Foliar spectral properties following leaf clipping and implications for handling techniques

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Abstract

After leaves are clipped their reflectance properties change over time at variable rates. Spectral change can in part be attributed to the changing water content of the leaf, which affects absorption in the VIS, NIR and the SWIR. Maintaining water volume within samples has been the motivation behind many leaf handling techniques. This study has assessed the time constraints between leaf collection and spectral measurement. Specifically the relationship between leaf water content and foliar spectra (350–2500 nm) was examined over time for five tropical trees (common guava (*Psidium guajava*), purple guava (*Psidium littorale*), weeping fig (*Ficus benjamina*), floss silk (*Chorisia speciosa*), and coffee (*Coffea arabica*)). This investigation was carried for leaves wrapped with moist gauze around their petiole (*treatment leaves*) and leaves with no treatment. Spectral measurements and mass measurements were repeated for each leaf once every hour for the first 12 h, then every 4–6 h for 18 h, followed by one measurement after 12 h, and finally once a day until the control samples became air-dry. Foliar reflectance in the visible spectrum was not immediately responsive to water content changes and did not change until wilting of the leaf was observed. The NIR and SWIR wavelength regions were affected immediately by small changes in water content. Thus, by the time wilting was first observed the NIR and SWIR foliar reflectance differed considerably from corresponding fresh leaf reflectance. No common time limit could be observed for leaf clipping and reflectance measurement. Leaves have a variety of water contents and dehydration rates hence measurement time constraints are dependent on the properties of the leaf or species. Rather than using a time limit it is recommended that leaf handling techniques be based upon managing leaf water content and leaf structure. The results of this study indicate that leaves with petioles wrapped in moist paper towel and placed within plastic bags will maintain leaf reflectance longer than equivalent leaves without treatment; samples tested here lasted a minimum of 7 days. θ and D indices ("angle difference" and "root mean square difference", respectively) revealed a stronger relationship between leaf water content and spectral shape than between leaf water and raw reflectance magnitude. The ratio of 1187/1096 nm, when compared with θ and D indices and individual reflectance bands, showed the highest coefficient of determination with leaf water content ($r^2 = 0.952$).
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1. Introduction

Remote sensing of forest canopies has possibilities for many practical applications such as assessing biomass, drought, stress and canopy chemistry (Peñuelas & Filella, 1998; Turner et al., 2003). Traditionally, in-depth tree canopy spectral analysis starts with the analysis of leaf spectral data (Asner, 1998).

Leaves contribute the most to canopy reflectance compared to background soil/litter reflectance or branch reflectance (Asner, 1998; Guyot et al., 1989). Due to the role leaves have in canopy reflectance many researchers have studied the relationships between leaf reflectance and biochemical or biophysical properties of leaves as part of scaling-up efforts (Blackburn, 1998; Buschmann & Nagel, 1993). Modelling canopy and leaf spectral properties has been a strategy for researching these systems (Dawson et al., 1998; Zarco-Tejada et al., 2004).

Whether being used as input to canopy models or for comparisons to canopy spectra acquired from remote platforms,

leaf spectra must be measured in situ or in a fresh state. Field measurements of in situ leaf reflectance are often impractical due to poor or highly variable lighting conditions and inaccessibility of portable spectral equipment. As an alternative, leaves are transported within variable time periods to the laboratory where measurements are performed (Asner, 1998; Horler et al., 1983). A benefit of laboratory environments is that leaves can be placed in the dark before measurement, which standardizes the light environment and reduces photochemical effects such as xanthophyll cycle pigment changes, and ultrastructural changes such as chloroplast movement, caused by variable light conditions (Gamon & Surfus, 1999; Haupt & Scheuerlein, 1990). A problem associated with this approach is that after leaves are clipped their reflectance properties change over time at variable rates (Hunt & Rock, 1989). Spectral change can in part be attributed to the water loss of the leaf, which affects absorption in the NIR and the SWIR (Carter, 1991; Hunt & Rock, 1989; Ripple, 1986). Carter (1991) outlined the theoretical "primary and secondary" effects of water content on leaf reflectance, where primary effects are direct water absorptions corresponding to the water absorption coefficient (Curcio & Petty, 1951) and secondary effects involve scattering properties of the leaf and efficiency of other absorbers (pigments) within the leaf. Carter (1991) tested his hypotheses by demonstrating that the spectral effects caused by drying out a *Liquidamber styraciflua* (sweetgum) leaf can be reversed by rehydrating it. He found that leaf rehydration ability was species dependent.

Maintaining water volume within samples has been the motivation behind many leaf handling techniques. Horler et al. (1983) for example, supplied water to samples and placed leaves within plastic bags to effectively maintain a humid microenvironment that reduces the vapour pressure gradient between leaf and air and reduces leaf dehydration. Other techniques include cutting leaves under water and cooling leaves. Cutting underwater has been shown to prolong photosynthesis in branches for up to 20 min (Clark, 1954) and may prolong freshness. The practice of artificially cooling/ chilling leaves is intended to reduce transpiration and has been applied by many authors including Lacaze and Joffre (1994), Cao (2000), and Sims and Gamon (2003). Richardson and Berlyn (2002) have attempted to assess dehydration effects on leaf spectral measurements at the branch scale and establish a methodology to minimize dehydration occurring between the sampling and measurement time. They concluded that the rate of spectral change was related to the ability of samples to retain water and that water loss can be reduced effectively with moist paper towel and plastic bags as a means to reduce transpiration.

This study builds on the findings of Richardson and Berlyn (2002) through improvements in experimental procedures and data analysis. Specifically, we increased the spectral range from between 306 nm and 1138 nm to between 350 nm and 2500 nm in order to correspond better with remote hyperspectral data from sources such as Hyperion (Pearlman et al., 2003; Vane & Goetz, 1993) that have broad spectral ranges and fine resolution. This study also utilized the full range of the reflectance data collected using Price's (1994) θ and D indices,

while Richardson and Berlyn (2002) limited their analysis to the Normalized Difference Vegetation Index, Chlorophyll Normalized Difference Index, red edge position and Photochemical Reflectance Index. As we were interested in direct relationships between water content and leaf spectral reflectance, unlike Richardson and Berlyn (2002), we measured the water content of our samples. The temporal resolution of the data points was increased from a minimum 12 h time interval to hourly in order to observe quicker rates of change in leaves. Finally, we attempted to reduce the intra- and inter-leaf variability in our data by limiting measurements to specific leaf spots; Richardson and Berlyn (2002) measured several leaves on several branches.

We endeavoured to assess time constraints between leaf collection and measurement. Furthermore we hypothesized that leaves cleanly cut across the petiole, supplied with water and placed in plastic bags will have consistent leaf reflectance longer than leaves without this treatment, at least for the species presented in this study. Leaf collection, for posterior laboratory spectral measurement, represents a daily and routine operation for some aspects of multi-scale research. Information presented in this paper aims to contribute to the development of standardized approaches for leaf handling, facilitating data sharing and data comparison within the SpecNet international association of collaborators. The examples shown are for leaves found in the tropics but their relevance is not limited to the tropics.

2. Methods

2.1. Sample acquisition

Leaf samples were chosen from five tropical trees at the Muttart Conservatory in Edmonton, Canada. The common guava (*Psidium guajava*), purple guava (*Psidium littorale*), weeping fig (*Ficus benjamina*), floss silk (*Chorisia speciosa*), and coffee (*Coffea arabica*) trees were selected because of their abundant leaves, and height (>1.5 m) within the conservatory, and their widespread distribution in tropical areas (Doggett & Parker, 2001).

A single branch from each tree was cut and transported to the laboratory within an hour. Two leaves were then cut from a branch, measured for reflectance and weighed. One leaf, herein referred to as the *treatment leaf*, was wrapped with moist gauze around its petiole and placed in a plastic bag. The other leaf, called the *control leaf*, was set upon the bench with no treatment. This process was repeated for each tree branch resulting in 5 control leaves and 5 leaves treated with water.

Spectral measurements and mass measurements were repeated for each leaf once every hour for the first 12 h, then every 4 or 6 h for 18 h, followed by one measurement after 12 h, and finally once a day until the control samples became air-dry. Control and treatment samples were left in the dark between measurements. The samples were deemed air-dry when the leaf weight had stabilized and leaves appeared brittle and lacked discernable change. Following the spectral measurements the leaves were oven dried for 48 h at 50 °C then weighed.

2.2. Leaf water content

Equivalent water thickness (EWT, water mass/leaf area) is a measure of absolute water content (Ceccato et al., 2001) and thus was deemed appropriate for comparing water content amongst several species. EWT was chosen over the relative water content scale (RWC; Ceccato et al., 2001) as the leaves were not saturated with water (turgor) following excision, a requirement for measuring RWC. Various definitions of EWT are discussed by Downing et al. (1993).

2.3. Histological process

Two fresh leaves of each species were collected in order to create images of microscopic leaf cross-sections. One of each set of leaves was allowed to air-dry until it began to show visible signs of wilting; this state will be referred to as "partially dried". Small cutouts of the fresh leaves and partially dried leaves were immediately treated with botanical histology practices described by Johansen (1940) and Purvis et al. (1966). The fixative used was formalin-aceto-alcohol. The dehydration and embedding processes were performed within the Fisher Model 166 Histomatic Tissue Processor using ethanol solutions, toluene, and paraffin wax. Paraffin blocks were sliced into layers 5 μm in thickness using a microtome and then mounted on slides using albumen. The paraffin was removed from the slides with toluene and the slides were stained using Harris' haematoxylin. Lastly, slip-covers were mounted with DPX (Distyrene, Plasticiser and Xylene Mixture). Pictures of the completed slides were taken using a light microscope (20× magnification) and a digital camera (Nikon DXM 1200 CCD).

2.4. Instrument set-up and reflectance measurements

Spectral measurements were made using an Analytical Spectral Devices Fieldspec FR spectrometer, which operates from 350 to 2500 nm, where full width half maximum is 3 nm at 700 nm and 10 nm at 1400 and 2100 nm. The sampling interval is 1.4 nm between 350 nm and 1050 nm and 2 nm between 1000 nm and 2500 nm. The light source was a 50 W quartz halogen lamp, was located at 45° from the normal to the surface and the sensor was at nadir. The sensor field of view was set to 1.4 cm^2. Leaf reflectance was calculated by dividing leaf radiance by that of a 99% reflectance panel (Spectralon, Labsphere, North Sutton) measured and illuminated under the same conditions. Each measurement lasted a few seconds precluding heating of the leaf surface. Leaves were measured against a 2% reflectance spectralon panel to minimize the contribution of background reflectance. Each leaf was placed between two black sheets of paper with matched openings. This measure gently flattened the leaf and minimized changes in spectral amplitudes introduced by curvature of the leaf surface. It also provided a means to ensure that the same area of each leaf was continually measured minimizing the effect of intra-leaf variability.
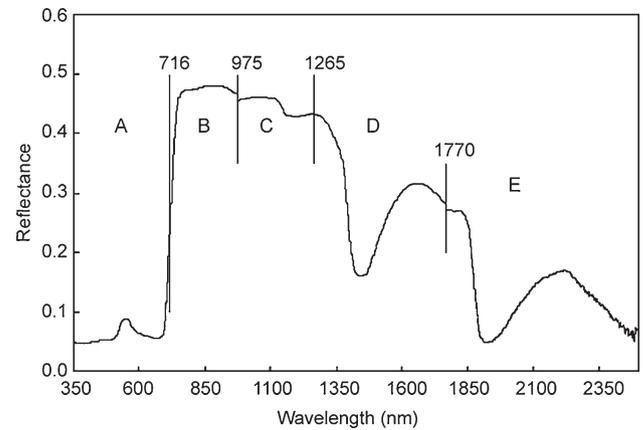

Fig. 1. Wavelength regions chosen for calculation of Sharp's θ and D. Divisions are marked by vertical lines between regions A, B, C, D, and E.

2.5. Spectral data manipulation

To quantify changes in spectral shape and magnitude with time, we computed θ and D indices as described by Price (1994):

(1) Shape index

$$\theta = a\cos\left(\frac{\sum(S_r S)}{(\sum S_r^2)(\sum S^2)}\right)$$

(2) D index

$$D = \text{sqrt}\left(\frac{\sum(S - S_r)^2}{N}\right)$$

where N is the number of bands, S is the sample spectrum, and S_r is the reference spectrum consisting of a straight horizontal line with an amplitude of one.

The θ value represents the angle between the reference and the sample spectrum, calculated using a vector dot product. This index is amplitude independent due to the denominator (Price, 1994). The D value calculates the root mean square difference between the sample spectrum reflectance amplitudes and the reference spectrum amplitudes.

The θ and D indices were calculated for the following wavelength regions (Fig. 1): 350 to 2500 nm (VIS, NIR, and SWIR), 350 to 716 nm (visible, A), 717 to 975 nm (near infrared, B), 976 to 1265 nm (near infrared, C), 1266 to 1770 (short-wave infrared, D), and 1771 to 2499 nm (short-wave infrared, E). The near infrared and the short-wave infrared were divided according to the wavelength limits of individual detectors.

3. Results

3.1. Leaf water content

The common guava, purple guava, and weeping fig control group leaves dehydrated at different rates. The common guava

dried out within a day while coffee, floss silk, and purple guava took two days (Fig. 2). The weeping fig did not reach a dry state until day seven. With the exception of coffee and floss silk each species displayed a unique dehydration rate. The treatment group leaves increased in water content in the first hour except the floss silk leaf, which displayed a steady decline over the 7 day period reaching 71% of its original water content (Fig. 2). The purple guava displayed minor fluctuations in water content and maintained its original value after 7 days. The common guava, coffee, and weeping fig never had values below their original water content and reached 115%, 111%, and 103% of their original water content, respectively, by the end of the experiment.

As the control leaves dehydrated, their surfaces displayed notable visible signs of wilting/curling and discoloration progressively from their edges towards their centers. Grey or black discoloration occurred on the leaf surface subsequent to wilting/curling. The veination also became more apparent as time progressed. The curling at the leaf edge was first observed 11 h after clipping for the common guava, at 21 h for the floss silk leaf, after a day and 7 h for the coffee leaf, after a day and 18 h for the purple guava and after 2 days for the weeping fig. For the treatment group, we did not observe leaf discoloration for the coffee and purple guava during the 7 day experiment. Discoloration around the leaf edge was observed 1 day and 18 h after clipping for the floss silk, 1 day and 19 h for common guava, and 2 days and 18 h for weeping fig. The discoloration did not progress as quickly as for the control samples. None of the treatment samples demonstrated wilting/curling of the leaf structure.

3.2. Histology

Cross-sections contrasting fresh leaves and partially air-dried leaves (Fig. 3) show that with dehydration, cells tend to contract noticeably. The contraction of the dried leaf cells contributes to the overall reduction in leaf thickness clearly visible for the common guava, purple guava, and weeping fig, a phenomenon associated with an increase in NIR reflectance (Fig. 4). The unsynchronized shrinking of the mesophyll cells in the purple guava, coffee and floss silk led to the disfigurement of the leaf shape.

3.3. Reflectance as a function of time

Spectra obtained for the treatment samples display low variability compared to that of the control samples (Fig. 4) consistent with the minor water content fluctuations observed during the course of the experiment (Fig. 2). For the control samples, several general observations can be made to describe spectral trends observed during dehydration of leaves. Most apparent is the gradual increase in NIR reflectance in contrast with the abrupt change in visible reflectance, the latter occurring some time after observation of physical signs of wilting (e.g. curling, discoloration) around the leaf edge. The spectral shape of drying leaves became more complex through time with the attenuation of water absorption bands near 1190 nm, 1450 nm, and 1940 nm and the appearance of lignin, cellulose, starch, and protein absorptions near 1690 nm, 1900 nm, 2130 nm and 2300 nm (Curran, 1989).

A spectral peak, extending upward along the red edge and into the NIR at 740 nm to 800 nm, can be seen in both treatment and control leaf spectra (Fig. 4). This feature corresponds to fluorescence (Gamon & Surfus, 1999), the emission of light from photosynthetic tissue in the red and far-red wavelengths in order to dissipate excess input energy (Peñuelas & Filella, 1998). Maximal fluorescence occurs within seconds of dark-adapted tissue exposure to a bright light source and is an indicator of photochemical efficiency (Gamon et al., 1990). The leaves in this study were consistently dark adapted prior to exposure to a bright halogen lamp; thus changes in fluorescence features over time should roughly correspond to changes in photochemical activity. Fig. 5 shows temporal changes of the fluorescence peak near 738 nm normalized to the reflectance at 570 nm. For the treatment spectra no variations are observed for all species. For the control spectra there is an observed decrease in fluorescence with time for all species likely indicative of a decrease in chloroplast activity.
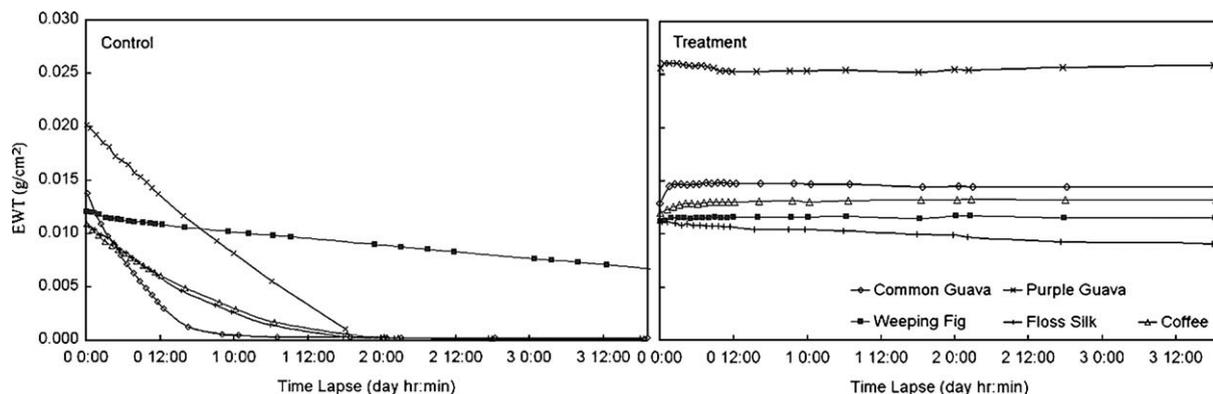

Fig. 2. Water content of leaves through time. Control leaves were left to air dry, while treatment leaves were wrapped with moist gauze and placed in sealed plastic bags.
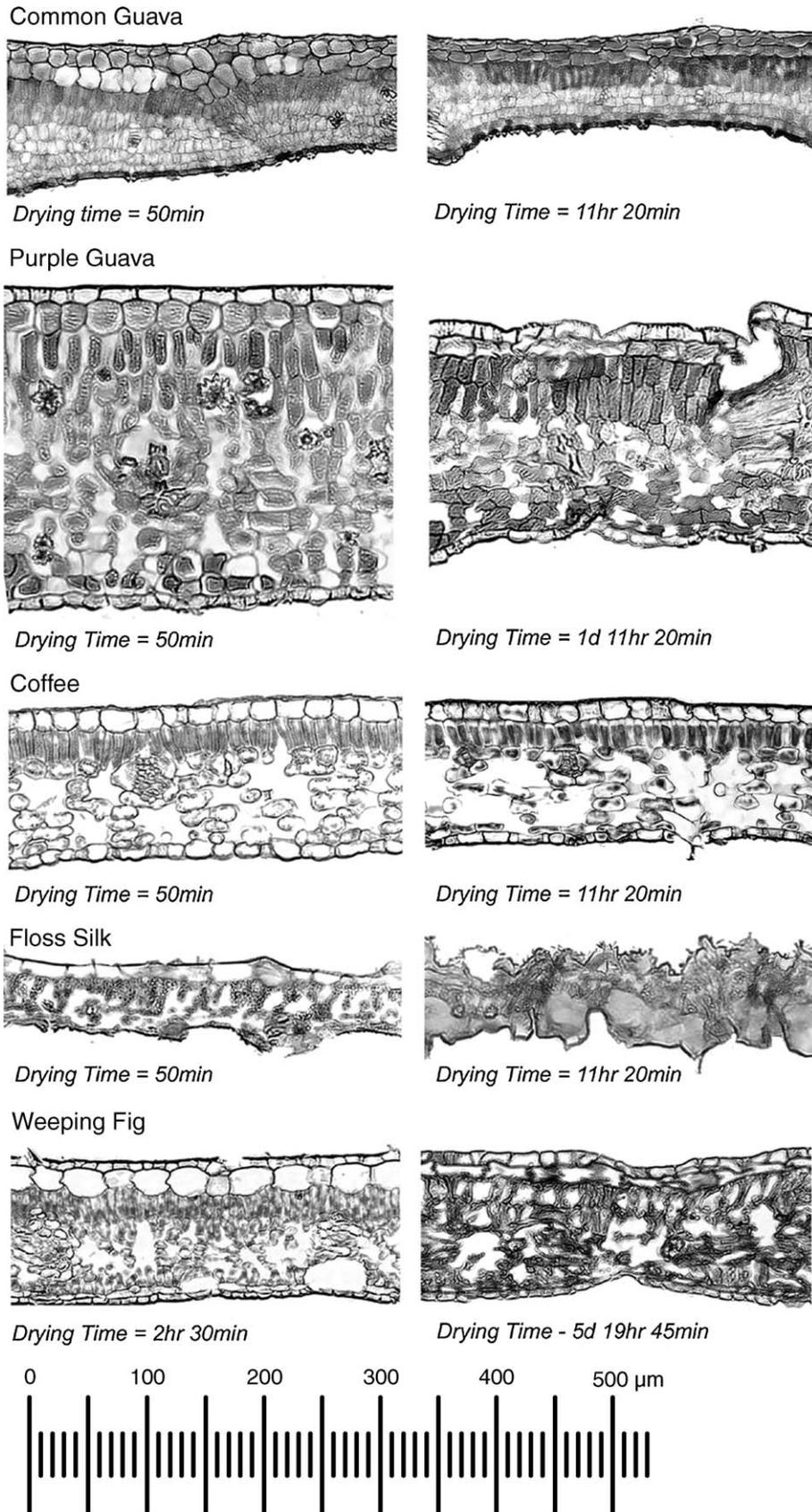
Common Guava

Drying time = 50min   Drying Time = 11hr 20min

Purple Guava

Drying Time = 50min   Drying Time = 1d 11hr 20min

Coffee

Drying Time = 50min   Drying Time = 11hr 20min

Floss Silk

Drying Time = 50min   Drying Time = 11hr 20min

Weeping Fig

Drying Time = 2hr 30min   Drying Time - 5d 19hr 45min

0   100   200   300   400   500 µm

Fig. 3. Leaf cross-sections produced via histological procedures. Sections on the left are fresh samples and sections on the right are partially dried samples.
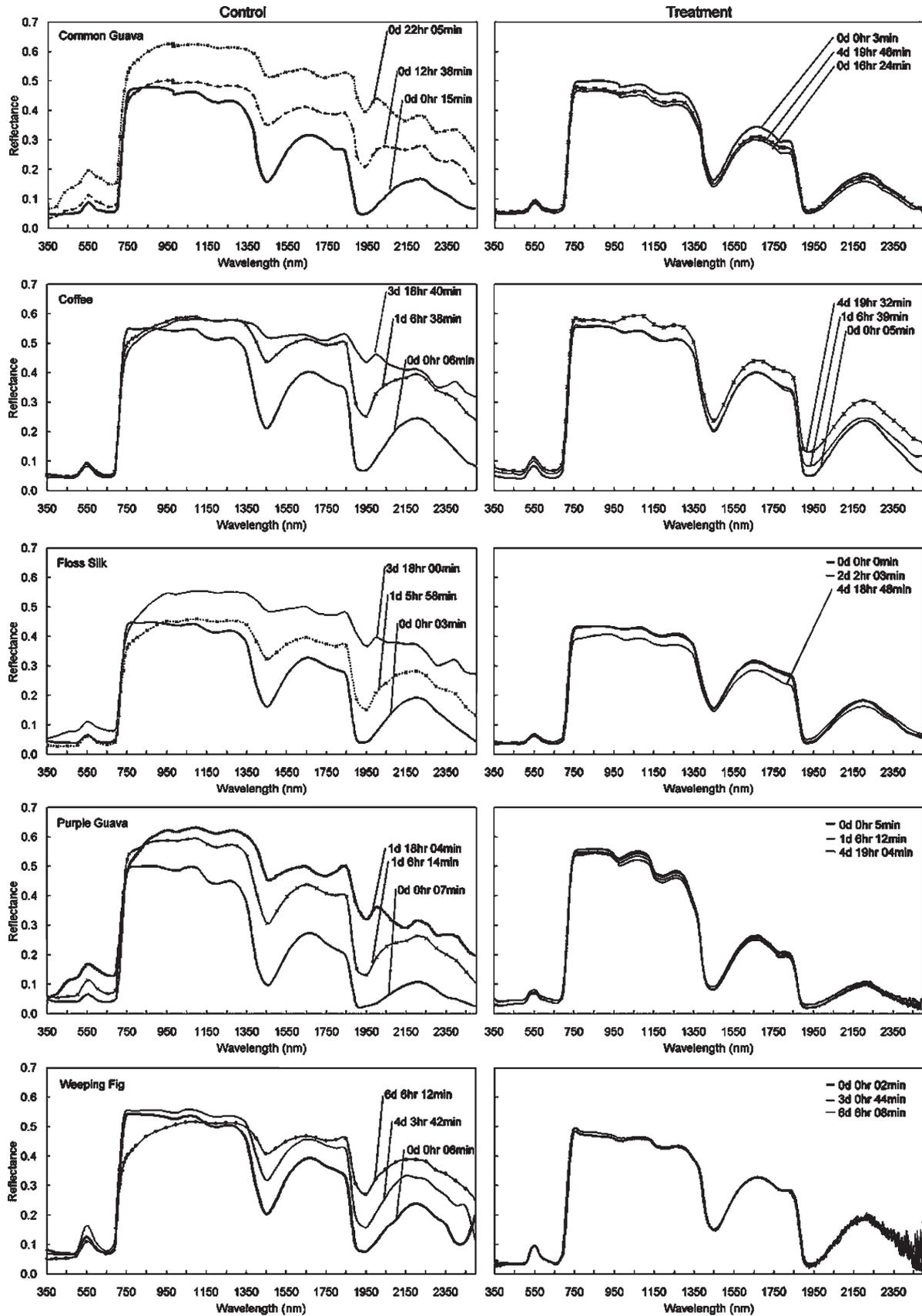
Fig. 4. Resultant leaf spectra from control (left column) and treatment (right column) leaf measurements. Spectral labels indicate the time lapse since clipping and measurement in days, hours, and minutes (d, h, and min). Small jumps in reflectance can be observed at 950 nm and 1750 nm where the internal sensor boundaries exist. This was caused by slightly asynchronous FOVs for each fiber optic cable corresponding to each internal sensor and by a bumpy or wrinkled leaf surface. Although efforts were made to flatten the leaf surface progressive wilting combated this.
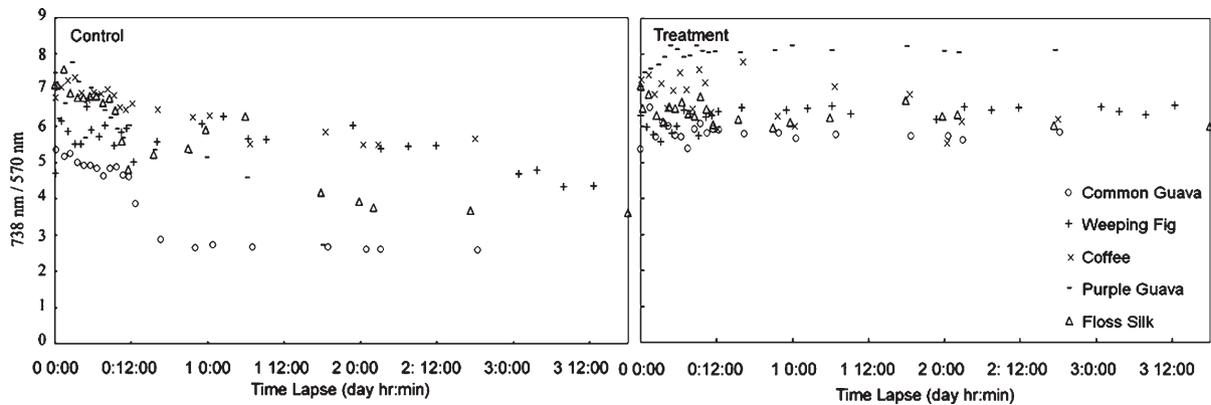
Fig. 5. Fluorescence peak, quantified by 738 nm/570 nm ratio, through time for both control and treatment leaves.

3.4. D and θ as a function of time and water content

D and θ calculated from reflectance within 350 to 2500 nm revealed temporal trends on a per species basis (Fig. 6). D and θ values gradually decreased over time for the control samples while the treatment values remained relatively constant. Decreasing D values represent an increase in reflectance magnitude while decreasing θ represent flattening of the spectral shape, trends which are visible in Fig. 4. For the control samples the coefficient of determination (r^2) between time and D (including all species) was 0.392 and 0.384 for time and θ; rather low considering that time is serially autocorrelated, which likely artificially increased the r^2 values. The r^2 values for the treatment samples were not significant (0.009 and 0.006 for D and θ). The corresponding relationships of D and θ (350 to 2500 nm) with equivalent water thickness (EWT) (not shown) were stronger than with time, even though time is serially autocorrelated. For the control samples, r^2 was 0.864 between EWT and θ and 0.529 between EWT and D. The treatment samples displayed r^2 values of 0.884 and 0.21 between EWT and θ and D, respectively. To further examine these EWT relationships, spectral indices θ and D were calculated for the five wavelength regions identified in Fig. 1. The division between regions B and C, corresponding to a detector boundary, bisects a moderate water absorption band, and reduces the significance of that band in the analysis. The values per wavelength regions are displayed in Fig. 7 as a function of EWT. θ_A, and θ_B show weak relationships ($r^2 < 0.103$) with EWT for all species for both the control and treatment data, an observation consistent with abrupt changes in control VIS reflectance for common guava and purple guava observed in Fig. 4. The relationship between θ_C, θ_D and θ_E and EWT is much improved with r^2 values of 0.953, 0.960, and 0.718, respectively (control and treatment combined). The r^2 values for relationships with D and wavelength regions within the SWIR (D and E) are 0.603, and 0.672 compared to low (<0.19) values for regions A, B, and C.

We endeavoured to test the significance of the D and θ values by creating an index that correlated highly with water content. This was achieved by analyzing the spectral areas with high correlations of θ or D with EWT. It was found that θ_C and θ_D had the highest correlations with EWT for all the samples, with

θ_C being the highest for control samples. These results are logical due to the water absorptions at 1187 nm and 1450 nm. The moderate absorption band 1187 nm was chosen in preference to the band at 1450 nm due to fewer overlapping leaf chemical absorptions (Curran, 1989) and higher atmospheric transmission when considered from a remote sensing perspective (Valley, 1965). The shape of the area was accounted for by calculating a ratio where the absorption center reflectance, at 1187 nm, was divided by a nearby reference reflectance at 1096 nm. The linear coefficient of determination for this relationship was 0.952 ($p < 0.001$) (Fig. 8). This ratio had a stronger relationship with water than any single reflectance band between 350 to 2500 nm, where the maximum r^2 value was 0.766 for reflectance at 2135 nm.

4. Discussion

An objective of this study was to assess time constraints between leaf collection and measurement. It was found that the broad spectral features studied here did not respond equally over time for all species. This means that a universal (for all species) time restriction for collecting samples and measuring leaf reflectance prior to spectral degradation is non-existent. However, it should be noticed that there are other far more dynamic and subtle optical signals associated with physiological regulation that were not part of this investigation, but reportedly change on much shorter time scales (Gamon & Surfus, 1999). In accordance with previous research (Aldakheel & Danson, 1997; Carter, 1991; Hunt & Rock, 1989; Ripple, 1986) we also found that leaf reflectance was more strongly related to leaf water content than time-lapse. Leaves have a variety of water contents and dehydration rates (Hunt & Rock, 1989; Fig. 2) hence measurement time constraints are dependent on the properties of the leaf or species. Factors such as cuticle thickness, leaf water conductance, stomatal density and stomatal control all likely affect leaf dehydration or transpiration rates. These properties vary between species and will probably result in similar drying rates within species sample groups (Bacelar et al., 2004).

The VIS, NIR and the SWIR spectral regions respond differently to water content changes. The researcher will need to consider these responses when choosing an appropriate time of
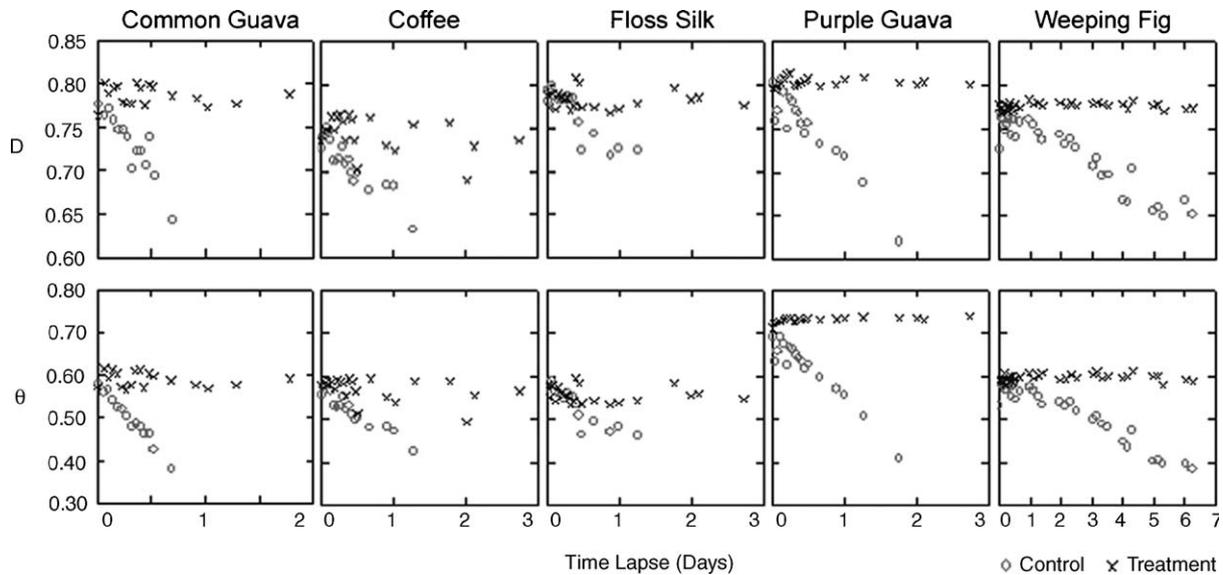
Fig. 6. Indices D and θ, calculated for wavelengths 350 nm to 2500 nm, plotted against time since clipping.

measurement and handling technique. The visible region for instance was not immediately responsive to water content changes and did not change until wilting of the leaf was observed. Carter (1991) noted a similar observation. The NIR and SWIR wavelength regions, identified in Fig. 1, were affected immediately by small changes in water content, as indicated by the control samples in Fig. 7. Thus, by the time wilting was first observed, the NIR and SWIR reflectance differed considerably from corresponding fresh leaf reflectance. Researchers should not use wilting time as a time limit for NIR or SWIR measurements.

Responses of the NIR and SWIR reflectance to water content were caused by structural changes within the leaf and by changes in light absorption by water. As leaf water volume decreases, leaf cells shrink and warp to accommodate water reduction (Fig. 3). In this process air, water, and leaf material interfaces will change in number and position (Knipling, 1970). Air–water and air–cell wall interfaces have higher angles of refraction than water–cell wall interfaces and likely contribute the most to the scattering of light (DeLucia et al., 1996; Kumar & Silva, 1973). Most of the measured reflectance in the NIR is a result of the scattering properties of the leaf; also apparent is the moderate influence by water and other absorbers (Gates et al., 1965; Gausman et al., 1969; Sinclair et al., 1971; Slaton et al., 2001). The SWIR, on the other hand contains strong water absorptions amongst other weaker absorptions such as cellulose, lignin, starch, protein, and nitrogen (Curran, 1989). Consequently the NIR is a spectral region better able to illustrate scattering/reflectance differences (Gausman et al., 1969) caused by structural variation in response to drying within leaves. The plot of D for the NIR, an approximation of reflectance magnitude, versus water illustrates various D behaviour depending upon species (Fig. 6).

An index or ratio that can predict water content successfully in a sample group with various internal structures must remove scattering effects of the leaves from the calculation. The 1187/ 1096 nm ratio managed scattering properties of the NIR by including the 1096 nm band, which captured scattering variations caused by variable leaf structure between species and by dehydration as illustrated in Fig. 4. This strategy appeared to be effective considering that the ratio had a strong relationship ($r^2 = 0.95$) with water content and applied to all species of this study. The high correlations of water to this ratio and θ indices in the NIR and SWIR are indicative of the practical value of spectral shape over raw reflectance magnitude. Some examples of successful analytical techniques capitalizing upon spectral shape are derivatives (Tsai & Philpot, 1998), principle component analysis (Bell & Baronoski, 2004), and band depth analysis (Kokaly & Clark, 1999).

The 1187/1096 nm ratio could be beneficial for determining canopy water content. Two important properties of this ratio are that it uses a moderate water absorption band that is sensitive to greater quantities of water than stronger water absorptions and it exists within an atmospheric window where atmospheric water is less absorbent (Valley, 1965). Despite the difference in scale (leaf versus canopy) our data support the findings of studies exploiting this water band for canopy water content (Rollin & Milton, 1998; Sims & Gamon, 2003). Rollin and Milton used an index called the relative depth index (RDI), centered upon 1150 nm and normalized with the 1116 nm band, to correlate CASI (Compact Airborne Spectrographic Imager) data and grass canopy water content. They found that the RDI ratio was the most effective and least sensitive to spectral smoothing when compared to first order derivative techniques applied to the 400 nm to 1300 nm range. Sims and Gamon (2003) conducted a thorough investigation of water content for thick and thin tissues encompassing a variety of plant species. They concluded that 1150–1260 nm (in addition to 1520–1540 nm) was an effective range for determining canopy water content due to water's weak absorption coefficient there. Sims and Gamon (2003) also combined these moderate water bands with a chlorophyll index in the form of the Canopy Structure Index
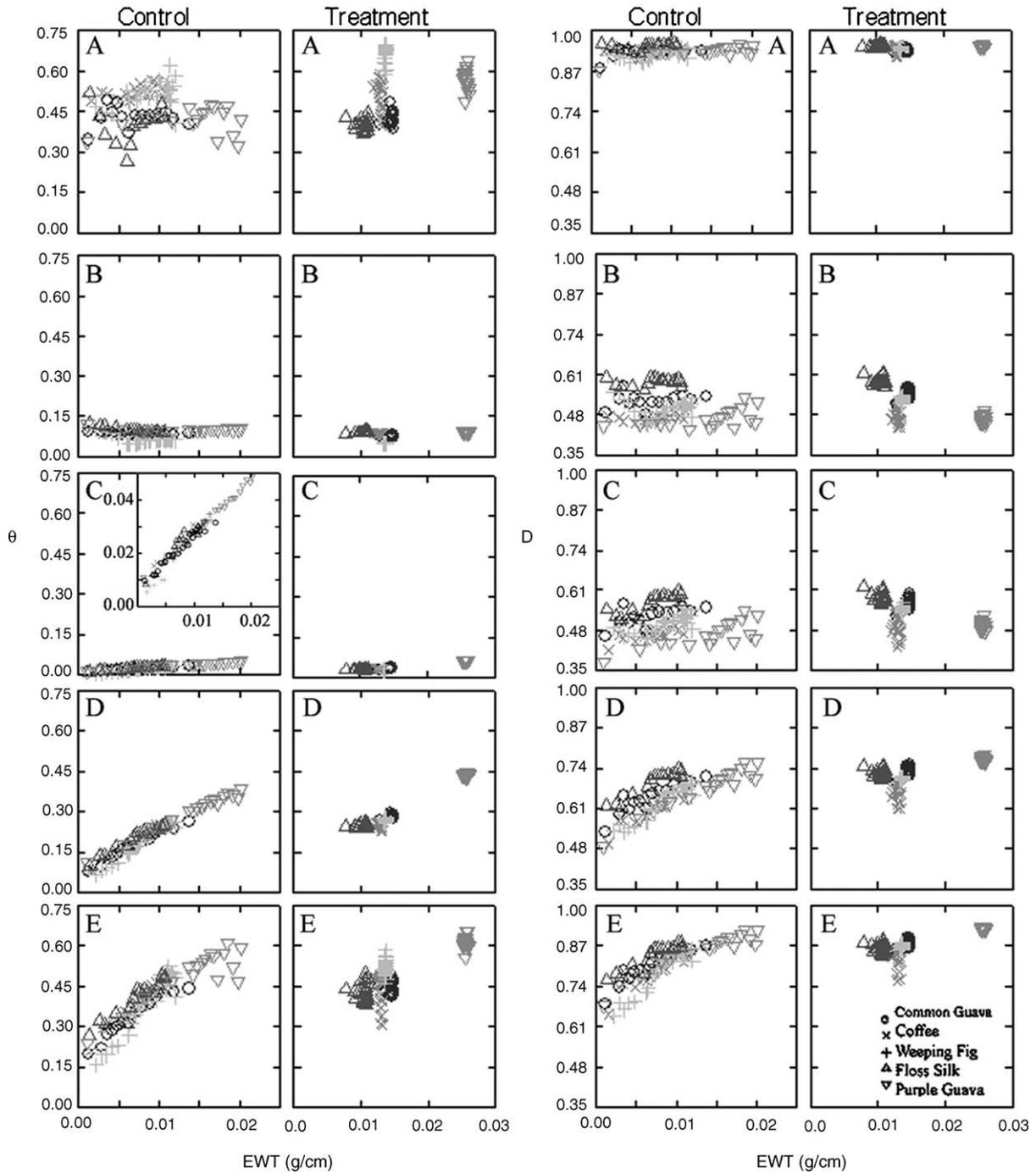
Fig. 7. Indices θ and D plotted against EWT. Indices were calculated for each wavelength region A, B, C, D, and E (labeled in plot areas; see Fig. 1). R^2 values calculated using control and treatment data combined were <0.19 for regions A and B for both indices; they were 0.953, 0.960, and 0.718 for regions C, D, and E (respectively) for θ; <0.0001, 0.603, and 0.672 for regions C, D, and E (respectively) for index D.

(CSI), which improved their ability to determine photosynthetic tissue area.

The results of this study illustrate that proper handling and storage techniques following leaf excision are essential for the preservation of broad spectral features between 350 and 2500 nm. We found that wrapping sample leaf petioles with moist gauze and placing them in plastic bags was an effective technique, which prolonged the life of all of the samples including the common guava whose freshness was extended

from 1 day to 7 days. Unfortunately, artificial water increases observed with this technique may present a problem for researchers interested in in situ leaf water content, therefore the findings presented in this paper should not be extrapolated when in situ water content is desired. The effectiveness of other techniques such as cutting underwater and cooling leaves was not tested here but they could not have substantially improved the retention of water in leaves, considering that the water content was above 100% for all
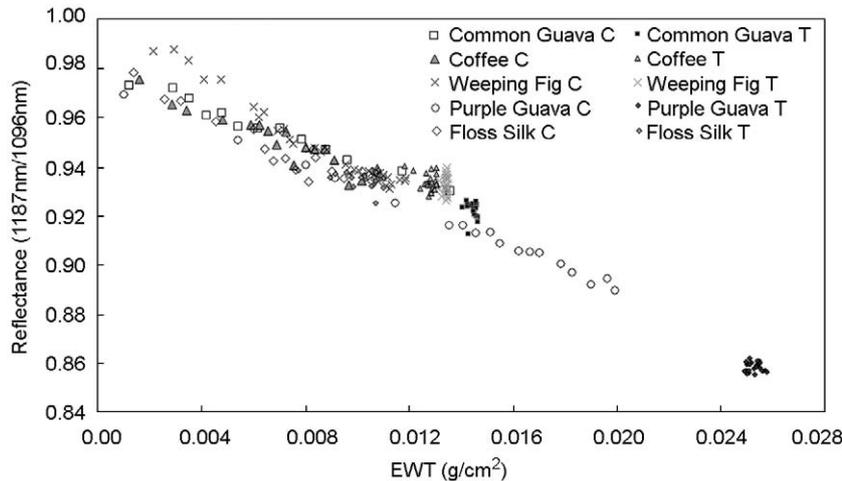
Fig. 8. Reflectance ratio 1187/1096 nm plotted against water content (EWT). Control and Treatment samples indicated by C and T, respectively, in the legend.

of the treatment samples except floss silk. Cooling of the leaf could be an effective technique for reducing evapotranspiration but expansion of freezing leaf water is likely to cause damage to leaf structure and affect reflectance, therefore leaves should be kept well above 0 °C. Furthermore, physical manipulation of the leaves should be done with care in order to reduce structural damage.

5. Conclusion

We concluded that there is no typical time limit for all leaves to be sampled and measured for reflectance. For studies concerned with broad spectral features we recommend that leaf handling techniques be based upon managing leaf water content and leaf structure. Our results clearly show that leaves with petioles wrapped in moist paper towel and placed within plastic bags will maintain leaf reflectance longer than equivalent leaves without this treatment; samples tested here lasted a minimum of 7 days.

The analysis using Price's (1994) θ and D indices revealed a stronger relationship between leaf water content and spectral shape than between leaf water and raw reflectance magnitude. The 1187/1096 nm ratio had the highest coefficient of determination with leaf water content ($r^2 = 0.95$), because the effects scattering on the spectra are normalized using the 1096 nm band. The high r^2 of this ratio in combination with its placement within an atmospheric window suggests that the 1187/1096 nm ratio may be effective for determining canopy water content. Further studies testing the applicability of this ratio for canopy water research are recommended.

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