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VARIABILITY IN LEAF OPTICAL PROPERTIES OF MESOAMERICAN TREES AND THE POTENTIAL FOR SPECIES CLASSIFICATION[1]

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Leaf traits and physiological performance govern the amount of light reflected from leaves at visible and infrared wavebands. Information on leaf optical properties of tropical trees is scarce. Here, we examine leaf reflectance of Mesoamerican trees for three applications: (1) to compare the magnitude of within- and between-species variability in leaf reflectance, (2) to determine the potential for species identification based on leaf reflectance, and (3) to test the strength of relationships between leaf traits (chlorophyll content, mesophyll attributes, thickness) and leaf spectral reflectance. Within species, shape and amplitude differences between spectra were compared within single leaves, between leaves of a single tree, and between trees. We also investigated the variation in a species' leaf reflectance across sites and seasons. Using forward feature selection and pattern recognition tools, species classification within a single site and season was successful, while classification between sites or seasons was not. The implications of variability in leaf spectral reflectance were considered in light of potential tree crown classifications from remote airborne or satellite-borne sensors. Species classification is an emerging field with broad applications to tropical biologists and ecologists, including tree demographic studies and habitat diversity assessments.

Key words: classification; hyperspectral; interspecific; intraspecific; leaf level; Mesoamerica; tropical trees.

An extensive body of literature on leaf optics describes the interaction of light with light-harvesting pigments and leaf structural components (e.g., Gates et al., 1965; Woolley, 1971; Gausman, 1985; Vogelmann, 1993). Three primary regions emerge from the reflectance spectrum of a leaf, the first (400–700 nm) is predominantly due to light absorption by pigments (especially chlorophyll), the second (700–1200 nm) is influenced by light scattering at air–cell-wall interfaces and characterized by high reflectance and transmittance, and the third (1200–2500 nm) is governed by water absorption (Hoffer and Johannsen, 1969). Furthermore, scientists have examined perturbations to leaf absorption, reflection, and transmission of light by external influences such as light availability (Gausman, 1984), disease (Knipling, 1967), freezing (Woolley, 1971), stress (Carter, 1993), and senescence (Gitelson and Merzlyak, 1994). The spectral properties of different plant types (Gates et al., 1965) or species (Allen et al., 1970; Gausman and Allen, 1973; Knapp and Carter, 1998) have also been compared. In more recent years, our understanding of leaf optics has been extended to larger-scale applications in the field of remote sensing, for assessing physiological performance of plant canopies (e.g., Gamon et al., 1990) and distinguishing between land cover vegetation types (e.g., Salem et al., 1995). In effect, results from leaf-level studies are often used as a basis for establishing techniques for retrieving information from canopy reflectance measured by remote sensors.

Little is known about the leaf optical properties of tropical trees or about the potential for identifying tropical tree species using remote sensing. Among the few exceptions are studies by Lee and Graham (1986) and Lee et al. (1990), who compared leaf optical properties of tropical sun and shade species, as well as Avalos et al. (1999), who compared leaf optical properties of tropical dry forest trees and lianas. Fung and Siu (1998) and Cochrane (2000) assessed reflectance of leaf or branch samples for differentiating species of tropical trees. More recently, Clark et al. (2005) was successful in discriminating seven tropical tree species at the leaf, pixel, and crown levels, publishing the first report on automated tropical rain forest tree classification using high spatial resolution hyperspectral imagery, in which spectral reflectance is measured over numerous contiguous bands of narrow width (typically ≤ 10 nm). The study represents an important breakthrough for identifying tropical tree species. Operational species classification could have numerous applications, including monitoring endangered or commercial tree species, characterizing biodiversity in ecologically important habitats such as reserves and biological corridors, and monitoring changes in species composition over time and changes in tree demography associated with global environmental changes (Clark et al., 2005).

The precept for species discrimination is that interspecies variability in spectral reflectance exceeds intraspecies variability. This presents a challenge for species identification due to inherent similarities in vegetation characteristics among species, including leaf biochemistry and anatomy, which result in similarities in leaf spectral reflectance. After posing the

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TABLE 1. Summary of characteristics of study areas.

Location	Latitude/Longitude	Holdridge life zone	Mean annual precipitation (mm)
1. Chamela-Cuixmala Biosphere Reserve, Mexico	19°30′ N, 105°03′ W	tropical dry forest	707
2. Los Inocentes, Costa Rica	11°01′ N, 85°30′ W	transition between basal tropical moist forest and premontane tropical moist forest	2098
3. Santa Rosa National Park, Costa Rica	10°48′53″ N, 85°36′54″ W	tropical dry forest and tropical dry transition to moist	1588
4. Los Horizontes, Costa Rica	10°45′39″ N, 85°38′6″ W	tropical dry forest	2378 (1995 data only)
5. El Rodeo, Costa Rica	9°31′60″ N, 84°4′0″ W	tropical dry forest	1896
6. Parque Natural Metropolitano (PNM), Panama	8°59′ N, 79°33′ W	tropical dry forest	1740
7. Fort Sherman (FTS), Panama	9°17′ N, 79°58′30″ W	tropical wet forest	3300

question "How unique are spectral signatures?," Price (1994) determined that while some are clearly distinct, others may not be separable at all. In reality, despite the advantages of hyperspectral technologies, classification of plant species may be difficult and, at times, unfeasible. A complicating factor is the dynamic nature of plant spectral signatures. Natural cycles such as leaf flush and senescence, as well as environmental factors affecting mineral nutrition, health, light availability, and water supply, may contribute to changes in a species' spectral signature over time or at a given position in the canopy (Gausman, 1984, 1985; Carter, 1993). To date, even the studies from temperate regions have not fully evaluated the seasonal dynamics of change in leaf spectral characteristics on tree classifications, nor do they explore the stability of tree spectral signatures over multiple sites with a range of climatic and edaphic conditions. Intuitively, these factors, which lead to within-species variability in reflectance spectra, could lead to significant confusion for species recognition using hyperspectral data. Although Clark et al. (2005) tested within-species variability for seven tropical tree species at one site, the degree of within-species variability over a large number of tropical tree species and a range of environmental conditions has not been adequately addressed.

With image-based, automated tropical tree species classification in its preliminary stages of development, we believe it to be constructive at this time to provide a comprehensive assessment of intraspecific variability of leaf spectral reflectance and its potential to influence tree species classifications. The main objective of this paper, therefore, was to evaluate within-species and between-species variability in leaf spectral reflectance for a wide variety of tropical trees found at seven sites in southern Mexico and Central America. We also investigated crown-level spectra of five tree species from Fort Sherman, Panama. In effect, we asked, "How unique are spectral signatures of tropical tree species?" A large part of our study focuses on the leaf level for several reasons: (1) by necessity, because high spatial resolution (<5 m) hyperspectral data was not available for most of our sites at the time of data collection, (2) the use of leaf spectra allows for a more controlled assessment of variability at multiple levels, and (3) spectral reflectance from leaves is usually the dominant contribution to tree crown reflectance. To evaluate variability in leaf reflectance of tropical trees, spectral metrics that describe reflectance shape and amplitude (Price, 1994) are compared at various levels within and between species. To explore the potential to identify species, we also classified the spectral data from the different sites using forward selection of features and a set of classifiers described in Castro-Esau et al. (2004). Lastly, we examined the correspondence between selected leaf traits (chlorophyll content, leaf thickness, and leaf histological features) and leaf spectral reflectance characteristics for one site to further our understanding of differences in leaf spectra between species.

MATERIALS AND METHODS

Study sites—Leaf samples were gathered from seven sites in Mexico, Costa Rica, and Panama (Table 1). Five of the sites support tropical dry forest ecosystems, where our research group is concurrently conducting studies on leaf area index and forest complexity and their ties with remote detection of secondary growth vs. mature forest. These sites are part of an international research consortium called TROPI-DRY that aims to understand the biophysical characteristics of neotropical dry forests and human interactions with these forests. Tropical dry forests undergo significant phenological changes through dry–wet seasonal cycles and typically contain species with varying degrees of deciduousness (Frankie et al., 1974; Bullock and Solis-Magallanes, 1990; Avalos and Mulkey, 1999). In addition, tropical dry forests can present high rates of species endemism (Lott et al., 1987; Kalacska et al., 2004).

Parque Natural Metropolitano, Panama, is the site of our case study to correlate leaf traits and leaf spectral reflectance. This 100–150-yr-old secondary forest has a pronounced dry season from January through April, with annual precipitation averaging 1740 mm.

Crown spectra were recorded from a canopy crane at Fort Sherman, Panama, a tropical wet forest site.

Leaf sample collection—At each site, 3–15 sun leaves were collected per tree (Table 2). One to five trees were sampled per species. In general, leaves were collected from the distal or middle portion of the branch. At some sites, it was not logistically feasible to sample more than one tree per species, such as in Parque Natural Metropolitano and Fort Sherman, where we were restricted to trees within reach of a canopy crane and by institutional regulations regarding the size of samples that can be collected from each tree. A full list of the species sampled is provided in Table 3. The samples were acquired using one of the following methods: pole pruner (Mexico and Costa Rica), sling shot (Costa Rica), or canopy crane (Panama). Leaves were collected, placed in plastic bags containing moistened paper towels, labeled, and placed in a larger bag or cooler containing ice. Samples were transported to an indoor laboratory, and leaf spectral reflectance was analyzed the same day. Maintaining leaf moisture prevented significant changes in leaf reflectance during this period (Foley et al., in press).

Crown spectra—Crown spectral measurements were obtained for five fairly flat, uniform crowns at Fort Sherman (Panama) by positioning the canopy crane gondola above the center of each crown and fitting the bare optic of a UniSpec (PP Systems, Amesbury, Massachusetts, USA) spectrometer through the floor grating (see *Measurements of bidirectional spectral reflectance*). The five species were *Cordia bicolor* (Boraginaceae), *Dussia munda* (Fabaceae), *Jacaranda copaia* (Bignoniaceae), *Tapirira guianensis* (Anacardiaceae), and *Vochysia ferruginea* (Vochysiaceae) (Fig. 1, Table 3).

Leaf trait data—At Parque Natural Metropolitano (Panama), ancillary data on pigmentation, leaf thickness, internal leaf structure, as well as diffuse reflectance and transmittance were collected in October 2003.

TABLE 2. Summary of data collected at each site.

Location	Date	Season	Instrument	Tree species	Trees per species	Leaves per tree	Measurements per leaf
Chamela-Cuixmala Biosphere Reserve, Mexico	Aug 2003	wet	UniSpec	27	1	15	1
Los Inocentes, Costa Rica	Nov 2002	wet	FieldSpec HH	10	1–3	3–5	1
	Mar 2003	dry	UniSpec	5	1	5	3
Santa Rosa National Park, Costa Rica	Dec 2002	wet	FieldSpec HH	2	3	5	3
Los Horizontes, Costa Rica	Oct 2002	wet	FieldSpec HH	8	3–5	3	3
El Rodeo, Costa Rica	Dec 2002	wet	FieldSpec HH	4	3	5	3
Parque Natural Metropolitano (PNM), Panama	Mar 2003	dry	UniSpec	6	1	3	6
	Oct 2003	wet	UniSpec	10	1	10	4
Fort Sherman (FTS), Panama	Mar 2003	dry	UniSpec	15	1	3	6
	Oct 2003	wet	UniSpec	15	1	10	4

Leaf chlorophyll content and thickness—Five 2.0-cm^2 leaf cores per tree species, each from a different leaf, were analyzed for chlorophyll content at Parque Natural Metropolitano. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll content were determined using the dimethyl sulphoxide extraction method (Hiscox and Israelstam 1979). A Smartspectro spectrophotometer (LaMotte, Chestertown, Maryland, USA) was used to determine absorbance at 645 and 663 nm. Pigment content was computed using Arnon's (1949) relationships and converted to a per area basis. In addition, chlorophyll content was nondestructively assessed using a SPAD 502 (Minolta Camera, Osaka, Japan) chlorophyll absorbance meter. The SPAD measures absorbance at 650 and 940 nm and yields a relative chlorophyll content, or "chlorophyll index" value (Markwell et al., 1995; Richardson et al., 2002). Leaf thickness was measured for 5–10 leaves per species using a Nikon (Tokyo, Japan) digital micrometer (to nearest 0.001 mm). Five measurements were taken per leaf, avoiding large veins.

Leaf histology—Small pieces of leaf blade (approximately 1 cm long and 2–3 mm wide) were collected at Parque Natural Metropolitano in October 2003 for histological analysis. The leaf pieces were fixed under vacuum for 2 wk in a formalin aceto-alcohol (5% formalin) solution. After fixation, the samples were run through an ethanol processing center (Fisher Histomatic 166, Hampton, New Hampshire, USA) and embedded in paraffin molds. Thin sections (5 μm) were cut from the mounted samples using a microtome. Slides were mounted and stained (Harris' hematoxylin stain), and viewed with a Leica (Wetzlar, Germany) DM RXM light microscope (200× total magnification). Digital photos were taken of the stained samples. The percentage of air space in the spongy mesophyll was determined by classifying the photos for cells vs. air space. Spongy mesophyll thickness and the ratio of spongy mesophyll to total leaf thickness were calculated using a 100-μm scale in each photo, from which a new ruler was constructed with 10-μm increments.

Leaf diffuse reflectance and transmittance—In addition to measurements of bidirectional reflectance of leaves described next, diffuse reflectance and transmittance were recorded using an 1800–12S external integrating sphere (LI-COR, Lincoln, Nebraska, USA) in conjunction with a UniSpec spectrometer. The spectrometer fiber optic was inserted through a port on the sphere. To record diffuse reflectance, the sphere illuminator was directed toward the adaxial side of the leaf sample in the sample port, and the fiber optic was directed to the sphere wall. To record transmittance, the leaf was reversed so that the underside faced the sphere interior. The sphere illuminator was directed through the leaf sample, and the spectrometer fiber optic was directed to the wall. Dark scans were performed regularly, and white reference scans (using barium sulfate) were repeated for every leaf sample. Both diffuse reflectance and transmittance were calculated as a ratio in relation to the white reference data.

Measurements of bidirectional spectral reflectance—Bidirectional leaf spectral reflectance was measured at all sites, using one of two spectrometers: (1) a UniSpec Spectral Analysis System (PP Systems, Amesbury, Massachusetts, USA) or (2) a FieldSpec HandHeld spectrometer (Analytical Spectral Devices, Boulder, Colorado, USA). The 2002 and 2003 data sets employed the FieldSpec HandHeld (FieldSpec HH) and the UniSpec, respectively. All crown spectra (Fort Sherman, Panama) were measured using the UniSpec.

UniSpec spectral analysis system—The Unispec spectral analysis system VIS/NIR relies on a 256-element photodiode array. The spectral range is 350–1100 nm, with a sampling interval of 3.3 nm and a spectral resolution of <10 nm. A bifurcated fiber optic delivers light from an internal 7.0 W halogen lamp via one branch and receives reflected light via the other.

To measure spectral reflectance of leaves, we employed a leaf clip that holds the foreoptic at 60° and maintains a 2.3 mm diameter field of view (FOV). Sample reflectance was measured by comparing leaf reflectance to reflectance of a white standard (barium sulfate). Ten scans were averaged per recorded spectrum. Dark scans and white reference scans were performed frequently to detect instrument drift on the spectra.

At Fort Sherman (Panama), crown spectra were recorded mid-morning under sunny conditions. White reference measurements were taken before measurements for each species as well as intermittently if we sensed the light conditions changed. Care was also taken to avoid taking measurements if the shadow of the gondola fell within the estimated FOV. Spectra were recorded at a height of 5 m above each crown, determined by lowering a measuring tape from the gondola. Using the bare UniSpec fiber-optic probe (40° FOV), the diameter of the viewed crown was approximately 3.6 m. Six measurements were taken from the same position above each crown and averaged.

FieldSpec HandHeld—The FieldSpec HandHeld spectrometer relies on one 512-element photodiode array. Its range is 325–1075 nm, with a sampling interval of 1.6 nm and spectral resolution of 3.5 nm at 700 nm. The FOV of the bare fiber-optic probe is 25°.

To obtain leaf spectral reflectance measurements, an external 50 W halogen lamp was directed at the leaf at an illumination angle of 45°. Leaves were placed against a black minimal (2%) reflectance panel. The FieldSpec HandHeld fiber-optic probe, inserted into a mounting gun attached to a tripod, was positioned directly above the sample. In all cases, height was adjusted so that the FOV had a diameter of approximately 11 mm. Reflectance was determined by standardizing the sample data to white reference (Spectralon, Labsphere, North Sutton, New Hampshire, USA) data under the same illumination and viewing conditions. White reference scans were recorded frequently. Ten scans were averaged per reflectance spectrum.

Spectral analysis—Spectra collected with the two spectrometers were analyzed separately rather than collectively due to differences between spectra measured for the same objects as described in Castro-Esau et al. (in press).

D and θ—Two spectral metrics described by Price (1994) enabled comparisons between pairs of spectra as follows:

$$D = \left[\frac{1}{\lambda_b - \lambda_a}\int_{\lambda_a}^{\lambda_b}[S_1(\lambda) - S_2(\lambda)]^2 d\lambda\right]^{1/2} \qquad (1)$$

$$\theta = \cos^{-1}\left[\frac{\int S_1(\lambda)S_2(\lambda)d\lambda}{\left[\int S_1(\lambda)^2 d\lambda\right]^{1/2}\left[\int S_2(\lambda)^2 d\lambda\right]^{1/2}}\right] \qquad (2)$$

D represents the difference in amplitude between two spectra and is computed as the root mean square difference between a pair of spectra (S_1 and S_2) averaged over the spectral range of interest (λ_a to λ_b). The metric θ determines

TABLE 3. Species sampled for this study.

Family and species	Field sites
Anacardiaceae	
Amphipterygium adstringens (Schlecht.) Schiede.	CH
Anacardium excelsum (Bertero & Balb. Ex Kunth) Skeels	$LI_{N/M}$, $PM_{M/O}$
Astronium graveolens Jacq.	CH, LI_{N}, $PM_{M/O}$
Spondias purpurea L.	CH
Tapirira guianensis Aubl.	FS_{CROWN}
Annonaceae	
Annona spraguei Saff.	$PM_{M/O}$
Apocynaceae	
Aspidosperma spruceanum Benth. Ex Müll. Arg	$FS_{M/O}$
Plumeria rubra L.	CH
Bignoniaceae	
Astianthus viminalis (HBK.) Baill.	CH
Jacaranda copaia (Aubl.) D. Don	FS_{CROWN}
Tabebuia donnell-smithii Rose.	CH
Tabebuia rosea (Bertol.) DC.	CH
Boraginaceae	
Cordia alliodora (Ruiz & Pav.) Oken	$PM_{M/O}$
Cordia bicolor A. DC.	$FS_{M/O}$, FS_{CROWN}
Cordia elaeagnoides DC.	CH
Brassicaceae	
Crateva graminacea	CH
Burseraceae	
Bursera simaruba (L.) Sarg.	ER, $LI_{N/M}$
Cochlospermaceae	
Cochlospermum vitifolium (Willd.) Spreng.	LH
Combretaceae	
Conocarpus erectus L.	CH
Terminalia amazonia (J. F. Gmel.) Exell	$FS_{M/O}$
Convolvulaceae	
Ipomoea wolcottiana Rose.	CH
Clusiaceae	
Marila laxiflora Rusby	$FS_{M/O}$
Euphorbiaceae	
Croton sp.	CH
Piranhea mexicana	CH
Sapium sp.	CH
Fabaceae (Caesalpinoideae)	
Caesalpinia platyloba S. Watson	CH
Caesalpinia sclerocarpa Standl.	CH
Hymenaea courbaril L.	LH, LI_{N}
Tachigali versicolor Standl. & L. O. Williams	$FS_{M/O}$
Fabaceae (Mimosoideae)	
Enterolobium cyclocarpum (Jacq.) Griseb.	CH, LI_{M}, PM_{O}
Pithecellobium dulce (Roxb.) Benth.	CH
Fabaceae (Papilionoideae)	
Dalbergia retusa Hemsl.	LH, LI_{M}
Diphysa americana (Mill.) M. Sousa	LH, LI_{M}
Dussia munda C. H. Stirt.	FS_{CROWN}
Gliricidia sepium (Jacq.) Kunth ex Walp.	ER, LH, LI_{M}
Lonchocarpus heptaphyllus (Poir.) DC.	$FS_{M/O}$
Malpighiaceae	
Byrsonima crassifolia (L.) Kunth	ER, LH, LI_{N}
Malvaceae	
Bombacopsis quinata (Jacq.) Dugand	LH, LI_{N}
Ceiba aesculifolia (Kunth) Britton & Baker	CH
Ceiba grandiflora Rose.	CH
Ceiba pentandra (L.) Gaertn.	CH
Guazuma ulmifolia Lamarck	CH, ER, LH, $LI_{N/M}$
Heliocarpus pallidus Rose.	CH
Luehea seemannii Triana & Planch.	$PM_{M/O}$
Pseudobombax septenatum (Jacq.) Dugand	PM_{O}
Meliaceae	
Carapa guianensis Aubl.	$FS_{M/O}$
Cedrela odorata L.	LI_{N}, SR
Swietenia humilis Zucc.	CH
Swietenia macrophylla King	LI_{N}
Moraceae	
Brosimum utile (Kunth) Oken	$FS_{M/O}$
Castilla elastica Sesse	PM_{O}
Ficus insipida Willd.	$PM_{M/O}$
Ficus nymphaeifolia Mill.	$FS_{M/O}$
Myristicaceae	
Virola surinamensis (Rol. ex) Rottb. Warb.	$FS_{M/O}$
Nyctaginaceae	
Guapira linearibracteata (Heim.) Standl.	CH
Rubiaceae	
Tocoyena pittieri (Standl.) Standl.	$FS_{M/O}$
Sapindaceae	
Matayba apetala Radlk.	$FS_{M/O}$
Thouinidium decandrum (Humb. & Bonpl.) Radlk.	CH
Sapotaceae	
Chrysophyllum cainito L.	PM_{O}
Manilkara bidentata (A. DC.) Chev.	$FS_{M/O}$
Simaroubaceae	
Simarouba amara Aubl.	LI_{N}, $FS_{M/O}$
Urticaceae	
Pourouma bicolour Mart.	$FS_{M/O}$
Verbenaceae	
Avicennia germinans (L.) L.	CH
Vochysiaceae	
Vochysia ferruginea Mart.	FS_{CROWN}

Notes: CH, Chamela; ER, El Rodeo; FS, Fort Sherman; LH, Los Horizontes; LI, Los Inocentes; PM, Parque Natural Metropolitano; SR, Santa Rosa. Subscripts: CROWN indicates species for which crown spectra were taken at Fort Sherman (Panama); M, N, and O refer to the month (March, November, and October, respectively) in which the data were obtained for locations with multitemporal data. Taxonomy is based on the updated Angiosperm Phylogeny Group (APG II, 2003) classification for orders and families of flowering plants.

the angle between two spectra and may be interpreted as the difference in shape between the pair of spectra. It is computed as a vector dot product for the pair of spectra, with amplitude dependence removed.

The spectral range used to calculate D and θ was 445.9–949.6 nm for both the UniSpec and FieldSpec Handheld. In this region, noise from both instruments was low. To compute D and θ for the same spectral range and interval, FieldSpec HandHeld data were interpolated to 3.3-nm intervals to match the sampling intervals of the UniSpec. No smoothing was performed.

D and θ were compared at the following levels for leaf spectra (numbers 1–6) or crown spectra (number 7):

1. Within-leaf. D and θ were calculated where multiple measurements were

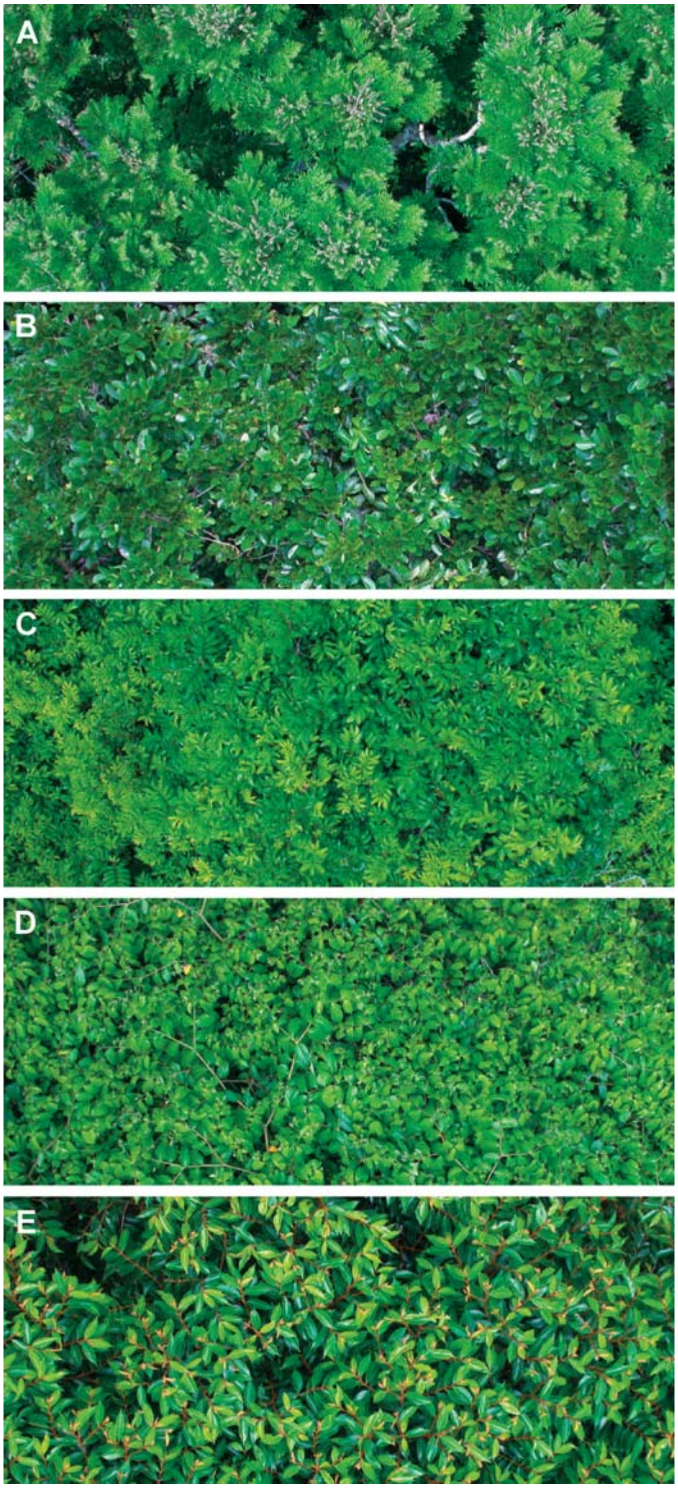

Fig. 1. Partial tree crowns, as viewed from a canopy crane at Fort Sherman, Panama, at a height of 5 m. The species are (A) *Jacaranda copaia*, (B) *Dussia munda*, (C) *Tapirira guianensis*, (D) *Cordia bicolor*, and (E) *Vochysia ferruginea*.

made per individual leaf. For example, six measurements per leaf were recorded for the Parque Natural Metropolitano March 2003 data set (Table 2). Therefore, D and θ were assessed for all 15 possible pairs of the six spectra (called pairwise combinations hereafter).

2. Within tree. Where multiple leaves were sampled per tree, average leaf reflectance was computed for each leaf, and D and θ were then calculated on all pairwise combinations of spectra from those leaves.
3. Between tree and within species, site, and season. An average tree spectrum was computed from all leaf spectra for each tree. Where multiple trees were sampled per species, D and θ were computed for all pairwise combinations of the mean tree spectra.
4. Between season and within species and site. Pairwise comparisons of D and θ were made between mean tree spectra of the same species measured in the dry season and wet season. These comparisons were possible at the Parque Natural Metropolitano and Fort Sherman (Panama) sites only where sunlit leaves were collected from the same trees and measured with the same spectrometer in both seasons (Table 2). This comparison was not performed for Los Inocentes (Costa Rica) since the November 2002 and March 2003 data sets were obtained using different spectrometers.
5. Between site and within species and season. D and θ comparisons were possible for species sampled at more than one site. There were seven such species, all measured with the FieldSpec HandHeld: *Bombacopsis quinata* (Malvaceae), *Bursera simaruba* (Burseraceae), *Byrsonima crassifolia* (Malpighiaceae), *Cedrela odorata* (Meliaceae), *Gliricidia sepium* (Fabaceae/Papilionoideae), *Guazuma ulmifolia* (Sterculiaceae), and *Hymenaea courbaril* (Fabaceae/Caesalpinoideae).
6. Between species and within site. Within a site, all pairwise combinations of tree spectra of different species were made. For example, the Chamela (Mexico) August 2003 data set provided possible combinations for 27 species.
7. Between species and within site (crown level). D and θ were computed for all pairwise combinations of the five mean crown spectra at Fort Sherman, Panama.

A Wilcoxon rank sum test was used to determine whether levels 3 and 6 differed significantly. This test indicated whether comparisons of individual conspecific tree crowns differed from comparisons among species. The test was performed using mean values of D and θ for comparisons of conspecific trees (level 3) and comparisons of heterospecific species pairs (level 6).

Classification—Beyond examination of shape and amplitude differences between spectra, we determined whether it was possible to discriminate between species based on reflectance spectra. Steps involved in the classification included feature selection, classifier training, and classifier testing.

Forward stepwise feature selection reduced the number of variables entered into the classification. Stepwise feature selection has proven useful for studies involving species classification from hyperspectral data (van Aardt and Wynne, 2001; Clark et al., 2005), and initial testing of our data sets led to higher classification accuracies than using principal components analyses as described in our earlier work (Castro-Esau et al., 2004) for discriminating liana and tree species. The approach for forward feature selection involves selecting the single best feature and consecutively adding those features that improve performance the most. In this case, the criterion used for the procedure was first nearest neighbor leave-one-out classification performance (Duin, 2000). By using alternate spectra, we split the data array in half into training and testing sets. Feature selection was performed using the training set only.

Spectra were classified to species using a selection of supervised parametric and nonparametric classifiers, previously described in Castro-Esau et al. (2004). The classifiers are part of a pattern recognition toolbox, PRTools, developed by Duin (2000) for use within Matlab (MathWorks, Natick, Massachusetts, USA). Parametric classifiers included a logistic linear classifier (loglc) and a normal density-based quadratic classifier (qdc). Nonparametric classifiers included a decision tree classifier (treec), a neural network classifier (based on back-propagation and Levenberg-Marquardt gradient descent) (lmnc), and a k-nearest neighbor classifier (knnc).

Classification involved several steps. Initially, training data were labeled according to species and used to train the classifiers. Secondly, the trained classifiers classified the test data set to validate the robustness of the model (Defernez and Kemsley, 1997). Error estimation was determined for both the training and testing data, which constituted the percentage of incorrectly classified spectra in each case. For each data set, classifications were run several times using multiples of 10 selected features. The highest classification accuracies were reported. The classifications involved all leaf spectra for each species (a minimum of five spectra, but in most cases 10+). Classifications were run on noninterpolated data in the range 445.9–949.6 nm for the UniSpec and 445.5–950.7 nm for the FieldSpec HandHeld.

A final analysis was run to determine the potential for classifying increasing numbers of tropical forest tree species. A data set of 50 species with 10 spectra each was compiled from Parque Natural Metropolitano, Fort Sherman, and Chamela, all using UniSpec 2003 wet season data. Classifications were run on 10 000 sets each of 5, 10, 15, 20, 25, 30, 35, 40, and 45 species, where each set of species was chosen randomly from the 50 total species. A single classification was run on the full set of 50 species. For this analysis, we used the logistic linear classifier and 30 previously selected features, the top 10 features from the within-site classifications for each of the three sites. Ten thousand repetitions were considered a representative sample of all possible species combinations since variation around mean classification errors stabilized by this point, as determined by testing with greater numbers of repetitions.

RESULTS

D and θ—With respect to the goal of classifying trees according to species, it is evident that the magnitude of variability in amplitude (D) and shape (θ) within each species presents a challenge (Figs. 2 and 3). Levels of comparison to the left of the between-species category are measures of intraspecific variability. While there is a general increase in mean D (Fig. 2) and θ (Fig. 3) with increasing level of comparison or "scale" from within-leaf measurements through to between-species measurements (with the exception of the between-sites category for the FieldSpec HH data), there is a high degree of overlap between each of these levels. Even so, a significant difference ($P < 0.05$) was found between level 3 (between tree) and level 6 (between species) with the Wilcoxon rank sum test, indicating that for one site at one date, variability between species is greater than variability between individuals of the same species. At the crown level, amplitude differences between all possible pairwise combinations of spectra of the five species were much higher than for any other category (Fig. 2a). This was particularly evident in the near-infrared region (Fig. 4).

The high variability within species but over multiple sites observed in Figs. 2b and 3b (between sites FieldSpec HH data) would appear to preclude classification of leaf reflectance spectra at one site based on training data from another site or from a library of hyperspectral signatures of tropical trees. Figure 5 shows average reflectance spectra collected during the wet season of 2002 (October–December) for two species encountered at three sites each. Leaves of the same species collected at multiple sites may exhibit different characteristics with regard to leaf moisture content, leaf area, and chlorophyll content (represented by the SPAD value; Table 4).

Similarly, leaf spectra vary widely across seasons. For the majority of tree species sampled at Parque Natural Metropolitano, Panama, higher reflectance in the visible region of the spectrum during the dry season (March 2003) was a manifestation of lower chlorophyll content as compared to the wet season (October 2003; data not shown). For example, average total chlorophyll content ($N = 5$ samples) varied from 465.63 ± 28.10 μmol/m^2 in the dry season to 652.00 ± 97.01 μmol/m^2 in the wet season for *Astronium graveolens* (Anacardiaceae). Average reflectance at 550 nm was 7.4% and 6.5%, respectively, for the two seasons. Differences in leaf age and stress levels from season to season may have caused additional variation in the leaf spectra.

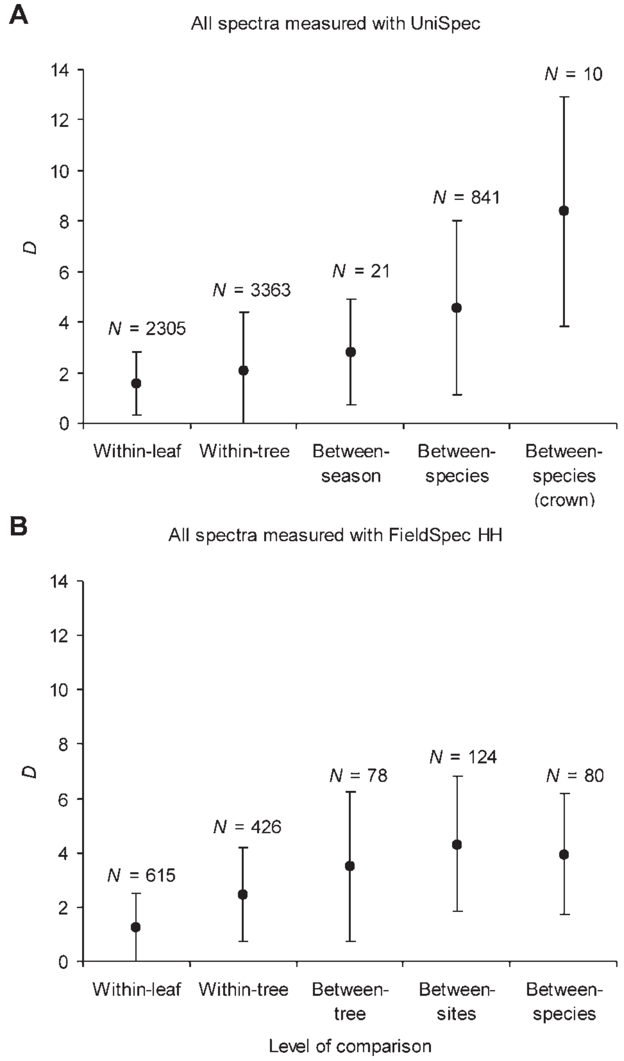

Fig. 2. Mean amplitude (D) (±1SD) of leaf reflectance spectra of tropical trees at sites in Mexico, Costa Rica, and Panama for various levels of comparison. Average D was calculated for UniSpec data (A) and ASD HandHeld data (B) (see Table 2). Number of samples used to compute each mean is indicated and includes all the pairwise combinations of spectra per respective level.

Classification—Species from one site and season were classified with high accuracy (Table 5). Not unexpectedly, the highest classification error accompanies the site for which the largest number of species was sampled (Chamela, Mexico, 27 species), indicating a potential problem for complex tropical dry forest ecosystems in which species density can exceed 50–70 species per ha (Kalacska et al., 2004). Still, accuracy on the test data was 80%.

For our case study site (Parque Natural Metropolitano, Panama), classification error on the test data (47 of the total 95 leaf spectra for the site, representing 10 species) was 8.51% (Table 5). Misclassified test spectra included one leaf spectrum (of 4 or 5 test spectra per species) for each of the following species: *Anacardium excelsum* (Anacardiaceae), *Luehea seemannii* (Malvaceae), *Cordia alliodora* (Boraginaceae), and *Enterolobium cyclocarpum* (Fabaceae/Mimosoideae). Since no

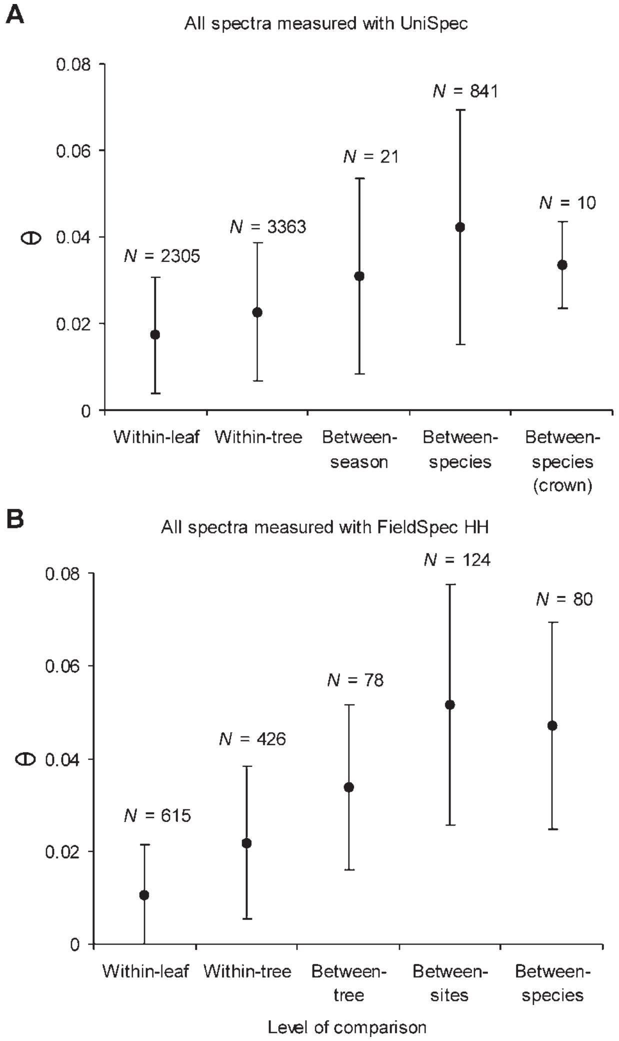

Fig. 3. Mean shape (θ) (±1SD) of leaf reflectance spectra of tropical trees at sites in Mexico, Costa Rica, and Panama for various levels of comparison. Average θ was calculated for UniSpec (A) and ASD HandHeld data (B) (see Table 2). Number of samples used to compute each mean is indicated and includes the pairwise combinations of spectra per respective level.

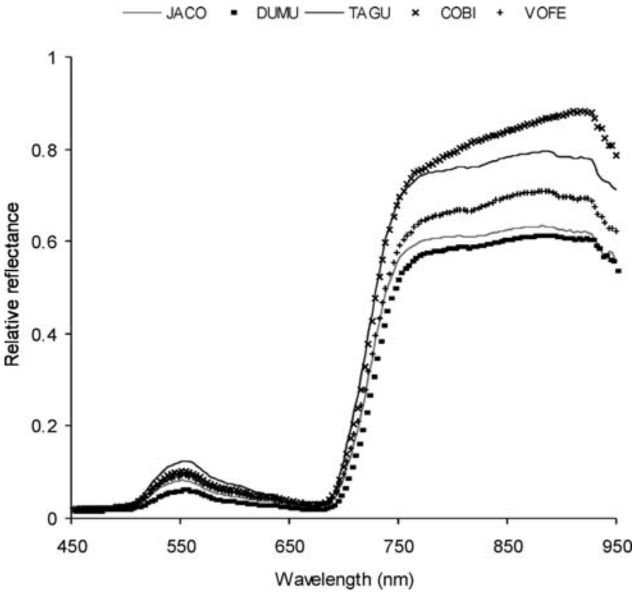

Fig. 4. Average crown reflectance spectra (field of view = c. 3.6 m) for five species at Fort Sherman, Panama. Spectra correspond to species pictured in Fig. 1 as follows: *Jacaranda copaia* (JACO), *Dussia munda* (DUMU), *Tapirira guianensis* (TAGU), *Cordia bicolor* (COBI), and *Vochysia ferruginea* (VOFE).

single species was confused with greater frequency than these, all species were generally well classified.

Accuracy was dependent on number of features and classifier. Highest classification accuracies were obtained with 20–40 features, beyond which classification accuracy eroded due to overfitting. The frequency at which wavebands in various regions of the spectrum were selected for the various species-level classifications, based on all classified sites and the top 20 selected features per site, was higher for visible (400–700 nm) bands than for near-infrared (700–950 nm) bands (Fig. 6). Classifiers also affected accuracy. For example, using 40 wavebands and a logistic linear classifier produced the lowest overall classification error of 2.67% for the Fort Sherman site (Table 5). The second lowest error for the same site, however, was 14.67% for a *k*-nearest neighbor classifier ($k = 2$; not shown). In general, however, reasonably accurate classifications by multiple classifiers at each site lends support for the inherent distinguishability of the species. For four of the six sites, the species-level classification using the logistic linear classifier produced the lowest classification errors.

Two additional classifications were run to determine the potential for classifying species across seasons and across sites. At Parque Natural Metropolitano, there were six species for which we had bidirectional reflectance data in both dry (March 2003) and wet (October 2003) seasons. Data from the wet season (October 2003, 10 samples/species) were used to train classifiers to test on the dry season (March 2003, 3 samples/species) data set. In this case, best-case scenario classification error on the test data was high (28%). To classify species across sites, we used data from the seven species sampled using the FieldSpec HandHeld at multiple locations. For the pooled data set, there were 10–43 samples (spectra) per species (mean = 29), and each species was sampled at 2–3 locations. Data from one site per species were used to train the classifiers, and data from the remaining site(s) were reserved for testing. Classification was poor again, with best-case scenario classification error on the test data at 51.75%.

The classification analysis using repeated classifications on random sets of species in multiples of 5, up to a total of 50 species, indicated that, within this range, mean classification error on the test data rises linearly with number of species (Fig. 7). Classification error on the training data was 0% in all cases. For the test data, it ranged from 13.79% for five species to 22.40% for 50 species. Variability was greater with fewer species since, from the random selection of species, entirely different sets of species were selected often. With a higher number of species, the same species would have entered into multiple classifications. It was noted that, below five species, the linear trend shown in Fig. 7 was not followed (e.g., for two classes, test data error was only 3.30%).

Case study including leaf trait data: Parque Natural Metropolitano—For each leaf trait, species at Parque Natural

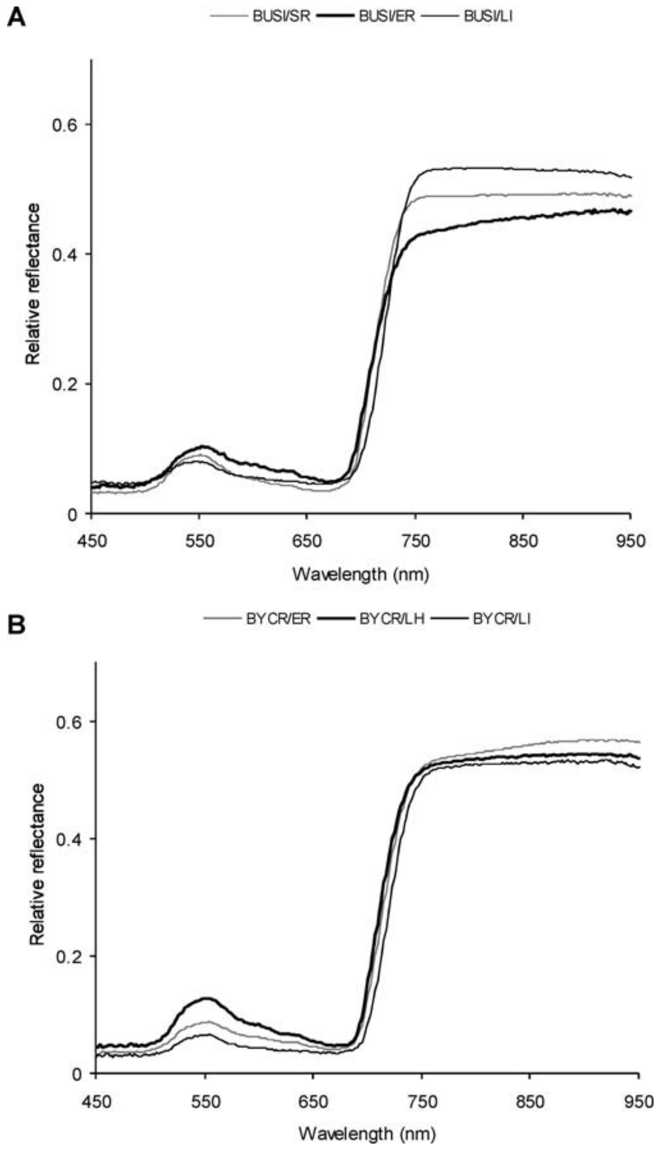

Fig. 5. Average relative reflectance spectra of (A) *Bursera simaruba* (BUSI) and (B) *Byrsonima crassifolia* (BYCR) each at three sites in Costa Rica (site abbreviations: SR, Santa Rosa; ER, El Rodeo; LI, Los Inocentes; LH, Los Horizontes). Accompanying leaf trait data are in Table 4.

TABLE 4. Leaf characteristics of *Bursera simaruba* and *Byrsonima crassifolia* at multiple sites.

Species/site	Moisture content (%)	Leaf area (cm^2)	Chlorophyll content
BUSI/SR	0.75 ± 0.03	286.15 ± 95.31	44.40 ± 6.06
BUSI/ER	0.65 ± 0.05	227.35 ± 86.48	42.37 ± 5.85
BUSI/LI	0.64 ± 0.05	not available	41.89 ± 5.00
BYCR/ER	0.55 ± 0.01	42.54 ± 13.20	48.60 ± 3.90
BYCR/LH	0.53 ± 0.002	56.80 ± 21.52	42.80 ± 4.70
BYCR/LI	0.58 ± 0.01	46.98 ± 18.94	52.78 ± 6.76

Notes: BUSI, *Bursera simaruba*; BYCR, *Byrsonima crassifolia*; SR, Santa Rosa; ER, El Rodeo; LI, Los Inocentes; LH, Los Horizontes. For moisture content and leaf area, $N = 15$ samples (3 trees × 5 leaves). For SPAD, $N = 15$ samples × 5 measurements/leaf = 75 measurements (exceptions: BYCR/LH and BYCR/LI, for which only three samples (3 trees × 1 leaf) were averaged. In those cases, 15 SPAD measurements were averaged (3 samples × 5 measurements/leaf)). Chlorophyll content was measured with SPAD, a hand-held chlorophyll absorbance meter (Markwell et al., 1995; Richardson et al., 2002).

Metropolitano were ranked from lowest to highest value in Table 6. In particular, *Astronium graveolens* (Anacardiaceae) is exceptional for its low reflectance in both visible and near-infrared regions, accompanied by high transmittance in the same regions (Table 6, Fig. 8). In thin sections, leaves had an unusually thin, compact spongy mesophyll for this species (Fig. 9). *Astronium graveolens* had both a low percentage of intercellular space and low near-infrared reflectance, whereas *Ficus insipida* (Moraceae) had a high percentage of intercellular space, a wide spongy mesophyll, and high near-infrared reflectance (Table 6, Fig. 9).

Correlation analyses between variables listed in Table 6 confirm associations between leaf pigmentation and/or structural features and leaf reflectance and/or transmittance. In addition to 548.5 and 800.7 nm, given in Table 6, the wavebands 445.9, 532, 571.6, 680, 706.2, 748.6, and 959.3 nm were also tested in the correlation analyses, for both diffuse reflectance and transmittance as well as bidirectional reflectance. Transmittance in near-infrared regions was negatively correlated ($P < 0.05$) with spongy mesophyll width (at 748.6 and 800.7 nm) and highly negatively correlated ($P < 0.01$) with percentage of intercellular space (at 706.2, 748.6, 800.7, and 959.3 nm; Table 7). While relationships between reflectance wavebands and chlorophyll content are generally nonlinear, there was a strong linear correlation ($P < 0.01$) between chlorophyll content (chlorophyll *a*, chlorophyll *b*, and total chlorophyll, measured in micromoles per square meter) and a common spectral index used for chlorophyll estimation, the simple ratio (R_{750}/R_{705}; Gitelson and Merzlyak, 1994). To test these relationships, chlorophyll data from individual samples ($N = 5$ samples per species for six species) were paired with bidirectional reflectance data for the same sample. (UniSpec spectral reflectance measurements were taken of the leaf cores that were then designated for chlorophyll analysis.) Leaf thickness was significantly correlated ($P < 0.05$) with bidirectional reflectance at 532, 548.5, 571.6, and 706.2 nm (not shown).

DISCUSSION

Leaf spectral reflectance is a rich data source for understanding differences in leaf traits among species and for assessing the potential for species discrimination. Differences in leaf traits are the basis for differences in leaf optical properties that permit species discrimination at the leaf level, which within sites and within seasons, appears promising when a limited number of species are present. Because classification accuracy degrades with increasing numbers of species, it is more likely that, for species-rich tropical forests, a portion of species with distinctive reflectance characteristics will be distinguishable from the larger community. At the crown scale, in the first tropical rain forest tree discrimination study using high spectral and spatial resolution imagery, Clark et al. (2005) also found good potential (92% accuracy) for analyzing a limited number of emergent tree species (seven) from a single-date (1998) HYDICE image of La Selva, Costa Rica, using a linear discriminant analysis and 30 wavebands. These efforts represent the initial stages of operational tropical tree species identification by hyperspectral remote sensors, which

TABLE 5. Best species-level classification results.

Site	Date	No. species	Samples/species	Top classifier	No. features	Training error (%)	Testing error (%)	Overall error (%)
El Rodeo, Costa Rica	Dec 02	4	15	lmnc	20	0	6.67	3.34
Los Horizontes, Costa Rica[a]	Oct 02	4	10	loglc	30	0	0	0
Los Inocentes, Costa Rica[a]	Nov 02	9	5–15	knnc	20	0	12.73	6.37
Parque Natural Metropolitano, Panama	Oct 03	10	7–10	loglc	20	0	8.51	4.26
Fort Sherman, Panama	Oct 03	15	10	loglc	40	0	5.33	2.67
Chamela-Cuixmala, Mexico	Aug 03	27	15	loglc	30	0	19.37	9.69

Notes: lmnc, neural network classifier; loglc, logistic linear classifier; knnc, *k*-nearest neighbor classifier.

[a] Due to insufficient data, some species were omitted from the classification.

will provide an important tool to tropical biologists and ecologists for a variety of applications.

D and θ (leaf level)—Use of Price's (1994) metrics *D* and θ show that variability in a species' reflectance begins within individual leaf blades (Figs. 2 and 3). Multiple spectral measurements taken over the leaf blade show variation, particularly over leaf veins (we avoided the main vein). Similarly, multiple measurements from the SPAD chlorophyll absorbance meter of the same leaf also vary, indicating uneven chlorophyll distribution across the leaf blade (also documented in other species; e.g., Hew et al., 1998). This variation increases when comparing multiple leaves from the same tree and is compounded further when examining spectra of leaves of different conspecific trees from a single site. Greater differences yet are seen for between-season and between-site spectra of the same species (Fig. 5).

Classification—Classification for each site was successful despite high intraspecific variability, with greater than 80% test data accuracy at all sites. These results may be attributed to the selection of a set of highly discriminating features (wavebands) from the spectral range available and the use of a variety of powerful tools for pattern recognition.

The most frequently selected wavebands in our analyses were in the blue region (400–499 nm), which is strongly influenced by absorption of chlorophylls and carotenoids (Jensen, 2000). The blue-green edge leading to the green peak (500–549 nm) and chlorophyll absorption leading to the red edge (650–699 nm) were also important (Fig. 6). This finding is similar to Fung et al. (1999), who used stepwise linear discriminant analysis for feature selection and found selected bands to lie mainly in the green peak and red edge regions. Overall, however, the bands selected depend on the data used and therefore differ from one suite of species to the next to optimize separability in each case. Wavebands beyond the range 400–950 nm, related to nitrogen and lignin concentrations, as well as O-H stretching, may be useful in future tree discrimination studies, as was the case for Martin et al. (1998), van Aardt and Wynne (2001), and Clark et al. (2005).

The logistic linear classifier, a parametric classifier, provided the best species-level classification results at four of six sites (Table 5). The *k*-nearest neighbor classifier, a nonparametric classifier, produced the second best results overall. The quadratic classifier generally performed the worst, possibly due to correlations between features. Of the nonparametric classifiers, the decision tree classifier generally performed

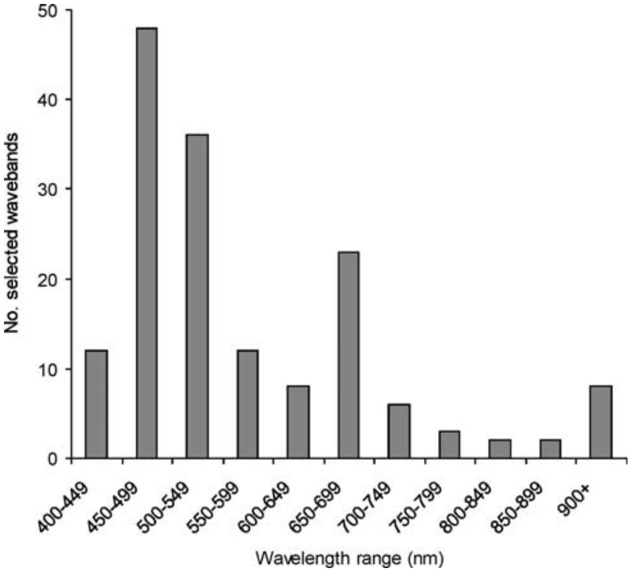

Fig. 6. Distribution of wavebands chosen in the forward feature selection process based on the within-site species classifications (Table 5). The top 20 wavebands per classification were tallied for this histogram.

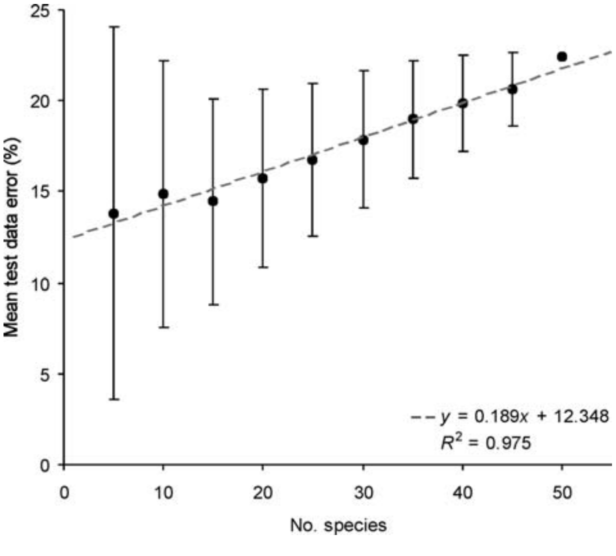

Fig. 7. Classification analysis for 50 species from three sites, Parque Natural Metropolitano, Fort Sherman, and Chamela. Data were gathered in the 2003 wet season using the UniSpec. Mean test data error ±1SD was computed from 10 000 classifications each per set of 5, 10, 15, 20, 25, 30, 35, 40, and 45 species. Sets of species were selected at random for each classification.

TABLE 6. Leaf trait data for species in the Parque Natural Metropolitano October 2003 data set.

Species	Chl_a (μmol m^{-2})	Chl_b (μmol m^{-2})	Chl_{total} (μmol m^{-2})	R_{550}(%)	T_{550} (%)	R_{800} (%)	T_{800} (%)	Thick (mm)	Space (%)	SMW (μm)	SM/T
ANEX	715.2 (6)	340.1 (6)	1055.3 (6)	11.33 (6)	6.23 (5)	49.83 (3)	40.27 (5)	0.268 (4)	12.21 (4)	93.5 (6)	0.54 (6)
LUSE	570.2 (5)	144.8 (4)	715.0 (5)	11.24 (4)	1.35 (1)	60.87 (8)	26.32 (1)	0.348 (7)			
ASGR	529.9 (3)	134.3 (3)	664.2 (3)	9.10 (1)	10.96 (8)	42.22 (1)	49.45 (9)	0.164 (1)	5.40 (1)	31.0 (1)	0.22 (1)
COAL	390.7 (1)	105.5 (2)	496.2 (1)	10.90 (3)	6.22 (4)	49.90 (4)	38.52 (4)	0.290 (5)	15.47 (6)	37.0 (2)	0.35 (2)
ANSP	544.8 (4)	145.4 (5)	690.2 (4)	9.17 (2)	8.00 (6)	50.09 (5)	43.88 (8)	0.259 (3)	9.86 (2)	58.5 (3)	0.41 (3)
CAEL				11.31 (5)	8.00 (6)	51.38 (6)	41.13 (6)	0.458 (9)	10.18 (3)	65.0 (4)	0.47 (5)
PSSE				11.70 (7)	9.24 (7)	48.37 (2)	43.32 (7)	0.256 (2)	12.70 (5)	78.5 (5)	0.42 (4)
FIIN	415.0 (2)	87.9 (1)	502.9 (2)	12.34 (8)	2.70 (2)	53.57 (7)	31.97 (2)	0.378 (8)	29.30 (7)	146.5 (7)	0.42 (4)
CHCA				15.36 (9)	4.91 (3)	60.92 (9)	35.01 (3)	0.299 (6)			

Note: ANEX, *Anacardium excelsum*; LUSE, *Luehea seemannii*; ASGR, *Astronium graveolens*; COAL, *Cordia alliodora*; ANSP, *Annona spraguei*; CAEL, *Castilla elastica*; PSSE, *Pseudobombax septenatum*; FIIN, *Ficus insipida*; CHCA, *Chrysophyllum cainito*. Chl_a, Chl_b, Chl_{total} are averages based on 5 samples/species. R_{550} refers to diffuse reflectance at 548.5 nm (average of 4 spectra/species); T_{550}, transmittance at 548.5 nm; R_{800}, diffuse reflectance at 800.7 nm; T_{800}, transmittance at 800.7 nm; Thick, average leaf thickness from 5–10 leaves (5 measurements/leaf); Space (%), percentage of intercellular space, based on the classification of one internal leaf morphology image per species; SMW, spongy mesophyll width; SM/T, ratio of spongy mesophyll width to total leaf width. SMW and SM/Total are both based on five measurements from a single leaf thin section. Species are ranked according to lowest to highest values in each column (numbers in parentheses). Missing data indicate data not available. There are no leaf trait data for a 10th Parque Natural Metropolitano species, *Enterolobium cyclocarpum* (reflectance only), which has been omitted from this table.

poorly, likely due to complex trees and the tendency toward overfitting. The decision tree classifier is not well suited to features that have a large number of possible values (Mitchell, 1997), as was also observed when discriminating between lianas and trees (Castro-Esau et al., 2004).

The spectrometer used for data collection may possibly influence classification accuracy as well. Instruments with higher spectral resolution will better capture fine spectral details that may be used for discriminating species. In this case, the FieldSpec HandHeld had a finer spectral resolution (3.5 nm at 700 nm) than the UniSpec (<10 nm), but there was no obvious advantage observed in our classification results (Table 5).

Based on our findings, which are at this point mainly limited to the level of the leaf, the potential for using a database of spectral signatures of species for classifying across sites and seasons appears low. The leaf-level spectral signature of a species from one site did not serve effectively to classify the same species at a second site (Fig. 5). Differences in leaf age, phenology, soil mineral nutrition, moisture availability, temperature, and other stresses (Carter, 1993) could certainly lead to the differences observed in spectral reflectance of the same species over multiple sites with varying edaphic and climatic conditions. In a similar way, progressively increasing stress with increasing elevation has been noted in the spectral reflectance properties of *Picea rubens*, *Abies balsamea* (Richardson et al., 2001), and *Betula papyrifera* (Richardson and Berlyn, 2002). Another possible cause of intersite differences in conspecific leaf spectra is that genetic differences between populations can contribute to spectral differences. Winter (1998), for instance, was able to distinguish between two different varieties of avocado (*Persea americana*) in an airborne TRWIS III image. Differences in the spectral characteristics of different genotypes of crops such as barley (e.g., Fetch et al., 2004) have also been detected. However, more investigation is required in this area with respect to tropical trees.

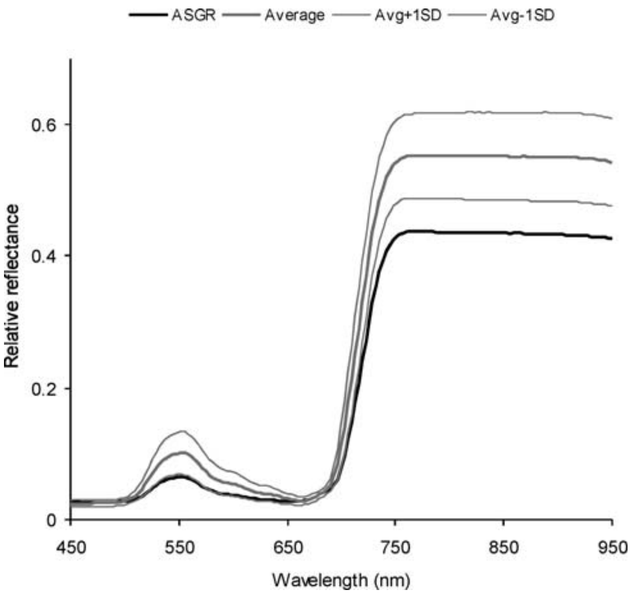

Fig. 8. Average reflectance spectra for *Astronium graveolens* (ASGR) compared to average reflectance spectra ±1SD for all species at Parque Natural Metropolitano, Panama, for October 2003.

Differences in leaf optical properties due to seasonality (wet/dry cycles) could be detrimental or beneficial to tree species identification. Temporal phenomena such as flowering, leaf flush, or senescence could be confusing if not well understood, but could become useful in detecting particular species at particular times of the year if understood. In a similar way, the timing of imagery is important in crop discrimination studies (Collins, 1978; Congalton et al., 1998; Murakami et al., 2001). Thus, a creative combination of image processing and classification with in-depth knowledge of tree ecology and phenology will likely be necessary for a successful species-level classification.

Based on our leaf-level classification analysis of 50 species, there is potential for classification of up to 20 species with approximately 85% test data accuracy and approximately 80% test data accuracy for up to 45 species (Fig. 7). Fung et al. (1999) achieved a similar level of overall accuracy (89%) for 25 subtropical tree species using a set of 13 bands selected using hierarchical clustering (as compared to 83.2% test data accuracy and 91.6% overall accuracy for 25 species, based on our data). For our analysis, five spectra were used for training

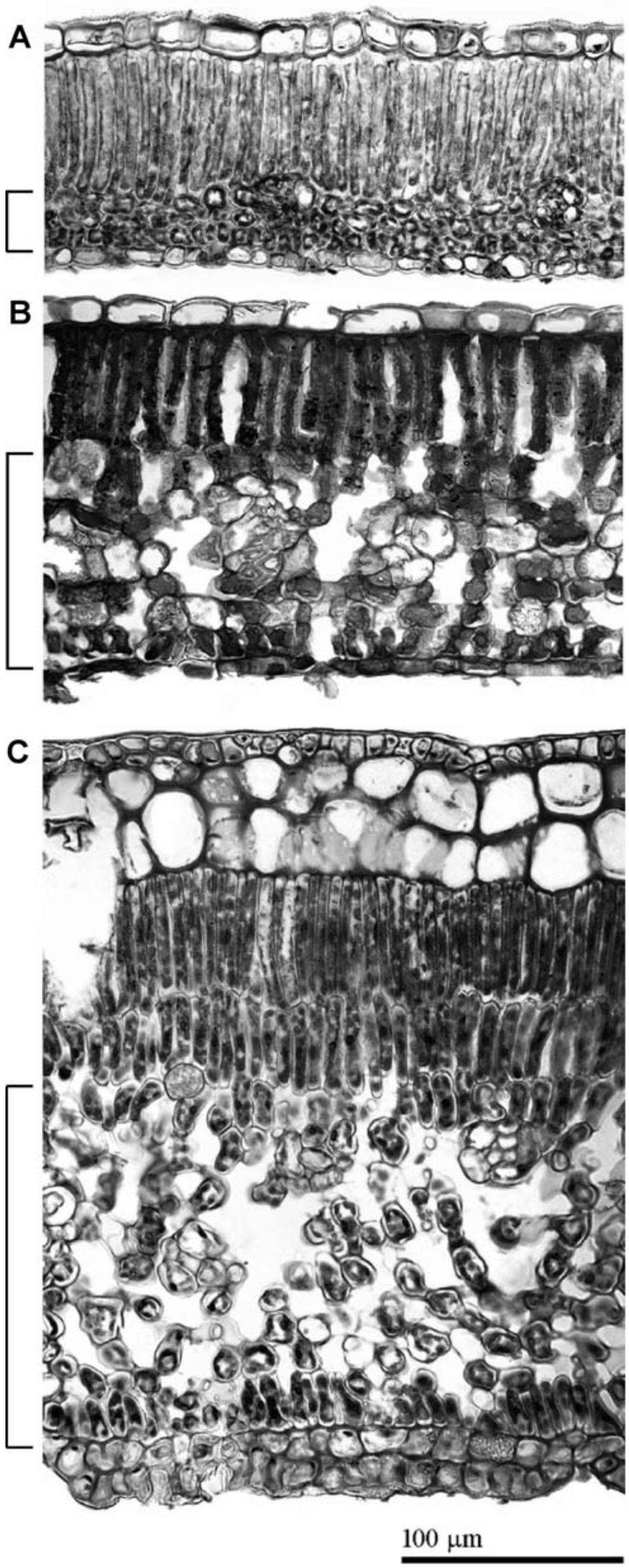

Fig. 9. Leaf cross sections of three species from Parque Natural Metropolitano, Panama. (A) *Astronium graveolens*, (B) *Anacardium excelsum*, (C) *Ficus insipida*. Bracketed area indicates approximate width of the spongy mesophyll. The 100-µm scale is applicable to all cross sections.

TABLE 7. Correlation coefficients and *P* values for relationships between leaf optical properties and leaf internal mesophyll structure, Parque Natural Metropolitano (October 2003 data set).

	Space (%)		SMW (µm)	
Waveband	*r*	*P*	*r*	*P*
$R_{706.2}$	0.760*	0.048	0.823*	0.023
$R_{748.6}$	0.626	0.133	0.632	0.128
$R_{800.7}$	0.723	0.066	0.684	0.090
$R_{959.3}$	0.786*	0.036	0.710	0.074
$T_{706.2}$	−0.879**	0.009	−0.728	0.063
$T_{748.6}$	−0.938**	0.002	−0.768*	0.044
$T_{800.7}$	−0.932**	0.002	−0.773*	0.042
$T_{959.3}$	−0.875**	0.010	−0.725	0.065

Notes: R, reflectance; *T*, transmittance; Space (%), percentage of intercellular space; SMW, spongy mesophyll width. Correlation coefficients determined for $N = 7$ species; $*P \leq 0.05$; $**P \leq 0.01$.

classifiers and five for testing the classifiers. With greater amounts of training data, such as using a cross-validation approach, classification accuracies could have been higher yet.

Provided the linear relationship in Fig. 7 continues, we may be able to classify test data for 75 species with 73% accuracy, and 100 species with 69% accuracy. Classification accuracy erodes further beyond 100 species. At the leaf level, therefore, it would not be possible to classify all species in species-rich neotropical forests where the number of tree species can exceed 100 even in 0.1-ha plots (Gentry, 1991), although potential for discrimination would likely be improved using a spectrometer with a greater range (e.g., to 2500 nm). Also, it remains to be determined whether a similar trend holds at the crown level. If it does, classification could still be successful for less species-rich tropical forests or for detecting smaller numbers of tree species with distinct features from the remainder of the canopy.

Spectra and leaf traits—While this paper does not attempt to explore a comprehensive suite of leaf traits, the selected traits measured for species at Parque Natural Metropolitano (chlorophyll content, leaf thickness, percentage of intercellular space, spongy mesophyll width) provide an illustration of leaf feature variability among nine tropical dry forest tree species, which supports the differences observed in leaf reflectance for these same species (Table 6). Where two species differ in one or more of these traits, the resultant differences in spectral reflectance at one or multiple wavebands provide the opportunity for their discrimination.

Pigment content plays a large role in visible reflectance. The range of total chlorophyll content for the selected species at Parque Natural Metropolitano was 496-1055 µmol/m^2 and for reflectance at 550 nm, 9.10–15.36% (Table 6). Overall, visible wavebands (400–700 nm) were key to species identification and were emphasized in feature selection (Fig. 6). Leaf thickness and internal leaf morphology appear to control near-infrared reflectance (Gates et al., 1965; Knipling, 1970; Knapp and Carter, 1998). From our samples, histological analysis of leaf thin sections (Fig. 9) can provide unique insight into the relative magnitude of reflectance in the near-infrared. The most striking link encountered was between the low percentage of intercellular space, spongy mesophyll width, and leaf thickness of *Astronium graveolens* and the species' uniquely low near-infrared reflectance. The leaf thin section of this species revealed a very thin, compact spongy mesophyll layer, allowing little opportunity for interactions of light at air–cell-wall interfaces. In fact, this same species transmitted more light through the leaf than any other species sampled. Stronger correlations with near-infrared bands might have been observed using the ratio of mesophyll cell surface area exposed to intercellular spaces per unit leaf surface area (Slaton et al., 2001), which provides a better description of air–cell-wall interfaces in the mesophyll than does the percentage of air space (Knipling, 1970). Water content was not studied here, but would probably correlate with leaf thickness, intercellular space, and infrared reflectance and transmittance (Sims and Gamon, 2003).

In general, accurate classification is closely associated with the presence of distinctive leaf features within the spectral range considered, as seen from the example of Parque Natural Metropolitano. Distinctive leaf spectral features that facilitate species classification will be tied to leaf traits such as (1) low or high levels of chlorophyll per unit area, (2) thick or thin leaves, and/or (3) low or high percentage of air space in the spongy mesophyll relative to other species.

Crown spectra—Although this discussion focused mainly on leaf spectra and their discrimination, the primary application for this study will be towards tree crown identification. How then, can knowledge about intraspecific variability in leaf reflectance be applied to future crown-level tree classifications from airborne or satellite-borne imagery? First, our results will provide a partial basis for interpreting variability in tree crown reflectance spectra within and between sites and seasons, since leaf reflectance often comprises the major component of an overall crown reflectance spectrum (with exceptions, especially in dry-season conditions in which many trees are deciduous). Unlike leaf spectra, tree crown spectra obtained from airborne or satellite-borne sensors are influenced by leaf area index (leaf density), leaf angle distribution, crown shape and shading, and background signals such as tree bark and soil. These additional factors may help or hinder tree crown classification. For species that have unique and predictable combinations of these factors, their overall crown spectral signatures may be even more distinguishable than their leaf spectral signatures. However, this has not been substantiated yet, and recent work (J. L. Zhang, B. Rivard, G. A. Sánchez-Azofeifa, and K. L. Castro-Esau, unpublished manuscript) indicates that intra-crown variation within species may pose a challenge. In a tropical environment, visual inspection of the crown spectra we recorded at Fort Sherman, Panama, indicate that those five crowns would be distinguishable without difficulty, mainly due to large differences in spectral amplitude and, to a lesser extent, differences in shape (Figs. 1 and 4).

Classification at the canopy level is clearly the next step in determining whether tropical tree species are differentiated and requires airborne or satellite-borne imagery with a combination of both high spectral and spatial resolution. Clark et al. (2005) presented pioneering work in hyperspectral tropical rain forest tree species discrimination at leaf to crown scales, which will likely lead to a burgeoning of research in this field. Additional efforts so far, using aerial photographs (Trichon, 2001) and high spatial resolution multispectral data (reflectance measured over a small number [four] of broad bands, each typically >50 nm wide) such as IKONOS (1 m and 4 m resolution) and Quickbird (0.7 m and 2.8 m resolution; Read et al., 2003; Wang et al., 2004), also support the possibility of separating a portion of the total number of tropical species. Combined with LiDAR (Light Detection And Ranging, an active remote sensor that measures return time for laser pulses), which can provide information on tree location and height as well as crown diameter and shape, the possibilities may extend even further (Gillespie et al., 2004). As these types of data become increasingly available for tropical forests, scientists may draw upon techniques developed for automated tree isolation (Wulder et al., 2000; Leckie et al., 2003) and for tree species classifications in ecosystems with more limited species diversity, such as arid environments (Lewis, 2000; Lewis et al., 2001) and temperate forests (Martin et al., 1998; Key et al., 2001; Roberts et al., 2004). The ability to accurately map tree species in tropical ecosystems will represent a significant advance that will facilitate ecosystem characterization, tree demographic studies, mapping endangered or endemic species, identifying important food sources for wildlife, and quantifying carbon pools and carbon sequestration rates.

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