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Leaf Handling Methodology and Species Classification of Tropical Tree Leaf Spectral Reflectance

by

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Abstract

After leaves are clipped their reflectance properties change over time at variable rates depending upon species and wavelength. This study indicates that Visible reflectance from leaves does not respond until wilting is observed and that NIR and SWIR reflectance respond immediately. Wrapping leaves in moist paper towel and placing them within plastic bags maintained leaf reflectance longer (greater than 7 days) than equivalent leaves without treatment. Strong relationships between leaf water content and spectral shape were defined via θ ("angle difference") and the ratio 1187/1096 nm.

Leaf reflectance of twenty tropical tree species can be classified accurately (79% to 97%) using linear discriminant analysis. The species studied were classified best using bands or indices correlated with leaf water content, followed by pigmentation properties. While wavelengths between 350 to 2500 nm were important for overall classification accuracy, certain species were classified accurately using only one range (VIS or SWIR) of wavelengths.

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Chapter One

1.0 Introduction

Molecules selectively absorb energy from specific wavelengths of light or electromagnetic radiation (EMR) and reflect others (Read, 1980). The discipline of remote sensing, exploits this fact with the use of sensors that quantify light coming from target substances at the earth surface such as minerals, and vegetation (Clark and Roush, 1984).

Vegetation, in particular, is very sensitive to light because of photosynthesis. Due to this sensitivity spectroscopy has been able to retrieve information from reflected light about plant biochemical and biophysical traits. Biochemical compounds such as chlorophyll and other photosynthetic pigments maximize energy accumulation by absorbing it from visible (VIS, 400 to 700 nm) wavelengths (Gamon and Surfus, 1999; Knipling, 1970), the most abundant energy source at the earth's surface (Valley, 1965). Due to this strong absorption, measured VIS wavelengths have a marked decrease in reflected light. Near infrared (NIR, 700 to 1300 nm; Knipling, 1970) wavelengths demonstrate much higher reflectance and very little absorption of light compared to the VIS. High NIR reflectance has been theoretically linked to scattering of light caused by refraction and reflection at boundaries within plant tissues (Slaton et al, 2001). In longer wavelengths called the short-wave infrared (SWIR, 1300 nm to 3000 nm; Peñuelas and Filella, 1998), leaf water content absorbs light strongly resulting in low measured reflectance (Carter, 1991). These spectral properties are characteristic of plants (Gates et

al, 1965) and continue to be important in vegetation studies using spectroscopic techniques.

Scientists have been aware of general vegetation spectral properties as early as the mid 1960's. Since then research has developed analytical techniques linking biophysical/biochemical properties to reflectance, and also used those links to establish greater understanding of natural variability of those properties (e.g., Datt, 1999). Sensor technology has also evolved with research so that current sensors are designed to maximize extractable information and allow progression of research (Nieke et al, 1997). Perhaps some of the greatest advances have been the placement of sensors on airborne and satellite platforms (henceforth called remote sensors) and the aggregation of spectral data into images. More recently, remote sensors have changed from multispectral systems to hyperspectral ones, which means that some sensors can now measure hundreds of EMR wavelengths at finer resolutions (Pearlman et al, 2003). Spatial resolution of each spectral measurement or pixel has also improved so that smaller (<1 m) earth features can now be resolved (Nieke et al, 1997). Potential applications of high spatial and spectral resolution imagery are numerous.

One application, in particular is the exploitation of spectral differences amongst tree species for classification of imagery (Martin et al, 1998). Classifications or maps of tree species can be used to assist habitat conservation efforts. An important motivation of this thesis was the desire to assist habitat conservation efforts for the Great Green Macaw (*Ara ambigua*). The Great Green Macaw has been labelled an indicator or umbrella species, which means that the success of this macaw infers the success of other coexisting species (Bjork and Powell, 1995). Powell et al (1999) observed thirty seven tree species

within the Great Green Macaw's habitat. For this thesis I sought to determine the potential for mapping the 20 indicator species noted by Powell (1999) by first analyzing their leaf reflectance.

While data sources enable investigation at various scales including leaf, canopy, and ecosystem scales, analysis often begins at the leaf scale because leaves are fundamental contributors to canopy reflectance (Asner, 1998). Due to variable field conditions and other logistical problems sample leaves are often transported and measured in controlled laboratory environments. Chapter two focuses on how a handling technique commonly used during transportation, and time lapse between collection and measurement can affect measured leaf reflectance. The results from chapter two provided methodological information for chapter three and can be informative for other researchers who are using leaf samples for remote sensing or spectroscopy research. Chapter three adheres more closely (than chapter two) to the original motivating factor and investigates the potential for classification of forests using the 20 tropical tree species (at the leaf level). Linear discriminant analysis was used and assessed for selection of important indices or wavelengths for classification. Chapter three includes an assessment of the usefulness of VIS, NIR, and SWIR wavelengths for classification.

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Chapter Two[1]

2.0 Foliar spectral properties following leaf clipping and implications for handling techniques.

2.1 Introduction

Remote sensing of forest canopies has possibilities for many practical applications such as assessing biomass, drought, stress and canopy chemistry (Peñuelas and Filella, 1998; and 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 (Guyot et al, 1989; and Asner, 1998). 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 (Buschmann and Nagel, 1993; and Blackburn, 1998). Modelling canopy and leaf spectral properties has been a strategy for studying these systems (Dawson et al, 1998; and 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 (Horler et al, 1983; and Asner, 1998). 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

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xanthophyll cycle pigment changes, and ultrastructural changes such as chloroplast movement, caused by variable light conditions (Haupt and Scheuerlein, 1990; and Gamon and Surfus, 1999). A problem associated with this approach is that after leaves are clipped their reflectance properties change at variable rates over time (Hunt and 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 (Ripple, 1986; Hunt and Rock, 1989; and Carter, 1991). Carter (1991) outlined the theoretical "primary and secondary" effects of water content on leaf reflectance, where primary effects are direct water absorption corresponding to the water absorption coefficient (Curcio and 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 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 to reduce the vapour pressure gradient between leaf and air and to reduce 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 minutes (Clark, 1954) and might prolong freshness. The practice of artificially cooling/chilling leaves is intended to reduce transpiration and has been applied by many researchers 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 towels and plastic bags which can 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 (Vane and Goetz, 1993; and Pearlman et al, 2003) 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 hour 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 a single circular area for each leaf; 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 would have consistent leaf reflectance longer than leaves without this treatment, at least for the species included 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 species included are found in the tropics but their relevance is not limited to this region.

2.2 Methods

2.2.1 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 (>1.5m) within the conservatory, and their widespread distribution in tropical areas (Doggett and Parker, 2001).

A single branch from each tree was cut, placed in a cooler, 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 hours, then every 4 or 6 hours for 18 hours, followed by one measurement after 12 hours, 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 hours at 50 degrees Celsius then weighed.

2.2.2 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). The differences between dried leaf mass and each mass measurement throughout the experiment were used as water mass values. Leaf area was measured by scanning the dried leaf after the experiment, digitizing the leaf boundaries and calculating leaf area using a geographic information system.

2.2.3 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 cut-outs 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 um 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 (20X magnification) and a digital camera (Nikon DXM 1200 CCD).

2.2.4 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 watt quartz halogen lamp, was located at 45 degrees from the normal to the surface and the sensor was at nadir. The sensor field of view was set to 1.4 cm^2 on the flattest area of each leaf not covering the main leaf vein. 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.

2.2.5 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

$$\theta = \text{acos}\left(\frac{\Sigma(S_r S)}{(\Sigma S_r^2)(\Sigma S^2)}\right)$$

(2) D index

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

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 (Figure 2-1): 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.

2.3 Results

2.3.1 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 (Figure 2-2). 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 (Figure 2-2). 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 hours after clipping for the common guava, at 21 hours for the floss silk leaf, after a day and 7 hours for the coffee leaf, after a day and 18 hours 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 hours after clipping for the floss silk, 1 day and 19 hours for common guava, and 2 days and 18 hours 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.

2.3.2 Histology

Cross-sections contrasting fresh leaves and partially air-dried leaves (Figure 2-3) 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 (Figure 2-4). The unsynchronized shrinking of the mesophyll cells in the purple guava, coffee and floss silk led to the disfigurement of the leaf shape.

2.3.3 Reflectance as a function of time

Spectra obtained for the treatment samples display minor changes in reflectance, compared to that of the control samples, (Figure 2-4) proportional to the minor water content fluctuations observed during the course of the experiment (Figure 2-2). 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 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 absorption 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 (Figure 2-4). This feature corresponds to fluorescence (Gamon and Surfus, 1999), the emission of light from photosynthetic tissue in the red and far-red wavelengths in order to dissipate excess input energy (Peñuelas and 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, then within a few seconds spectral response was measured; thus changes in fluorescence features over time should correspond to changes in photochemical activity. Figure 2-5 shows temporal changes of the

fluorescence peak near 738 nm normalized to the reflectance at 570 nm. For the treatment leaves fluorescence had weak relationships with time lapse (r^2values were less than 0.43 for all species). For the control spectra there was an observed decrease in fluorescence with time for all species likely indicative of a decrease in chloroplast activity.

2.3.4 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 (Figure 2-6). 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 Figure 2-4. 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 Figure 2-1. 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 Figure 2-7 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 Figure 2-4. 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 ($p<0.001$) (Figure 2-8). 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.

2.4 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 and Surfus, 1999). In accordance with previous research (Ripple, 1986; Hunt and Rock, 1989; Carter, 1991; and Aldakheel and Danson, 1997) 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 and Rock, 1989; Figure 2-2) 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 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 Figure 2-1, were affected immediately by small changes in water content, as indicated by the control samples in Figure 2-7. 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 (Figure 2-3). 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 (Kumar and Silva, 1973; and DeLucia et al, 1996). 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; and 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 (Figure 2-6).

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 Figure 2-4. 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 and Philpot, 1998), principle component analysis (Bell and Baronoski, 2004), and band depth analysis (Kokaly and 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 absorption 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 and Milton, 1998 and Sims and 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 in that range. Sims and Gamon (2003) also combined these moderate water bands with a chlorophyll index in the form of the Canopy Structure Index (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 analysis time 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 can 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 greater than the original water content for all of the treatment samples except floss silk (Figure 2-2). 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 degrees Celsius. Furthermore,

physical manipulation of the leaves should be done with care in order to reduce structural damage.

2.5 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 of 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 can be effective for determining canopy water content. Further studies testing the applicability of this ratio for canopy water research are recommended.

2.6 Figures

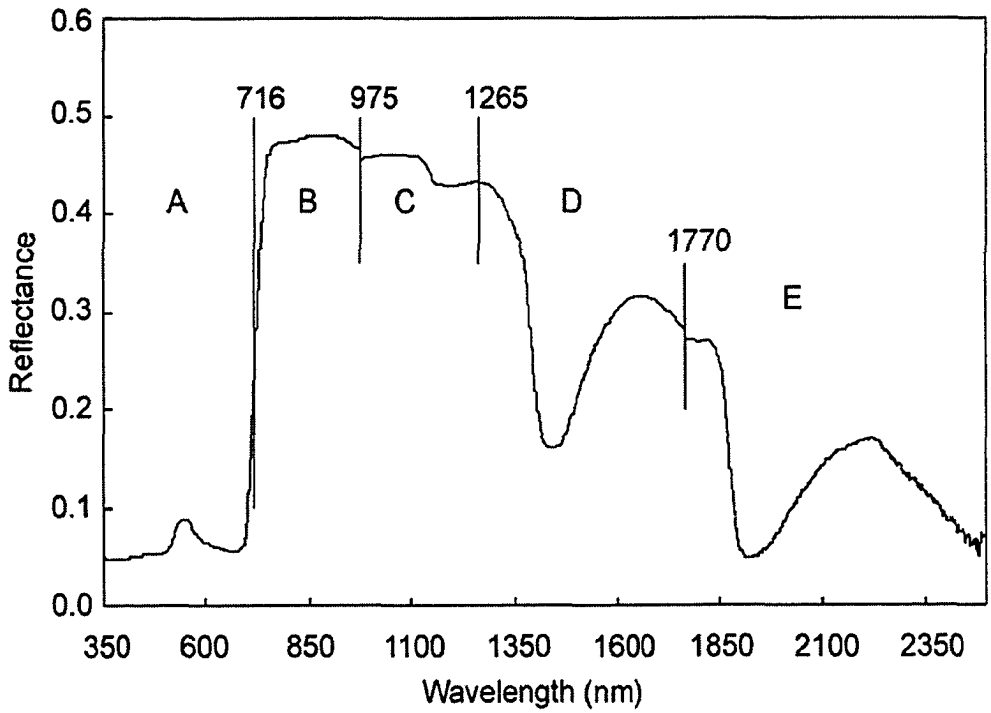

Figure 2-1: Wavelength regions chosen for calculation of Price's (1994) θ and D. Divisions are marked by vertical lines between regions A, B, C, D, and E.

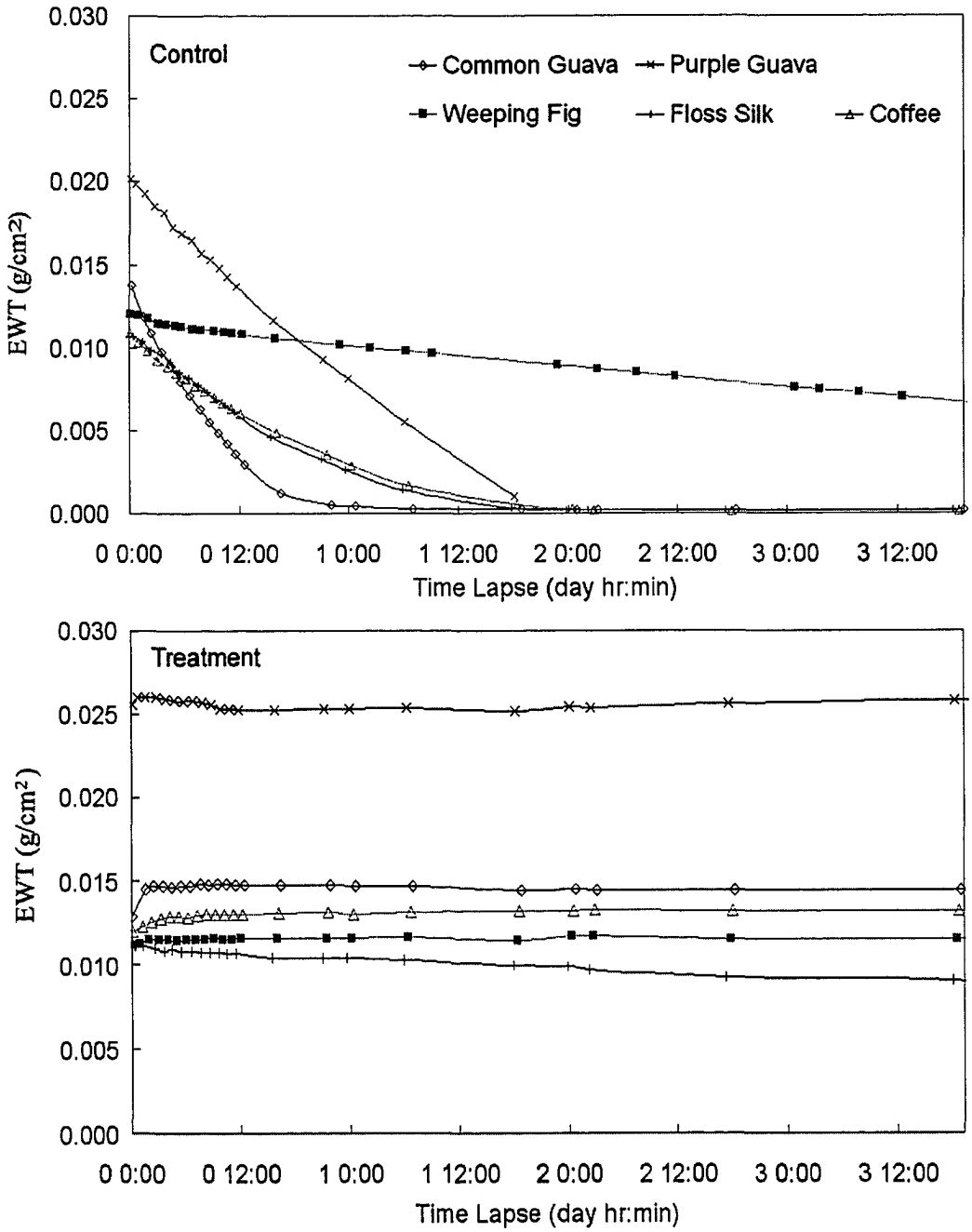

Figure 2-2: Water content of leaves through time. Control leaves were left to air dry, while treatment leaves were wrapped with moist gauze and placed in sealed plastic bags.

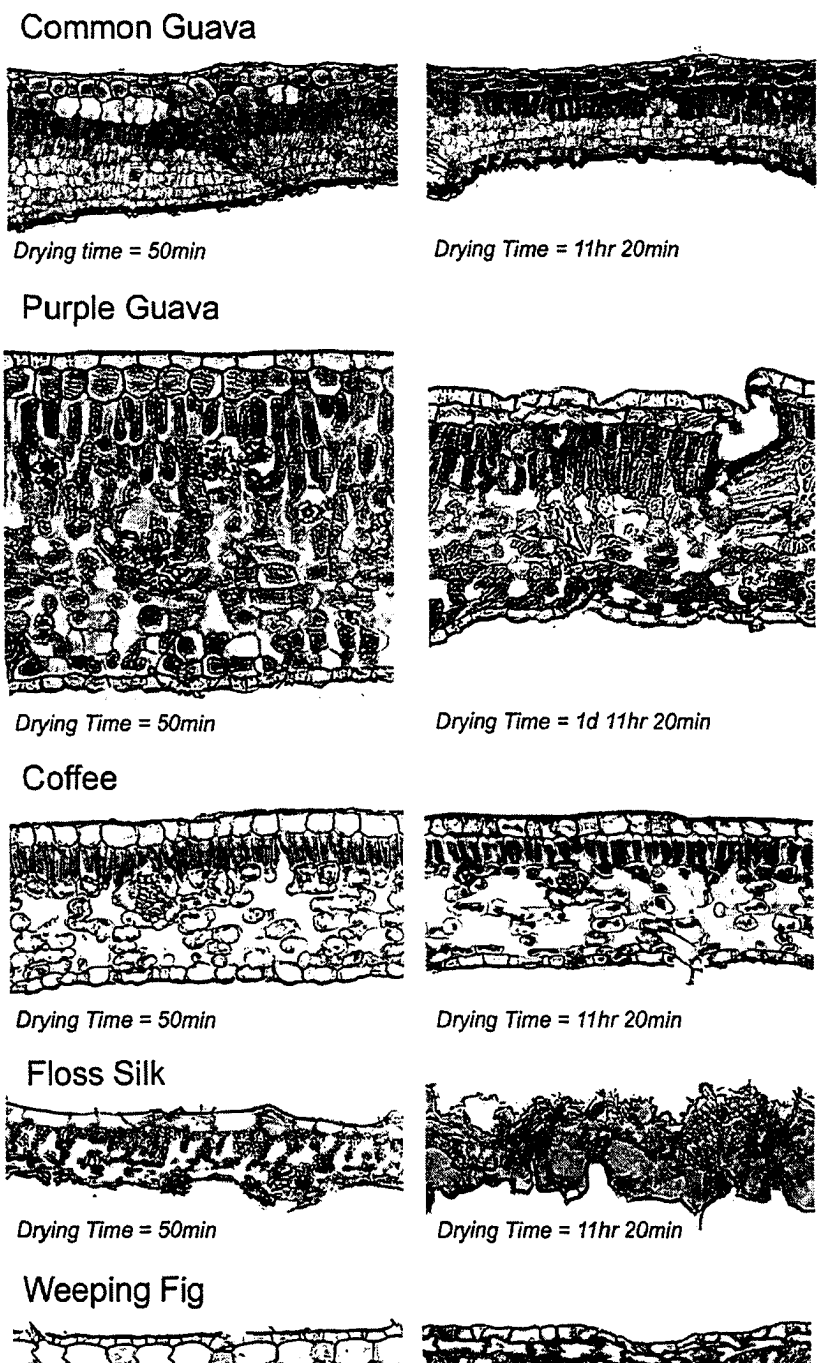

Figure 2-3: Leaf cross sections produced via histological procedures. Sections on the left are fresh samples and sections on the right are partially dried samples.

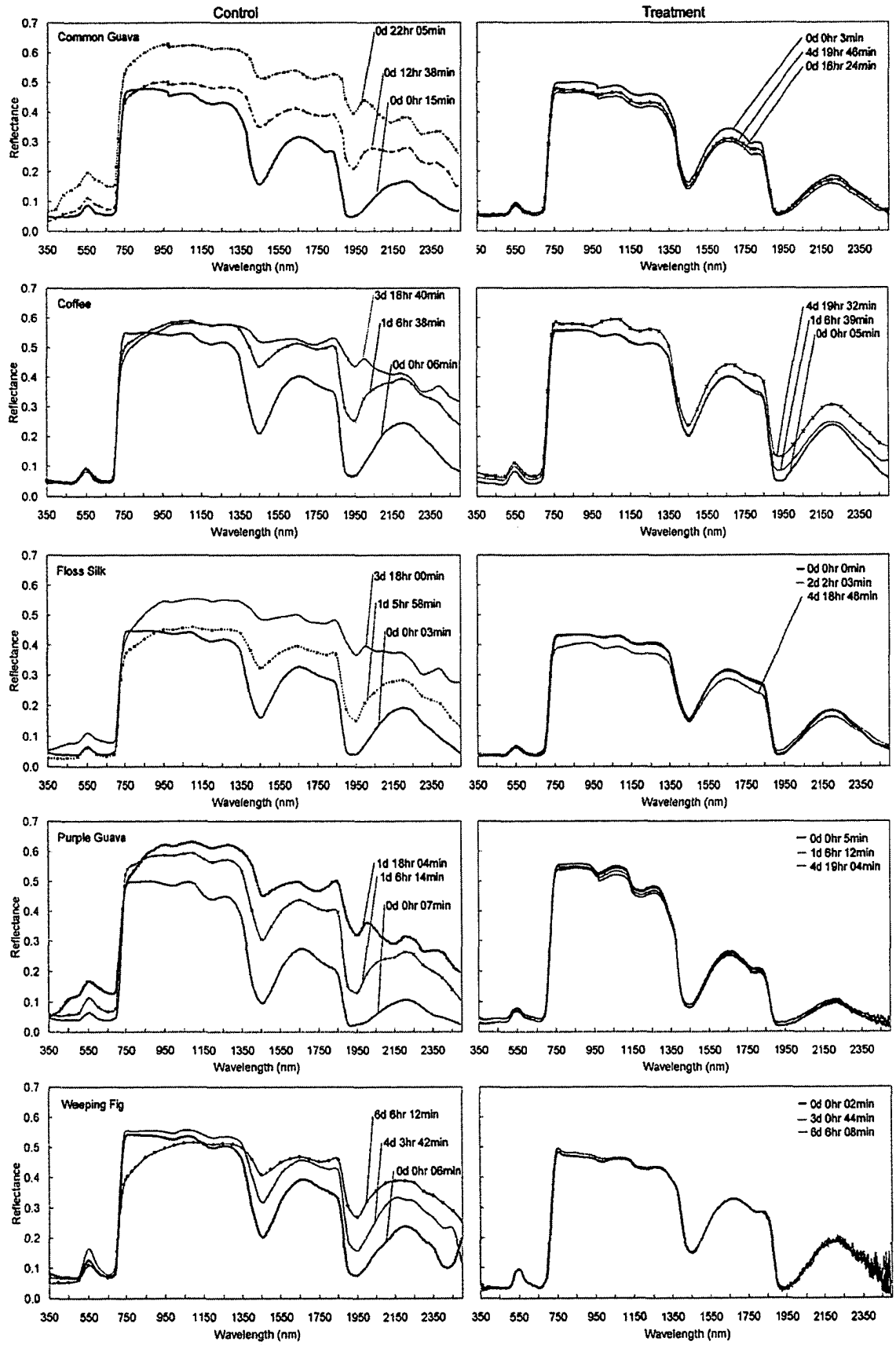

Figure 2-4: Resultant leaf spectra from control (left column) and treatment (right column) leaf measurements. Spectral labels indicate the time lapse since clipping and measurement in days, hours, and minutes (d, hr, and min). Small jumps in reflectance can be observed at 950 nm and 1750 nm where the internal sensor boundaries exist. This was caused by slightly asynchronous FOV's 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.

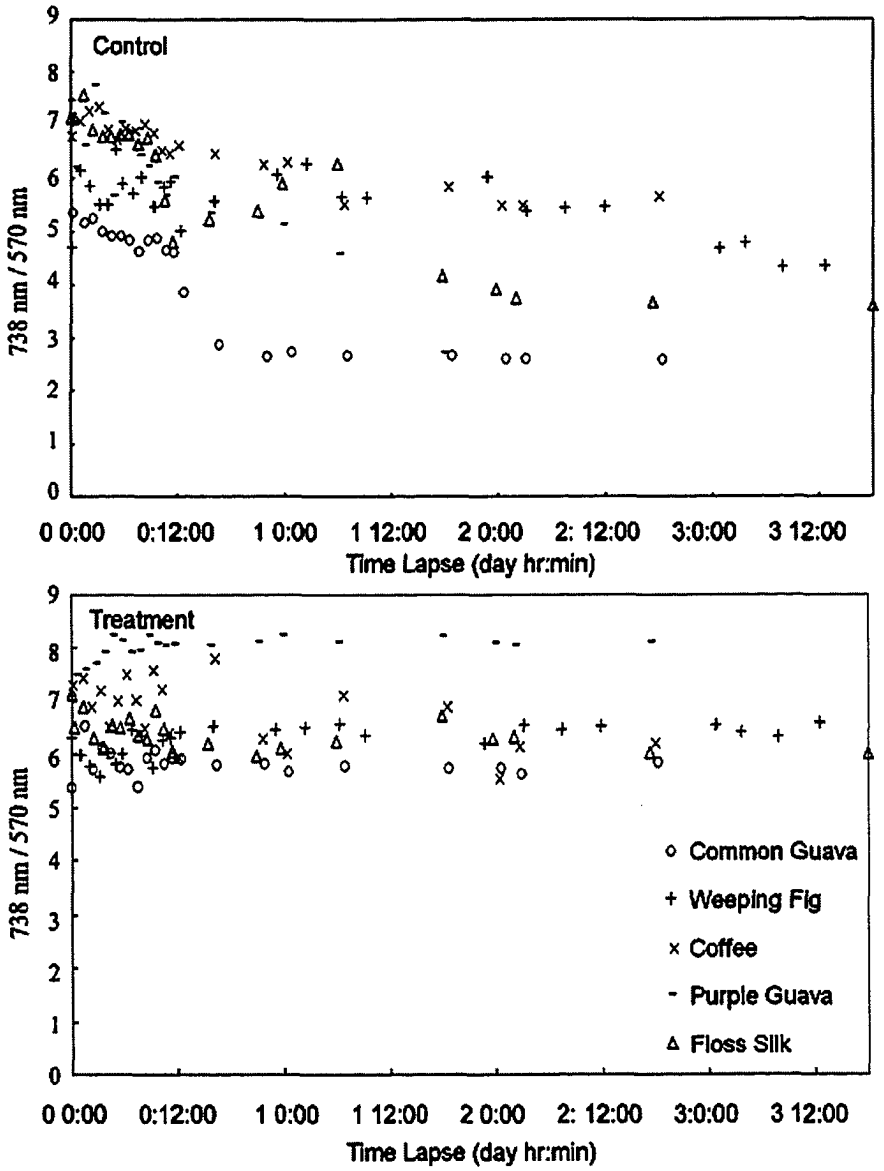

Figure 2-5: Fluorescence peak, quantified by 738 nm/570 nm ratio, through time for both control and treatment leaves. Control leaf fluorescence when related with time lapse had r^2 values of 0.65, 0.71, 0.77, 0.79, and 0.83 for Common Guava, Coffee, Floss Silk, and Weeping Fig leaves respectively. Treatment leaf fluorescence had poorer relationships with time lapse where r2 values were 0.03, 0.05, 0.20, 0.33, and 0.43 for Common Guava, Purple Guava, Weeping Fig, Floss Silk, and Coffee respectively.

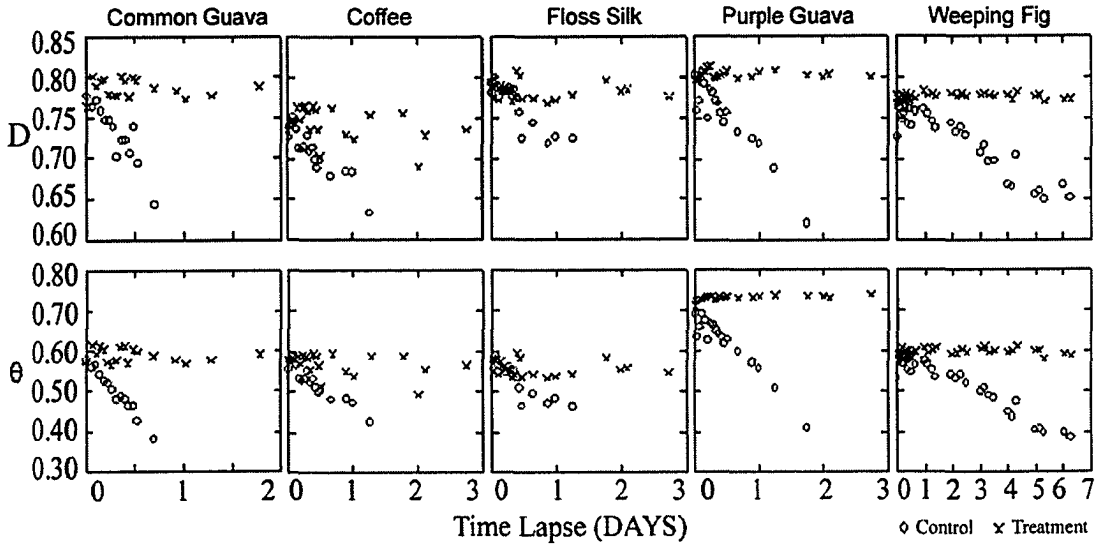

Figure 2-6: Indices D and θ, calculated for wavelengths 350 nm to 2500 nm, plotted against time since clipping.

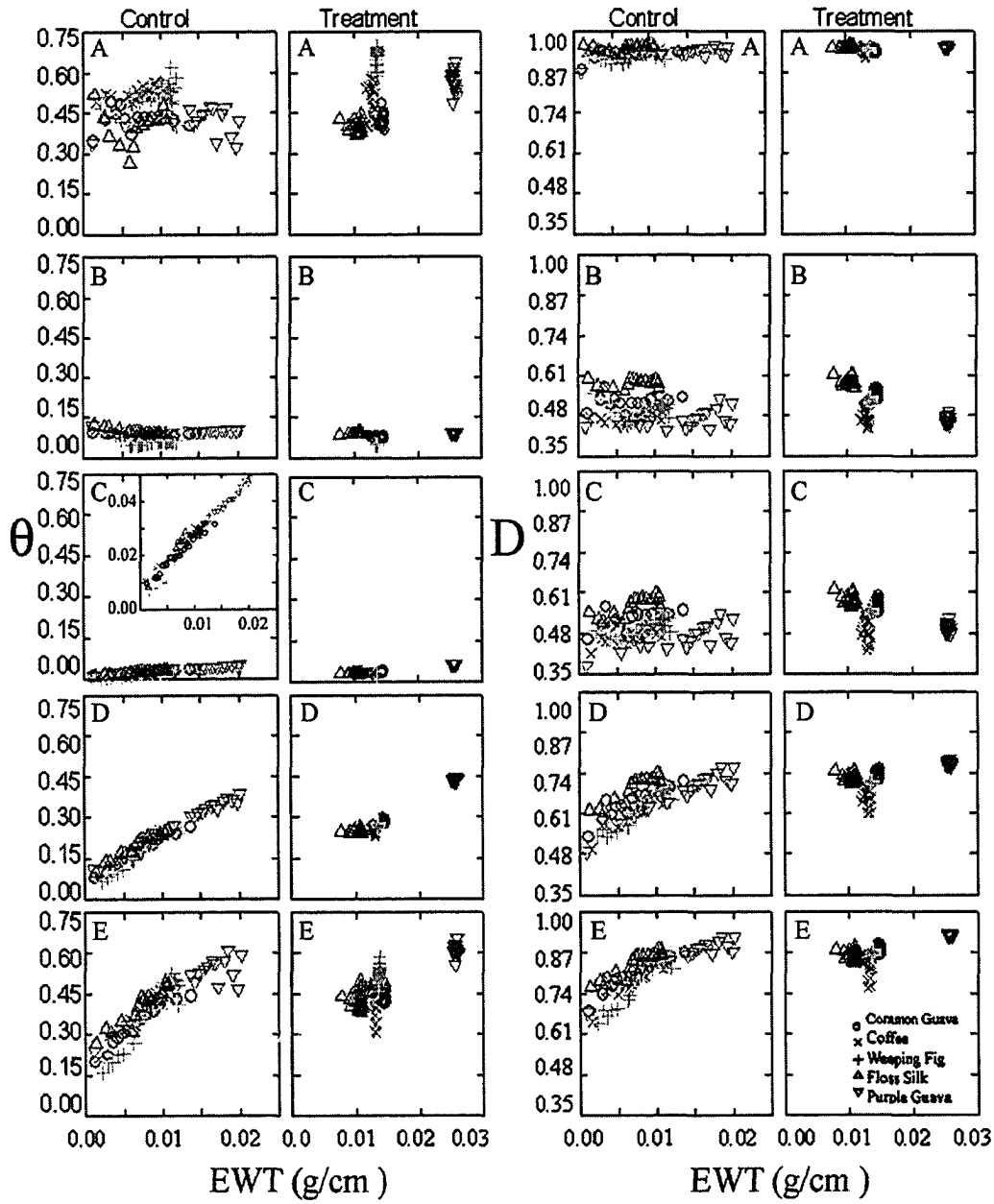

Figure 2-7: Indices θ and D plotted against EWT. Indices were calculated for each wavelength region A, B, C, D, and E (labeled in plot areas; see Figure1). R^2 values calculated using control and treatment data combined were <0.19 for regions A and B for both indices; they were 0.953, 0.960, and 0.718 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.

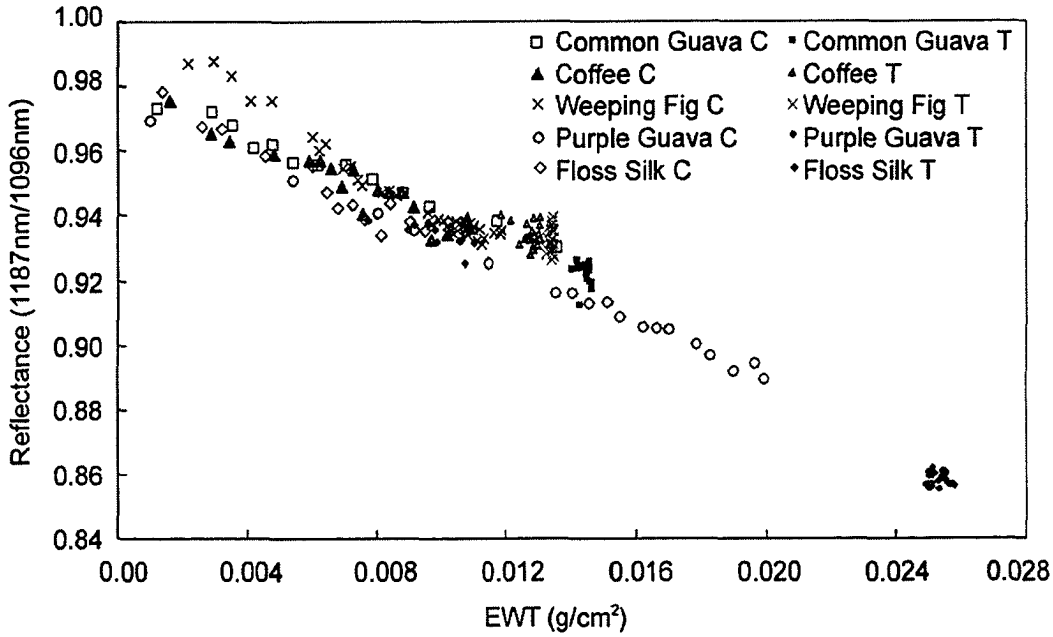

Figure 2-8: Reflectance ratio 1187/1096 nm plotted against water content (EWT).

Control and Treatment samples indicated by C and T respectively in legend.

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Chapter Three

3.0 Classification of Tropical Tree Leaf Reflectance and Band Selection

3.1 Introduction

Few studies have focused on classification of tree species from spectral data despite the many potential applications in conservation biology and forest conservation/management (Peñuelas and Filella, 1998; and Turner et al, 2003). Even fewer studies (e.g, Fung and Siu, 1998; and Cochrane, 2000) have examined classification of tropical tree species due to their spatial extent and high biodiversity and one (Clark et al, 2005) has investigated shortwave infrared wavelengths (SWIR) for this purpose.

Successful classification of boreal forest, crops and other features using remote imagery encourages continued research into the statistical discrimination of tree species. Martin et al (1998) for example was able to achieve an accuracy of 75% when classifying 11 North American forest cover types using airborne visible/infrared imaging spectrometer (AVIRIS) imagery. Other studies looking at boreal forest species differentiation include Van Aardt and Wynne (2001), who studied three pine and three deciduous tree species; Gong et al (1997), who investigated 6 coniferous species; and Shen et al (1985), who studied 3 coniferous and 2 deciduous species.

It is clear that plants have dominant spectral properties controlled by pigment content, water content and leaf structure (Gates et al, 1965) but it is not clear for tropical species which characteristics differ enough to enable remote detection. While it is understood that environmental variables, such as photon flux, temperature, nutrient availability, humidity, and precipitation can result in increased spectral variability within species

groups (Curran et al, 1992; Carter and Young, 1993; and Cao, 2000) it is questionable whether or not intra-specific variation surpasses inter-specific variation.

Quantification of variability is more attainable using imagery with high spatial and spectral resolution from sensors such as Hyperion (Pearlman et al, 2003), and AVIRIS (Vane et al, 1993). Such imagery is more suitable for studies of high diversity tropical forests because they allow measurement of inter/intra-specific variability at sub-canopy spatial scales and fine spectral feature scales. High spectral resolution also introduces challenges for data processing techniques; most notably the large number of bands requires more data processing time and storage space (Bell and Baranoski, 2004).

Strategies that have been taken to manage the large number of bands include pre-analysis processing such as conversion to principle components (Bell and Baranoski, 2004), derivatives (Tsai and Philpot, 1998), wavelets (Kaewpijit et al, 2003), and filtered data (Van Aardt and Wynne, 2001). Bands can also be chosen using statistical methods such as 1-way ANOVA (Luther and Carrol, 1999), correlation analyses (Shaw et al, 1998), and linear discriminant analysis (Gong et al, 1997; and Fung and Siu, 1998).

Discriminant analysis is particularly suited for classification of species because it reduces the number of bands but more importantly selects bands that contain maximal differences among species groups and minimal differences within those groups. It is more useful than PCA for example which chooses eigenvectors containing the maximum amount of variability, whose results can vary depending upon non diagnostic features and will actually hinder classification. Eigenvectors can capture variation caused by sensor noise, for example, which would be detrimental for discrimination of species.

Determination of bands or indices with high discriminatory power is essential for classification of remote sensing imagery.

The main objective of this study was to investigate the potential use of leaf reflectance to discriminate between twenty tropical tree species at the leaf scale. We hypothesized that the selected 20 tree species have different leaf spectroscopy properties and consequently using reflectance data these species can be differentiated. The relevance of these results for the interpretation of airborne imagery and the investigation of canopy scale species differentiation would represent the next step of this research. Even though twenty species is a relatively high number to study when compared to Van Aardt and Wynne's (2001) study of six boreal species or Cochrane's (2000) study of 11 tropical species we must point out that our study can not be considered representative of a diverse tropical forest where hundreds of tree species can exist (Hartshorn and Hammel, 1994).

In order to select/limit the number of bands used for classification of leaf spectral data, collected from these twenty tropical species, we applied Wilk's stepwise discriminant analysis. Four sample sets were analysed to test the robustness of this technique, given that two assumptions of discriminant analysis were not met: 1) the number of available bands (e.g. 2150) exceeds the the sample size though the number of bands selected does not, 2) the species groups did not display equal variance Five different band sets (band ranges and indices) were analysed to determine how sensor limitations, caused by atmospheric interference for example, affects selected bands. The chosen bands were compared against each other and evaluated based upon classification accuracy of a test dataset.

The tree species tested in this study were all found in the Great Green Macaw's (*Ara ambigua*) habitat and are of paramount importance to the survival of this bird in the Caribbean lowlands of Costa Rica (Powell et al, 1999). The Great Green Macaw is an umbrella species, meaning that it shares habitat with many other animals and plants and its welfare indicates the welfare of these other coexisting species. Currently, this species is listed as vulnerable by the International Union for Conservation of Nature and Natural Resources (Birdlife International, 2004) and is under severe pressure due to the extent of its habitat deforestation and forest fragmentation. Current efforts to conserve this emblematic species include the establishment of a new National Park and the consolidation of the proposed San Juan-Braulio Carrillo biological corridor. In this context the ability to map even one of the species would benefit these conservation efforts, especially if the detectable species was important for nesting or feeding of the Great Green Macaw; in such a case areas including detected trees could be protected as habitat.

3.2 Study Area

This study was conducted in INBioparque and in the La Selva biological station, Costa Rica. Both sites were chosen to acquire leaf samples due to their diverse vegetation, readily available facilities, and their affinity for supporting research. INBioparque is an eco-tourist location near the capital San Jose, Costa Rica. It has a botanical garden that contains trees representative of several ecosystems native to Costa Rica such as the Tropical Dry Forest, Central Valley Forest, Humid Forest, Tropical Rain Forest and Wetlands. La Selva is a biological station (longitude: 84°00'12.959"W,

latitude: 10°25'52.513"N) bordering the foothills of the central volcanic chain and the Caribbean coastal plain and is within the San Juan-Braulio Carrillo Biological Corridor that protects representative habitat of the Great Green Macaw. In general INBioparque trees were smaller than La Selva's and therefore had fewer leaves that could be sampled. Both sites had readily accessible laboratories enabling quick measurements following sampling.

3.3 Methods

3.3.1 Sample Acquisition

The tree species (Table 3-1) chosen for sample collection were selected as the most important species for the survival of the Great Green Macaw (*Ara ambigua*) (Powell et al, 1999). *Sacoglottis trichogyna* and *Dipteryx panamensis* trees are the most important food sources during the reproductive stage of this bird, particularly the *D. panamensis* tree which is the only tree species used for nesting. A total of 20 trees, 1 per species, were selected within INBioparque, and La Selva.

Leaves were sampled from the well-lit upper portion of tree crowns to avoid sample variability introduced by light environment. Delucia et al (1996) have shown that light environment is a significant factor affecting leaf structure. Sun leaves are more relevant for the remote sensing context of this study because at the top of a tree canopy they strongly influence remote spectral measurements. Leaves at INBioparque were measured immediately in the laboratory while leaves collected from La Selva trees were put in bags and brought for measurement in the laboratory within 2 hours. Two hours was an acceptable time frame to preserve leaf freshness (Chapter 2). None of the leaves were

intentionally dark adapted but La Selva leaves were collected at dawn and may have been partially dark adapted prior to measurement.

Based on expectations tests presented in the results section, a minimum of 15 leaves were collected from each tree at both sites. Exceptions include the *D. panamensis* tree (13 leaves) sampled at Inbioparque due to the small size of the available tree and corresponding reduced number of leaves available without damaging the tree. Three of the sampled *D. panamensis* leaves appeared to be less than fully mature; analysis procedures were performed with and without these sample measurements.. *Sloanea geniculata* leaves had holes and scars likely caused by insects; these were avoided during measurements but reduced the sample size to 12. *Conceveiba pleiostemona*, and *Inga pezizifera* leaves were damp from rain and the leaf surfaces were gently dried with gauze.

3.3.2 Spectral Measurements

Leaf sample reflectance was measured using the Analytical Spectral Devices Fieldspec FR spectrometer, which is composed of 3 internal sensors ranging from 350nm to 975nm, 976nm to 1,770nm and 1,771nm to 2,500nm with spectral resolutions of 3 nm at 700nm and 10 nm at 1,400 nm and 2,100 nm. The sensor tip was positioned at nadir above each leaf resulting in a 3.55 cm^2 field of view (FOV); this area was selected to fit the smallest leaf sampled at INBioparque. The FOV for the *Lecythis ampla* leaves was smaller, 1.4 cm^2, in order to fit within the perimeter of the small leaves. A 50 watt quartz halogen lamp was oriented at 45 degrees from the normal to the surface and aimed directly at the FOV of the sensors. A black wooden panel measuring less than 5% reflectance was placed below each leaf to absorb light transmitted through the leaves. A

99% reflectance panel (Spectralon, Labsphere, North Sutton) was used to calculate leaf reflectance by dividing leaf radiance by that of the panel's.

Each leaf was measured in 3 to 7 (3 for most species) spots along an axis perpendicular to the main leaf vein except for palm leaves which were measured on a central axis parallel with the length of the leaves. For small leaves the cumulative field of view from the three measurements covered most of the leaf surface area. For larger leaves the minimum of 3 spots per leaf was determined using expectation tests presented in the results section. At each spot 10 measurements were averaged. For each leaf this process lasted a few seconds precluding heating of the leaf surface.

3.3.3 Linear Discriminant Analysis

The spectral measurements data were formatted for Wilk's linear stepwise discriminant analysis. This type of discriminant analysis functions by first selecting the band with the lowest Wilk's lambda value, which indicates group separability, then at each subsequent step the band that best increases species group separation is added to the model (Huberty, 1994). Four random 50% samples of leaf spot reflectance measurements were chosen in order to test the consistency between the four resultant models; the remaining 50% was used to evaluate the classification accuracy of the models. SAS (Statistical Analysis System) and SPSS (Statistical Package for the Social Sciences) were used to perform linear discriminant analyses upon each 50% sample labelled A, B, C, and D. SAS was chosen because of its ability to run stepwise discriminant analysis with all 2,150 bands as inputs. The top 30 bands selected for each sample by SAS were grouped together, resulting in a maximum of 120 bands, and then

entered into SPSS for a rerun of Wilk's stepwise discriminant analysis. SPSS was used as a secondary step because of its comprehensive output and flexible options.

The above methodology was applied to indices (Table 3-2) and band sets (Table 3-3) in order to model sensor scenarios and to determine wavelength regions of importance for separating species. Band set 1 includes all bands from 350 to 2500 nm; band set 2 is band set 1 minus bands that would be affected by atmospheric absorption in an airborne or satellite remote sensing situation; band set 3 includes bands from 1076 to 2470 nm without atmospheric bands; band set 4 includes bands that can be measured by the Analytical Spectral Devices HH spectrometer (350-1075 nm); and lastly band set 5 includes indices listed in Table 3-2, and bands also in band set 2. Chlorophyll indices were selected from a recent comparative study by Le Maire et al (2004), while water indices, the hyperspectral normalized difference vegetation index (NDVI) and hyperspectral simple ratio (SR) were selected from a comparative study by Sims and Gamon (2003). These indices were complemented by the photochemical reflectance index of Peñuelas et al (1995b), carotenoid-chlorophyll a ratio of Peñuelas et al (1995a), the normalized difference water index (NDWI) of Gao (1996), the moisture stress index (MSI) of Rock et al (1986) and finally the cellulose absorption index (CAI) of Nagler et al (2003).

3.4 Results

3.4.1 Expectation Tests

The minimum number of spots per leaf and leaf sample size per species were determined at INBioparque using expectation tests (Richard, 1979) for Near Infrared

(NIR) wavelengths (Figure 3-1). In this test, an adequate sample size is determined when the average reflectance approaches a constant value as more measurements are included. Figure 3-1 displays the average reflectance with increasing number of spots measured on one leaf for each of the six species. For small leaves (*D. panamensis, Cedrela odorata, and Tabebuia guayacan*) no more then 3 spots could be measured. For each species results are shown for three wavelengths of highest standard deviation. With the exception of *Pentaclethra macroloba* the average reflectance approached a constant value with the measurement of 3 spots, thus this number was used as a minimum number of measurement per leaf. *P. macroloba* is a compound leaf presenting gaps in leaf surface area therefore finding locations where the leaf material filled the field of view was difficult and leaf material could have overlapped. In Figure 3-2 expectation test results for leaf sample number are displayed for the same species. For all species the change in the average reflectance is smaller than 1% following seven leaf measurements and less than 0.5% for five of the six species following 14 measurements. Consequently, wherever possible, the minimum number of leaf samples per tree was 15.

3.4.2 Species Mean Reflectance

Mean reflectance for the six species in the VIS region was similar but there was increased separation at longer wavelengths (Figure 3-3). Interspecies reflectance relationships change across the spectrum, for example, *Vochisia ferruginea* and *Dussia macroprophyllata* have very close mean reflectances at 900 nm but differed at 1,660 nm. NIR and SWIR wavelengths can in some instances provide enhanced discriminating power (Figure 3-3).

3.4.3 Discriminant Analysis Models Per Band Set

The bands selected by discriminant analysis for each sample and band set are summarized in Table 3-3, and Figure 3-4. In general the bands selected early for each model are the most consistent between the four sample groups. Bands selected for all models were considered robust to small changes between samples. Band differences between band set models resulted in classification accuracy differences up to 15% (Table 3-3).

The models derived from wavelengths 350 nm to 2500 nm (band set 1) and for sample sets A, B, C and D are composed of 5 to 15 bands. Models with the least number of bands (1C and 1D) also had the lowest classification accuracies. The first three bands added to each model were 1440 nm, 1316 nm, and 1662 nm. Common bands that were not necessarily selected in the same order are 1720 nm, 735 nm, and 390 nm. The average classification accuracy of models 1A to 1D was 90%, the third highest value when compared to the averages for the other band sets.

For Band set 2, bands were removed in order to approximate remote hyperspectral imagery affected by atmospheric absorption. The four resulting models had 11 to 14 bands; the first five bands added were approximately 2326 nm, 1090 nm, 730 nm, 707 nm, and 739 nm. Of the remaining selected bands 37% were VIS bands. Reoccurring bands or narrow wavelength regions (<20nm) that were selected at different times for each model were 1500 nm (100%), 1661 nm (75%), 1770 nm (50%), 1240 nm (75%), and 600 nm (100%). These models have a 95% classification accuracy average, the highest when compared to the other band set model averages.

For band set 3 atmospheric bands were removed again to represent realistic atmospheric effects on remote imagery and VIS bands were removed to observe if classification results are different without the VIS bands. The number of bands in each model ranged from 7 to 10. The first two bands selected for these models (2326 nm and 1090 nm) were the same as the bands selected for models from band set 2. The order of the bands selected was slightly variable but the next four bands included were 1963 nm, 1500 nm, 1661 nm, and 1729 nm. Average model classification accuracy was 84%, one of the two lowest averages when compared to the other band set model averages.

Band set 4 was intended to model data from sensors such as the Analytical Spectral Devices HH that only measures light from 350 nm to 1075 nm. Six to eight bands were chosen for each model. The first four bands selected in each model were 911 nm, 737 nm, 711 nm, and 722 nm to 747 nm. Out of the remaining 12 bands five were between 650 nm and 700 nm, two were between 550 nm and 600 nm, one was 526 nm, and four were less than 450 nm. These models had a classification average of 84%, sharing the lowest average value with that of band set 3.

Several indices (Table 3-2) were added to band set 2, creating band set 5, in order to determine if indices linked to biochemistry are important for differentiating between these species. The number of bands/indices selected (13 to 22) increased compared to the other model types and several different indices were selected before any individual bands. The moisture stress index (MSI), Datt's (1999) chlorophyll index (DattB), and Sims and Gamon's (2002) chlorophyll index (SGA) were selected first for all four models. The fourth band selected for each model was either the Water Index developed by Peñuelas et al (1993; 900/970) or the Car/ChlA index (Peñuelas et al, 1995a).

Following the indices the first band selected was approximately 1785 nm for all four models then the consistency between the models ceased and different bands were included in each model. The differences between each of the models do not impact the overall classification results much because they range only from 93.1% to 95.7%. The average classification value is 94%, a slightly lower value than the average accuracy of band set 2.

3.4.4 Selected Band Variance

The distribution of species data was compared to other species and described as interspecies relationships. Figure 3-5 depicts distributions of species data for several bands and indices belonging to the models (Table 3-3). The first bands selected for Band Set 1, 2, and 3 models were 2326 nm or 1440 nm (Figure 3-5); these bands in addition to selected bands 1963 nm, 1930 nm, and 1500 nm show similar inter-species relationships; for example in all of these bands the *C. pleiostemona* mean is slightly higher than the *D. panamensis* mean, while the *V. ferruginea* mean is the lowest of all 20 species. The second bands selected for Band Set 1, 2, and 3 models were NIR bands 1316 nm and 1090 nm, which including 912 nm are the second group sharing inter-species commonalities (1090 nm is plotted in Figure 3-5 as an example). Band 1661 nm was selected third for many of the models and demonstrated an inter-species pattern similar to 1715 nm, and 1787 nm, but different from previous bands mentioned (Figure 3-5). *V. ferruginea* and *T. guayacan* have distinct values for band 1661 nm compared to the rest of the species. Band 730 nm (Figure 3-5) contains the fourth inter-species pattern also demonstrated by band 747 nm and 737 nm. The 730 nm reflectance values appear (in

Figure 3-5) to have better separation for more species subsets than 1661 nm (e.g, *Byrsonima crispa* versus *Sterculia apetala*).

The models derived from indices and individual bands (band set 5) show the importance of specific spectral features for differentiating species. The first variable selected from band set 5 was MSI, thus it has greater separation of species compared to the other input bands such as 2326 nm (Figure 3-5). The second variable selected, a chlorophyll index developed by Datt (1999; DattB), clearly shows separation of *T. guayacan* from the other species (Figure 3-5). Sims and Gamon's (2002; SGA) chlorophyll index was selected third and when compared with Datt's (1999; DattB) chlorophyll index shows a slight shift for *Welfia regia* and *I. pezizifera* species (Figure 3-5). These two indices are composed of different bands (Table 3-2) but are highly correlated with one another (r = 0.97). The next variable chosen was either the carotenoid-chlorophyll ratio (Peñuelas et al, 1995a) or WI_{970}, developed by Sims and Gamon (2003). WI_{970} shows greater group separation than the Car/Chl ratio as shown in Figure 3-5, and was found to have an inverse correlation coefficient of r = 0.85 with MSI. The subsequent variables were not as consistent between the four models (5A to 5D) but resulted in a maximum 2.6% difference in classification accuracy.

3.4.5 Species Level Classification Results

In general, all of the species, except for *D. panamensis*, were identified correctly most of the time (Figure 3-6). *D. panamensis* was confused mostly with *Laetia procera* by all of the models. As mentioned in section 3.4.3 and shown in Table 3-3, models for band

sets 2 and 5 had the highest overall accuracies. Species results show that they also have the highest accuracies for *D. panamensis*, especially for band set 5 models.

The classification of some species was not affected by the restriction to SWIR (band set 3) or VIS (band set 4) bands. The SWIR models (3A to 3D) maintained high accuracies for six species, while five different species had high accuracies when classified using VIS models (4A to 4D; bold on Figure 3-6). The high classification accuracy for these species indicates that they do not require more bands for differentiation. *S. trichogyna* was the most noticeably affected by the loss of SWIR bands, as its values dropped from 100% (model 2A) to 55% (model 4A; Figure 3-6). Other species classification accuracy values dropped for both SWIR (3A to 3D) and VIS (4A to 4D) band models (*L. procera* and *B. crispa*) suggesting at least some benefit from using bands from both regions.

The models derived from indices and bands differentiated the species very well (Figure 3-6e). Each of the species had classification accuracies greater than 94% for at least one sample, even *D. panamensis*, which had values ranging from 52% to 100%. The remaining species had values greater than 76% with the sum of their four classification accuracies being greater than 345%.

3.5 Discussion

The main objective of this study was to determine if 20 tropical tree species, important to the habitat of the Great Green Macaw, could be differentiated using leaf spectral properties. All of the species were differentiated with greater than 90% accuracy by at least one model. One of the species, *D. panamensis*, was the most difficult to

classify, but this was likely due to the immaturity (light coloring and structural flexibility) of three of the thirteen sample leaves. Despite this difficulty the *D. panamensis* leaves were classified with 100% accuracy when indices were used as variables within the classification model. Other species such as *W. regia* and *T. guayacan* also showed VIS pigment variations but were differentiated accurately.

Four slightly different samples were used to evaluate the robustness of Wilk's stepwise discriminant analysis for selecting variables from the wavelength region 350 nm to 2500 nm, a relatively large data set with 2150 input bands. Results indicate that the first bands selected were the most consistent among samples and overall classification results varied by a maximum of 15% among samples (Table 3-3). Thus stepwise discriminant analysis is robust to small amounts of variability for the first bands selected but remaining band combinations will likely be affected and can also affect classification results.

A shortcoming of this discriminant analysis is that when applied to reflectance data the spectral shape, which can include diagnostic features (Curran et al, 2001) is not exploited to its full extent. This is evident by the subtle increase in classification accuracy when indices, which exploit these diagnostic features, were added to the classification models. Also, indices were selected before individual bands, meaning that they had better discriminatory ability. Another shortcoming to this analysis was indicated by the selection of several correlated chlorophyll indices within single models. This problem can be managed by altering the tolerance criteria within SPSS; for this study the band selection constraints were kept constant for all of the models.

The spectral range of input bands were manipulated in order to create classification models more appropriate for data affected by atmospheric absorption and sensor wavelength limitations. The resulting models were used to interpret capabilities of indices and VIS and SWIR wavelengths separately or in combination for classification. When SWIR bands were included resultant models first included bands associated with water absorption. In particular, bands were repeatedly selected near or at the 1430 nm water absorption feature. MSI, which was correlated with canopy moisture stress by Rock et al (1986), was selected first from all of the indices and bands listed for band set 5 (Table 3-3). Thus it seems that for the species of this study leaf water content was the best individual variable for differentiating the species. Similarly, Roche et al (2004) found that leaf water content (% of total leaf weight or its inverse leaf dry matter content) proved to be the most reliable variable for differentiating 10 plant species at 8 sites in southern Mediterranean France when compared to eight other leaf traits (area, fresh weight, dry weight, volume, density, thickness, specific leaf area, leaf nitrogen content). Leaf water content was also correlated with average minimum temperature for each site. Leaf water content and climate variable relations can be different for tropical regions although Geeske (1994) concluded that elevation affected leaf size and leaf mass per area (LMA) for trees in Hawaii.

Models with only VIS and NIR bands up to 1075 nm were insufficient to separate all 20 species but despite the lack of strong water bands some species were separated accurately (Figure 3-6). The best model derived from this region contained a band in the NIR plateau, several bands along the red edge and some bands near the green peak (526 nm – 558 nm). NIR and red edge bands were the most consistently selected and

classifications without them had some of the lowest accuracies (models 1C, 1D, 3A, 3B, 3C and 3D). NIR plateau bands, which were selected first from this band set and second from many other band sets, have been associated with scattering of light from within the leaf (Slaton et al, 2001) thus species with leaf internal structure differences can be the cause of these band selections. Datt (1999) concluded that red edge bands were strongly associated with chlorophyll concentration therefore variation in chlorophyll concentrations caused by speciation can be the cause for selection of these bands; the inclusion of chlorophyll indices in other models reaffirms this point.

Models with only SWIR and NIR bands, like models with only VIS and NIR bands, also had lower differentiation accuracies, but again not for all species. In fact, the list of species with low differentiation accuracies was different for each model type; thus when VIS, NIR and SWIR bands were combined models had higher overall classification accuracies (models 2A to 2D). Models derived from all bands and indices linked to biochemical features including water content, and pigment content had the second highest differentiation accuracies and the highest accuracies for the otherwise poorest classifying species *D. panamensis*. It appears for this group of species that several bands or indices representing water absorption, pigment absorptions, and NIR scattering are necessary to differentiate all of the species in this study accurately, and that these representations can be accomplished with several combinations of bands and/or indices.

For other groups of species different ranges of bands have been found to be useful for species separation. Fung and Siu (1998) tested wavelengths 400 nm to 900 nm for classifying tree leaves from 12 subtropical (Hong Kong) tree species and found that 7 out of 12 were identified with accuracies greater than 90%. Gong et al (1997) also tested

VIS and NIR bands for differentiation, but of tree canopies for 6 conifer species and found that VIS bands had higher discriminatory power than NIR bands. Van Aardt and Wynne (2001), who tested bands 350 to 2500 nm, found that different band sets were useful for separating canopies of 3 pine tree species, 3 hardwood tree species and for separating the pine from the hardwood species. The hardwood trees, which were most similar to the 20 species used for this study compared to coniferous trees, were separated with bands largely in the VIS and NIR with few bands in the SWIR regions; notably there was not a preference for water bands. These classification examples, in addition to the results from this study, demonstrate several wavelength combinations that are possibly only relevant for the species under investigation. Some of the wavelength selection differences could also result from differences between leaf and canopy measurements.

3.6 Conclusion

The results from this leaf scale investigation indicate that Wilk's linear discriminant analysis is an effective technique for selecting reliable bands/indices for classification of leaf data. Differentiation of 20 tropical tree species using these selected bands/indices resulted in high accuracies ranging from 79% to 97%. It was found that models with the highest accuracies included bands or indices correlated with water content (especially 1450 nm band and MSI) followed by pigmentation properties (red edge bands, green peak and chlorophyll indices were selected). While accuracies were high when the 350 to 2500 nm spectrum was exploited, some species were identified accurately (up to 100%) using only NIR and SWIR bands (1076 -2500 nm) or using only VIS and NIR bands (350 - 1075 nm). Furthermore, published investigations of other species groups

found different band/indices significance patterns for identification suggesting that spectral differences can vary among species groups. Future studies investigating species differentiation should analyze bands/indices within the entire 350 nm to 2500 nm region as this study suggests that exclusion of any of these wavelengths may reduce identification accuracy.

It was found that indices linked to leaf water content, and chlorophyll concentration had better discriminating potential than individual bands between 350 nm to 2500 nm. Index based models resulted in improved identification for some species and high overall differentiation accuracies. Consequently continued research focusing on leaf/canopy biochemical and biophysical indices/variables should also increase species detection capabilities. The ability to detect species would have significant ramifications for the field of ecology, conservation biology, and forest conservation and management.

3.7 Figures and Tables

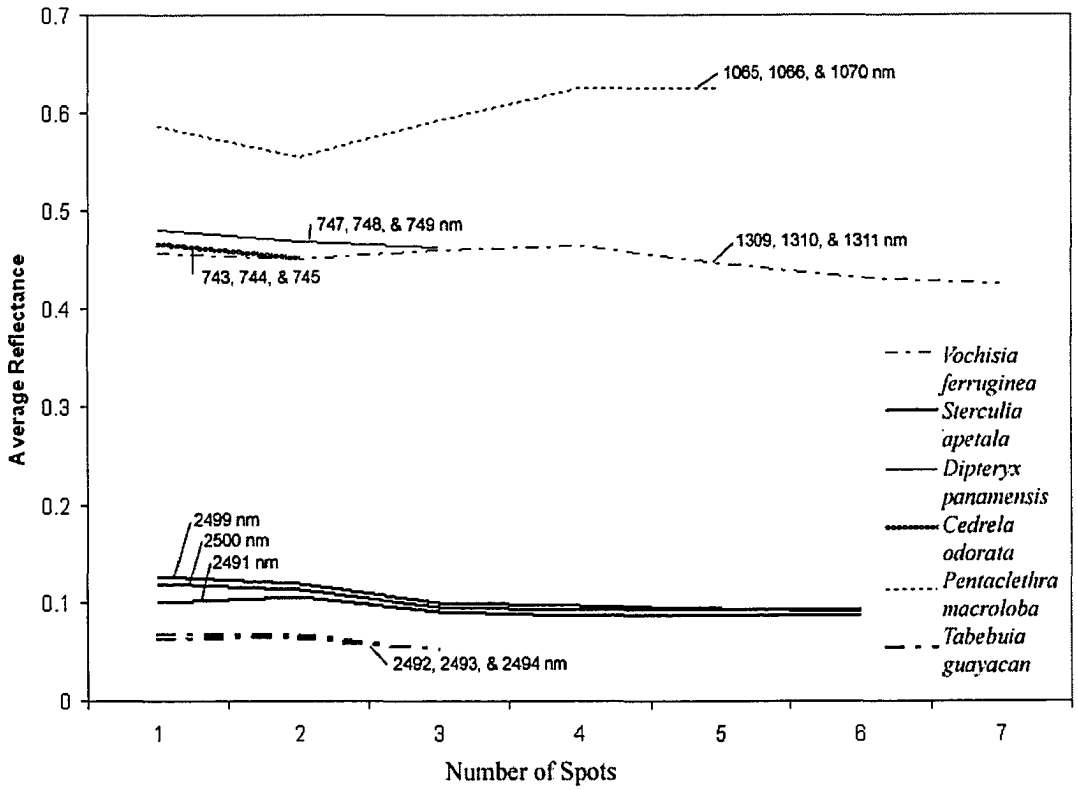

Figure 3-1: Expectation test results, which were used to determine the minimum number of measurements per leaf. Plot represents changing average reflectance values as sample spot number increases. Leaves of some species were too small for more than 3 measurements.

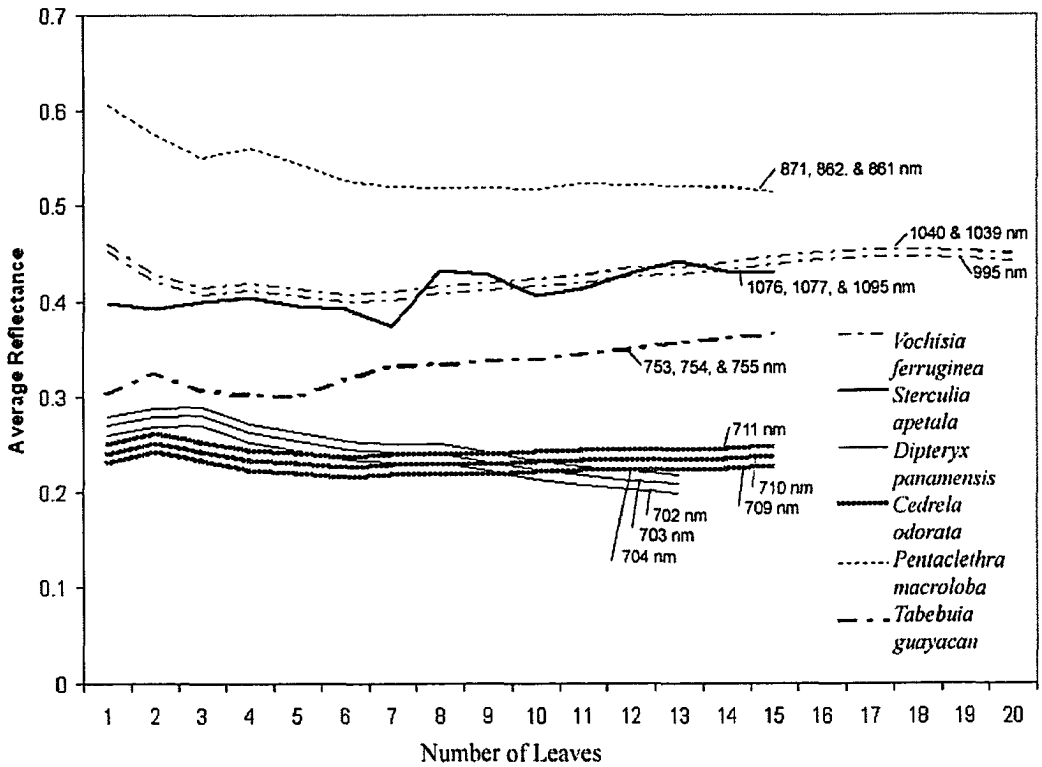

Figure 3-2: Expectation test results, which were used to determine the minimum number of leaf measurements for each species. Numbers in nm associated with lines indicate the wavelength at which the test was conducted. 15 leaf measurements were chosen as the minimum number of measurements based upon slope values.

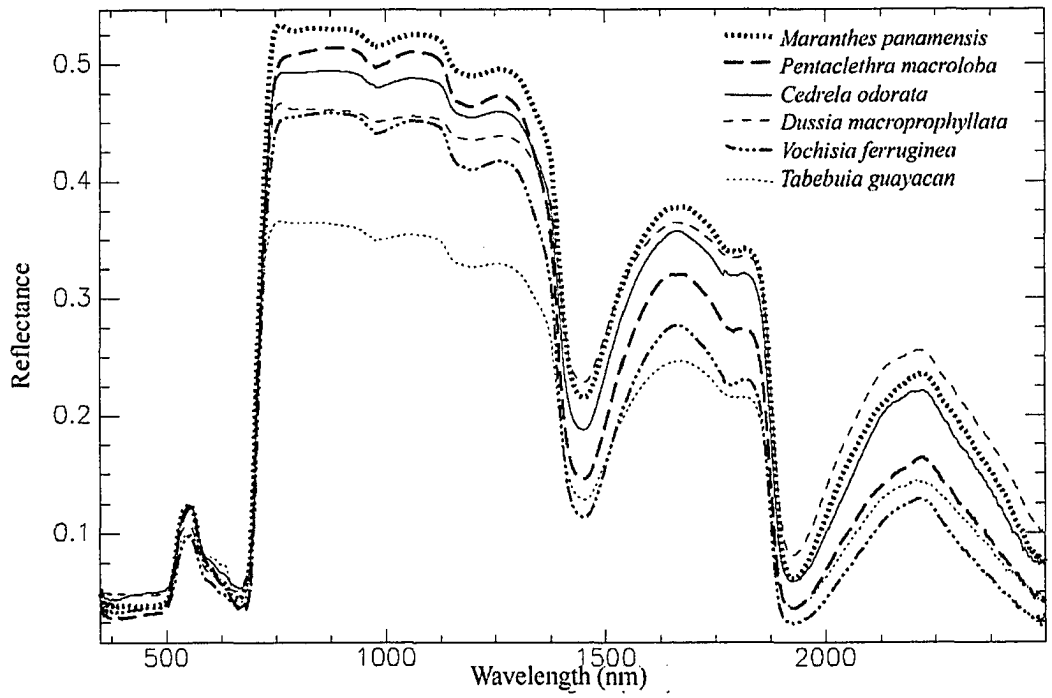

Figure 3-3: Mean spectral reflectance for selected species. Note the fluorescence peak near 738 nm (red edge and near infrared plateau convergence) on the *Maranthes panamensis* spectral line and the change in relative position of species reflectance between 1000 nm, 1450 nm, 1600 nm, and 2200 nm. In order to preserve clarity not all species are shown.

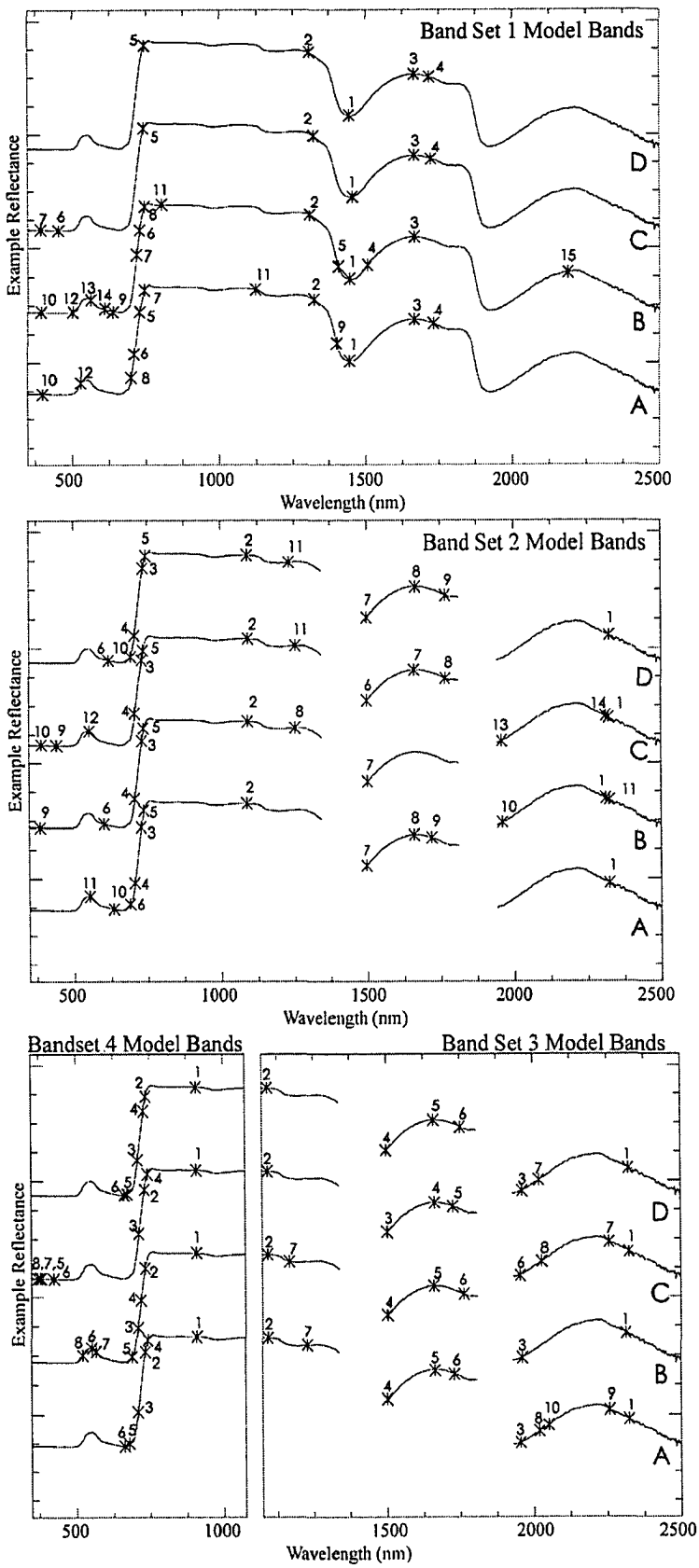

Figure 3-4: Model bands selected from band sets 1 to 4. Numbers indicate order of band selection. Letters on the right side of each plot indicate the input sample for each model.

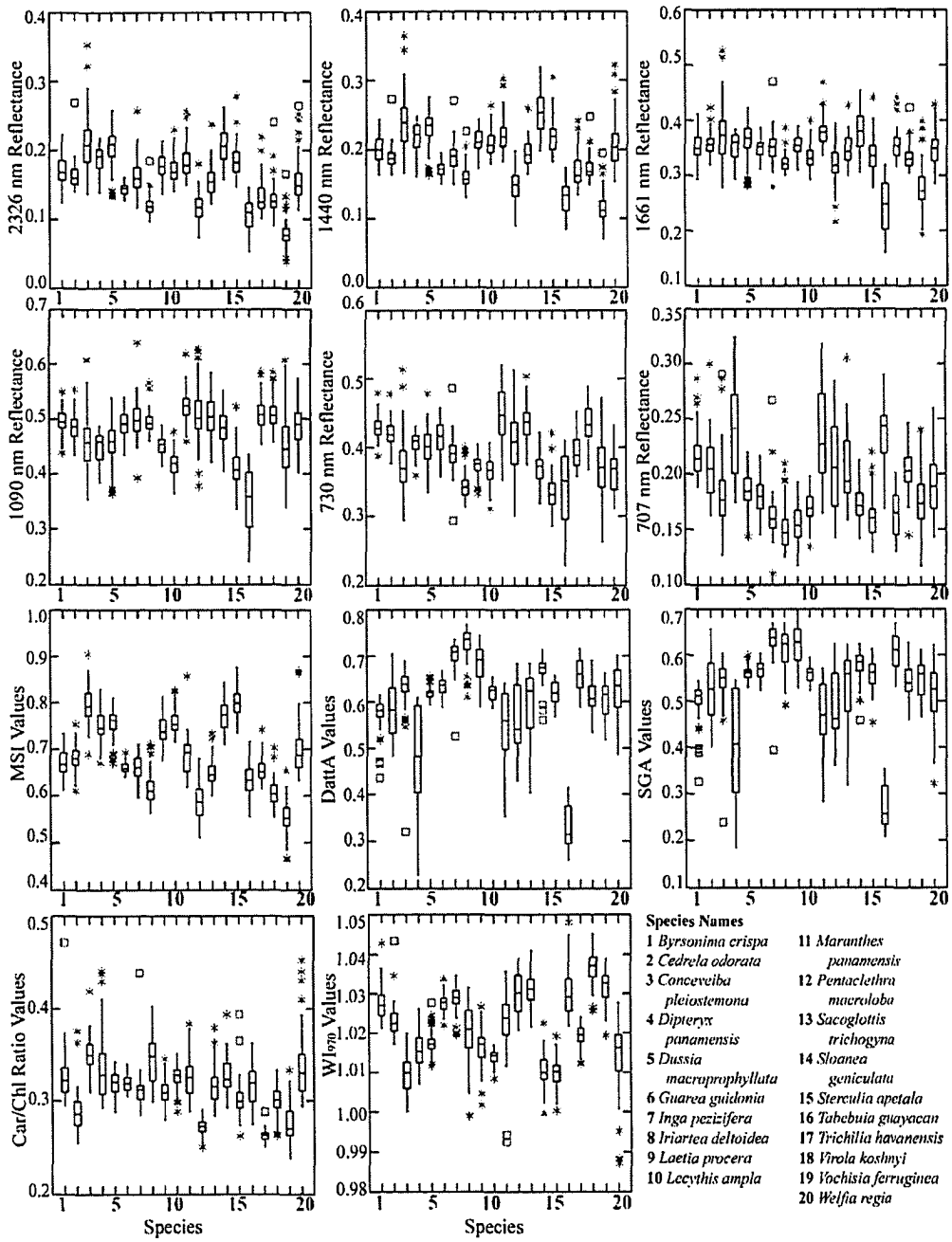

Figure 3-5: Box-plots of selected variables showing relative distributions of species data. Species numbers correspond to x-axis numbers. Note similar positioning of species data for 2326nm and 1440nm and also note different positioning of species data for the other bands and indices shown here.

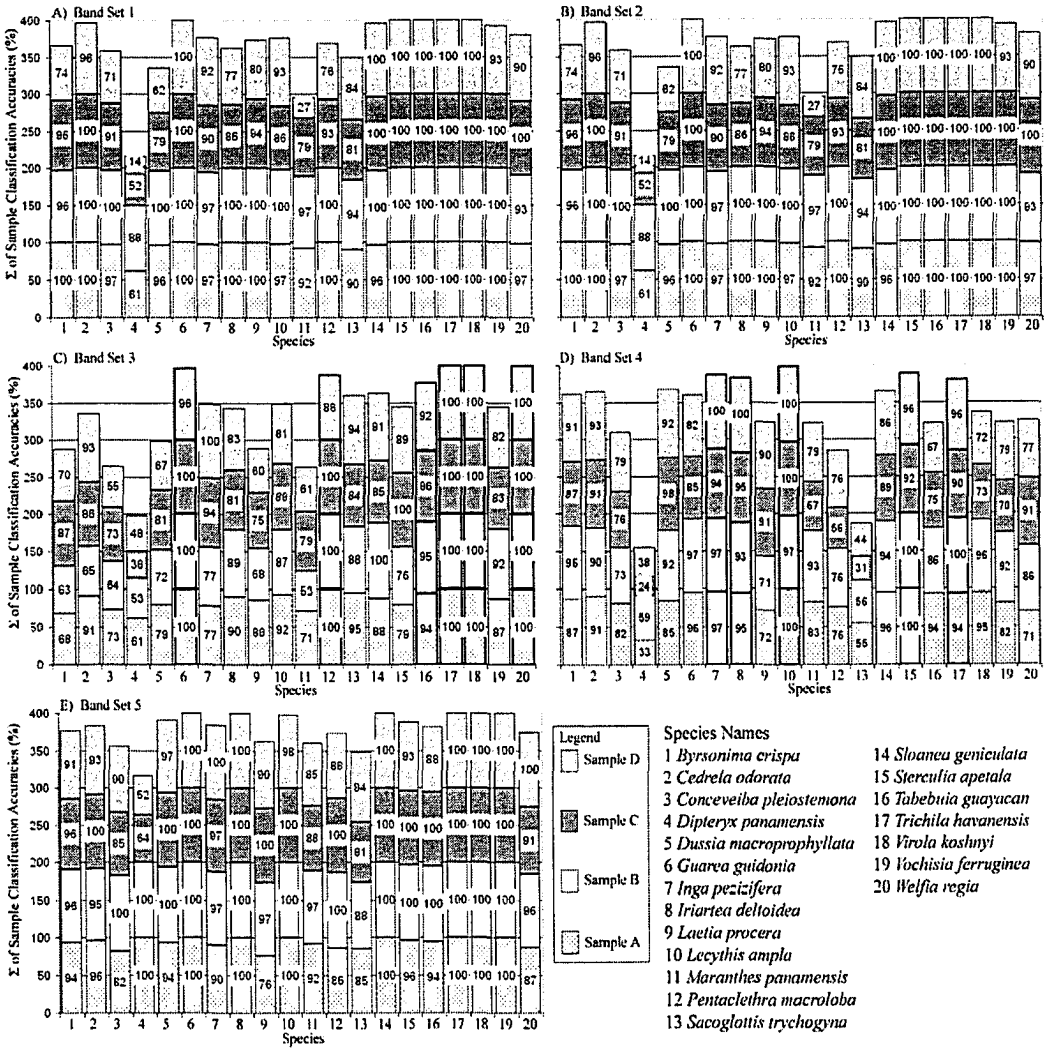

Figure 3-6: Species level differentiation results for band set 1 to 4 models. Numbers within each bar represent the identification accuracy for that specified species and sample. Bars in bold highlight species that were classified accurately despite the limited range of bands belonging to those identification models.

Family	Genus	Species	Common Name	# of Leaves	Site
Malpighiaceae	*Byrsonima*	*crispa*	nance	15	La Selva
Meliaceae	*Cedrela*	*odorata*	cedro amargo	15	INBIO
Euphorbiacea	*Conceveiba*	*pleiostemona*	algodón	15	La Selva
Papilionaceae	*Dipteryx*	*panamensis*	almendro	13	INBIO
Papilionaceae	*Dussia*	*macroprophyllata*	sangrillo	17	La Selva
Meliaceae	*Guarea*	*guidonia*	cocora	15	La Selva
Fabaceae	*Inga*	*pezizifera*	guavilla	15	La Selva
Arecaceae	*Iriartea*	*deltoidea*	palmito	17	La Selva
Flacourtiaceae	*Laetia*	*procera*	manga larga	15	La Selva
Lecythidaceae	*Lecythis*	*ampla*	jícarro	18	La Selva
Chrysobalanaceae	*Maranthes*	*panamensis*	pejiballe	19	La Selva
Fabaceae	*Pentaclethra*	*macroloba*	gavilán	15	INBIO
Humiriaceae	*Sacoglottis*	*Trichogyna*	titor	15	La Selva
Elaeocarpaceae	*Sloanea*	*geniculata*	paleta	12	La Selva
Sterculiaceae	*Sterculia*	*apetala*	panamá	15	INBIO
Bignoniaceae	*Tabebuia*	*guayacan*	corteza	15	INBIO
Meliaceae	*Trichilia*	*havanensis*	uruca	15	INBIO
Myristicaceae	*Virola*	*koshnyi*	fruta dorada	15	La Selva
Vochysiaceae	*Vochisia*	*ferruginea*	botarrama	20	INBIO
Arecaceae	*Welfia*	*regia*	corozo	15	La Selva

Table 3-1: Tree species used in this study and the number of leaves sampled. Only one tree per species was sampled. Species are sorted alphabetically by genus. Species descriptions can be found in Gentry (1993), and Mabberly (1990).

Name	Index	Name	Index
Chlorophyll Index (DattA) Datt 1999 & Maccioni et al 2001	$\frac{R_{780} - R_{710}}{R_{780} - R_{680}}$	Photochemical Reflectance Index (PRI) Peñuelas et al 1995	$\frac{R_{531} - R_{570}}{R_{531} + R_{570}}$
Chlorophyll Index (DattB) Datt 1999	$\frac{R_{850} - R_{710}}{R_{850} - R_{680}}$	Water Index (WI_{1180}) Sims & Gamon 2003	$\frac{R_{900}}{R_{1180}}$
Chlorophyll Index (SGA) Sims & Gamon 2002	$\frac{R_{750} - R_{705}}{(R_{750} + R_{705} - 2R_{445})}$	Water Index (WI_{970}) Peñuelas 1993	$\frac{R_{900}}{R_{970}}$
Chlorophyll Index (SGB) Sims & Gamon 2002	$\frac{R_{750} - R_{445}}{R_{705} - R_{445}}$	Normalized Difference Water Index (NDWI) Gao 1996	$\frac{R_{860} - R_{1240}}{R_{860} + R_{1240}}$
Normalized Difference Vegetation Index (NDVI)	$\frac{R_{800} - R_{680}}{R_{800} + R_{680}}$	Moisture Stress Index (MSI) Rock et al 1986	$\frac{R_{1600}}{R_{820}}$
Simple Ratio (SR)	$\frac{R_{800}}{R_{680}}$	Cellulose Absorption Index (CAI) Nagler et al 2003	$0.5\,(R_{2000} + R_{2200}) - R_{2100}$
Carotenoid Chlorophyll A Ratio (Car/Chl) Peñuelas 1995	$4.44 - 6.77 \exp[-0.48\,(R_{800} - R_{445})/(R_{800} - R_{680})]$		

Table 3-2: Various indices tested with discriminant analysis.

Band Set (nm)	Model Name	Classification Accuracy of Test Data	Bands Selected (in order of selection) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1A	96.7	1442,	1316,	1662,	1729,	723,	707,	739,	694,	1397,	396,	1117,	526			
350 to 2500	1B	91.2	1441,	1303,	1663,	1503,	1402,	723,	711,	739,	633,	390,	798,	497,	559,	606,	2185
	1C	90.9	1450,	1316,	1662,	1719,	735,	449,	390								
	1D	81.1	1440,	1303,	1661,	1715,	744,										
2	2A	97.2	2326,	1089,	730,	707,	739,	691,	1500,	1661,	1723,	636,	557				
350-1341,	2B	90.7	2313,	1090,	732,	707,	739,	604,	1503,	1256,	390,	1963,	2326				
1500-1798, &	2C	96.3	2326,	1090,	731,	708,	737,	1503,	1661,	1770,	445,	396,	1235,	557,	1963,	2313	
1954-2470	2D	96.0	2326,	1089,	734,	707,	747,	620,	1500,	1661,	1770,	698,	1235				
3	3A	85.9	2326,	1089,	1959,	1500,	1661,	1729,	1226,	2022,	2259,	2053					
1076-1341,	3B	81.9	2313,	1090,	1963,	1503,	1661,	1763,	1163								
1500-1798, &	3C	86.3	2326,	1090,	1503,	1661,	1729,	1959,	2259,	2030							
1954-2470	3D	81.8	2326,	1089,	1963,	1500,	1661,	1753,	2022								
4	4A	84.8	912,	737,	711,	747,	682,	666									
350 to 1075	4B	87.7	911,	737,	711,	722,	693,	558,	571,	526							
	4C	79.0	912,	736,	713,	747,	386,	429,	385,	377							
	4D	82.5	912,	740,	711,	732,	678,	666									
5	5A	93.1	MSI,	DattB,	SGA,	WI970,	DattA,	1787,	724,	750,	1500,	392,	483,	525,	696		
Indices &	5B	95.7	MSI,	DattB,	SGA,	CarlChl,	SGB,	WI970,	1789,	637,	1500,	380,	664,	2156,	1723,	1341,	753,
350-1341,			733,	712,	702,	1961,	2296,	SR680,	393								
1500-1798, &	5C	93.6	MSI,	DattB,	SGA,	CarlChl,	1787,	722,	743,	1667,	1723,	712,	616,	SR680,	NDWI,	559,	CAI
1954-2470	5D	94.7	MSI,	DattB,	SGA,	WI970,	CarlChl,	SGB,	1778,	1500,	2156,	2296,	1961,	559,	1723,	1628,	SR680,
			525,	380,	2141												

Table 3-3: Spectral bands selected by discriminant analysis and categorized by input band sets and input sample letter. Bands and indices are listed in order of selection. Full index names and abbreviations are shown in table 2.

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Chapter 4

4.0 Conclusions

This thesis provides information about data collection and analysis for the purpose of tropical tree differentiation from remote sensing data. The first paper (chapter two) is in press and examines the spectral effects resulting from two leaf handling techniques. The second paper (chapter three) contains an assessment of band and index importance for classification of tropical leaf reflectance using linear discriminant analysis.

4.1 Leaf Handling Technique

Leaf reflectance data are collected for botanical studies and remote sensing studies (Peñuelas, and Filella, 1998); in both cases it is often essential that reflectance data be acquired from fresh leaves. Chapter two shows that reflectance is affected by leaf handling technique and time-lapse between leaf detachment and measurement. This information is beneficial for researchers developing or assessing leaf handling methodology. At present, there are several handling techniques used by researchers and ensuing variations in spectral reflectance relate to leaf hydration, temperature, and light exposure (e.g, Sims and Gamon, 2003; and Cao, 2000). Time-lapse between leaf detachment and measurement is also variable between studies and can depend upon remoteness of sampling sites (e.g, Asner, 1998; and Horler et al, 1983).

Chapter two shows the effects of two leaf handling techniques and how time delay can affect reflectance. It is evident that leaf water content is a dominant factor that can be managed to minimize spectral change. Placing leaves in plastic bags, with petioles wrapped in moist paper towel kept leaf water content stable and also kept reflectance

stable for a minimum of 7 days. Management of leaf water content is advisable for studies interested in broad spectral features.

Spectral shape had a stronger relationship than raw reflectance amplitude with water content. The ratio, 1187/1096 nm was developed based upon the strong relationship with spectral shape in the NIR, and resulted in the highest coefficient of determination (r^2=0.95) with water content. An unexpected benefit is that this ratio could potentially be used for canopy water detection.

4.2 Classification of Leaf Reflectance

Creation of species distribution maps from remote airborne/satellite imagery is becoming more feasible with improved sensor technology and data availability (Turner et al, 2003). At present few studies have explored spectral differences between tree species (e.g, Martin et al, 1998), and fewer have focused on tropical tree species (e.g, Clark et al, 2005). Many more studies have examined relationships between reflectance and leaf biochemical and biophysical properties (e.g, Curran et al, 2001; Slaton et al, 2001; and Lacaze, and Joffre, 1994) resulting in indices defining these relationships (e.g, Nagler et al, 2003; and Peñuelas et al, 1995). As demonstrated in chapter three indices are beneficial for classification of leaf reflectance into species classes, this is because they usually correlate better to certain vegetation properties than the individual bands that make them; for example, the ratio developed in chapter two has a higher correlation with water content than it's composite bands. When studied they increase understanding of vegetation property variability between and within species groups, which inevitably improves understanding of species identification potential.

Chapter three shows that leaf reflectance of twenty tropical tree species can be differentiated very accurately (79% to 97%). Discriminating bands and indices were selected using linear discriminant analysis, an effective technique that can result in high identification accuracy. This group of species (listed in table 3.2) was differentiated best using bands or indices correlated with leaf water content (especially the 1450 nm water band and MSI), followed by pigmentation properties (red edge, green peak, and chlorophyll indices were selected). Chapter three shows that some species subgroups can be classified very well by using only a particular spectral region (VIS or SWIR) therefore it is unlikely that all species groups will share the same variable requirements for classification. In general, overall identification accuracy was greatly improved by the consideration of all bands from 350 to 2500 nm; future studies should consider that exclusion of any of these bands can hinder identification results.

4.3 Leaf Freshness and Classification

The studies presented in chapter two and three are linked by practicality as the results presented in chapter two were particularly useful for defining the methodology for chapter three. Unexpectedly the conclusions from chapter three reaffirm the importance of handling methodology. Leaf water content can be an important variable for species identification while chapter two shows that leaf handling technique can affect leaf water content. As water content is a potential discriminating variable useful for classification extra precaution must be taken when handling leaves. Improperly handled leaves can result in altered water content thus resulting in detrimental effects upon selection of discriminating variables and upon identification results.

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Appendix

A.1 Canonical functions

The following canonical functions are derived from discriminant analysis classification model 2C (Table3-3) which had the second highest classification accuracy. To facilitate visualization of species clusters, scatterplots of canonical function pairs are shown in the next section. Immediately below are the canonical functions for which values are plotted in the following figures.

F1 = V396*-0.830 + V445*0.479 + V557*0.070 + V708*0.696 + V731*-2.852 + V737*2.485 + V1090*0.778 + V1235*0.864 + V1503*2.317 + V1661*-21.304 + V1770*19.332 + V1963 *-1.80 + V2313*0.310 + V2326*-0.361

F2 = V396*-0.049 + V445*-0.591 + V557*-0.432 + V708* 4.725 + V731*-21.942 + V737*18.920 +V1090*-2.155 + V1235*4.896 +V1503*-1.134 +V1661*-10.682 + V1770*10.020 + V1963*0.299 +V2313*-0.699 + V2326*-0.274

F3 = V396*0.412 + V445*-1.164 + V557*-0.161 + V708*3.303 + V731*-18.823 + V737*16.743 + V1090*1.195 + V1235*-5.859 + V1503*1.482 + V1661*16.010 +V1770*-13.034 + V1963*-0.503 + V2313*0.439 + V2326*0.476

F4 = V396*2.471 + V445*-2.283 + V557*-0.563 + V708*0.359 + V731*-2.540 + V737*3.072 + V1090*3.146 + V1235*-5.312 + V1503*-4.629 + V1661*-0.622 + V1770*6.245 + V1963*-0.027 + V2313*0.471 + V2326*0.233

F5 = V396*1.573 + V445*-2.038 + V557*1.40 + V708*-2.571 + V731*4.043 + V737*-3.017 + V1090*0.304 + V1235*1.887 + V1503*3.649 + V1661*-1.956 + V1770*-2.911 + V1963*-0.034 + V2313*-0.647 + V2326*0.345

A. 2 Figures

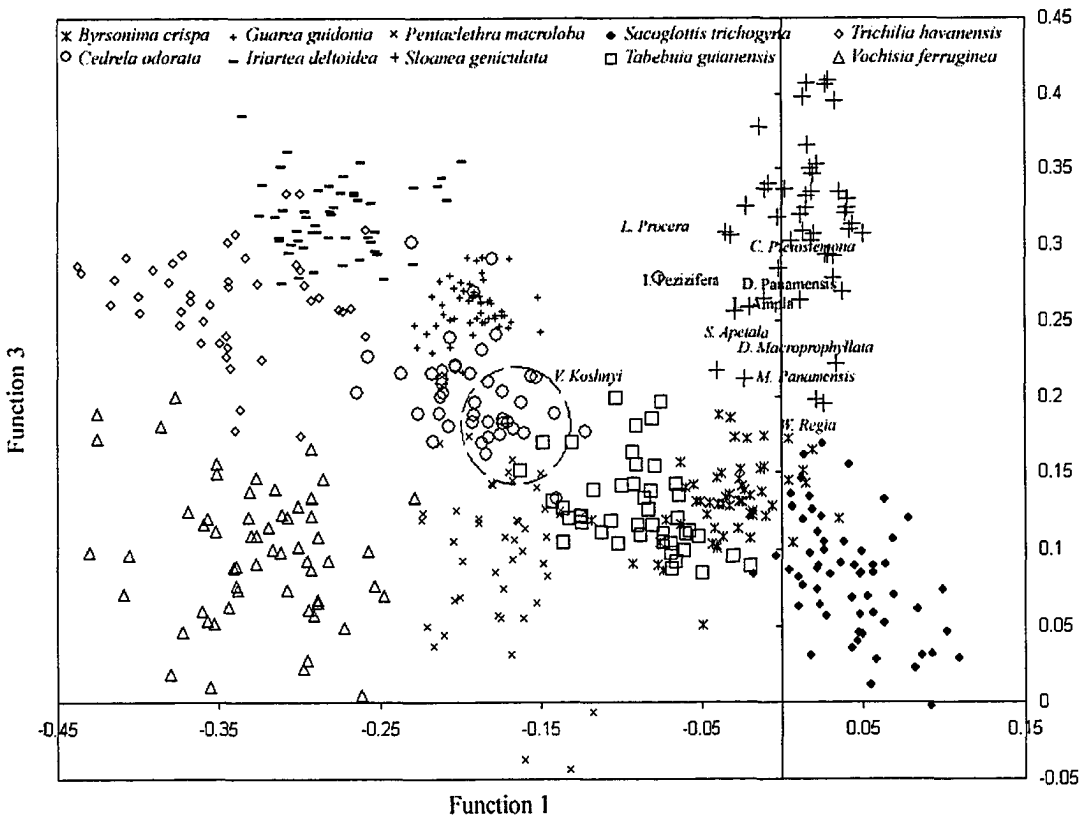

Figure A-1: Scatterplot of values for canonical functions 1 and 3 illustrating clustering by species. Functions are derived from classification model 2C (Table3-3) which had the second highest classification accuracy and showed the clearest separation of species in this two-dimensional scatterplot. The circle represents two standard deviations from the mean for *Virola Koshnyi* . The species labels on the plot represent the mean location for each respective species; these species are better discriminated in the following figures.

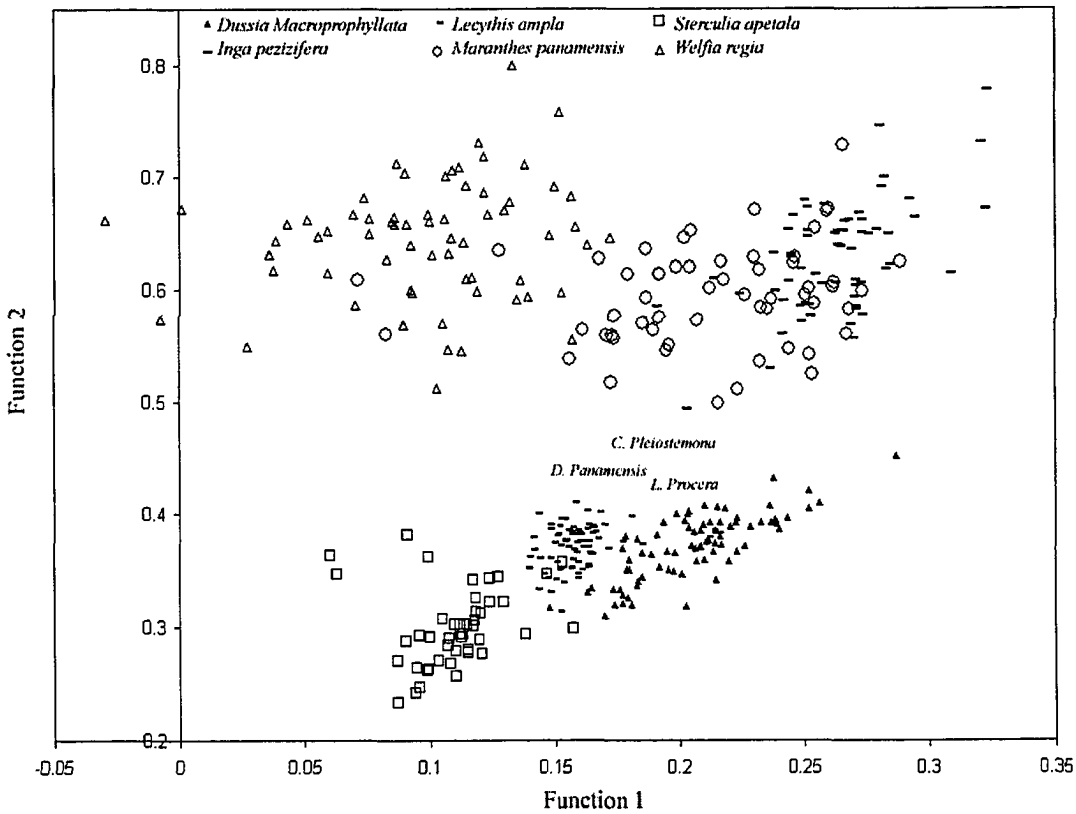

Figure A-2: Scatterplot of values for canonical functions 1 and 2 illustrating clustering by species. The species labels on the plot represent the mean location for each respective species.

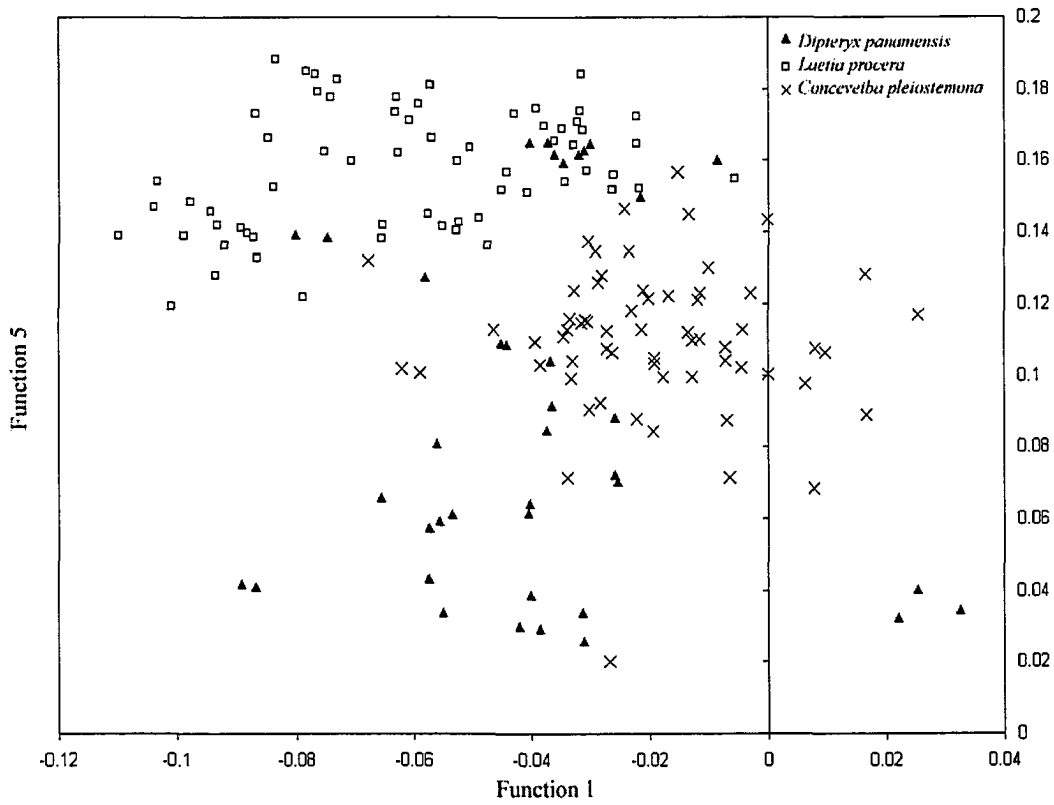

Figure A-3: Scatterplot of values for canonical functions 1 and 5 illustrating clustering by species. Note the scatter of *D. panamensis* points, which had the lowest classification accuracy.