Subtropical Plant Science, 63:54-64.2011.

Effects of Leaf Excision and Sample Storage Methods on Spectral Reflectance by Foliage of Giant Reed, *Arundo donax*

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ABSTRACT

Research was conducted to evaluate the effects of leaf excision and sample storage methods on spectral reflectance by foliage of giant reed, *Arundo donax*, an invasive weed which has caused extensive damage in many areas of the Rio Grande Basin in Texas and Mexico. Within 24 hours of excision, *A. donax* leaves exposed to ambient laboratory conditions (room temperature under natural lighting conditions) exhibited two trends indicative of physiological stress: 1) small but significant increases in reflectance of blue and red wavelengths (400-500 nm and 600-700 nm, respectively) and 2) a substantial reduction in reflectance of near-infrared (NIR) wavelengths (700-1,100 nm). A similar but less pronounced trend was evident among leaf samples held within conventional paper sacks. Leaf samples held within sealed plastic bags (Glad-Bags) under two types of lighting conditions (natural light and artificial darkness) and temperature regimes (room temperature vs artificially cooled) exhibited slight but significant increases in both visible and NIR wavelengths (a trend that was also evident in attached foliage), although no evidence of physiological stress was detected during a 96-hour observation period. These trends indicate that accurate spectral measurements may be obtained from samples of *A. donax* foliage under for periods up to 72 - 96 hours following excision if such samples are transported and maintained in suitable containers designed to minimize effects of desiccation.

Additional Index Words: Carrizo cane, sampling, spectroradiometry, remote sensing.

Giant reed, *Arundo donax*, an invasive weed native to the Mediterranean region, has caused extensive damage in many areas of the Rio Grande Basin and is currently the target of a major biological control effort in Texas and Mexico (Goolsby and Moran 2009). A variety of remote sensing technologies have been used in this program, including aerial color-infrared (CIR) photography and high-resolution satellite imagery for mapping *A. donax* infestations in the Rio Grande Basin (Everitt et al. 2004, 2005). As in most similar programs, most of the spectral reflectance data and multispectral imagery for *A. donax* infestations has been acquired *in situ* or from aircraft and/or high-resolution satellite platforms.

Under certain conditions, *in situ* spectral measurements and/or aerial multispectral or hyperspectral imagery may be difficult or impossible to obtain and/or may not provide critical spectral information required for a given study. For example, giant reed plants typically grow to heights approaching or exceeding 5-6 m and commonly occur in dense inpenetratable stands that may cover extensive areas (Fig. 1). Under such conditions, the transport of conventional field spectroradiometers to selected sample points within such infestations may be logistically difficult, and reflection of incident electromagnetic radiation (EMR) from numerous plants within the canopy (path radiance) may seriously degrade the accuracy of spectral measurements for a given target plant or even preclude calibration of the instrument itself. In such cases, the collection of leaf samples in the field for subsequent spectral analysis in the laboratory may provide the only feasible means of acquiring meaningful spectral data for certain types of comparisons (e.g., correlation between concentrations of certain elements within leaf tissues and reflectance of EMR of various wavelengths).

The feasibility of acquiring accurate spectral

Fig. 1. One of the authors (J. Goolsby) near stand of *Arundo donax* (a) and extensive infestation bordering Rio Grande near Laredo, Texas (b). Photos by J. Goolsby, USDA-ARS, Weslaco, TX.

measurements from excised leaf samples of a given species is dependent on the effects of excision on the physiology of leaves which may profoundly affect reflectance of EMR in various waveband regions (Foley et al. 2006; Gandy et al. 2011). Since magnitude of these effects may vary among plant species (Foley et al. 2006), we conducted research designed to evaluate 1) the effects of excision on spectral reflectance by foliage of *A. donax*, and 2) the effectiveness of several types of sample holding containers commonly used to transport leaf samples from the field to laboratory facilities.

MATERIALS AND METHODS

Potted *A. donax* plants obtained from the USDA-ARS Kika de la Garza Agricultural Research Center in Weslaco, TX, were transported to the Department of Biology at the University of Texas – Pan American where they were maintained in a laboratory at room temperature (23-25^{o} C) under natural lighting conditions for several days prior to the experiment. Prior to excision, spectral measurements of selected leaves were collected using a FieldSpec VNIR spectroradiometer equipped with a Remote Cosine Receptor (for measuring irradiance) and an 18^{o} IFOV target probe (Analytical Spectral Devices, Inc., Boulder, CO). Spectral measurements were collected under an artificial lighting source (700-w quartz halogen lamp, Commercial Electric, Inc.; Summy et al. 2004) and the instrument was calibrated prior to each series of measurements by use of a white Spectralon® reference plate (Analytical Spectral Devices, Inc., Boulder, CO).

Following initial spectral measurements of attached foliage, selected leaves of *A. donax* plants were excised with small scissors. Spectroradiometer measurements of each such leaf were collected immediately after excision (5 measurements per leaf) and samples were placed in groups of 3 within either of two types of holding containers – 31.4x15.5 cm paper sacks (Wal-Mart Stores, Betonville, AR) or 17.7x19.7 cm plastic zip-loc Glad-Bags® (Glad Products C., Oakland, CA). An additional sample of excised *A. donax* leaves was exposed to ambient laboratory conditions by placing such leaves near, but not within, paper bags (Fig. 2). Four of the plastic sample bags were maintained under natural lighting conditions (50%) and the remainder were protected from visible light by covering container with tin foil (50%). Within each of the latter groups, 50% of containers were maintained at room temperature (22-25^{o} C) while the remaining 50% were maintained in translucent plastic boxes containing cooling devices that lowered temperatures to those equivalent to that of an ice chest (5-10^{o} C). Spectral measurements of leaves within each container were collected immediately after excision and at subsequent intervals of 24, 48, 72 and 96 h post-excision. Similar measurements were also collected at similar intervals for selected leaves of a live *A. donax* plant maintained under similar conditions and a group of excised leaves which were not enclosed within any type of container.

Spectral measurements for each treatment group were processed using ViewSpec Pro® software (Analytical Spectral Devices, Inc., Boulder, CO). Means and 95% confidence intervals for selected wavelengths in the blue (460 nm), green (550 nm), red (680 nm) and near-infrared regions (850 nm) were calculated for selected time intervals (0, 24, 48, 72 and 96 hours post-excision) using Systat 10 (SPSS, Inc., 1996). Means for reflectance of each wavelength during time intervals 24, 48, 72 and 96 hours were compared to the mean reflectance for that particular sample at the time of excision (time 0), and rejection or acceptance of the null hypothesis of no difference was based on whether the 95% confidence limits overlapped (no difference) or were distinct (significance at 5% probability level).

RESULTS AND DISCUSSION

Intact (attached) foliage of *A. donax* exhibited minimal changes in the blue, green and red (visible) regions of the spectrum (<1%; $P>0.05$), and small but significant increases ($P<0.05$) in NIR reflectance (Fig. 3; Table 1). The series of spectral curves for attached foliage were typical of those for healthy vegetation in general (i.e., relatively low levels of reflectance in the blue and red regions, a slight peak in green reflectance, and a significant increase in NIR reflectance) and provided no evidence of physiological stress during the 96-h observation period. Indeed, the progressive increases in NIR reflectance suggested a positive response by live plants to the laboratory environment which was considerably milder than outside ambient conditions under which plants had been maintained prior to the experiment (maximum daily temperatures generally approached or exceeded 38^{o}C before and during the study period).

Excised leaves exposed to ambient laboratory conditions and counterparts enclosed within paper bags both exhibited two trends that are commonly associated with pronounced plant stress (Fig. 4; Table 2). First, small but significant ($P<0.05$) increases in reflectance of photosynthetically active radiation (or conversely, decreases in absorption of blue and red wavelengths) were evident in both sample categories and are generally considered evidence of a disruption or cessation of photosynthetic processes (Campbell, 2006). Second, leaf samples of both categories

Fig. 2. Sample holding containers for foliage of *Arundo donax* evaluated during study included 17.9 x 19.7 cm plastic Glad-Bags (left) and 31.4 x 15.5 cm paper bags (right).

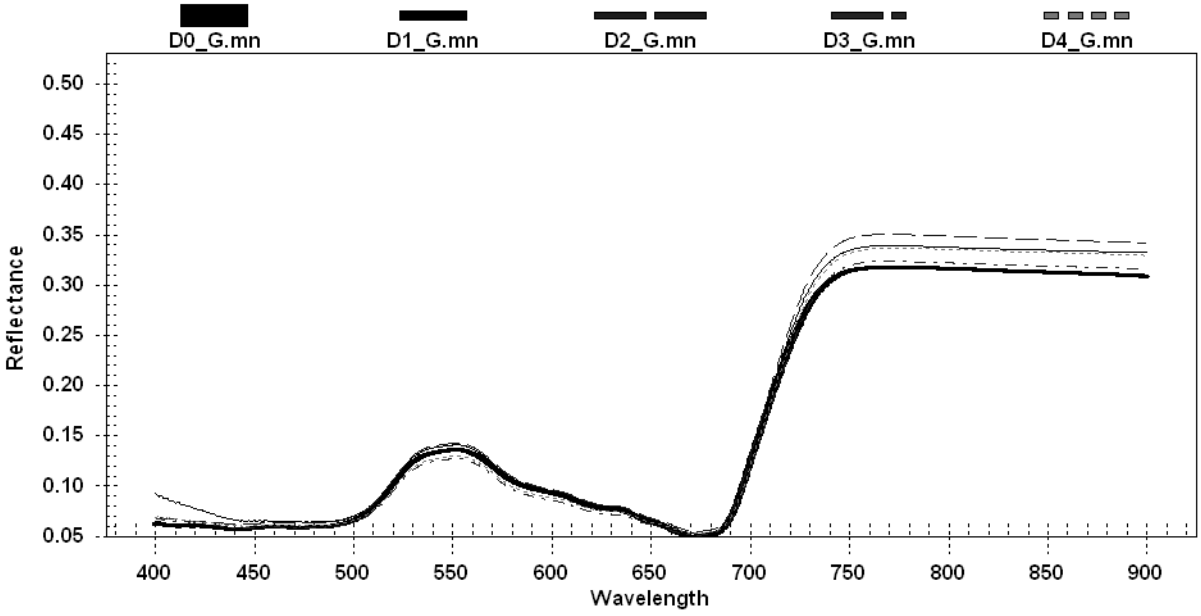

Fig. 3. Spectral reflectance curves for attached (live) foliage of *A. donax* maintained under natural lighting conditions in the laboratory during a 96-h observation period.

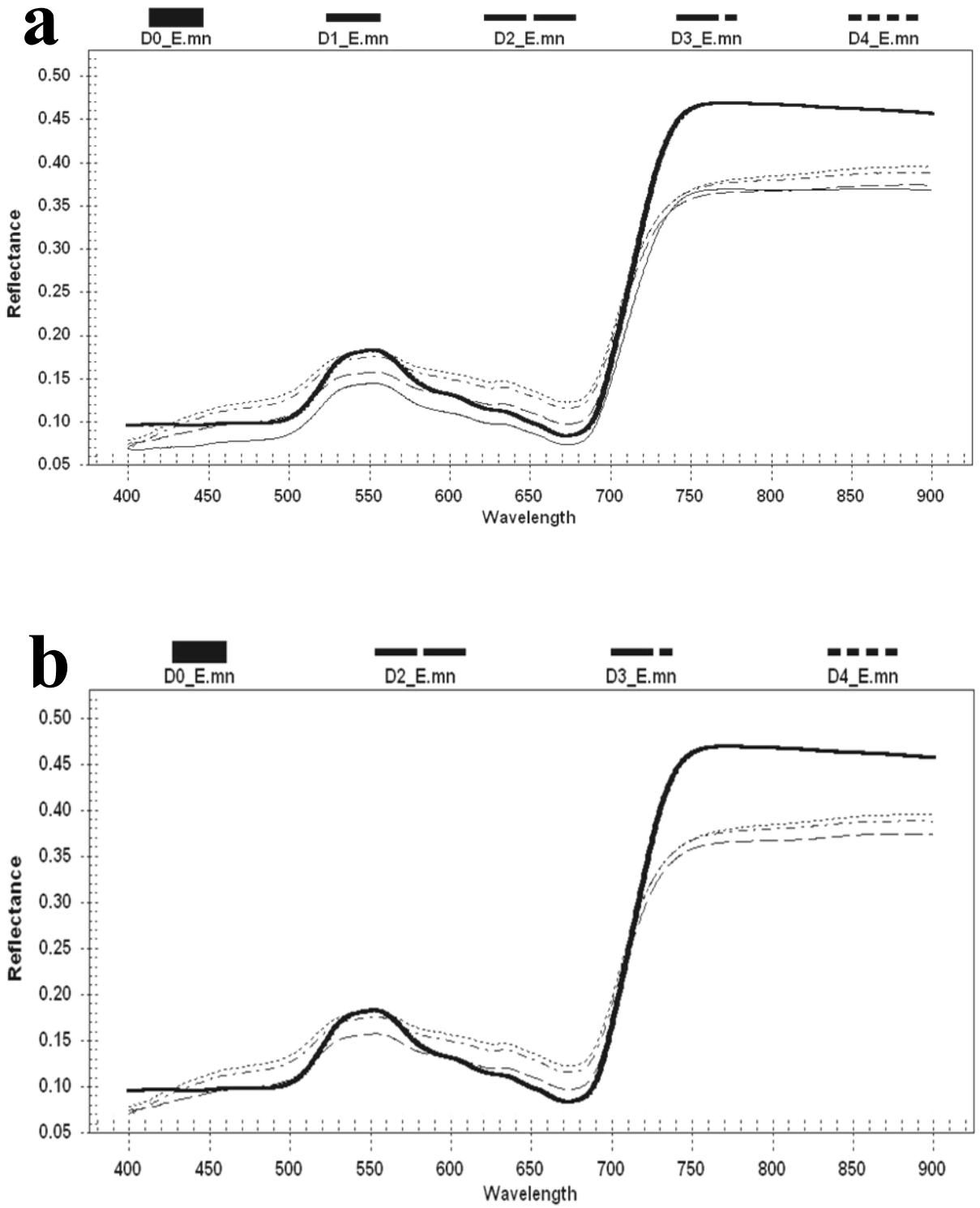

Fig. 4. Spectral reflectance curves for excised leaves of *A. donax* exposed to ambient laboratory conditions (a) and maintained within 31 x 15 cm paper bags (b) during a 96-h observation period.

Table 1. Spectral reflectance by attached foliage of *Arundo donax* under natural lighting conditions in the laboratory.

	Spectral Reflectance (Mean± CL_{95})			
Time post excision (h)	460 nm	550 nm	680nm	850nm
0	5.94 ±0.34	13.6 ±1.7	5.04 ±0.29	31.28 ±0.50
24	6.54 ±0.22 *	14.1 ±1.51	5.35 ±0.21	33.46 ±1.27 *
48	6.36 ±0.44	14.3 ±1.66	5.65 ±0.47	34.50 ±1.61 *
72	6.11 ±0.31	13.2 ±1.03	5.42 ±0.35	33.16 ±1.17 *
96	6.23 ±0.26	13.6 ±2.25	5.55 ±0.33	35.18 ±1.16 *

* denotes significant change compared to 0hr post excision

Table 2. Spectral reflectance by excised foliage of *Arundo donax* maintained within paper sample bags (upper) and exposed to ambient laboratory conditions under natural lighting conditions(lower).

	Spectral Reflectance (Mean±CL95)			
Time post excision (h)	460 nm	550 nm	680nm	850nm
		Bagged		
0	9.8 ±0.28	19.6 ±1.64	8.6 ±0.32	45.2 ±0.96
24	9.3 ±0.15 *	20.1 ±1.04	8.9 ±0.16	48.3 ±0.82 *
72	10.5 ±0.58 *	17.9 ±0.94	11.6 ±0.71 *	41.4 ±2.16 *
96	11.5 ±0.59 *	17.9 ±0.88	12.2 ±0.77 *	39.8 ±1.82 *
		Unbagged		
0	9.8 ±0.23	18.3 ±0.46	8.7 ±0.19	46.3 ±0.85
24	7.8 ±0.35 *	14.7 ±0.97 *	7.6 ±0.38 *	37.6 ±1.85 *
48	9.7 ±0.52	15.7 ±0.82 *	9.9 ±0.58 *	37.3 ±1.83 *
72	11.3 ±0.74 *	17.5 ±1.36	11.8 ±0.90 *	38.7 ±3.48 *
96	11.8 ±0.85 *	17.9 ±1.23	12.6 ±1.05 *	38.8 ± 2.53 *

* denotes significant change compared to 0 h post excision.

exhibited progressive reductions in reflectance of NIR wavelengths which is typically associated with changes in leaf structure caused by senescence or stress (Campbell, 2006). In both cases, the principal stress factor involved appears to have been desiccation of leaf tissues, which became evident within 24 h in both sample categories and had become pronounced by 72 h post-excision (Fig. 2).

Excised leaf samples enclosed within plastic GladBags® generally exhibited slight increases in reflectance of both visible and NIR wavelengths (P<0.05) within a period of 24 h post-excision (Figs. 5-6; Tables 3-4). However, the relative proportions of visible and NIR wavelengths reflected by excised foliage remained stable (i.e., the basic shapes of the spectral curves did not change) and leaf samples exhibited none of the classic symptoms associated with plant stress (typically manifested by significant increases in red and blue wavelengths and decreases in green and NIR wavelengths) during the remainder of the 96-h

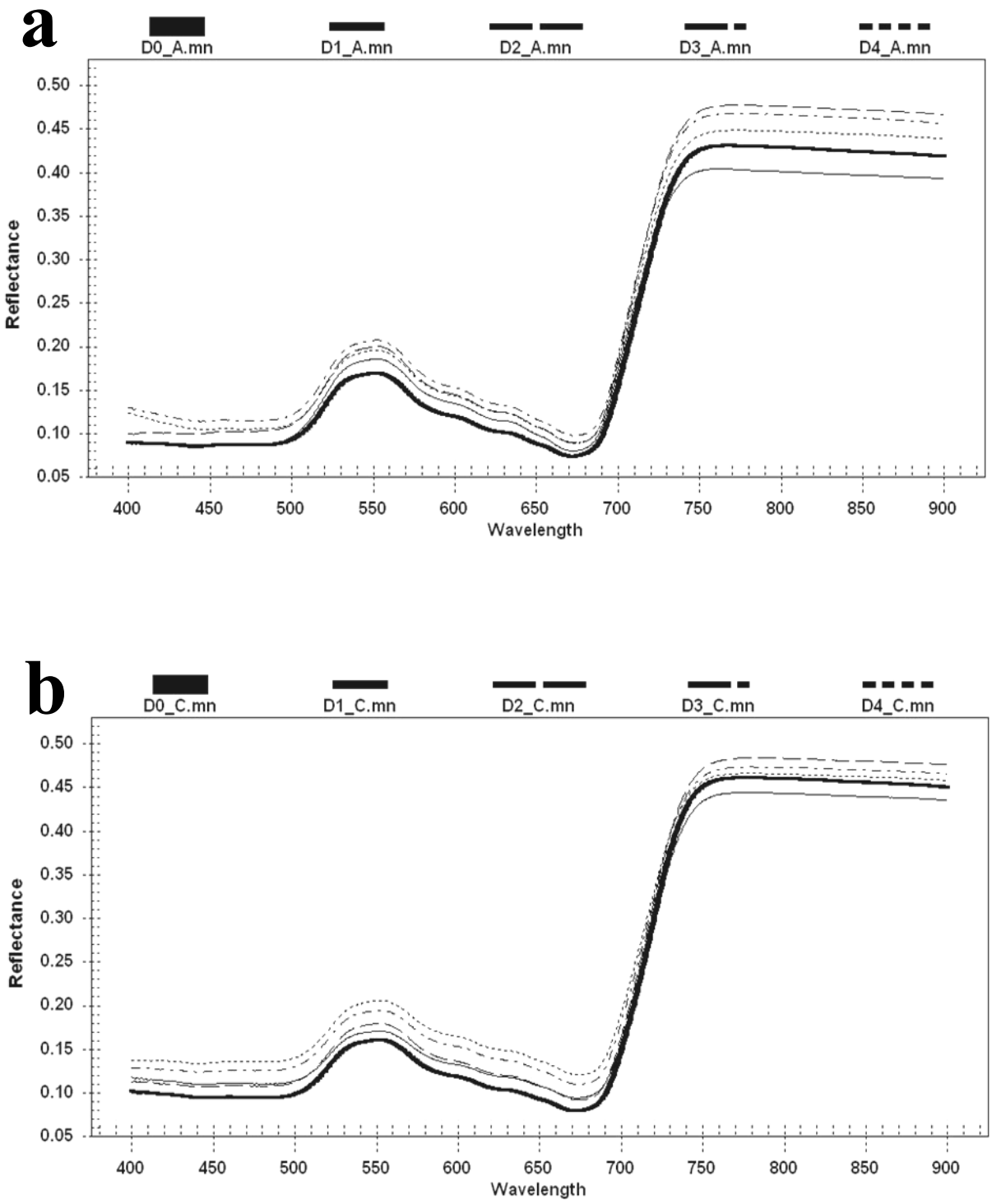

Fig. 5. Spectral curves for *A. donax* leaf samples held within plastic zip-lock bags under natural lighting conditions in the laboratory at room temperature (a) and reduced temperatures (b) during a 96-h observation period.

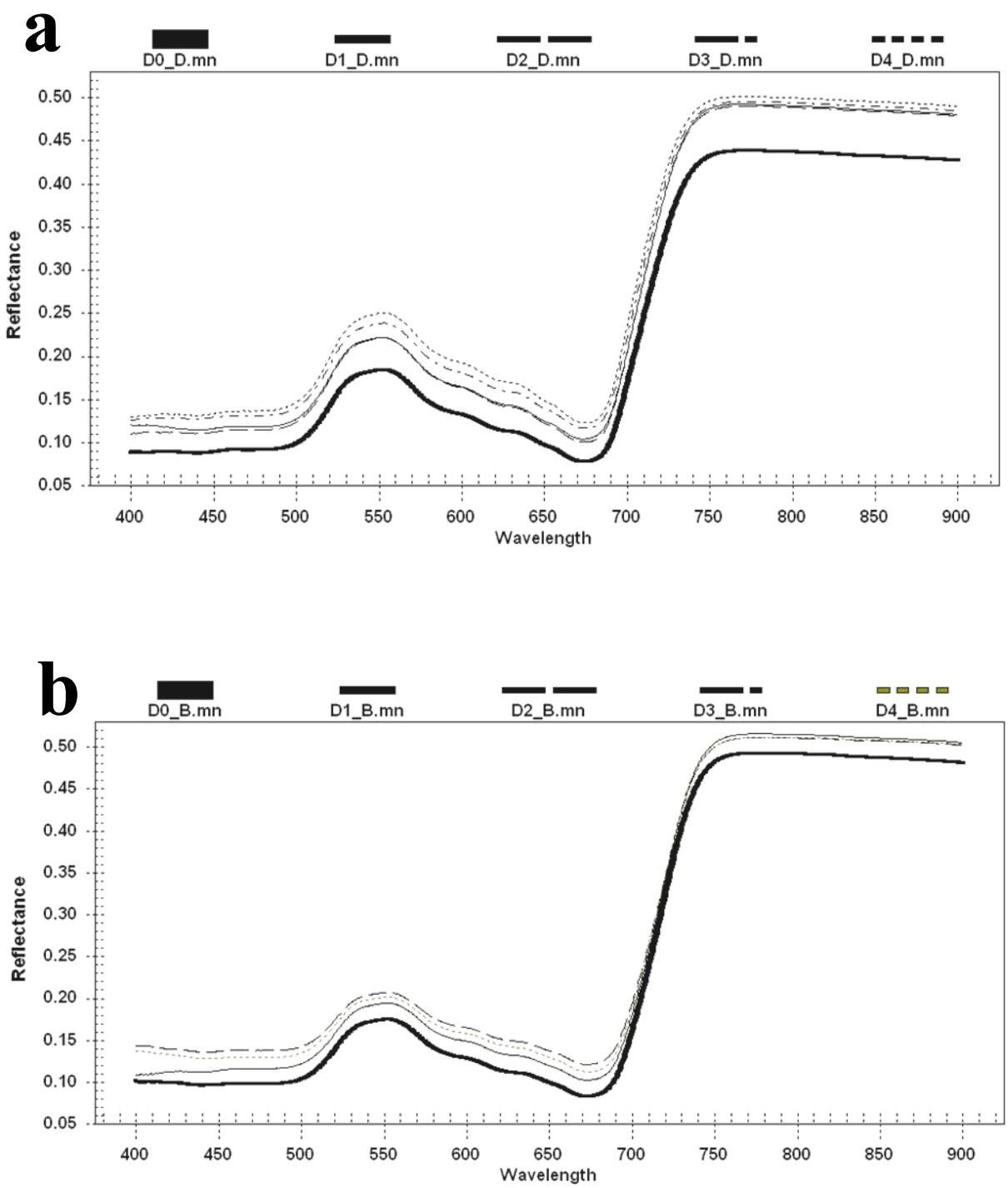

Fig. 6. Spectral curves for *A. donax* leaf samples held within plastic zip-lock bags under conditions of artificial darkness and room temperatures (a) and reduced temperatures (b) during a 96-h observation period.

Table 3. Spectral reflectance by excised foliage of *Arundo donax* maintained within plastic zip-lock bags under natural lighting conditions at room temperature (upper) and reduced temperatures (lower).

Time post excision (h)	Spectral Reflectance (Mean± CL_{95}) 460 nm	550 nm	680nm	850nm
		Room Temperature		
0	8.8 ±0.45	16.9 ±0.49	7.7 ±0.45	42.4 ±2.41
24	10.2 ±0.45 *	19.3 ±0.97 *	9.0 ±0.39 *	46.2 ±2.11
48	10.3 ±0.42 *	20.0 ±1.02 *	9.1 ±0.50 *	47.2 ±1.84 *
72	11.6 ±0.90 *	20.7 ±1.71 *	9.9 ±0.95 *	46.3 ±2.14
96	10.6 ±0.52 *	19.6 ±1.34 *	9.1 ±0.59 *	44.4 ±2.83
		Reduced Temperature		
0	9.6 ±0.58	16.1 ±1.18	8.2 ±0.49	45.6 ±2.12
24	11.1 ±0.42 *	17.1 ±0.91	9.6 ±0.44 *	43.9 ±2.04
48	10.8 ±0.29 *	17.9 ±0.78	9.3 ±0.26 *	48.0 ±1.62
72	12.6 ±1.30 *	19.5 ±1.56 *	11.2 ±1.42 *	47.1 ±2.32
96	13.6 ±1.30 *	20.6 ±1.35 *	12.2 ±1.44 *	46.3 ±1.79

* denotes significant change compared to 0hr post excision

Table 4. Spectral reflectance by excised foliage of *Arundo donax* maintained within plastic zip-lock bags under conditions of artificial darkness at room temperature (upper) and reduced temperatures (lower).

Time post excision (h)	Spectral Reflectance (Mean± CL_{95}) 460 nm	550 nm	680nm	850nm
		Room Temperature		
0	9.2 ± 0.23	18.6 ±1.54	8.1 ±0.15	43.5 ±1.64
24	11.3 ±0.47 *	22.2 ±1.71 *	10.7 ±0.36 *	48.6 ±2.32 *
48	11.4 ±0.31 *	22.1 ±1.75 *	10.3 ±0.279 *	48.4 ±1.58 *
72	13.1 ±1.05 *	23.8 ±1.36 *	11.9 ±0.95 *	49.0 ±1.79 *
96	13.6 ±1.24 *	25.0 ±1.69 *	12.5 ±1.31 *	49.6 ±1.15 *
		Reduced Temperature		
0	9.9 ±0.62	17.5 ±0.95	8.6 ±0.47	48.8 ±2.54
24	11.6 ±0.39 *	19.4 ±0.63 *	10.5 ±0.40 *	51.1 ±1.92
48	12.1 ±0.28 *	19.7 ±0.31 *	11.1 ±0.26 *	50.2 ±1.52
72	13.8 ±1.07 *	20.7 ±1.19 *	12.4 ±1.06 *	50.8 ±2.59
96	13.0 ±0.48 *	20.2 ±0.75 *	11.5 ±0.46 *	50.9 ±2.08

* denotes significant change compared to 0hr post excision

Fig. 7. Samples of *A. donax* leaves maintained in plastic zip-lock bags (l) and conventional paper sacks (r) following a 96 –h observation period.

observation period. This trend was evident among samples held under natural lighting conditions and variable temperatures (Fig. 5) and also among contemporaries held under conditions of artificial darkness and variable temperatures (Fig. 6; Table 4).

CONCLUSIONS

An important implication of these results is that the acquisition of accurate spectral measurements for excised foliage of *A. donax* under laboratory conditions is feasible provided that leaf samples are transported in storage containers designed to minimize desiccation (Foley 2006; Gandy et al. 2011). Of the containers evaluated herein, plastic Glad-Bags® or equivalents appear to be most suitable for transport and storage of leaf samples, while conventional paper bags are clearly unsuitable because of problems with desiccation (Fig. 7). Although the time frame for this study was arbitrarily defined as 96 hours post-excision, most sampling programs for *A. donax* would probably include accommodations for the processing of leaf samples within a period of 24-48 hours after collection. As excised *A. donax* foliage does not appear to be adversely affected by brief periods of darkness and reduced temperatures, the conventional means of transporting leaf samples (i.e., enclosed within suitable containers inside cooled ice chests) appears to be entirely suitable for the purposes discussed in this paper.

ACKNOWLEDGMENTS

The research reported herein was supported by the U. S. Department of Agriculture, Agricultural Research Service, and was funded in part by a grant from the U. S. Department of Homeland Security – Customs and Border Protection .

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