# SPECTRAL SEPARABILITY ANALYSIS OF FIVE SOYBEAN CULTIVARS WITH DIFFERENT OZONE TOLERANCE USING HYPERSPECTRAL FIELD SPECTROSCOPY

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## **ABSTRACT**

In this contribution, we examine the potential of using field spectroscopy to discriminate the responses of five soybean cultivars to background ozone concentration. Statistical analysis of hyperspectral data including one-way analysis of variance (ANOVA) and spectral instability analysis (ISI) were used to identify the most effective wavelengths in mapping and differentiating the five cultivars with different tolerance to ozone damage. Our results show several distinctive spectral regions that can be used for effective crop type mapping within species level, and quantifying the effects of ozone damage at leaf and canopy scales. This work demonstrates that hyperspectral remote sensors soon become available from government and private sector satellites offer a new set of high-resolution spectral data that will help to quantify impacts background ozone concentrations due to climate change on food security.

*Index Terms*— Hyperspectral imaging, field spectroscopy, soybean, background ozone

#### 1. INTRODUCTION

New measurement capabilities from space within the next several years promise to offer a new set of high-resolution spectral data that will help the farming community better understand the mechanisms behind the decrease of local, regional and national decline in crop yield due to increasing ambient ozone concentrations under climate change.

In summer 2014, we set up an experimental site in Maryland Heights, MO (38.719551, -90.447467), USA. Five soybeans genotypes including AK-HARROW, PI88788, DWIGHT, PANA, and WILLIAMS82 were planted on April 23, 2014, and harvested on October 1, 2014. AK-HARROW and PI88788 were found to be relatively sensitive [1] while DWIGHT, PANA and WILLIAMS82 were identified as relatively tolerant to ozone [2,3]. Plant biophysical variables including gas exchange and fluorescence, leaf area index (LAI) were collected throughout the growing season along with field based spectroscopy measurements and hyperpsectral imaging. The objectives of this study were to identify distinct spectral wavelengths that can differentiate

the responses of soybean cultivars to background ozone concentrations, which is imperative to determine the effects of background ozone on soybean crops.

#### 2. DATA AND METHODS

## 2.1. Remote sensing data

We used high resolution full range PSR-3500 (Portable Spectroradiometer, Spectral Revolution, Inc., Lawrence, MA, USA). The wavelength range of PSR-3500 is 350-2500 nm with a resolution of 3.5 nm in the 350-1000 nm range, 10 nm in the 1000-1900 nm range and 7 nm in the 1900-2500 nm range.

#### 2.2. Methods

# 2.2.1. Statistical analysis

A one-way analysis of variance (ANOVA) was used to examine whether differences exist between among genothypes in response to background ozone concentrations. The day of the year was considered as a fixed effect. A multiple comparisons test using Bonferroni adjusted t test, was carried out in order to determine which pairs of genotype means differ. We applied the Bonferroni multiple comparisons procedure with  $\alpha$ = 0.01 to the data. Additionally, scaled photochemical reflectance index (sPRI) [4] and chlorophyll index (CI) [5] were tested for their potential to discriminate the study organisms.

## 2.2.2. Spectral instability analysis

The spectral separability of the different species at a specific growth period was quantified using a Instability Index (ISI) (also called Separability Index) [6,7]. The Instability index is defined as the ratio of the between-class variability and the within-class variability:

$$ISI_{i} = \frac{\Delta_{within,i}}{\Delta_{between,i}} = \frac{m}{m(m-1)} \sum_{z=1}^{m-1} \sum_{j=z+1}^{m} \frac{1.96 \times (\sigma_{z,i} - \sigma_{j,i})}{|R_{mean,z,i} - R_{mean,j,i}|}$$
(1)

where i is the wavelength, m represents the number of endmember classes (soybean cultivars),  $R_{mean}$  is the mean reflectance of an endmember, and  $\sigma$  is the standard deviations at the same wavelengths of class m, respectively.

#### 3. RESULTS

### 3.1. ANOVA analysis

To understand the potential of remote sensing based separation of soybean genotypes, field measured leaf spectra were analyzed for 1st half, 2nd half and entire growth period. The purpose of this study is to see if a unique spectral signature of ozone damage can be determined by comparing soybean cultivars that are known to be sensitive to ozone with

cultivars that are relatively ozone-tolerant. We then analyze these measurements using the principle of spectral separability analysis. Therefore, ANOVA test with  $\alpha$ =0.01 was carried out on three groups of full range (350 - 2500 nm) spectra, respectively (Fig. 1). The gray shading indicates the wavelength where the spectral reflectance of soybean genotypes is statistically unique within five species with confidence level (p-value) of 0.01-0.05. In the 1st half of the growing season spectral differences between the genotypes were highly significant across the 404 -736 nm, 1395-1577 nm and 1877-2405 nm (Fig. 1a). In the 2nd half, spectrally separable bands in visible portion of the spectra reduced substantially compared to the 1st half of the growth period (Fig. 1c).

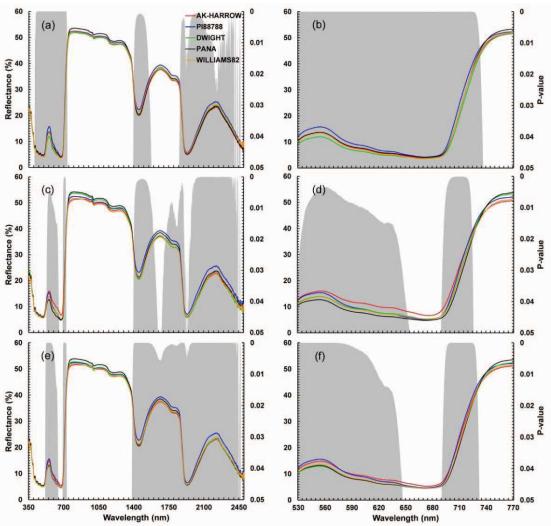


Fig. 1. Mean leaf spectra of five soybean genotypes in  $1^{st}$ -half growth period (a),  $2^{nd}$ -half growth period (c), all growth period (e) and corresponding close-up of 530-770 nm region for each growth period (b), (d) and (f), with ANOVA tests showing significant differences in gray shading (p-value  $\leq 0.05$ ). The experimented genotypes can be spectrally separated in green, red and shortwave infrared (SWIR) portions of the spectrum.

In contrast, spectrally distinct bands were expanded in shortwave infrared (SWIR) region together with increase in

their statistical significance. Distinct spectral signatures corresponding to the 2nd half of the growth period when

plants were exposed to increased ozone concentrations were located in 528-655 nm, 690-725 nm, 1416-1642 nm and 1679-2469 nm. Similar results were found with the leaf spectra of the whole growing period (Fig. 1e), but with somewhat more and continuous wavelength-specific differences in SWIR region than found in the 1st and 2nd half of the growth cycle. We identified three spectrally unique regions 516-646 nm, 690-732 nm, and 1381-2467 nm for the whole growth period. Fig. 1b, d, f were the enlargements of visible and near infrared regions including 530-770 nm wavelength region. In all cases, shorter visible region of 530-646 nm and 690-725 nm, which is considered within red-edge region, were found as distinguishable spectral signatures. There was no spectral uniqueness detected in 350-400 nm and around 2470-2500 nm due to the weak radiant energy of the halogen lamp light source.

The consistent performance of SWIR regions in spectral separability during the growth cycle might be associated with changes in leaf water content [8,9]. In contrast, the green and red portion of the spectrum is mostly related to biophysical status (i.e., health) of plants [1]. The results of ANOVA test indicated that the visible and red-edge regions of the spectra were crucial for detecting abiotic stress (e.g., due to background ozone), and a spectral index capable of capturing the spectral variability in this wavelength should have a good potential of monitoring plant health.

## 3.2. Instability Index

Fig. 2 shows temporal variation of ISI (Instability Index) at each growth stage plotted against wavelength. An ISI value that is below one indicates the between-species spectral

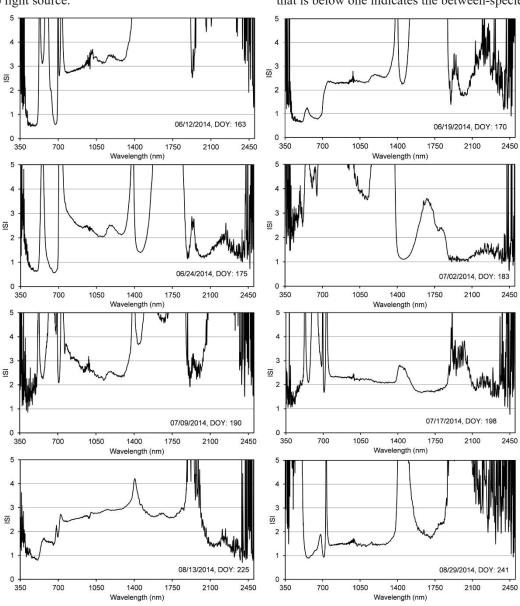


Fig. 2. Temporal variation of ISI (Instability Index) at each growth stage plotted against wavelength

variability exceeds the within-species variability, while an ISI value which is above one denotes the opposite trend. Wavelengths with an ISI value below one are expected to contain useful information for spectral signature separation and should be selected for plant response detection. During the early growth stage (DOY: 163, 170 and 175), low ISI (< 1) values were found mainly for blue and red spectral regions, which are closely related with amount of chlorophyll in the leaf. At the early growth stages of plants, the significant difference in chlorophyll content is expected as different species takes different period of time to reach a certain growth stage. At the middle of the growth stage (DOY: 183, 190 and 198), the ISI values were mostly larger than one. This spectral similarity implies that soybean species are in similar physiological state and yet to experience significant damage. At the end of the growth stage (DOY: 225 and 241), low ISI were detected mainly in blue, green and far red spectral regions and shortwave infrared region. These dissimilarities are associated with different responses of soybean species to intensifying background ozone by decreased chlorophyll, photosynthesis and leaf water content. NIR regions did not show low ISI for entire growth stage. This may imply that there was no significant variation among the soybean species in terms of leaf internal structure.

# 3.3. Leaf spectral indices

One-way ANOVA analysis of sPRI and Chlorophyll Index (CI) (Table 1) are effective indicators of ozone induced changes to plant physiology during the entire growth cycle. The results are significant at the 99% significance level (P<0.0001). Based on the statistical analysis, CI appears to be the most reflective indicator of plant physiology, which is closely followed by sPRI.

# 4. CONCLUSION

In this work, we examined spectral differences among five soybean cultivars with different ozone tolerance. The objective was to identify hyperspectral wavelength regions that capable of characterizing the responses of common soybean cultivars to background ozone by using remotely sensed spectral signatures, and foliar biophysical and biogeochemical properties. Our results show that there a number of bands within visible and near infrared regions of the spectrum that has the potential of obtaining plant biophysical variables from leaf reflectance spectra. The study was conducted at an agricultural test site located in Maryland Heights MO during the Summer 2014 where five different soybean cultivars were planted. The outcome of this research has potential implications for development of space based observation of large-scale crop responses to ozone damage, as well as for biotechnological breeding efforts to improve ozone tolerance under future climate scenarios.

TABLE 1. ONE-WAY ANOVA OF SPRI AND CHLOROPHYLL INDEX (CI) (A=0.01) DERIVED FROM LEAF REFLECTANCE SPECTRA.

Growth period	sPRI		CI	
	F-stat	P-value	F-stat	P-value
1 <sup>st</sup> -half	4.37	0.0020	24.48	< 0.0001
2 <sup>nd</sup> -half	6.49	< 0.0001	6.81	< 0.0001
All	8.31	< 0.0001	13.17	< 0.0001

#### 5. ACKNOWLEDGEMENTS

Funding for this work was provided by NASA (NNH09ZDA001N, NNX13AB23A), National Science Foundation (IIA-1355406) and Center for Sustainability at Saint Louis University.

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