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Spectral characteristics of leafy spurge (*Euphorbia esula*) leaves and flower bracts

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Leafy spurge can be detected during flowering with either aerial photography or hyperspectral remote sensing because of the distinctive yellow-green color of the flower bracts. The spectral characteristics of flower bracts and leaves were compared with pigment concentrations to determine the physiological basis of the remote sensing signature. Compared with leaves of leafy spurge, flower bracts had lower reflectance at blue wavelengths (400 to 500 nm), greater reflectance at green, yellow, and orange wavelengths (525 to 650 nm), and approximately equal reflectances at 680 nm (red) and at near-infrared wavelengths (725 to 850 nm). Pigments from leaves and flower bracts were extracted in dimethyl sulfoxide, and the pigment concentrations were determined spectrophotometrically. Carotenoid pigments were identified using high-performance liquid chromatography. Flower bracts had 84% less chlorophyll *a,* 82% less chlorophyll *b,* and 44% less total carotenoids than leaves, thus absorptance by the flower bracts should be less and the reflectance should be greater at blue and red wavelengths. The carotenoid to chlorophyll ratio of the flower bracts was approximately 1:1, explaining the hue of the flower bracts but not the value of reflectance. The primary carotenoids were lutein, β-carotene, and β-cryptoxanthin in a 3.7:1.5:1 ratio for flower bracts and in a 4.8:1.3:1 ratio for leaves, respectively. There was 10.2 $\mu g\ g^{-1}$ fresh weight of colorless phytofluene present in the flower bracts and none in the leaves. The fluorescence spectrum indicated high blue, red, and far-red emission for leaves compared with flower bracts. Fluorescent emissions from leaves may contribute to the higher apparent leaf reflectance in the blue and red wavelength regions. The spectral characteristics of leafy spurge are important for constructing a well-documented spectral library that could be used with hyperspectral remote sensing.

Nomenclature: Leafy spurge, *Euphorbia esula* L. EPHES.

Key words: Carotenoids, chlorophyll, fluorescence, reflectance, spectral libraries.

Leafy spurge is a noxious perennial weed, which infests 1.2- to 2-million hectares of land in North America (Anderson et al. 2003; Lajeunesse et al. 1999) and causes severe economic impacts (Bangsund et al. 1999; Leitch et al. 1996). Biologically based integrated pest management is now practical for the control of leafy spurge (Anderson et al. 2003). Location and monitoring of populations is a requirement for management of all weeds (DiTomaso 2000). Remote sensing has a possible role in fulfilling this requirement (Hall et al. 2000; Radhakrishnan et al. 2002).

Remote sensing has been successfully used to locate and monitor leafy spurge distribution because of the unique coloration of the flower bracts (Anderson et al. 1996, 1999; Everitt et al. 1995; Hunt et al. 2003; Parker Williams and Hunt 2002, 2003). The reflectance in the yellow-green portion of the visible spectrum allows the flowering shoots of leafy spurge to be distinguished from those of co-occurring species with hyperspectral imagery (Parker Williams and Hunt 2002). In northeastern Wyoming, determination of the presence or absence of leafy spurge, while flowering, was 95.2% accurate with hyperspectral imagery (Parker Williams and Hunt 2003). Nonflowering shoots of leafy spurge have a similar reflectance spectrum as other vegetation, and nonflowering leafy spurge could not be distinguished using remote sensing (Parker Williams and Hunt 2003).

Our overall research goal was to develop methodologies using remote sensing to locate and monitor existing leafy spurge populations as part of an integrated pest management program.

The objective of this study was to determine the physiological basis for the unique remote sensing signature of flowering leafy spurge by comparing the pigment concentrations and fluorescence spectra of leaves and flower bracts (Carter and Knapp 2001; Gates et al. 1965; Knipling 1970). Understanding the physiological basis for spectral signatures can lead to a better understanding of when and where remote sensing can be used to locate and monitor populations of other weed species.

Materials and Methods

Leafy spurge was collected in the field at two locations, one near Devils Tower National Monument in northeastern Wyoming (44°32′49″N, 104°48′12″W) and the other near the city of Fort Collins in northeastern Colorado (40°36′9″N, 105°5′49″W). The Wyoming site was on a hillslope, which was used for cattle grazing. The vegetation community is a northern mixed-grass prairie with a mixture of sagebrush (*Artemisia tridentata* Nutt.) and grasses (Parker Williams 2001). Biological control agents (*Aphthona lacertosa* and *A. nigriscuis*, Coleoptera: Chrysomelidae) were released nearby in 1998; there were some *A. lacertosa* found at the site by sweeping an insect net through the leafy spurge

(Parker Williams 2001). Collections were made during peak flowering in late June 2000, a year with normal amounts of precipitation (Parker Williams 2001).

The Colorado site was in a riparian area dominated by cottonwoods (*Populus* spp.) and an understory of downy brome (*Bromus tectorum* L.) and western wheat grass [*Pascopyrum smithii* (Rydb.) Á. Löve]. The presence of *Aphthona* spp. was not determined. Collections were made during peak flowering in June 2003, a year with above-normal amounts of precipitation, which followed a year with severe drought conditions.

Entire shoots with numerous leaves and flowers of different ages were excised and sealed in a cooler with cold packs to keep the interior chilled and were shipped overnight to Beltsville, MD. Ten healthy shoots with both leaves and flowers were selected from each site. Leaves and flower bracts were excised from a single shoot and combined for one sample of leaves and one sample of flower bracts. For the Wyoming site, each sample was used first for reflectance measurements, then for fluorescence measurements, and last for pigment extractions. For the Colorado site, only the pigments were extracted; furthermore, the remainder of the leaves and flower bracts were combined into single samples for high-performance liquid chromatography (HPLC). All measurements and pigment extractions were performed within 48 h after collection (EPA-ERT 1994).

Reflectances from 400- to 850-nm (violet to near-infrared) wavelength at 1-nm wavelength intervals were measured using an ASD FieldSpec Pro FR spectroradiometer.[1] Leaves and flower bracts were placed in the sampling port of an LI 1800-12 integrating sphere[2] for total hemispherical reflectance. Individual leaves and flower bracts were too small for the integrating sphere port, so four leaves or bracts were held in place, without overlap, using black electrical tape, to fill the field of view for reflectance measurements. As a result of using the black electrical tape, transmittances could not be measured. Corrections for the fraction of nonleaf material were made according to the methods of Daughtry et al. (1989). We tested the reflectance properties of the black electrical tape and found negligible effects on the reflectance measurements when the tape was carefully placed to avoid specular reflectance.

Excised leaves and flower bracts (0.1 g fresh weight) were used to extract pigments in 4 ml of dimethyl sulfoxide (DMSO) in sealed cuvettes for 36 h in the dark at 25 C. Absorption spectra from 400- to 800-nm wavelength were obtained using a dual-beam Perkin–Elmer Lambda 40 UV/VIS spectrometer[3] on these plant extracts. Chlorophyll *a,* chlorophyll *b,* and total carotenoids were determined by equations using the absorbances at 665-, 649-, and 470-nm wavelengths (Lichtenthaler 1987; Wellburn 1994). The extracts did not have any absorption features associated with chlorophyll degradation products (Hendry et al. 1987).

A Fluorolog II spectrofluorometer[4] as described by Corp et al. (2003) and McMurtrey et al. (1994) was used to collect fluorescence excitation and emission spectra on leaves and flower bracts held in place with the black electrical tape. Excitation radiation centered at 355 nm (ultraviolet) was used to detect fluorescence of various plant pigments and other compounds. The emission spectrometer was attached to a photon-counting photomultiplier tube that is electronically corrected to obtain a linear response throughout the instrument's emission wavelength range of 290 to 850 nm. We tested the fluorescent properties of the electrical tape, and found that it was not a source of emitted radiation.

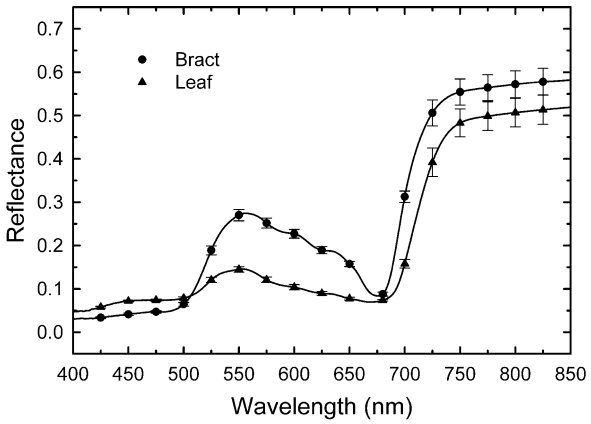

FIGURE 1. Reflectance spectra of leaves and flower bracts of leafy spurge from the Wyoming site. Error bars at various wavelengths are ± 1 SE of 10 samples.

Carotenoids in the DMSO extracts were identified using a HPLC system (Edwards et al. 1999; Fordham et al. 2001). The method of Bieri et al. (1979) was modified and used on a Hewlett Packard 1050 HPLC[5] with a column of C-18 reverse phase, 4.6-mm diameter and 250-mm length, operated at a temperature of 20 C. About 1 g fresh weight of leaves or flower bracts from many shoots was used to extract pigments into 12 ml of DMSO. Then, two portions of 12 ml hexane were used to remove the carotenoids from the DMSO. The hexane extract was evaporated to dryness and redissolved in methylene chloride. Three concentrations of the methylene chloride solution were made, 200-µl of an internal standard solution was added to each concentration, and the extracts were evaporated to dryness again. The extracts with the internal standard were dissolved in 200 µl of the mobile phase solvent, and two samples of 50 µl each were injected into the Hewlett Packard 1050 HPLC for each of the three dilutions. The retention factors and absorbance spectra were compared with a reference library for identification, which was focused primarily on carotenoids with vitamin A activity.

A two-sample Student's *t* test was used for all statistical analyses to test the alternative hypothesis that the difference between means was significant. For reflectance and fluorescence spectra, various wavelengths were selected for the statistical tests, and we assumed that the values at these wavelengths were independent of the values at other wavelengths.

Results and Discussion

The mean reflectance spectrum of flower bracts compared with leaves was lower in the blue wavelengths from 400 to 500 nm and greater in the green, yellow, and orange wavelengths from 525 to 650 nm (Figure 1). Also, reflectance of the flower bracts was greater at 700-nm wavelength. The mean reflectance spectrum of flower bracts was not different from that of leaves at near-infrared wavelengths from 725 to 850 nm and at red wavelengths centering at 680-nm wavelength (Figure 1).

The total chlorophyll ($a + b$) of flower bracts was 81.3 mg g^{-1} fresh weight (Table 1), which is approximately 1.8

TABLE 1. Pigment concentrations of leaves and flower bracts determined spectrometrically. Data are mean and standard error from 10 samples from each of the two sites. Significance was determined using Student's *t* test.

	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Car:Chl[a]
	μg g^{-1} fresh weight			
Bracts	61.5 ± 4.0	19.8 ± 2.0	77.1 ± 2.3	0.96 ± 0.09
Leaves	387.6 ± 11.4	109.9 ± 5.2	138.0 ± 4.4	0.28 ± 0.03
Significance	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

[a] Carotenoid to Chlorophyll ratio.

nmol cm^{-2} using a mean flower bract weight–area ratio. The total chlorophyll of leaves was 497.5 mg g^{-1} fresh weight (Table 1), which is about 11 nmol cm^{-2} using a mean leaf weight–area ratio. The ratio of carotenoids to chlorophylls was approximately 1:1 in the flower bracts, whereas the ratio was nearly 1:4 in leaves (Table 1). The 1:1 ratio of chlorophyll and carotenoids explains the distinctive yellow-green hue of the flower bracts, using the intensity–hue–saturation color transformation (Franklin 2001). In leaves, the total carotenoid concentration was greater than in flower bracts; however, the 1:4 ratio of carotenoids to chlorophylls (Table 1) results in a green hue.

Whereas leaf reflectance at 680 nm is at a local minimum due to absorption by chlorophyll, the value of reflectance at 680 nm can be higher or lower depending on chlorophyll concentration. When chlorophyll concentration is greater than 10 nmol cm^{-2}, absorption at 680 nm is at the maximum and saturated (Gitelson and Merzlyak 1996). Therefore, leaf reflectance at 680 nm should be the lowest possible value. Because both carotenoids and chlorophyll have high absorption coefficients at blue wavelengths (Lichtenthaler 1987), the same logic holds for absorption and reflection at blue wavelengths (Gitelson and Merzlyak 1996). Given the measured chlorophyll and carotenoid concentrations of leaves and flower bracts (Table 1), predicted reflectances of the flower bracts at 680 nm and blue wavelengths were expected to be significantly higher than leaf reflectance. However, measured reflectances did not differ at 680 nm and were lower for flower bracts at blue wavelengths (Figure 1).

The main carotenoid isolated from both leaves and bracts was the xanthophyll, lutein, with some β-carotene and β-cryptoxanthin (Table 2). The ratios of lutein, β-carotene, and β-cryptoxanthin were 3.7:1.5:1 for flower bracts and 4.8:1.3:1 for leaves. These three carotenoids are frequently found in chloroplasts of higher plants (Bartley and Scolnik 1995; Bramley 2002; Britton 1995; Goodwin 1980;). Phytofluene, a colorless precursor to β-carotene (Bramley 2002; Goodwin 1980;), was present in flower bracts but not in leaves (Table 2). Accumulation of phytofluene is found in fruits, flowers, and other plant organs that are not photosynthetically competent (Goodwin 1980). Three additional carotenoids were found in both leaves and flower bracts in lower concentrations (data not shown). From the absorption spectra, these three unidentified carotenoids could be violaxanthin, antheraxanthin, and zeaxanthin of the photosynthetic xanthophyll cycle (Deming-Adams and Adams 1996; Deming-Adams et al. 1996). Without calibration of the retention factors, identity and concentrations of the three carotenoids are not certain. However, the xanthophyll cycle is an important protection mechanism of photosynthesis in higher plants (Deming-Adams and Adams 1996; Deming-Adams et al. 1996). Therefore, the presence of these three carotenoids in the leaves and flower bracts of leafy spurge is reasonable.

TABLE 2. Carotenoid concentration of leaves and flower bracts determined from high-performance liquid chromatography (HPLC). To obtain sufficient material, leaves and flower bracts from many different shoots obtained at the Colorado site were combined into one sample.

	Lutein[a]	β-Carotene[b]	β-Cryptoxanthin[c]	Phytofluene[d]
	μg g^{-1} fresh weight			
Bracts	41.9	16.8	11.2	10.2
Leaves	85.7	23.4	18.0	0

[a] (3R,3′R,6′R)-β,ϵ-carotene-3,3′-diol (xanthophyll).
[b] β,β-Carotene.
[c] (3R)-β,β-Caroten-3-ol.
[d] 7,8,11,12,7′,8′-Hexahydro-ψ,ψ-carotene.

The mean fluorescence spectrum of leaves was greater than the fluorescence spectrum of bracts at three of the four major emission bands (Figure 2): blue wavelengths (400 to 500 nm), red wavelengths (650 to 700 nm), and far-red wavelengths (700 to 725 nm). At the green emission band (500- to 550-nm wavelengths), leaves and flower bracts had similar fluorescence (Figure 2). Higher fluorescence at red and far-red wavelengths is related to higher chlorophyll concentrations in leaves (Buschmann and Lichtenthaller 1998; Buschmann et al. 2000; Chappelle 1999). Higher fluorescence at blue wavelengths is related to the compounds in the leaf epidermis (Buschmann and Lichtenthaller 1998; Buschmann et al. 2000). Yellow leaf pigments, such as lutein and β-carotene, have fluorescent emissions at the green wavelengths (Chappelle et al. 1999). Also, other plant compounds such as riboflavin, quercitin, berberin, and phyllo-

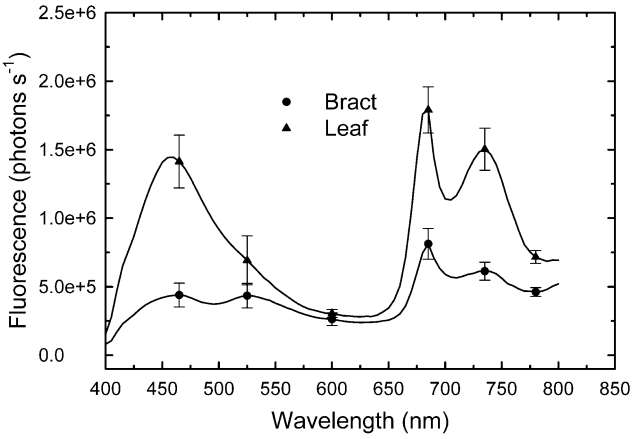

FIGURE 2. Fluorescence spectra of leaves and flower bracts of leafy spurge from the Wyoming site. Emission was caused by laser excitation at 355-nm wavelength. Error bars at various wavelengths are ± 1 SE of 10 samples.

quinone contribute to green fluorescence (Buschmann et al. 2000). The fluorescence spectra of leaves and flower bracts (Figure 2) are consistent with the measured chlorophyll and carotenoid concentrations (Table 1).

The total of reflectance, transmittance, and absorptance of incident radiation must equal 1 for a given wavelength by definition, and absorptance increases with the amount of absorbing material in accordance with the Beer–Lambert law (Gates 1980). Thus, larger quantities of an absorbing material, such as leaf pigments, will result in a greater absorptance and less transmittance and reflectance. The reflectances of flower bracts at red and blue wavelengths were less than predicted (Figure 1) for the measured chlorophyll and carotenoid concentrations (Table 1). What caused the decreased spectral reflectance for flower bracts of leafy spurge? Alternative hypotheses are (1) that transmittance through the flower bracts is extremely high relative to leaves, so absorption at these wavelengths is consistent with the pigment concentrations; (2) that there is a unique, nonchlorophyll pigment in the flower bracts that absorbs blue and red wavelengths thereby decreasing reflectance; or (3) that there is higher fluorescent emission of leaves at blue and red wavelengths, increasing the apparent reflectance. With fluorescent emission, the apparent sum of reflectance, transmittance, and absorptance of incident radiation would be greater than 1.

Because of the measurement procedure using black electrical tape, the first hypothesis could not be tested directly. However, most leaves have transmittances approximately equal to, or less than, the reflectances depending on leaf thickness (Gates et al. 1965). Furthermore, any changes in transmittance due to leaf structure apply to all wavelengths equally (Maas and Dunlap 1989), any differences in transmittance between leaves and flower bracts would be apparent at near-infrared wavelengths (725 to 850 nm), where there is little absorption. The equal reflectances in this region (Figure 1) do not support this alternative.

The second alternative hypothesis above is the possibility of another pigment in the flower bracts. Typical carotenoids are present in the bracts (Table 2), which approximately add up to the total carotenoid concentration measured by the spectrophotometer (Table 1), so there is no indication that unidentified carotenoid pigments should have been found in large concentrations. Furthermore, it is reasonable to expect that a pigment present in the flower bracts, which absorbs blue light, would have a greater green fluorescence emission (Chappelle et al. 1999). Therefore, the equal green fluorescence between leaves and flower bracts (Figure 2) provides indirect evidence that there is no another pigment in the flower bracts.

A third alternative is that leaf fluorescence at blue and red wavelengths (Figure 2) increases the apparent reflectance (Entcheva-Campbell et al. 2002; Kim et al. 1993; Zarco-Tejada et al. 2000, 2003). The contribution of fluorescence to reflectance is extremely difficult to measure in intact leaves because fluorescent emission is still a relative measure (Chappelle et al. 1999; Corp et al. 2003). Entcheva-Campbell et al. (2002), Kim et al. (1993), and Zarco-Tejada et al. (2000, 2003) have suggested that fluorescence can contribute about 3 to 5% reflectance measured at blue and red wavelengths. Therefore, this alternative is supported because of the greater fluorescent emission of leaves (Figure 2) and because it is consistent with work done with other species.

Understanding the physiological causes for leaf and flower bract spectra is not necessary for detecting leafy spurge by remote sensing. Pixels with similar spectra could be grouped together using one of many algorithms (classification), and by fieldwork, the groups of pixels could be assigned to various predefined categories (Franklin 2001), for example, having or not having leafy spurge. Based on species characteristics, individual species may be detected, but not all the species within a mixed plant community will be identified (Hunt et al. 2003). Other weed species are detectable by remote sensing during flowering (Everitt et al. 1992; Lass et al. 1996). Having numerous bands (the definition of hyperspectral) is not even required because most of the variability across an image can be encapsulated into a few bands (Price 1998). A major problem is that the classes may not be extrapolated in time or space because of differences in soil background reflectance, species composition, the leaf area index, the leaf angle distribution, atmospheric effects, and the solar elevation and azimuth (Franklin 2001). Each image may have to be analyzed as a unique occurrence of different factors (Price 1994).

However, with a variety of newly developed algorithms based on hyperspectral remote sensing (Kokaly et al. 2003; Parker Williams and Hunt 2002), species occurrence in imagery can be predicted using documented spectral libraries and without recourse to extensive fieldwork. This study of leafy spurge is a prototype for constructing a spectral library, in which chlorophyll and carotenoid concentrations are measured along with the reflectance spectra of leaves and flower bracts. However, there are limits to this prototype because pigment concentrations alone could not explain leaf and flower bract reflectance at visible wavelengths. Reflectances at other wavelengths in the near-infrared (725 to 1,100 nm) and shortwave infrared (1,100 to 2,500 nm) will be determined by leaf morphology and water content, respectively (Gates et al. 1965; Knipling 1970; Slaton et al. 2001), which were not measured in this study. More work is necessary to understand the relationships among physiology, genetics, and environment for the determination of robust, extrapolatable spectral signatures.

Once spectral libraries are developed, what then? There are a wide variety of sensors and platforms available for remote sensing, each sensor–platform combination has different spatial, spectral, radiometric, and temporal resolutions (Franklin 2001). Spectral library information are inputs to canopy radiation models, such as SAIL (Verhoef 1984), which predict canopy reflectance for specified species composition, soil background reflectance, the leaf area index, the leaf angle distribution, and the solar elevation and azimuth. From canopy reflectance, topography, atmospheric transmittance, and sensor characteristics, the expected data available from remote sensing data are easily calculated from physical laws (Gates 1980). These calculations could be used to determine which sensors are able to detect a given weed species in a specified environment. Part of the specified environment includes time of year, which not only directly affects solar elevation and azimuth but also leaf area index and leaf angle distribution based on plant phenology. In summary, with documented spectral libraries, it is possible to determine whether a weed species can be detected by

remote sensing and then to determine which sensors and platforms should be used to maximize detection accuracy and minimize cost of detection.

Sources of Materials

[1] ASD FieldSpec Pro FR spectroradiometer, Analytical Spectral Devices Inc., 5335 Sterling Drive, Boulder, CO 80301.

[2] LI 1800-12 integrating sphere, LICOR Inc., 4421 Superior Street, Lincoln, NE 68504.

[3] Perkin–Elmer Lambda 40 UV/VIS spectrometer, Perkin–Elmer Inc., 45 William Street, Wellesley, MA 02481.

[4] Fluorolog II spectrofluorometer, Spex Industries, 3880 Park Avenue, Edison, NJ 08820.

[5] HP 1050 HPLC, Hewlett Packard Inc., 3495 Deer Creek Road, Palo Alto, CA 94304.

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