

# **Relationships Between Pigment Composition Variation and Reflectance for Plant Species from a Coastal Savannah in California**

by

**Susan L. Ustin, Eric W. Sanderson, Yaffa Grossman, Quinn J. Hart,  
and Robert S. Haxo**

Department of Land, Air, and Water Resources  
University of California, Davis, CA 95616

## **Introduction**

Advances in imaging spectroscopy have indicated that remotely sensed reflectance measurements of the plant canopy may be used to identify and quantify some classes of canopy biochemicals (Wessman et al, 1988a ); however the manner in which differences in biochemical compositions translate into differences in reflectance measurements is not well understood. Most frequently, multiple linear regression routines have been used to correlate narrow band reflectance values with measured biochemical concentrations (e.g., see Wessman et al, 1988b, Card et al, 1988). Although some success has been achieved with such methods for given data sets, the bands selected by multiple regression are not consistent between data sets, nor is it always clear what physical or biological basis underlies the correlation (Curran, 1989).

To examine the relationship between biochemical concentration and leaf reflectance signal we chose to focus on the visible spectrum where the primary biochemical absorbances are due to photosynthetic pigments. Pigments provide a range of absorbance features, occur over a range of concentrations in natural samples, and are ecophysiologically important. Concentrations of chlorophyll, for example, have been strongly correlated to foliar nitrogen levels within a species (Evans, 1989) and to photosynthetic capacity across many species (Field and Mooney, 1986). In addition, pigments effectively absorb most of the photosynthetically active radiation between 400-700 nm, a spectral region for which silicon detectors have good signal/noise characteristics. Our strategy has been to sample a variety of naturally occurring species to measure leaf reflectance and pigment compositions. We hope to extend our understanding of pigment reflectance effects to interpret small overlapping absorbances of other biochemicals in the infrared region. For this reason, selected samples were also tested to determine total nitrogen, crude protein, cellulose and lignin levels. Leaf reflectance spectra measured with AVIRIS bandwidths and wavelengths were compared between species and within species and for differences between seasons, for changes in the shape of the spectra. We attempted to statistically correlate these shape changes with differences in pigment composition.

In parallel with our comparisons of pigment composition and leaf reflectance, we have modified the PROSPECT leaf reflectance model to test the contributions of pigments or pigment group concentrations (Jacquemoud and Baret, 1990, Jacquemoud, 1993). PROSPECT considers a leaf as a multi-layer dielectric plane with an uneven surface. Jacquemoud adapted the basic analysis of Allen (1973, 1968) for surface effects, a leaf thickness factor, and the absorption of water and chlorophyll (actually all pigments) and the plant matrix. Our modifications to PROSPECT in the forward direction include

breaking out the pigment concentration parameter into separate components for chlorophyll a and b and a number of xanthophylls and carotenes, and introducing a shift and convolution function to model the spread and shift from their *in vitro* measurements to their *in vivo* state. Further we have considered how the matrix elements (i.e., all biochemicals and structural effects not modeled explicitly) vary with species.

Currently we are inverting PROSPECT using our modifications to process measured leaf reflectance spectra from a wide variety of non-cultivated plant species for which pigment compositions and water contents are known. PROSPECT in the backward direction was embedded in an optimization routine which estimates concentration shift and spread parameters for each pigment based on measured spectra. The means by which PROSPECT might be scaled up to canopy level measurements like AVIRIS will be discussed.

### **Materials and Methods**

Leaf samples were collected from approximately fourteen species representing different ecological groupings commonly found in plant communities of the Central Coast Range of California to provide a wide range of reflectance and biochemical variation, including photosynthetic pigments and other important biochemicals. The individual plants were identified and resampled repetitively in different seasons over the course of one year. Reflectance and transmittance spectra of ten excised leaves for each sample were measured either with NIRS or Varian CARY 5E spectrometers between 400-2500 nm using bandwidths similar to AVIRIS.

Concurrently obtained bulk leaf samples were analysed in the laboratory for their pigment compositions (chlorophyll a and b, phaeophytin a and b, chlorophyllide,  $\beta$ -carotene, cis- $\beta$ -carotene, lutein, neoxanthin, zeaxanthin, violaxanthin, and antheraxanthin) by HPLC (Wright et al, 1991). Specific leaf absorption curves were determined for each of the pigments from the HPLC diode array detector. Some samples were also analysed for crude protein (Pierce, 1993), total nitrogen, hydrolyzed cellulose and non-acid hydrolyzable lignin contents (Effland, 1977). Both the bulk (biochemistry) samples and the leaf disks were assumed to be representative of the same population of leaves from a given plant sample.

### **Discussion**

Statistical comparisons (F test) of the reflectance spectra of the excised leaves and the first derivatives of the reflectance spectra show significant (98% confidence level above thin bar on figure) differences between the green foliage of different species, even species of the same genus. For example, *Quercus agrifolia* (Live Oak) and *Quercus lobata* (Valley Oak) (Figure 1). Differences in the visible region were detected in the derivative spectra indicating that pigment compositions has greater effect on spectral shape than albedo effects. Concomitant changes in pigment concentration were also detected (Figure 2). Parallel comparisons of the biochemical and leaf reflectance were made for the species and seasonal effects. The deciduous and evergreen oaks shown here are more similar than many other comparisons. The ranges of pigment concentration found across the sample

species are listed in Table 1. Similar statistical differences in spectral features in the SWIR region were found.

Figure 1: Leaf reflectance spectra for *Quercus lobata* (Valley Oak) and *Quercus agrifolia* (Live Oak) and values of F test of significance for comparisons of first derivative spectra.

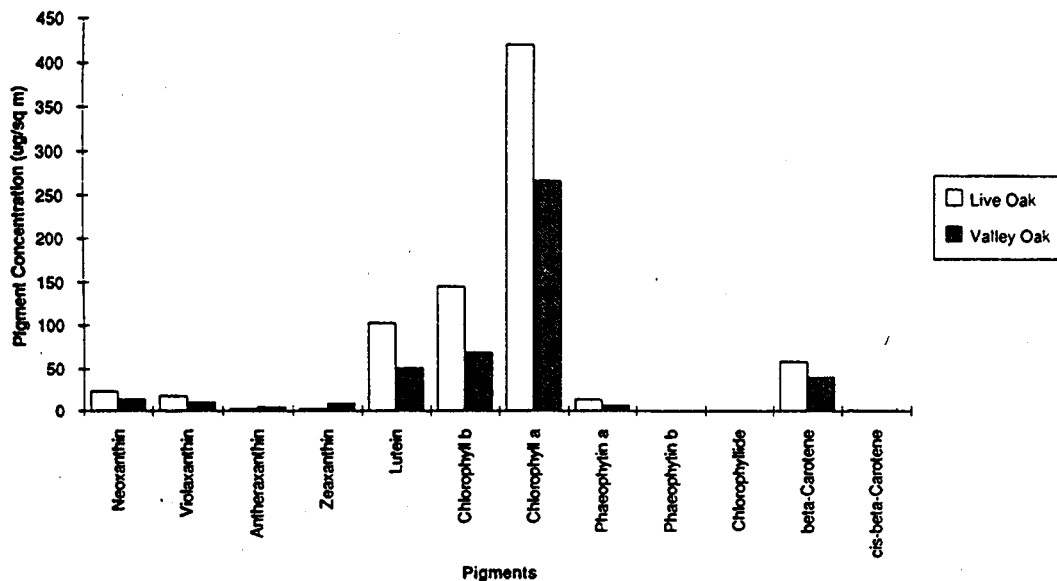
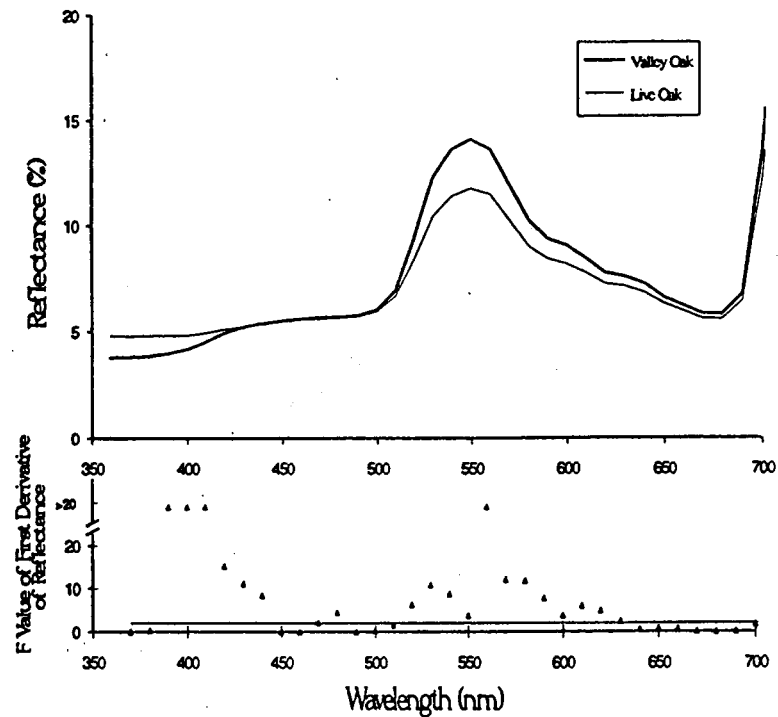


Figure 2. Pigment composition and concentration for mature leaves of *Quercus lobata* (Valley Oak) and *Quercus agrifolia* (live oak).

We adapted the PROSPECT leaf reflectance model to accept the coefficients of multiple pigments and have evaluated the contribution of each pigment to the spectrum using specific absorbance curves developed from our study. We are interested in how different reflectances/biochemistries are expressed between communities and whether ranges within a species are greater than ranges between similarly adapted species. Species differences

in biochemistry and reflectance appear to vary by ecological categories (e.g., community type or guild) and by season more than within species differences at a single point in time. The former differences result from evolutionary convergences of form and functional traits. The latter differences result from ecophysiological adjustments made in the foliage with regard to season and environmental conditions.

**Table 1. Concentration Ranges ( $\mu\text{mol}/\text{m}^2$ ) for Thirteen Leaf Pigments for Nine Species<sup>a,b</sup>**

	Concentration Range ( $\mu\text{mol}/\text{m}^2$ )	Fold Difference	Minimum Value Species	Maximum Value Species
Chlorophyll a	55-883	16	<i>Quercus lobata</i>	<i>Quercus douglasii</i>
Chlorophyll b	15-290	19	<i>Quercus lobata</i>	<i>Quercus douglasii</i>
Phaeophytin a	1-90	90	<i>Quercus lobata</i>	<i>Umbellularia californica</i>
Phaeophytin b	0-12	--	several	<i>Umbellularia californica</i>
Chlorophyllide	0-41	--	several	<i>Umbellularia californica</i>
Lutein	12-194	16	<i>Quercus lobata</i>	<i>Quercus douglasii</i>
Ncoxanthin	3-48	16	<i>Quercus lobata</i>	<i>Eriodictyon californicum</i>
Violaxanthin	1-41	41	<i>Acer macrophyllum</i>	<i>Eriodictyon californicum</i>
Antherxanthin	0.2-29	145	<i>Acer macrophyllum</i>	<i>Eriodictyon californicum</i>
Zeaxanthin	1-42	42	<i>Arbutus menziesii</i>	<i>Eriodictyon californicum</i>
$\beta$ -carotene	8-150	19	<i>Quercus lobata</i>	<i>Eriodictyon californicum</i>
cis- $\beta$ -carotene	0.1-3	30	<i>Quercus lobata</i>	<i>Eriodictyon californicum</i>

<sup>a</sup>Some species were sampled twice in different habitats. The sampled species were *Acer macrophyllum*, *Arbutus menziesii* (2x), *Eriodictyon californicum* (2x), *Heteromeles arbutifolia*, *Ludwigia pacifica*, *Quercus agrifolia*, *Quercus douglasii*, *Quercus lobata* (2x) and *Umbellularia californica*.

<sup>b</sup>Species sampled in late May 1993 at Jasper Ridge Biological Preserve, Stanford University.

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