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Gandy, Y., Mamachen, A., Mamachen, A., Lieman, J., Persans, M., Parsons, J., Ibrahim, E., Everitt, J., Little, C., & Summy, K. (2011). Techniques to Facilitate the Acquisition of Accurate Spectral Measurements and Multispectral Imagery of Plant Foliage Under Artificial Lighting Conditions. *Subtropical Plant Science*, 63, 45–53.

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Techniques to Facilitate the Acquisition of Accurate Spectral Measurements and Multispectral Imagery of Plant Foliage Under Artificial Lighting Conditions

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ABSTRACT

Spectral measurements obtained in the laboratory under artificial lighting sources have been used for many years to develop spectral ‘libraries’ for various soil types, rocks and minerals, and other inanimate features occurring on or near the earth’s surface. Quartz halogen lamps have been shown to emit all of the electromagnetic (EMR) wavelengths required for the acquisition of quality multispectral imagery, and various techniques have been developed to facilitate the acquisition of accurate spectral measurements using such artificial lighting sources. Our objectives in this study were to evaluate the several factors of critical importance in obtaining accurate spectral measurements for plant foliage in the laboratory, including the effects of leaf orientation, excision, and background reflectance on reflectance of visible and NIR wavelengths. Results demonstrated that accurate spectral measurements and imagery of various types of foliage may be obtained using quartz halogen lighting provided that excised leaf samples are placed on an NIR-absorbent background and samples are maintained in containers designed to minimize desiccation.

Additional Index Words: spectroscopy, spectral curves, remote sensing, artificial lighting.

Remote sensing technology has been used for many years to map the distribution and condition of agricultural crops, to identify and delineate stands of native vegetation, to identify and monitor crop diseases and damage caused by insect infestations, and a multitude of other practical applications (see reviews in Avery and Berlin 1992; Jensen 2000; Lillesand et al. 2004; Cracknell and Hayes 2007). In most cases, spectral measurements of various landscape features and imagery of the earth’s surface have been acquired either on the ground or from various types of platforms (e.g., aircraft or satellite systems) under natural lighting conditions. This approach has yielded a wealth of information relating to temporal changes on the earth’s land and water surfaces, but is subject to one major constraint - a requirement for suitable weather conditions (e.g., clear sunny skies) for measuring most types of reflected radiation.

In certain types of studies, the use of leaf samples (rather than whole plants) and artificial lighting

sources (rather than sunlight) may be required to obtain spectral data required to analyze certain variables or parameters. For example, Gandy (2010) evaluated the relationship between spectral reflectance by common sunflower (*Helianthus annuus*) and arsenic content of individual leaves. Since the experimental procedures used in this study involved the acquisition of spectral measurements for individual leaves under laboratory conditions followed by the immediate preservation of leaves for chemical analyses, preliminary studies were required to evaluate 1) the spectral properties of an artificial lighting source in relation to sunlight, 2) the spectral effect(s) of various background materials, and 3) effects of leaf excision and handling methods on reflectance of visible and NIR wavelengths. In this paper, we report the results of these studies and subsequent experiments designed to evaluate the feasibility of acquiring accurate spectral measurements of excised plant foliage in the laboratory under artificial lighting conditions.

MATERIALS AND METHODS

Measurements of incident and reflected electromagnetic radiation (EMR) were obtained using a FieldSpec Pro® VNIR spectroradiometer (Analytical Spectral Devices Inc., Boulder, CO) with sensitivity to wavelengths extending from 350 nm (ultraviolet) through the visible (400-700 nm) and near-infrared region of the spectrum (700-1,100 nm). The instrument was equipped with a target probe fitted with an 18° field-of-view adapter for measuring reflected EMR and a remote cosine receptor (RCR) designed to measure incident radiation or irradiance, recorded in units of watts/m² (Jensen 2005). Prior to each series of measurements, the spectroradiometer was optimized to the particular lighting source in use at the time, and the instrument was calibrated by obtaining a white reference measurement from a Spectralon® reference panel (Analytical Spectral Devices, Boulder, CO). Raw reflectance data were transformed in real time to percent reflectance using RS³ Dual® software and data were processed using ViewSpec Pro® software (Analytical Spectral Devices, Boulder, CO).

Irradiance measurements for a 500-W quartz halogen lighting source (Fig. 1) and reflectance measurements for each of the four quadrants of a standard calibration panel (Fig. 2) were obtained in the laboratory using the artificial lighting on 5 September 2011.

Equipment was then installed outdoors and similar measurements were obtained under natural lighting conditions, i.e., sunlight with ~50% cloud cover. Graphs of irradiance for natural and artificial lighting were plotted using ViewSpec Pro® software, and summary statistics for reflectance of selected wavelengths in the blue (460nm), green (550 nm), red (680 nm) and NIR region (850 nm) were calculated using SYSTAT 10 (SPSS 1996). The decision criterion for rejection or acceptance of the null hypothesis of no difference among means at each wavelength (H_0) was based on whether or not 95% confidence intervals overlapped (accept H_0) or were distinct (reject H_0).

Two procedures were used to evaluate spectral effects associated with background reflectance. The first method involved a comparison of spectral curves obtained for individual leaves attached to plants (*Philodendron scandens*) in close proximity to surrounding foliage, and spectral curves for these same leaves immediately after they had been excised and placed on a nonreflecting background material (Fig. 3). The second method involved a comparison of spectral curves obtained for excised leaves of *Arundo donax* placed on black cloth backgrounds, one of which was known to be NIR-absorbent and the other NIR-reflecting (see Fig. 3b). Spectral reflectance data were obtained and analyzed using procedures described previously, and color-infrared imagery of foli-



Fig. 1. Commercial Electric 500-w quartz halogen lamp used as an artificial lighting source for acquisition of spectroradiometer measurements and multispectral imagery under laboratory conditions (Summy et al. 2004).

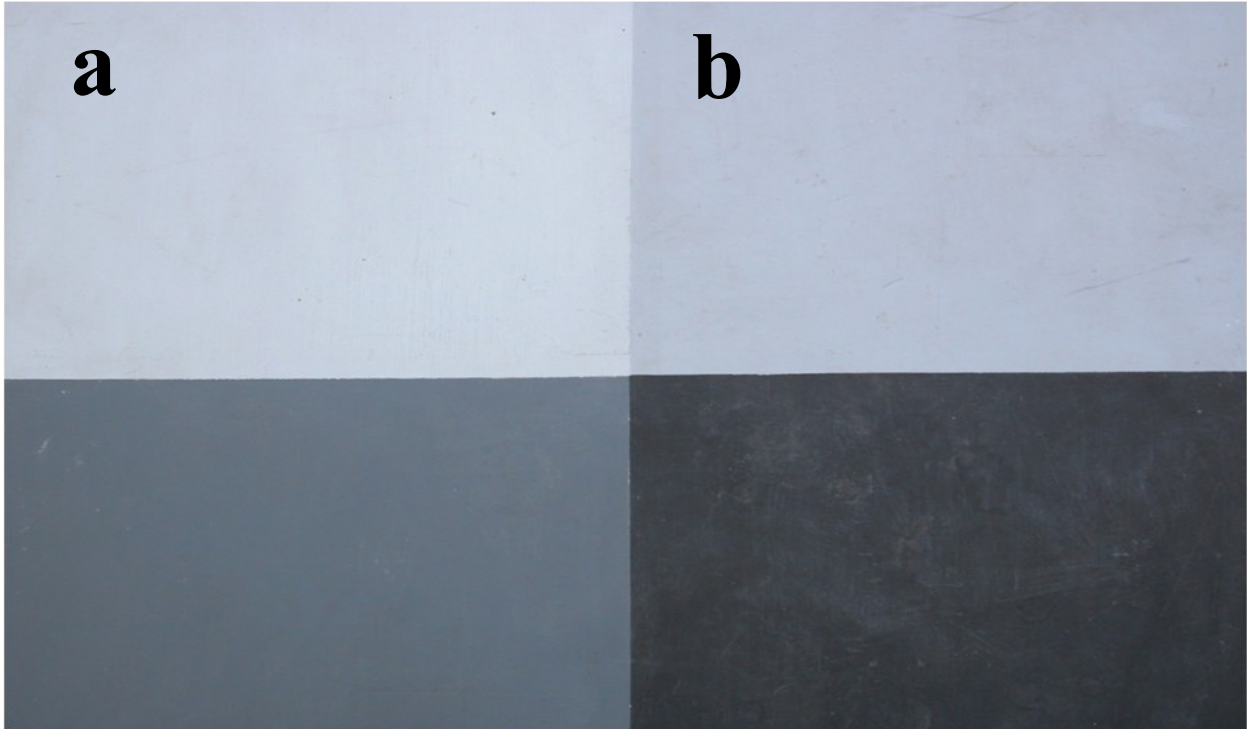


Fig. 2. Standard calibration panel used to measure reflectance of light grey (a), medium grey (b), dark grey (c) and black (d).

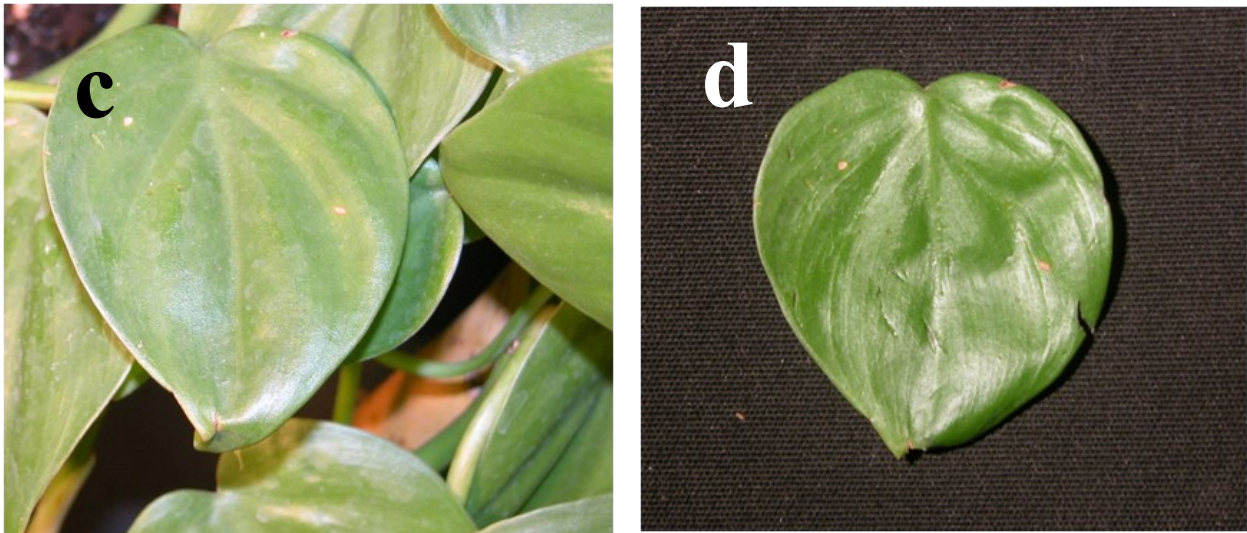


Fig. 3. Leaf of *Philodendron scandens* attached to plant (a) and detached on background (b).

age placed on each type of background was obtained using a DuncanTech MS3100 CIR camera system (DuncanTech, Auburn, CA) equipped with a NI 1424 frame-grabber (National Instruments, Austin, TX).

Physiological effects of leaf excision were evaluated by obtaining spectral measurements for individual leaves of *A. donax* before and immediately after excision, and at subsequent intervals of 1 h and 24 h (Fig. 4). Mean reflectance values for selected wavelengths in the blue (460 nm), green (550 nm), red (680 nm) and near-infrared (850 nm) were compared using statistical procedures described previously.

RESULTS AND DISCUSSION

Although levels of incident radiation were substantially greater under natural lighting conditions (sunlight) relative to the artificial lighting source (quartz halogen lamp), both lighting sources provided sufficient quantities of all EMR wavelengths (visible and near-infrared) required for accurate spectral measurements and quality multispectral imagery (Fig. 5). Although comparison of reflectances obtained from the four quadrants of the standard calibration panel revealed significant differences ($P < 0.05$) in 9 of 16 comparisons, the magnitude of these differences was minimal (0 – 2.4%) and for practical purposes, the accuracy of spectral reflectance measurements obtained using the artificial (quartz halogen) lighting source was equivalent to that obtained under natural lighting conditions (Table 1).

A comparison of spectral reflectance curves for individual leaves of *Philodendron scadens* obtained before and after excision (see Fig. 3) revealed substantial changes in reflectance of certain EMR wavelengths (Fig. 6). Prior to excision, leaves exhibited typical healthy plant spectral profiles with relatively low reflectances in the blue and red regions (~5% for both wavelengths), a slightly high reflectance of green (~16%) and a substantially higher reflectance of NIR wavelengths (~72%). The process of leaf excision was accompanied by no apparent change in reflectance of blue and red wavelengths, a significant decrease in green reflectance (from 16% to 11%), and a substantial decrease in reflectance of NIR wavelengths (from ~72% to ~38%). These apparent decreases had little or no physiological basis; they were primarily an effect of eliminating spectral ‘noise’ caused by reflectance and transmittance of EMR not absorbed for photosynthesis (primarily green and NIR) from adjacent and underlying foliage. This phenomenon is a serious consideration in the acquisition of spectral measurements under both natural and artificial lighting conditions (Campbell 2007) and provides an excellent rationale for the collection of spectral measurements from leaf samples (rather than from attached foliage) in certain situations.

Another subtle but potentially important source of background ‘noise’ was exemplified in a comparison of spectral reflectance curves for three potential background materials – a standard black laboratory table top and two types of black cloth, one of which was

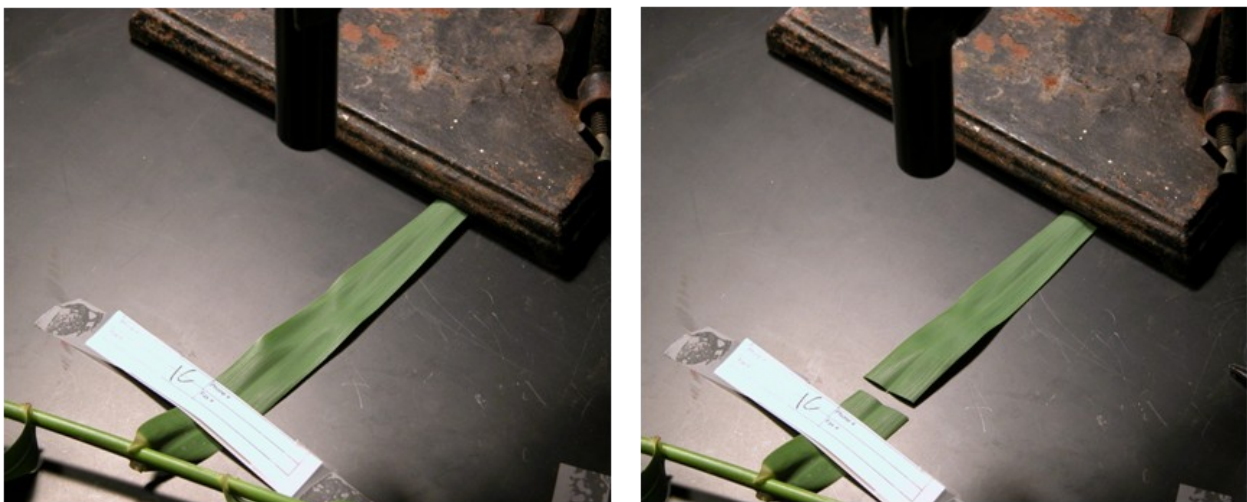


Fig. 4. Acquisition of spectroradiometer measurements for leaf of *Arundo donax* attached to plant (a) and after immediately after excision (b).



Fig. 5. Irradiance curves for sunlight and quartz halogen lamp used in the acquisition of spectroradiometer measurements under laboratory conditions. Data acquired at 11:00 am on 4 August 2011.

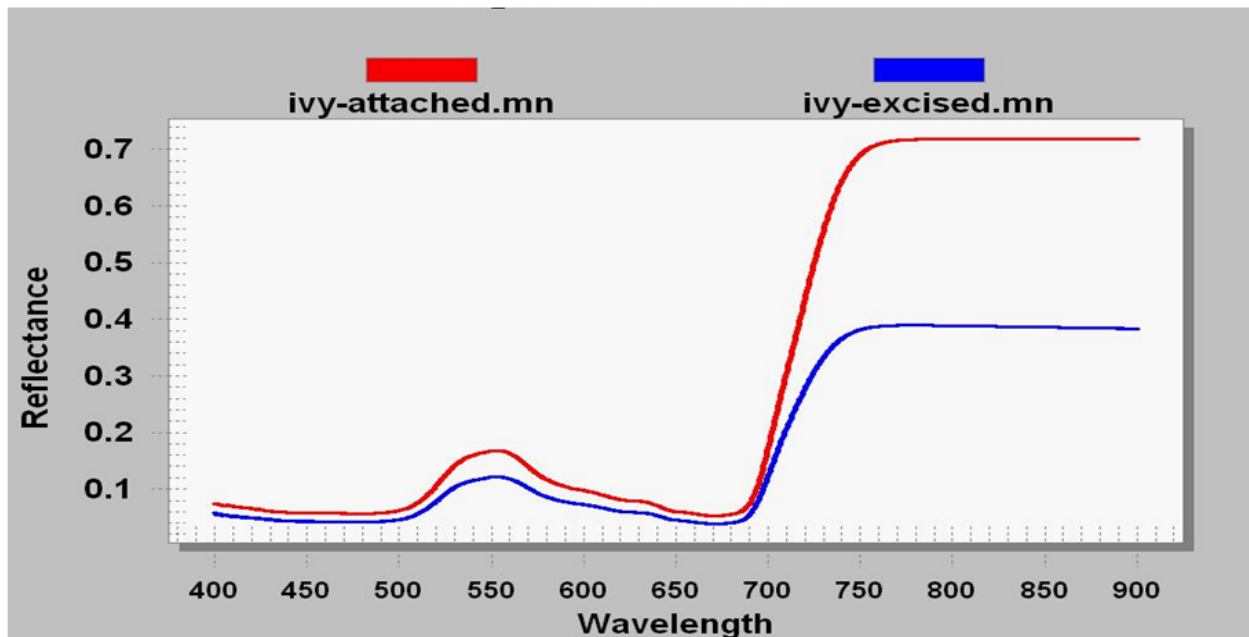


Fig. 6. Comparison of spectral reflectance curves for leaf of *Philodendron scandens* attached to plants in close proximity to surrounding foliage (red) and excised leaf placed on a NIR-absorbent black cloth background (blue).

Table 1. Spectral reflectance (mean \pm 95% CI) for calibration panels under natural and artificial lighting conditions.

Source	460 nm	550 nm	680 nm	850 nm
Light Gray				
Sunlight	47.6 \pm 0.6 <i>a</i>	44.9 \pm 0.6 <i>a</i>	40.7 \pm 0.4 <i>a</i>	35.5 \pm 0.4 <i>a</i>
Artificial	47.9 \pm 0.5 <i>a</i>	45.8 \pm 0.5 <i>a</i>	42.3 \pm 0.5 <i>b</i>	37.9 \pm 0.5 <i>b</i>
Medium Gray				
Sunlight	36.3 \pm 0.3 <i>a</i>	34.6 \pm 0.2 <i>a</i>	31.3 \pm 0.1 <i>a</i>	27.1 \pm 0.1 <i>a</i>
Artificial	35.6 \pm 1.2 <i>a</i>	33.6 \pm 1.2 <i>a</i>	30.8 \pm 1.1 <i>a</i>	27.2 \pm 1.0 <i>a</i>
Dark Gray				
Sunlight	14.9 \pm 0.3 <i>a</i>	13.3 \pm 0.3 <i>a</i>	11.4 \pm 0.2 <i>a</i>	9.7 \pm 0.2 <i>a</i>
Artificial	17.3 \pm 0.6 <i>b</i>	14.6 \pm 0.5 <i>b</i>	12.6 \pm 0.5 <i>b</i>	11.3 \pm 0.5 <i>b</i>
Black				
Sunlight	5.9 \pm 0.2 <i>a</i>	5.7 \pm 0.2 <i>a</i>	5.5 \pm 0.2 <i>a</i>	5.2 \pm 0.1 <i>a</i>
Artificial	7.8 \pm 0.1 <i>b</i>	5.9 \pm 1.2 <i>a</i>	6.6 \pm 0.1 <i>b</i>	6.4 \pm 0.1 <i>b</i>

Means within column for each category followed by same letter are not significantly different at <0.05

known to be NIR-absorbent and the other of which was known to reflect strongly in the NIR region (Fig. 7). Because of their black coloration, all three of the background materials absorbed 100% of the incident visible wavelengths. While the laboratory table top and the NIR-absorbent cloth material also absorbed 100% of the incident NIR wavelengths, the second type of cloth background (NIR-reflecting) reflected ~55% of the incident NIR at 850 nm (Fig. 8). Leaf segments of *A. donax* placed against the NIR-absorbent background materials reflected ~25% of incident NIR wavelengths (the correct reflectance) whereas the same leaf segments placed against the NIR-reflecting background indicated an NIR reflectance of ~65% NIR, most of which was due to background 'noise' (Figs. 9-10). These effects were clearly evident in a CIR image in which the NIR-absorbent cloth appeared black (indicating little or no reflectance of NIR wavelengths) while the NIR-reflecting black cloth appeared bright red, which is indicative of high NIR reflectance of the background material (Fig. 9).

Short-term physiological effects of excision on reflectance by leaves of *A. donax* appear to have been minimal (Table 2). Spectral curves obtained before and after excision indicated slight increases in reflectance of both visible and NIR wavelengths within 1 h of excision, and further increases during the subse-

quent 24 h period. However, the magnitude of these changes were small ($<2\%$) and leaves exhibited none of the classical symptoms associated with plant stress, i.e., significant increases in reflectance of blue or red wavelengths with concomitant decreases in reflectance of green and NIR wavelengths (Campbell 2007). Similar trends were reported previously by Gandy (2010) who noted no significant changes in reflectance by excised leaves of common sunflower, *Helianthus annuus*, during the first 30 min after excision. However, Foley et al. (2006) noted that spectral reflectance by excised leaves is very much dependent on properties of the leaf (i.e., may vary among species and within species) and may be profoundly influenced by water content and desiccation. Results of a recent study reported in this issue (Summy et al. 2011) indicates that excised leaves of *A. donax* may be stored for periods up to 72-96 h in clear plastic zip-lock bags without appreciable changes in spectral reflectance. Moreover, an evaluation of the spectral properties of such containers indicates that EMR wavelengths in the visible and NIR regions are readily transmitted through the plastic material without significant attenuation, and that reflectance measurements of leaf samples enclosed within such containers are equivalent to those obtained for exposed samples (Table 3).

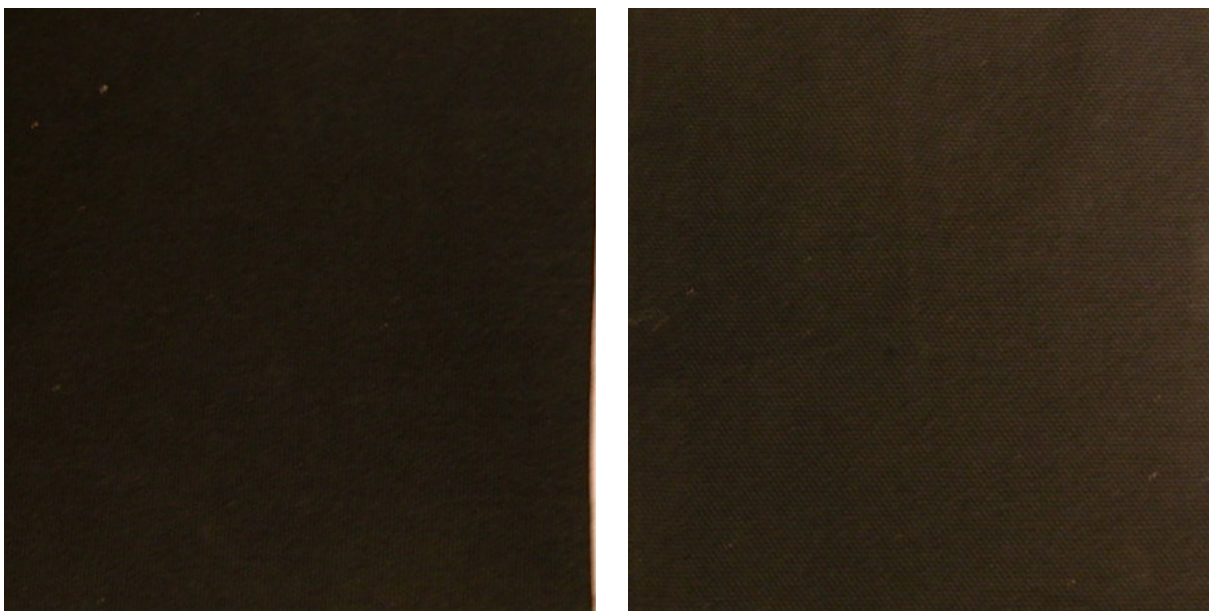


Fig. 7. Potential backgrounds for spectroradiometer measurements and multispectral imagery: NIR-absorbent (a) and NIR-reflecting (b) black cloth.

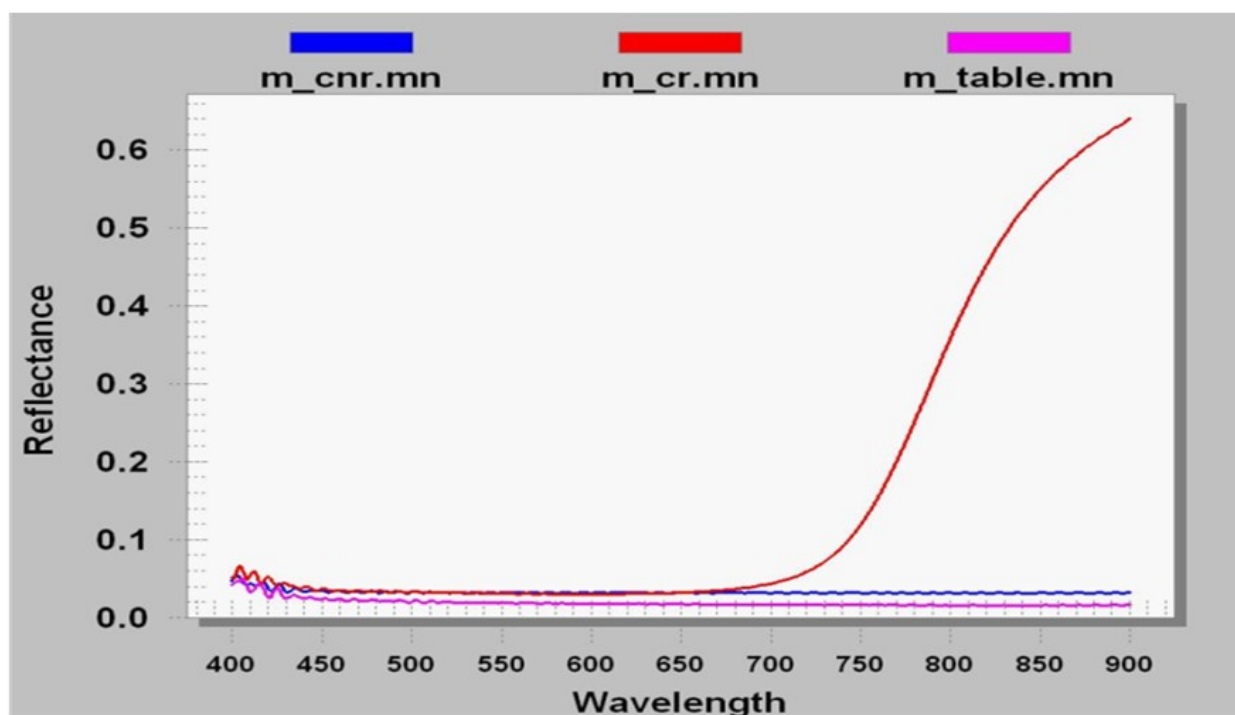


Fig. 8. Reflectance of NIR-absorbent cloth (blue), NIR-reflecting cloth (red) and standard black laboratory table top (magenta).

Fig. 9. Color infrared (CIR) image of excised leaves from *Arundo donax* on NIR absorbing cloth (a) and NIR reflecting cloth (b)

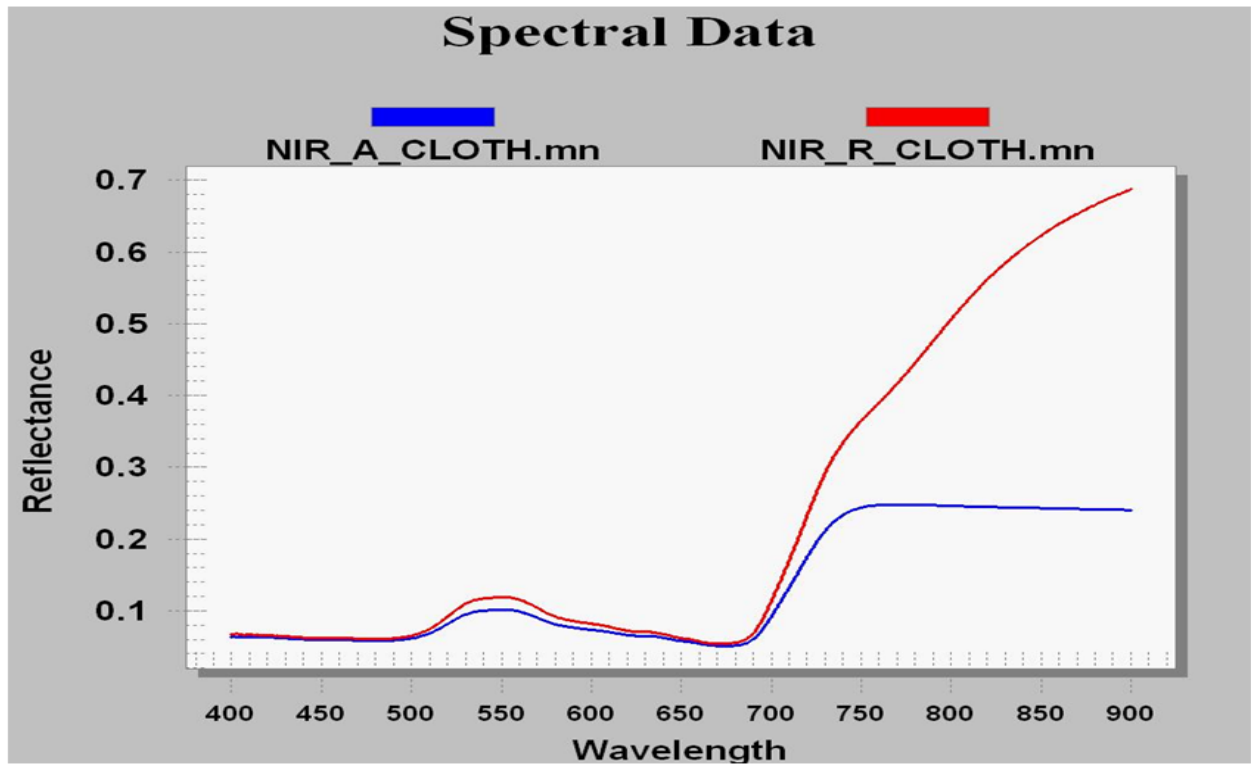
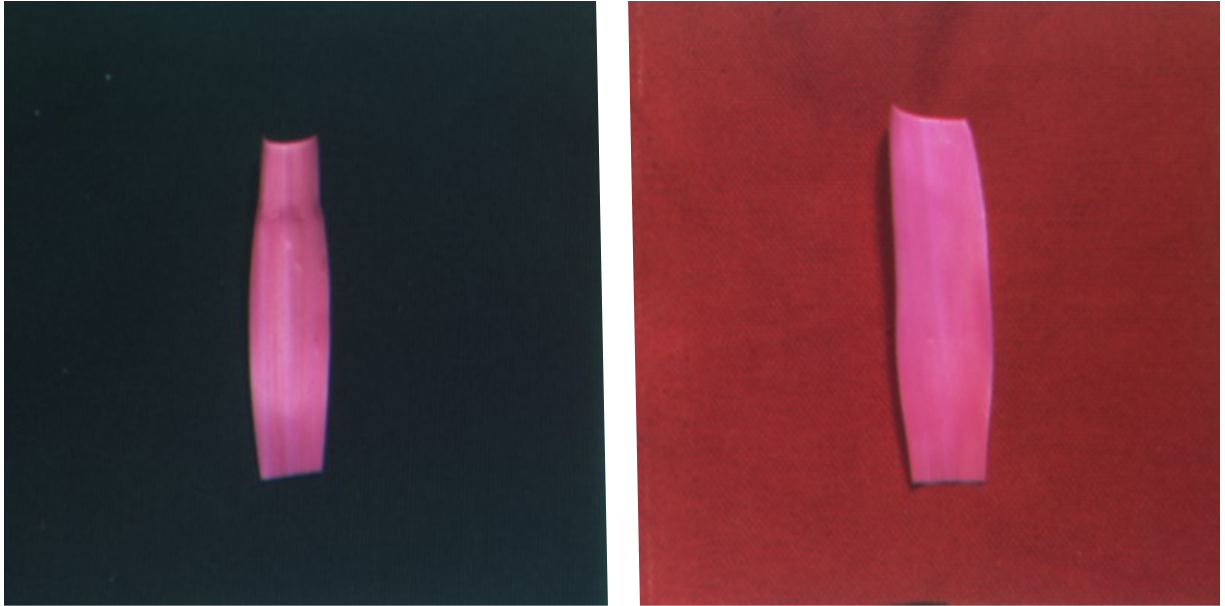


Fig. 10. Spectral reflectance curves for *Arundo donax* on NIR-absorbing (blue) and NIR-reflecting backgrounds (red).

Table 2. Spectral reflectance of foliage of *arundo donax* $\pm 95\%$ CI at selected wavelengths at time of excision (0 hr) and subsequent intervals of 1 hr and 24 hr.

Time post excision	% Reflectance			
	460 nm	550 nm	680 nm	850 nm
0 Hr	9.9 \pm 0.6	17.5 \pm 1.0	8.6 \pm 0.5	48.8 \pm 2.5
1 Hr	11.2 \pm 0.5 *	19.2 \pm 0.7	9.7 \pm 0.4 *	51.8 \pm 1.4
24 hr	11.6 \pm 0.4 *	19.4 \pm 0.6 *	10.5 \pm 0.4 *	51.1 \pm 1.9

* Means within column followed by asterisk are significantly different $P < 0.05$ from measurement at time 0

Table 3. Comparison of spectral measurements for unbagged and bagged (clear plastic) leaf segments of *Arundo donax* under quartz halogen lighting conditions.

Treatment	460 nm	550 nm	680 nm	850 nm
Exposed	9.6 \pm 0.1 <i>a</i>	18.7 \pm 0.3 <i>a</i>	8.5 \pm 0.2 <i>a</i>	47.8 \pm 0.4 <i>a</i>
Bagged	9.4 \pm 0.3 <i>a</i>	17.0 \pm 0.3 <i>b</i>	8.1 \pm 0.2 <i>a</i>	47.8 \pm 0.5 <i>a</i>

* Means within column for each category followed by same letter are not significantly different at $P < 0.05$

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