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Comparison of spectral indices obtained using multiple spectroradiometers

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Abstract

To determine the degree of comparability between three spectrometers (Analytical Spectral Devices FieldSpec Pro FR (FR), Analytical Spectral Devices HandHeld (HH), and UniSpec Spectral Analysis System (UN)), leaf spectra of three species (*Cafea arabica*, *Lantana camara*, *Eriobotrya japonica*), recorded from each instrument, were compared using two illumination, viewing, and field of view (FOV) scenarios. Scenario 1 eliminated differences due to illumination, viewing, and FOV conditions. Scenario 2 represented a 'typical' illumination and viewing set-up for each instrument. Six vegetation indices were computed from the raw spectra as well as spectra (1) interpolated to 1-nm intervals (the sampling interval of the FR) and (2) interpolated to 3.3 nm (the sampling interval of the UN). The spectra measured from the three instruments differed in both shape and amplitude, more so for scenario 2 than scenario 1. In many cases, indices obtained using one instrument differed significantly from the same indices obtained using the other two instruments (but the same leaves), regardless of scenario. The severity of these differences varied between indices. Interpolation was generally ineffective in 'matching' the spectra from the various instruments. Care should be exercised when comparing indices generated from spectra measured from different instruments.
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Keywords: Bandwidth; Field of view; Illumination and viewing geometry; Integrating sphere; Interpolation; Spectral indices; PRI; SIPI; SR705; mSR705; ND705; mND705; Spectral resolution; Spectroradiometer

1. Introduction

Vegetation indices such as the Normalized Difference Vegetation Index (NDVI=NIR−Red/NIR+Red) and Simple Ratio (SR=NIR/Red) are computed from both broadband sensors (e.g., Landsat, where NIR [TM band 4]=760–900 nm and Red [TM band 3]=630–690 nm) and narrow-band sensors, such as high spectral resolution spectroradiometers with a bandwidth of 1 nm to a few nanometers. These sensors may vary greatly in spatial resolution as well, from images with pixel dimensions of approximately 1 km^2 to less than 1 m^2, to laboratory spectra, with fields of view of several centimetres to a few millimetres. Within this range of spatial resolutions, the area of interest may range from homogeneous to highly heterogeneous with regard to reflectance properties and topography.

The spectral and spatial resolution of the instrument used to gather the data from which spectral indices are computed obviously impact the outcome and interpretation of such indices. Recently, the use of narrow-band indices has proliferated in studies involving either leaf-level (e.g., Richardson & Berlyn, 2002; Sims & Gamon, 2002; Tambussi et al., 2002; Thenot et al., 2002) or in situ canopy-level reflectance spectra (e.g., Stylinski et al., 2002; Trotter et al., 2002). These types of indices, such as the photochemical reflectance index (PRI), structure-insensitive pigment index (SIPI), and variations of the simple ratio (SR) and normalized difference index (the narrow-band formulation designated here as ND to distinguish it from the broad-band based NDVI) are generally used to detect subtle differences in vegetation pigment content, previously impossible with broad-band sensors.

The purpose of this paper is to present the results of a comparative study in which reflectance of the same leaves was recorded using three spectrometers, each with a unique spectral resolution and sampling interval. We discuss the variability exhibited by spectral indices (PRI, SIPI, SR_{705}, mSR_{705}, ND_{705}, mND_{705}) obtained using the different spectroradiometers under two scenarios: first, with a fixed illumination and viewing

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geometry and eliminating field of view effects between instruments, and second, using a 'typical' illumination and viewing configuration for each instrument. The three species used for the experiment, coffee (*Cafea arabica*), lantana (*Lantana camara*), and loquat (*Eriobotrya japonica*) represent a variety of leaf surface morphologies.

This research is intended to form part of the Spectral Network (SpecNet) data-sharing cooperative and addresses its theme of instrument comparison and standardization of sampling procedures. In particular, it has implications for reporting and interpretation of indices derived from multiple spectroradiometers.

2. Methods

2.1. Sample collection

The three species sampled for this study have contrasting leaf surface characteristics: (1) coffee (*C. arabica*, Family: Rubiaceae) has smooth, shiny, dark green leaves, (2) lantana (*L. camara*, Family: Verbenaceae) has rough, lighter green leaves, and (3) loquat (*E. japonica*, Family: Rosaceae) has thick, leathery leaves with a heavy greyish pubescence on the surface. If rubbed off, the leaf surface underneath is dark and shiny. Forty healthy, mature leaves were collected per species from an atrium at the University of Alberta. They were placed in sealable plastic bags with moistened paper towels and brought immediately (within 15 min) back to the spectroscopy laboratory for spectral measurements.

2.2. Instruments

Spectral reflectance of the 40 leaves was measured using three spectroradiometers under different set-up configurations described in Section 2.4. The three spectroradiometers employed in the study are the Analytical Spectral Devices FieldSpec Pro FR (Analytical Spectral Devices, Boulder, CO, USA) (abbreviated here as FR), Analytical Spectral Devices FieldSpec HandHeld, (Analytical Spectral Devices, Boulder, CO, USA) (abbreviated as HH) and UniSpec Spectral Analysis System (PP Systems, Amesbury, MA, USA) (abbreviated as UN). Instrument specifications are described in Table 1. It should be noted that the actual FieldSpec Pro FR wavebands (at 1.4 nm (VNIR) and 2 nm (SWIR) spacings, Table 1) are not reported, but undergo an automatic interpolation to 1-nm spacings. The interpolation is accomplished using a linear equation in the VNIR region and a polynomial equation in the SWIR.

In this paper, spectral resolution refers to the full-width at half maximum (FWHM) of the typical Gaussian-shape of the detector sensitivity (Jensen, 2000). There are typically >1 sampling intervals within the spectral resolution of the instrument, and this oversampling reduces degradation when spectra are resampled to match wavelengths of other sensor bands (van Aardt, 2000).

Additionally, a 1800-12S External Integrating Sphere (LI-COR Inc., Lincoln, NE, USA) was used to measure directional–hemispherical reflectance for this study. The integrating sphere is internally coated with barium sulfate, which is highly diffuse. There are six ports on the sphere. Four of these consistently hold the same item during diffuse reflectance measurements. These include the spectrometer fibre-optic port, the reference standard (barium sulphate) port, the leaf sample port, and the transmittance lamp port (this port is located behind the sample port and contains a hollow black plug during reflectance measurements). The remaining two ports contain either the sphere lamp, the 1800-12S illuminator, or a white plug. These two items are interchanged based on what is measured (dark reading, white reference reading, or sample reflectance). The reflectance reference is recorded while the sphere lamp is directed toward the barium sulphate reference standard. Diffuse reflectance of a sample is recorded when the light source is directed towards the sample, and a spectrometer fibre-optic, inserted in a sphere port, views the sphere wall.

2.3. Leaf freshness test

Prior to the main experiment, a freshness test was performed to determine if leaf reflectance changed while the leaf was held

Table 1
Characteristics of spectroradiometers used in this study and an overview of illumination and viewing scenarios 1 and 2

Instrument	Spectral range (nm)	Spectral resolution (FWHM)[a]	Sampling interval	Bare fibre-optic FOV (°)	Scenario 1	Scenario 2			
					Light source	Light source	Illumination angle (°)	Viewing angle	Diameter of field of view (mm)
ASD FieldSpec Pro FR (FR) (model FSP 350-2500P)	350–2500	3 nm at 700 nm 10 nm at 1400 and 2100 nm	1.4 nm (350–1050 nm) 2 nm (1000–2500 nm)[b]	25	LI-COR 1800-12S illuminator	External 50 W halogen lamp	45	Nadir	11
ASD FieldSpec HandHeld (HH) (model FSHH 325-1075P)	325–1075	3.5 nm at 700 nm	1.6 nm	25	LI-COR 1800-12S illuminator	External 50 W halogen lamp	45	Nadir	11
UniSpec Spectral Analysis System (UN) (model UniSpec, Serial No. 9742)	350–1100	<10 nm	3.3 nm	40	LI-COR 1800-12S illuminator	Internal 7.0 W halogen bulb	60	60°	2.3

[a] FWHM = full width at half maximum of an emission line.
[b] Both 1.4-nm and 2-nm sampling intervals are automatically interpolated to 1-nm intervals by this instrument.

in the sample port of the integrating sphere under constant illumination, as was necessary for scenario 1, described in Section 2.4. The test was performed using the UniSpec and the 1800-12S External Integrating Sphere. At the onset of the test, the UniSpec and sphere illuminator warmed up for 1 h. The UniSpec integration time was then adjusted followed by a dark scan and white reference (barium sulfate) scan. With a single leaf in the sample port, spectral reflectance was measured, first at 1-min intervals for the first 10 min, and then at 5-min intervals for the remainder of the time (1 h for the *C. arabica* leaf, 30 min each for *L. camara* and *E. japonica*). A new white reference scan was performed prior to each measurement.

2.4. Measurements of spectral reflectance

Spectral reflectance of the 40 leaves per species was recorded using two illumination and viewing scenarios. Leaves 1 to 40 were measured in the same order for each instrument and scenario. During the period when measurements were made, leaves were kept in the sealed bags with wet towels around the petioles to maintain freshness.

For both scenarios, instruments were turned on a minimum of 45 min prior to taking measurements.

2.4.1. Scenario 1

The first scenario was designed to compare diffuse reflectance of the 40 leaf samples using the three instruments under the same illumination and viewing conditions, while at the same time eliminating any possible effects associated to differences in FOV. This type of configuration was achieved by alternately inserting the fibre-optics of each instrument into the same port of the 1800-12S External Integrating Sphere (Fig. 1, top diagram).

Although the bare fibre-optic field of view (FOV) differs between the ASD instruments (25°) and the UniSpec (40°) (Table 1), the fact that each fibre-optic views diffusely reflected light on the sphere wall rather than viewing a portion of the sample directly eliminates any potential observation differences due to differing FOV. In other words, although they view different-sized areas of the sphere wall, since the energy per unit area on the wall is uniform, the fibre-optics receive the same photon flux.

Instrument optimization and reflectance reference measurements were performed prior to sample measurements. For the UniSpec, integration time was adjusted to the sphere conditions (UniSpec halogen lamp off, sphere lamp on) and tested periodically thereafter, but found not to change. A dark scan was taken for every one to two sample measurements and a white reference scan was taken before every sample measurement. Ten scans were averaged per sample measurement. Likewise, for the ASD FieldSpec HandHeld, the configuration was adjusted to 10 scans per dark current, white reference, and sample measurement. Integration time was adjusted with the fibre-optic exposed to white reference conditions, so that the spectrum would peak but not saturate. Periodic checks also indicated that it was unnecessary to change the integration time during scenario 1 measurements. A white reference measurement was taken prior to each leaf measurement. The white reference activates a dark current measurement, which was therefore taken for each leaf also. For the ASD FieldSpec Pro FR, configuration was adjusted to that described above for the FieldSpec HandHeld, and the instrument was optimized every one to two measurements. A white reference scan was taken before every leaf spectrum, which also activated a dark scan.

With the leaf inserted into the sample port, a reflectance measurement was taken using each spectrometer. During the period of these three measurements, the leaf was not moved, but remained inside the sample port for a total of 3.5–6 min. This sequence was repeated for each of the 40 leaves. To avoid potential systematic effects related to a combination of instrument order and/or declining leaf freshness, the order of spectrometers used to take measurements was reversed after each leaf for *C. arabica* (UN-HH-FR, then FR-HH-UN), and for *L. camara* and *E. japonica*, which were measured at a later date, rotated among all six possible combinations of the three instruments.

In all cases, reflectance spectra were obtained by determining the ratios of data acquired for a sample (an average of 10 scans) to data acquired for a white reflectance standard (barium sulphate). When using the 1800-12S External Integrating Sphere,

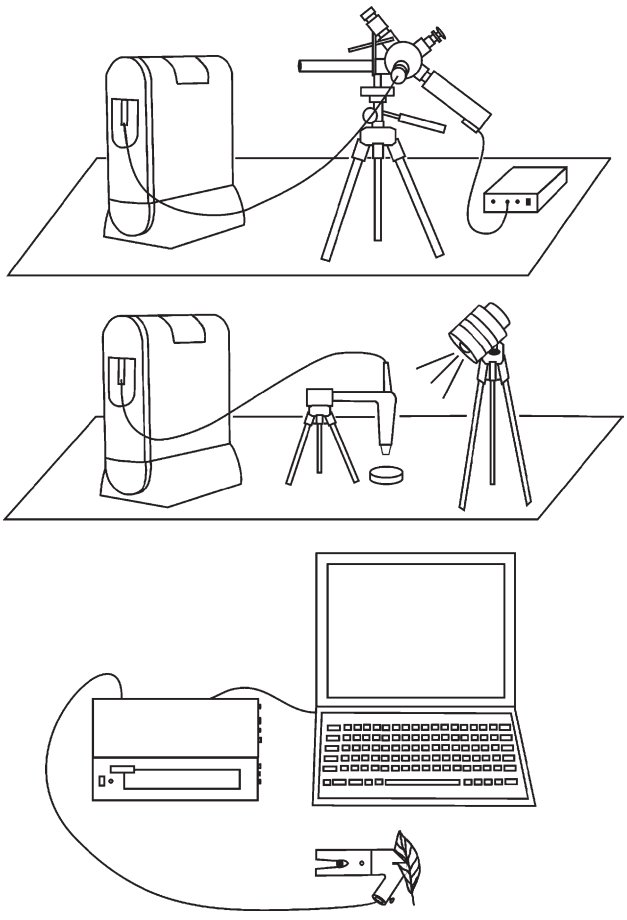

Fig. 1. Illustration of instrument set-up. Top figure shows scenario 1 configuration with the FR fibre-optic inserted into the port of the integrating sphere. Sphere lamp is shown on the right. Middle figure shows scenario 2 configuration for the FR. Bottom figure shows scenario 2 configuration for the UN, with the fibre-optic inserted into the leaf clip.

Table 2
Spectral indices computed in this study

Index		Computation	Significance	Source
Photochemical reflectance index	PRI	$(R_{531}-R_{570})/(R_{531}+R_{570})$	Correlated with the epoxidation state of xanthophyll cycle pigments and photosynthetic radiation use efficiency	Gamon et al., 1992
Structure-independent pigment index	SIPI	$(R_{800}-R_{445})/(R_{800}-R_{680})$	Correlated with the carotenoid/chlorophyll *a* ratio	Peñuelas et al., 1995
Simple ratio	SR_{705}	R_{750}/R_{705}	Estimation of chlorophyll content	
Modified simple ratio	mSR_{705}	$(R_{750}-R_{445})/(R_{705}-R_{445})$	Estimation of chlorophyll content	Sims & Gamon, 2002
Normalized difference index (also called 'chlorophyll index')	ND_{705}	$(R_{750}-R_{705})/(R_{750}+R_{705})$	Estimation of chlorophyll content	Gitelson & Merzlyak, 1994
Modified normalized difference index	mND_{705}	$(R_{750}-R_{705})/(R_{750}+R_{705}-2R_{445})$	Estimation of chlorophyll content	Sims & Gamon, 2002

white reference measurements were obtained with the lamp directed toward the reference standard. To obtain sample reflectance, the lamp was moved to a new position, directed toward the leaf sample in the sample port. The barium sulphate reference was mounted in the 1800-12S reference holder and was not removed during sample measurements. Procedure for obtaining reflectance from the three spectrometers was slightly different based on instrumentation requirements, but in all cases, a white reference measurement was obtained before recording each leaf spectrum for each instrument.

2.4.2. Scenario 2

The second scenario involved the collection of reflectance spectra of the same 40 leaves under conditions that would be considered fairly typical for each instrument. In this case, bi-directional configurations were used rather than employing an integrating sphere (Table 1).

For the ASD FR and HH, an external 50-W halogen lamp was set up with an illumination angle of 45°. Besides the lamp, there were no other sources of illumination in the laboratory and therefore no environmental contribution. The instrument fibre-optic was fit through a mounting gun attached to a tripod, and adjusted to a nadir viewing position (Fig. 1, middle diagram). Between the ASD FR and HH measurements, the same mounting gun was used and the lamp was not moved from its position. The distance from the fibre-optic to the sample was 2.5 cm, which translated to a FOV of approximately 11 mm (using a 25° bare fibre-optic). Prior to sample measurements, ASD HH integration time was adjusted as required, and found not to change throughout the time of the scenario 2 measurements. ASD FR optimization was performed at the same time as each white reference (every fifth leaf for both HH and FR). Instrument configuration was adjusted as in scenario 1, with 10 scans averaged per white reference, dark current, and sample reflectance spectrum. Reflectance spectra were recorded as the ratio of sample data to white reference (99% reflectance Spectralon panel) data under the same illumination and viewing conditions. Sample reflectance was recorded by placing a black 2% reflectance panel beneath the leaf. Reflectance of all 40 leaves was first measured using the ASD FR, and then the instruments were switched and all 40 were measured using the ASD HH. Leaves, which had been numbered 1 to 40, were measured in the same order for each instrument. The measurements were always taken to the same side of the leaf midrib, midway between the top and bottom of the leaf, to approximate the same FOV for the two instruments. Actual position of the FOV would have differed since placement of the leaves under each instrument fibre-optic was not an exact match, neither with respect to area of the leaf within the FOV nor the orientation of the leaf toward the sensor, since the leaf was not flat.

Bi-directional reflectance measurements with the UniSpec involved a notably different set-up involving a bifurcated fibre-optic. One branch delivers light from an internal 7.0-W halogen lamp, and the other returns the reflected light to the detector. A leaf clip was attached to the fore-optic, achieving several purposes: it shields the sample from ambient light, it limits the FOV to a constant 2.3 mm, and it holds the fore-optic at a constant 60° (Fig. 1, bottom diagram). Since the fore-optic provides the light

Table 3
Wavebands used for computing indices listed in Table 2

Waveband of interest	UniSpec			ASD HH			ASD FR		
	Nearest waveband	Interpolated to 1 nm	Interpolated to 3.3 nm[a]	Nearest waveband	Interpolated to 1 nm	Interpolated to 3.3 nm	Nearest waveband	Interpolated to 1 nm[a]	Interpolated to 3.3 nm
445	445.9	445	445.9	445.5002	445	445.9	445	445	445.9
531	532	531	532	530.7551	531	532	531	531	532
570	571.6	570	571.6	570.2251	570	571.6	570	570	571.6
680	680	680	680	680.7408	680	680	680	680	680
705	706.2	705	706.2	704.4227	705	706.2	705	705	706.2
750	748.6	750	748.6	750.2079	750	748.6	750	750	748.6
800	800.7	800	800.7	800.7294	800	800.7	800	800	800.7

[a] Data were not actually interpolated since their original sampling interval matched the interpolation sampling interval.

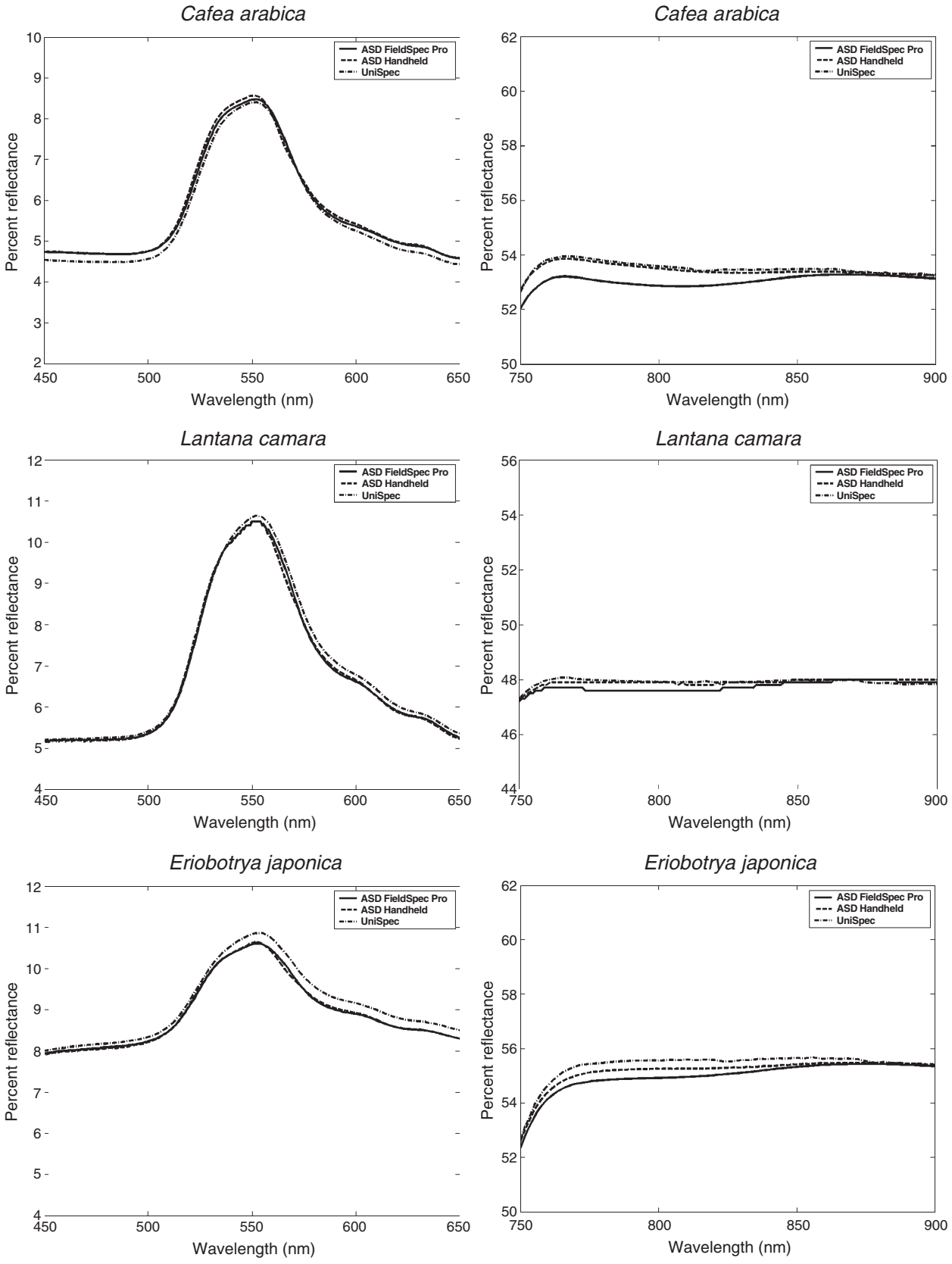

Fig. 3. Average visible (top three graphs) and near-infrared (bottom three graphs) reflectance spectra of 40 leaves of three species as measured by three spectroradiometers under scenario 1, which controlled for illumination, viewing, and FOV effects.

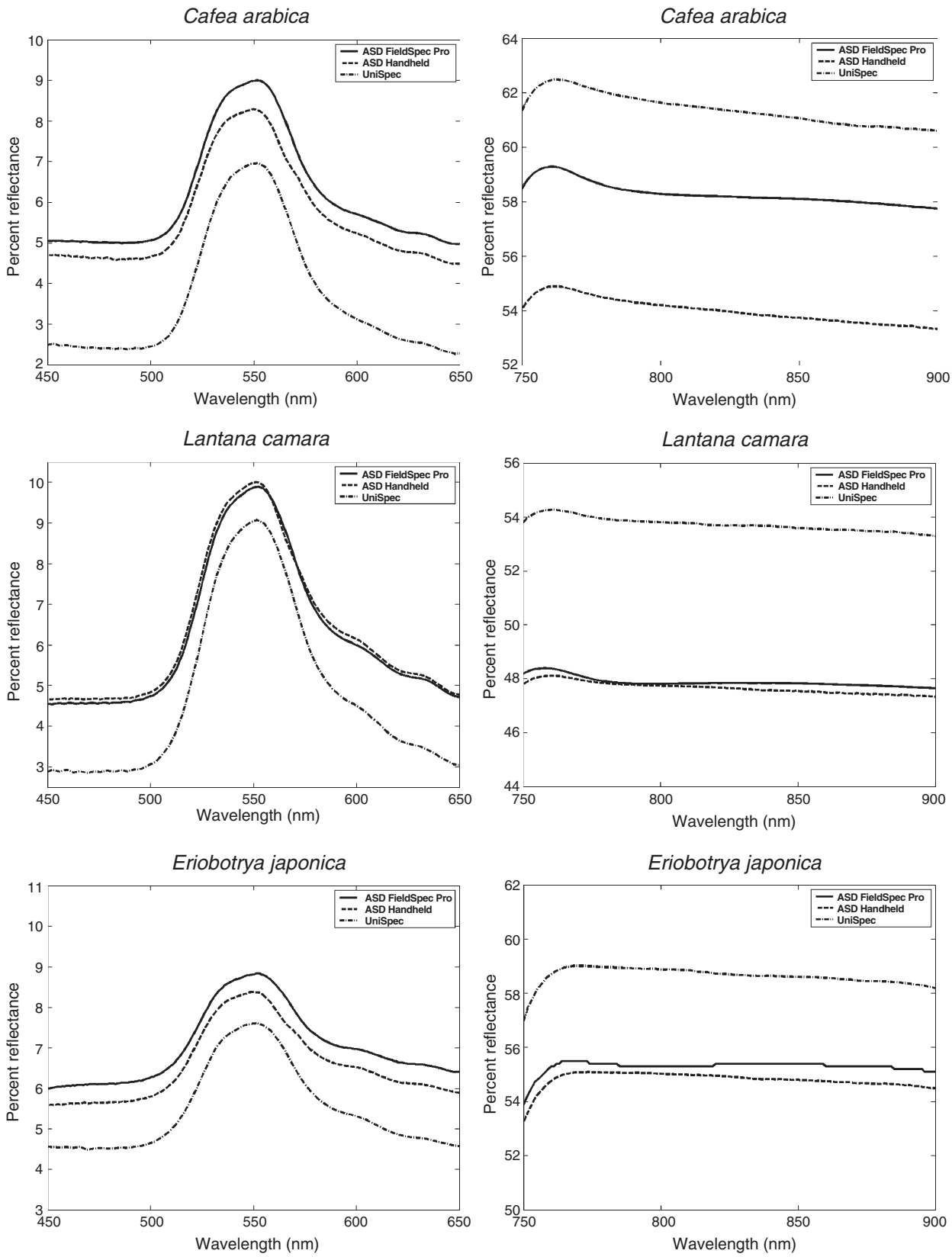

Fig. 4. Average visible (top three graphs) and near-infrared (bottom three graphs) reflectance spectra of 40 leaves of three species as measured by three spectroradiometers under scenario 2, a 'typical' instrument set-up.

Table 4
PRI computed with three spectroradiometers over two illumination and viewing scenarios

Interpolation and interval	Scenario 1			Scenario 2		
	Cafea arabica	*Lantana camara*	*Eriobotrya japonica*	*Cafea arabica*	*Lantana camara*	*Eriobotrya japonica*
No interpolation						
UN	0.0609±0.0076[a]	0.0306±0.0083[a]	0.0094±0.0077[a]	0.1052±0.0128	0.0672±0.0103	0.0501±0.0132
HH	0.0624±0.0075[a]	0.0325±0.0078[a]	0.0134±0.0082	0.0693±0.0234	0.0458±0.0094	0.0316±0.0092
FR	0.0504±0.0060	0.0247±0.0068	0.0101±0.0083[a]	0.0565±0.0160	0.0318±0.0083	0.0227±0.0086
Linear 1 nm						
UN	0.0389±0.0063	0.0084±0.0081	0.0006±0.0066	0.0695±0.0101[a]	0.0342±0.0084	0.0319±0.0094[a]
HH	0.0625±0.0073	0.0330±0.0077	0.0135±0.0081	0.0694±0.0231[a]	0.0460±0.0093	0.0316±0.0090[a]
FR	0.0504±0.0060	0.0247±0.0068	0.0101±0.0083	0.0565±0.0160	0.0318±0.0083	0.0227±0.0086
Cubic 1 nm						
UN	0.0400±0.0064	0.0092±0.0082	0.0009±0.0067	0.0714±0.0103[a]	0.0357±0.0085	0.0329±0.0097[a]
HH	0.0626±0.0074	0.0331±0.0077	0.0135±0.0082	0.0696±0.0233[a]	0.0462±0.0094	0.0319±0.0093[a]
FR	0.0504±0.0060	0.0247±0.0068	0.0101±0.0083	0.0565±0.0160	0.0318±0.0083	0.0227±0.0086
Linear 3.3 nm						
UN	0.0609±0.0076	0.0306±0.0083	0.0094±0.0077	0.1052±0.0128	0.0672±0.0103[a]	0.0501±0.0132
HH	0.0789±0.0083	0.0505±0.0077	0.0208±0.0092[a]	0.0861±0.0281[a]	0.0647±0.0103[a]	0.0402±0.0097
FR	0.0708±0.0068	0.0468±0.0071	0.0188±0.0097[a]	0.0798±0.0226[a]	0.0561±0.0094	0.0346±0.0114
Cubic 3.3 nm						
UN	0.0609±0.0076	0.0306±0.0083	0.0094±0.0077	0.1052±0.0128	0.0672±0.0103[a]	0.0501±0.0132
HH	0.0791±0.0083	0.0507±0.0078	0.0209±0.0092[a]	0.0862±0.0284[a]	0.0649±0.0104[a]	0.0402±0.0097
FR	0.0709±0.0068	0.0468±0.0071	0.0188±0.0097[a]	0.0799±0.0226[a]	0.0561±0.0094	0.0346±0.0114

Similar superscripts within an interpolation and interval scheme for a single species indicate indices that do not differ significantly, based on both a paired *t*-test ($\alpha=0.01$) and a Wilcoxon signed rank test.

To provide examples of between-species D and θ, in comparison to the within-species D and θ values reported for instrument-pair spectra, D and θ were also computed, using the ASD FR scenario 1 spectra only, for the three species pairs. The species pairs were (1) *C. arabica* and *L. camara*, (2) *C. arabica* and *E. japonica*, and (3) *L. camara* and *Eriobotry japonica*. It would be expected that within-species D and θ values (based on instrument-pair spectra) would be smaller than between-species D and θ (based on species-pair spectra), provided by these three examples. In fact, in scenario 1, within-species D and θ should be very close to zero, while deviations from zero indicate instrumental differences.

2.5.3. Statistical tests

Results from Section 2.5.1 were tested statistically to determine if computed indices differed based on the instrument used. The assumption of normality was assessed using normal probability plots of the data and appeared reasonable overall. Even so, we used both parametric (*t*-test) and nonparametric (Wilcoxon signed rank) statistical tests to lend support to the conclusions of the hypotheses.

Since the experiments presented in this paper involve paired data; that is, the same 40 leaves measured in the same order by each instrument for each illumination and viewing scenario, two-tailed *t*-tests for paired data were performed. The two-tailed *t*-tests were conducted to evaluate the mean difference between the pairs. Three sets of *t*-tests were required, one for each instrument-pair (FR:HH, FR:UN, and HH:UN). Secondly, the Wilcoxon signed rank test of equality of medians (also for matched samples) was executed for each instrument-pair. This test is the nonparametric alternative to the *t*-test for paired data and requires less stringent assumptions.

Where the two tests agreed, we could be very confident of our results (Ott, 1993). In fact, it was found that the results of the parametric test and non-parametric alternative agreed in the large majority of cases. In the few exceptions where they did not agree, the conservative result (i.e., do not reject the null hypothesis) was reported. The null hypothesis of each case stated that there was no significant difference ($\alpha=0.01$) between a spectral index measured on the same leaves but from two different spectrometers. All analyses were performed in Matlab Version 13.

3. Results

3.1. Freshness test

Over time, reflectance (measured by the UniSpec) of leaves maintained under illumination in the integrating sphere changed perceptibly (Fig. 2). The three species exhibited unique responses to exposure from the sphere lamp. Visible reflectance of *C. arabica* declined slightly during the period of exposure. During the first 10 min, the decrease in visible reflectance generally remained at or below 2% from the original, time zero reflectance. *L. camara* exhibited a noticeable rise in reflectance on either side of the green peak after a short (3–4 minute) drop. Of the three species, *L. camara* has the thinnest leaf, likely the most susceptible to wilt under the intense illumination of the sphere lamp. Lastly, the leathery *E. japonica* leaf exhibited slight

Table 5
SIPI computed with three spectroradiometers over two illumination and viewing scenarios

Interpolation and interval	Scenario 1			Scenario 2		
	Cafea arabica	*Lantana camara*	*Eriobotrya japonica*	*Cafea arabica*	*Lantana camara*	*Eriobotrya japonica*
No interpolation						
UN	1.0136±0.0078[a]	1.0091±0.0066[a]	1.0139±0.0122[a]	1.0276±0.0046[a]	1.0344±0.0046	1.0199±0.0052[a]
HH	1.0107±0.0044[a]	1.0057±0.0057	1.0111±0.0111[b]	1.0217±0.0083	1.0241±0.0094	1.0212±0.0085[a]
FR	1.0101±0.0069[a]	1.0096±0.0062[a]	1.0119±0.0108[a,b]	1.0295±0.0075[a]	1.0307±0.0068	1.0288±0.0075
Linear 1 nm						
UN	1.0135±0.0077[a]	1.0093±0.0065[a]	1.0141±0.0123[a]	1.0278±0.0046[a]	1.0347±0.0044	1.0199±0.0053[a]
HH	1.0099±0.0041[b]	1.0052±0.0058	1.0108±0.0111[b]	1.0203±0.0080	1.0228±0.0090	1.0202±0.0084[a]
FR	1.0101±0.0069[a,b]	1.0096±0.0062[a]	1.0119±0.0108[a,b]	1.0295±0.0075[a]	1.0307±0.0068	1.0288±0.0075
Cubic 1 nm						
UN	1.0135±0.0077[a]	1.0092±0.0065[a]	1.0141±0.0123[a]	1.0277±0.0046[a]	1.0346±0.0044	1.0199±0.0053[a]
HH	1.0099±0.0042[b]	1.0051±0.0058	1.0108±0.0111[b]	1.0203±0.0080	1.0229±0.0090	1.0202±0.0084[a]
FR	1.0101±0.0069[a,b]	1.0096±0.0062[a]	1.0119±0.0108[a,b]	1.0295±0.0075[a]	1.0307±0.0068	1.0288±0.0075
Linear 3.3 nm						
UN	1.0136±0.0078[a]	1.0091±0.0066[a]	1.0139±0.0122[a]	1.0276±0.0046[a]	1.0344±0.0046	1.0199±0.0052[a]
HH	1.0099±0.0041[b]	1.0050±0.0057	1.0106±0.0110[b]	1.0204±0.0080	1.0227±0.0091	1.0201±0.0084[a]
FR	1.0101±0.0069[a,b]	1.0095±0.0062[a]	1.0117±0.0108[a,b]	1.0294±0.0075[a]	1.0308±0.0069	1.0286±0.0074
Cubic 3.3 nm						
UN	1.0136±0.0078[a]	1.0091±0.0066[a]	1.0139±0.0122[a]	1.0276±0.0046[a]	1.0344±0.0046	1.0199±0.0052[a]
HH	1.0099±0.0042[b]	1.0050±0.0057	1.0106±0.0110[b]	1.0204±0.0080	1.0227±0.0092	1.0201±0.0084[a]
FR	1.0101±0.0069[a,b]	1.0095±0.0062[a]	1.0117±0.0108[a,b]	1.0294±0.0075[a]	1.0308±0.0069	1.0286±0.0074

Similar superscripts within an interpolation and interval scheme for a single species indicate indices that do not differ significantly, based on both a paired *t*-test ($\alpha=0.01$) and a Wilcoxon signed rank test.

initial drops in visible reflectance at 532 nm and 571.6 nm. At 680 nm, reflectance of all three species declined steadily. In the near-infrared wavelengths (748.6 nm and 800.7 nm), both *C. arabica* and *E. japonica* declined slightly over time, while *L. camara* exhibited a small initial decline in the first 2–3 min and then began to rise (data not shown). Initial fluctuations in the visible wavelengths may be related to changes in the relative amounts of xanthophyll cycle pigments upon sudden exposure to high light intensity; later on, the leaf may have suffered heat stress and dehydration. Since in the main experiment of interest for this study (scenario 1), leaves remained in the sphere for only 3.5–6 min, and the order of spectrometers was changed after each leaf, we consider the effect on spectral reflectance minimal.

3.2. Reflectance spectra

Average visible and near-infrared reflectance spectra of coffee leaves for scenarios 1 and 2 are shown in Figs. 3 and 4. Clearly, there is a much better match, as expected, between spectra obtained by the three instruments in scenario 1, where illumination, viewing, and FOV conditions were held constant using the integrating sphere, than in scenario 2, where a 'typical' instrument configuration was used. In scenario 2, illumination and viewing geometry were actually the same for the ASD FR and HH, but FOV was only approximated, which resulted in a notable difference between the average spectra of *C. arabica*, the shiny leaf, but less so for *L. camara* and *E. japonica*. In the case of the UniSpec, scenario 2, the same instrument recorded both the lowest average visible reflectance and the highest near-infrared reflectance for all species. This finding may be partly related to the different illumination and viewing configurations between the UniSpec and the other two spectrometers.

3.3. Indices

Indices computed from the three spectrometers differed significantly in the majority of cases, and attempts to reduce those differences using interpolation were largely unsuccessful. This was the case even under matched illumination, viewing, and FOV conditions (scenario 1). The use of $\alpha=0.05$ instead of $\alpha=0.01$ would have changed this finding only in a small minority of cases. Results for PRI and SIPI are given in Tables 4 and 5; these indices were the most and least affected by instrument, respectively. When compared, on a percentage difference from FR values basis, *C. arabica* PRI values from the UN and HH differed between −22% and +86%, depending on scenario and interpolation combination. For *L. camara*, PRI values differed between −66% and +111%, and for *E. japonica*, they differed between −99% and +121%. SIPI, however, consistently differed less than 1% between the three instruments (true for all three species). For the remaining four indices, SR_{705}, mSR_{705}, ND_{705}, mND_{705}, UN and HH index values typically differed between ±6% from FR values, and never more than ±15%.

Despite what appear to be fairly small differences between indices derived from different instruments (with the exception of PRI), statistical differences were still found in many cases. For scenario 1, this is likely due to the consistency of albeit small differences in reflectance spectra as measured by the three

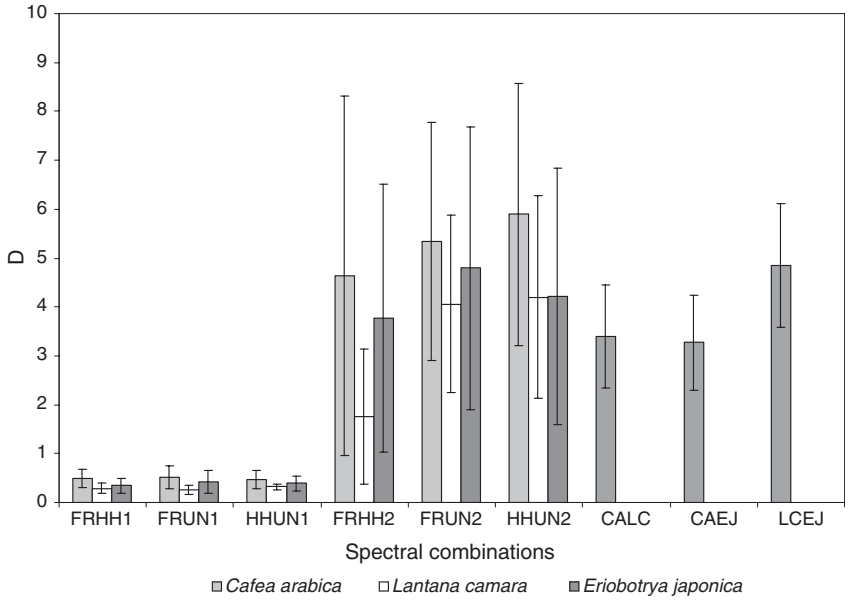

Fig. 5. Average $D \pm$ one standard deviation for the instrument-pairs over the two illumination, viewing, and FOV scenarios. Instruments/scenario are indicated along the *x*-axis in the following manner: e.g., FRHH1 = FR and HH data pair, scenario 1. Additional *D* values are given for three species pairs for comparison. CALC = *C. arabica*/*L. camara*, CAEJ = *C. arabica*/*E. japonica*, LCEJ=*L. camara*/*E. japonica.* Spectra for the species-pair *D* values were measured with the ASD FR spectrometer according to scenario 1 configuration (using the sphere).

instruments under controlled conditions. In scenario 2, where sample results were more variable than in scenario 1, larger differences in means would have been required to observe significant differences between instruments.

3.4. D and θ

Amplitude (D) and shape (θ) differences for instrument-pair spectra were minor in the case of scenario 1, for which reflectance measuring conditions were alike (Figs. 5 and 6). As expected, D and θ were much greater for instrument combinations in scenario 2, and variability was also greater. Even the FR:HH combination for scenario 2 exhibited fairly large D and θ, despite the fact that the illumination, viewing and approximate FOV were matched; however, D and θ were lower for the scenario 2 FR:HH combination than for either the FR:UN or HH:UN. With respect to the three species, *C. arabica*, the shiniest leaf, consistently had higher D and θ values for instrument-pair spectra than either *L. camara*

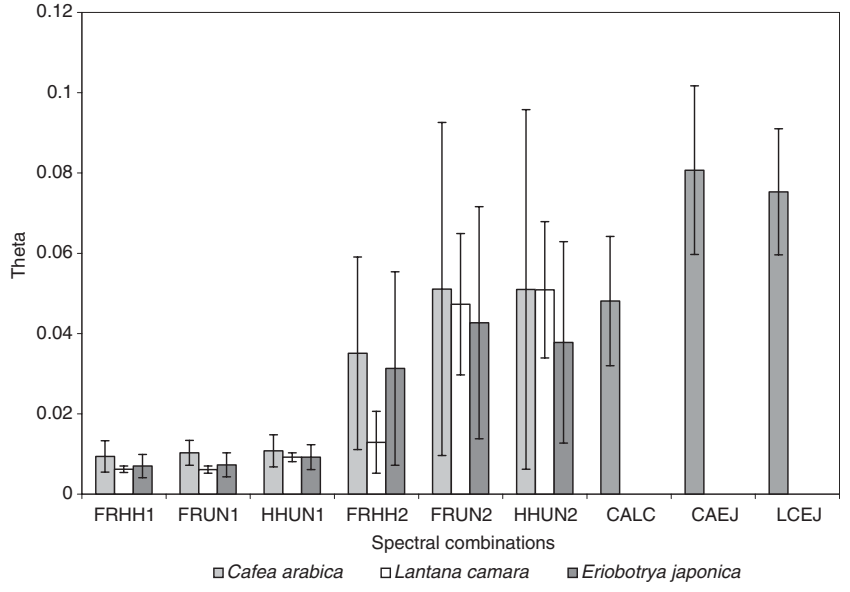

Fig. 6. Average $\theta \pm$ one standard deviation for the instrument-pairs over the two illumination, viewing, and FOV scenarios. Instruments/scenario are indicated along the *x*-axis in the following manner: e.g., FRHH1 = FR and HH data pair, scenario 1. Additional θ values are given for three species pairs for comparison. CALC = *C. arabica*/*L. camara*, CAEJ = *C. arabica*/*E. japonica*, LCEJ = *L. camara*/*E. japonica.* Spectra for the species-pair θ values were measured with the ASD FR spectrometer according to scenario 1 configuration (using the sphere).

or *E. japonica*, regardless of scenario or combination of instruments. The effect of scenario 2 on D and θ values was similar in magnitude to comparing entirely different plant species (species-pair spectra), rather than the same leaves of the same species (Figs. 5 and 6).

4. Discussion and conclusions

Same-leaf spectra recorded by multiple spectroradiometers differ, as indicated by this experiment. Interpolation to match the three sampling intervals was generally ineffective at bringing each index into agreement across instruments. While this was an expected result for scenario 2, in which illumination and viewing geometry differed between the UN and the ASD instruments, and FOV could not be matched exactly between the ASD FR and HH, it was unexpected for scenario 1, in which these effects were eliminated. For the majority of scenario 1 and two interpolations and indices, significant instrument effects ($P<0.01$) were observed for each species (Tables 4 and 5). These differences were particularly acute for PRI (Table 4).

Why do these differences occur for scenario 1? There are a number of possible reasons. Instrument sensitivities are clearly different, as shown in Table 1. Spectral resolution, a measure of the narrowest spectral feature that may be resolved by an instrument, is significantly poorer (<10 nm) for the UN than either the FR (3 nm) or the HH (3.5 nm), and the UN sampling interval is at least double that of the HH or FR. Small spectral features that would be well-defined by the ASD instruments would therefore not be detected by the UN. In fact, this was observed prior to the experiment when comparing spectra of calibration standards (dysprosium oxide, erbium oxide, and holmium oxide), which had sharp absorption features. Coarser resolution may partially explain the ineffectiveness of interpolating UN spectra to 1 nm, but it does not help explain the poor results of degrading FR and HH data to 3.3 nm. It should be kept in mind that while the apparent band spacing of the FR is 1 nm, the actual band spacing (1.4 nm in the VNIR, 2 nm in the SWIR regions) is lost due to an automatic interpolation performed by FR instrument software. This is an unknown that may be an additional source of error in our paper. In any case, the argument of differing spectral resolutions as the cause of differences in same-leaf spectra is lessened by the fact that there are no sharp spectral features in a leaf spectrum, but rather fairly gradual ones. Signal-to-noise ratios (SNR) will also differ between instruments. While SNR was not quantified in this experiment, we attempted to reduce inconsistencies by averaging 10 scans per spectrum for each instrument. Integration time, however, differed between the instruments, the longest of which was for the HH. Increasing number of scans averaged per specõtrum would have improved SNR for all instruments and could have influenced the results of the experiment slightly. However, within the spectral range used for this experiment (see Table 2 for index wavelengths, 450–900 nm for D and θ computation), spectra appeared smooth for all instruments, and improvements in SNR would not have eliminated all amplitude and shape differences between the spectra. Stray light in the integrating sphere was tested prior to the experiment using the FR fibre-optic and found to be minimal. While both the FR and HH fibre-optics fit snugly into the sphere port, the UN fibre-optic would slip if not held in place. Therefore, it was held in place for each measurement, to the point at which a wider portion of the fibre-optic cable rested flush with the entrance to the sphere port. The issue of declining leaf freshness while each leaf remained in the sphere port for 3.5–6 min should have been averaged out by changing the order of instrument with each successive leaf; however, in the case of *C. arabica*, the first species recorded, simply reversing the order of instrument signified that the HH was the middle measurement in all cases. While this could have been a source of error, the fact that discrepancies between spectra from the three spectrometers were observed for all three species seems to indicate that it is not an important one. In the case of PRI, another reason for the apparent discrepancies between instruments may be the fact that this index can be very dynamic, with actual values varying with time of light exposure and physiological state (Gamon & Surfus, 1999). While we made attempts to minimize error by varying the order of sampling, this could have been the source of some of the discrepancies between instruments when reporting PRI values. Although each of the possible sources of error mentioned above appear minor, in combination, they may have led to the small but significant differences observed in same-leaf spectra from the different spectrometers.

For scenario 2, as indicated, differences in spectra recorded by the three instruments were expected. The second scenario was intended to illustrate the magnitude of differences in leaf spectra that could be expected since, typically, different users will employ different set-up configurations, or, alternatively, set-up geometry may be limited by the instrument itself. UN spectra were recorded at a 60° illumination angle and 60° viewing angle geometry, with the leaf FOV and light source contained within a leaf clip. FR and HH spectra were recorded at a 45° illumination angle and nadir viewing geometry, while the leaf was positioned on a black non-reflective panel under the illumination of an external lamp. The differences in set-up geometry would be expected to have the greatest impact on the shiny (*C. arabica*) leaves, which would have been most influenced by specular reflection. The leaf FOV for which spectra were recorded, although approximated, differed between all three instruments. As such, non-homogeneity in the leaf surfaces, including features such as veins, would have translated into small differences in spectral reflectance as recorded by the three instruments. Of particular note, a small (2.3 mm) area of leaf was held relatively flat by the UN leaf clip. For the FR and HH, however, leaves were placed on a surface under the mounted fibre-optic. The leaves were not flat, although some attempt was made to position them as flat as possible within the fibre-optic FOV. Therefore, the differing leaf topographies with respect to orientation toward the sensors would have caused differences between the spectra recorded by the three instruments, particularly for the *C. arabica* leaves in which small changes in leaf positioning could cause notable changes in specular-type reflection on different parts of the leaf surface. This was also true for the *E. japonica* leaves, which often tended to have a convex curve with respect to the upper surface. The *L. camara* leaves, on the other hand, were relatively flat. Lastly, although Spectralon was used as the white reference standard for all scenario 2 measurements, one standard was used for the UN, and a different one

for the FR and HH. A spectrum of the UN Spectralon reference as compared to the laboratory Spectralon panel used for the FR and HH showed some incongruities towards shorter wavelengths that might have contributed to the spectral differences reported in scenario 2.

The observed differences in spectra between instruments have several implications for users. Since scenario 2 is more the reality than scenario 1, differences in reflectance spectra between instruments can be expected to be large (Fig. 4). The use of indices will reduce these effects, but not eliminate them. The most apparent implication is that care should be taken when comparing indices across instruments, such as in multiple published studies. The same is true for cases in which particular wavebands or vegetation indices are correlated with pigment content, such as chlorophyll and/or carotenoid concentration (e.g., Buschmann & Nagel, 1993; Merzlyak et al., 1999; Richardson et al., 2002; Sims & Gamon, 2002). Separate regressions may be required for different spectrometers. Sensor choice and measurement configuration may also influence which bands are selected as optimal for correlation with pigment concentrations. In the same way, spectral reflectance inputs to inverted radiative transfer models may require adjustments based on instrument. Leaf-level spectral libraries gathered using one model of spectrometer may not be highly useful for species classifications of leaf spectra gathered by another model of spectrometer. Fine spectral features, such as the first derivative double-peak observed by Zarco-Tejada et al. (2003), may be less clear in the coarser resolution spectrometers such as the UniSpec (although see Sims et al., 2006). Similarly, measurement of the 'blue shift' requires 1–5 nm resolution (Ustin et al., 2004) and thus may be more accurately tracked with the ASD HH or FR. To what degree instrument affects canopy-level spectra will also be important to determine.

For some applications, however, differences in spectra recorded by multiple spectrometers may not be important on a practical level. With the exception of PRI, the differences of within-index, between-instrument values (instrument-pair spectra), albeit significant in many cases, were fairly small, within a few percent from each other for scenario 1 and, surprisingly, even scenario 2. For example, *C. arabica* ND_{705} values computed on raw data were 0.5202, 0.5457, and 0.5416 for the UN, HH and FR under scenario 1, respectively. When linearly interpolated to 1 nm, the values became 0.5485, 0.5316, and 0.5416. Similarly, linear interpolation to 3.3 nm resulted in ND_{705} of 0.5202, 0.5016, and 0.5118, respectively. In each of the three cases, the ND_{705} from the different spectrometers was significantly different ($P<0.01$) based on both a paired *t*-test as well as a non-parametric alternative, the Wilcoxon signed rank test. The magnitude of differences in ND_{705} values between instruments for scenario 2 were similar to those from scenario 1 or slightly smaller or larger. However, whether or not the magnitude of these differences is important to an investigator wishing to compare results between studies is the question. If the overall range of values observed in a study is much greater than the differences in values observed between instruments, the instrument effect is likely trivial.

Of the six indices, however, PRI is an exception in the relatively drastic manner, it was affected by both instrument and interpolation method. The reason for this is likely related to the fact that both PRI wavebands, 531 and 570 nm, are in regions of slope change. Additionally, the 531 nm reflectance value has been shown to be very dynamic with physiological state and illumination history (Gamon et al., 1992). These factors render PRI unstable compared to other indices that have at least one waveband rooted in a fairly flat, stable area of the spectrum (e.g., 445 and 800 nm, both used in SIPI). Average PRI of the same 40 *C. arabica* leaves, measured under the same illumination and viewing configuration (scenario 1), was 0.0609, 0.0624 and 0.0504, respectively, for the UN, HH, and FR, when using non-interpolated data and the nearest waveband (Table 4). The UN and HH values differ >20% from the FR value. For the UN alone, PRI values, again of the same 40 leaves, were 0.0609, 0.0389, and 0.0400, respectively, for non-interpolated, linearly interpolated, and cubically interpolated data (to 1 nm). Again, note that wavebands in areas of slope change (such as those used to compute PRI) will be affected by interpolation much more so than wavebands in flat areas (e.g., see results for the interpolation of UN SIPI values, which barely change when interpolated). By interpolating the UN 3.3 nm data to 1 nm, matching the FR 1 nm data was attempted. However, the linear and cubic interpolated 1nm UN spectra resulted in PRI values of 0.0389 and 0.0400, respectively, which are no closer to the FR PRI of 0.0504 than the original non-interpolated UN PRI of 0.0609. Conversely, the reverse was attempted. The FR 1 nm spectra were degraded to the UN sampling interval of 3.3 nm. In this case, the FR PRI (originally 0.0504) became 0.0708 and 0.0709, respectively, using either linear or cubic interpolation, also overshooting the UN PRI of 0.0609 at the same sampling interval (3.3 nm). The sensitivity of PRI to instrument could potentially affect interpretations of plant pigment content and photosynthetic radiation use efficiency if instrument differences are not carefully considered.

In conclusion, we present the outcome of this experiment as a cautionary note against direct comparison of indices derived from reflectance spectra gathered from different spectrometers. In reality, investigators use a wider variety of instrument configurations for their studies than explored here, a fact that would likely amplify the differences observed in our experiment. Since spectral differences between instruments occurred for all three species, we do not consider the results to be an isolated phenomenon for a single leaf type. For the three instruments used in this study, there were minor to major shape and amplitude differences between spectra of the same leaves. To use indices effectively and confidently, they should be accompanied by context, for instance, an array of values, collected from the same instrument, that span a range of vegetation health, leaf maturity, functional/structural groups, or that serve to compare between species. Furthermore, authors who decide to interpolate (e.g., Sims & Gamon, 2002) or not (e.g., Trotter et al., 2002) should be well aware of the effect interpolation has on values of indices, which can be considerable. Whether spectral data are interpolated or not, and if not, what specific instrument channels are used to compute indices, should be reported in studies involving hyperspectral data.

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