measurements, for example, the ratio of reflectances at 520 and 720 nm.

[22] Our results quantify the increasing reflectance in visible spectrum caused by water stress. Although our results are based on greenhouse-grown maize at a particular point in its phenology, a number of studies [e.g., Carter, 1991, 1993; Yu *et al.*, 2000; Ceccato *et al.*, 2001; Aldakheel and Danson, 1997] qualitatively reported such effects in other species. Thus, our findings should be generalizable (perhaps with parametric adjustments) to other phenological states and species.

[23] The significance of our findings is twofold. First, we have identified a proxy for plant water stress based on the PAR spectrum. Second, the PAR spectrum plays an important role in surface/air energy interchange. The water deficit

induced increase in PAR albedo has, to our knowledge, not been recognized or applied in climate studies or models.

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