Characterizing Crop Responses to Background Ozone in Open-Air Agricultural Field by Using Reflectance Spectroscopy

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***Abstract*—We examine the spectral signatures and foliar biophysical and biogeochemical properties of different soybean cultivars that are known to be sensitive in varying degrees to background concentrations of ozone (O_3). Specifically, the potential of plant biophysical variables from leaf reflectance spectra, including chlorophyll index, photochemical reflectance index, and leaf area index, to detect foliar O_3 damage is explored. The study was conducted at an agricultural test site located in Maryland Heights, Missouri, during the summer of 2014 where five different soybean cultivars were planted. Our results show that the soybean cultivars demonstrated different sensitivity to background O_3 as demonstrated by spectral indices and plant biophysical measurements. The outcome of this research has potential implications for development of space-based observation of large-scale crop responses to O_3 damage, as well as for biotechnological breeding efforts to improve O_3 tolerance under future climate scenarios, as background O_3 concentrations are expected to increase through the twenty-first century.**

***Index Terms*—Background ozone (O_3), climate change, crop response, remote sensing, soybean.**

I. Introduction

GLOBAL demand for agricultural production is projected to increase by 50% in 2050 mainly due to population increase [1], [2]. However, altered water availability, changes in temperature, and increased concentration of pollutants in the air under changing climate may cause considerable effects on crop yield [3]. Background elevated ozone (O_3) concentrations have been identified as one of the few key threats to global food security [3], [4], but the responses of different crops to O_3 pollution in an open-air field environment are poorly understood.

The cost of air pollution to the agricultural community may be as high as tens of billions of dollars globally [5] and is predicted to become even more costly in the next several decades [6] because of degrading global air quality in the form of rising concentrations of background levels of tropospheric O_3 [1]. In the USA, observable damage to the soybean crop alone likely exceeds $1 billion [4]. New measurement capabilities from space within the next several years, however, will 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. These data, coupled with improved estimation of air pollutants from space, provide the framework for a new avenue of research that can significantly benefit global agricultural research.

In recent years, leaf and canopy spectroscopy measurements have been explored to quantify foliar injury from background O_3 levels [7], [8]. Remotely sensed photochemical reflectance index (PRI) [8] has been demonstrated as a promising index for assessing O_3 damage at leaf [10] and canopy [11] scales. Ainsworth *et al.* [12] also tested the potential of plant photosynthetic capacity derived from leaf spectra and gas exchange measurements to characterize O_3 damage. While obvious decreases in chlorophyll, nitrogen content, and, therefore, crop yield were reported at elevated O_3, they found no effect of O_3 on photosynthetic capacity, which might be attributed to insufficient temporal sampling of spectral measurements. Thus, more research comparing crop responses to varying degrees of O_3 concentrations in field growth environment is warranted. The goals of this contribution are as follows: 1) to characterize the spectral responses of soybean crops to background O_3 concentrations in field growth conditions and 2) to develop approaches to identify the traits of foliar injury at its early stage for protective crop management. We utilize spectral and structural indices, including chlorophyll index (CI), leaf area index (LAI), and PRI, collected during the growing season of the crops.

II. Materials and Methods

A. Study Site

The test site was located in Maryland Heights, Missouri (38.719551, −90.447467), at an elevation of 142 m from mean

Manuscript received October 30, 2014; revised December 17, 2014; accepted January 25, 2015. This work was supported in part by the NASA Applications Program through its Air Quality Applications Science Team (NNH09ZDA001N) and in part by NASA EPSCoR (NNX13AB23A).
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Digital Object Identifier 10.1109/LGRS.2015.2397001

sea level. Five different genotypes of noncommercial tolerant and nontolerant soybeans (AK-HARROW, PI88788, DWIGHT, PANA, and WILLIAMS82) were planted on April 23, 2014 and harvested on October 1, 2014. Although DWIGHT, PANA, and WILLIAMS82 were found to be relatively tolerant to O_3 [13], [14], no published studies systematically examined responses of AK-HARROW and PI88788 to background O_3 concentrations in contrast to the less sensitive species. For each genotype, we planted 100 seeds (0.015 kg) in Peers Silk Loam soil and applied some minor herbicide and no irrigation. Our test site is a 12 ft $\times$ 12 ft plot with five evenly spaced rows. Each row corresponds to a genotype.

B. Data

All of the measurements were made under clear-sky conditions between 10:00 A.M. and 2:00 P.M. local time once per week during the growing season. Leaf reflectance spectra were collected using high-resolution full-range PSR-3500 (Portable Spectroradiometer, Spectral Revolution, Inc., Lawrence, MA, USA). A reference spectrum was taken from a 99% Spectralon calibration panel (Labsphere, Inc., North Sutton, NH, USA) before target measurement. A leaf clip with a bifurcated fiber optic connected to both the device and the 5-W tungsten halogen lamp light source was used to record leaf reflectance readings with black background. PSR-3500 was configured to automatically average 40 spectra for each sampling. The raw spectra bandwidth was interpolated to 1 nm for further analysis.

The LAI-2200C Plant Canopy Analyzer (LI-COR Inc., Lincoln, NE, USA) was used to make nondestructive measurements of LAI throughout the growing season. With a critical upgrade to the system in 2014, the instrument allows measurements at any time of the day in full sun and no longer requires specific sun angles or cloud cover. LAI measurements were made along a pair of diagonal transects between the rows using the 45° view cap. Measurements were taken along and across the rows by transects, with two readings being taken from above the canopy at the beginning and end. Along with the above canopy reading, three below canopy readings along the transect at even spacing were taken. The sensor view direction was positioned parallel to the row in the first transect and perpendicular in the next transect. Extra sky radiation measurements were taken for scattering corrections.

Fifteen-minute average O_3 measurements were collected at the Forest Park Ozone Garden in St. Louis located 22 km southwest of the field site (http://go3project.com).

C. Spectral Reflectance Indices

Leaf reflectances were interpolated at 1-nm intervals. CI was calculated using $(R_{750} - R_{705})/(R_{750} + R_{705})$ [15], where R is the leaf reflectance spectra, and the subscripts represent wavelengths regions in nanometers (nm). Red reflectance at around 705 nm is low for green leaves with high levels of chlorophyll content and carotenoids, which sharply increases when chlorophyll content reached to a relatively low level. In contrast, the reflectances at 750 nm are maximal regardless of chlorophyll concentration levels and plant senescence [15]. Therefore, CI is directly proportional to chlorophyll content, i.e., a higher CI value corresponds to the higher chlorophyll content and carotenoids, or *vice versa*.

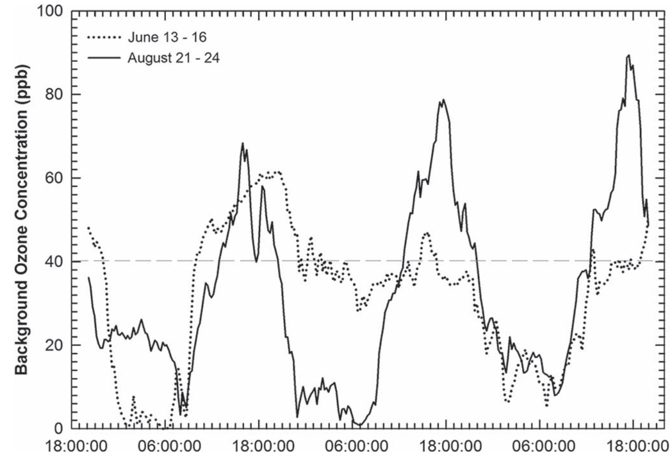

Fig. 1. Ozone (O_3) measurements representative of the beginning and end of the growing season during 2014 are shown. Measurements plotted in this depiction are 15-min averages measured at the Forest Park Ozone Garden in St. Louis [19]. Even when extremely clean air is present, values often exceeded 40 ppb during daytime hours, which is considered a threshold that can cause damage to plants. Furthermore, O_3 concentrations this high were not commonplace before the middle twentieth century [19].

The PRI derived from narrow-band spectral reflectances [9] has been increasingly used as an indicator of plant photosynthetic efficiency, as well as functional convergence of biochemical, physiological, and structural components affecting leaf, canopy, and ecosystem carbon uptake efficiencies. A synthesis study of multiple scales indicated that the PRI accounted for 42%, 59%, and 62% of the variability of radiation use efficiency at the leaf, canopy, and ecosystem, respectively, with unique exponential relationships for all the vegetation types studied [16]. To avoid negative values, we adopted the scaled PRI (sPRI), as shown in the following equation [17]:

$$\mathrm{sPRI} = \frac{\frac{R_{531}-R_{570}}{R_{531}+R_{570}} + 1}{2} \tag{1}$$

where R refers to the reflectance at 531 and 570 nm. Additionally, the ratio of the reflectance at the wavelengths 801 nm (NIR) and 670 nm (VIS) were explored due to its sensitivity to LAI and other plant biophysical variables.

III. Results and Discussion

A. Background O_3 Profile

Exposure to cumulative O_3 concentrations over 40 ppb causes significant damage to plant growth [12], [18], thereby decreasing photosynthesis and crop yield [4], [14]. Fig. 1 shows the 15-min representative O_3 concentration for early (dashed line) and late summer (solid line) in 2014. Although background O_3 concentrations in 2014 were some of the lowest on record in June and early July, values after 15 July through September were representative of values typically measured in the Midwest in the twenty-first century [4]. Most importantly, the threshold value 40 ppb, which can cause damage to plants, was exceeded for nearly all of the days from June through September 2014.

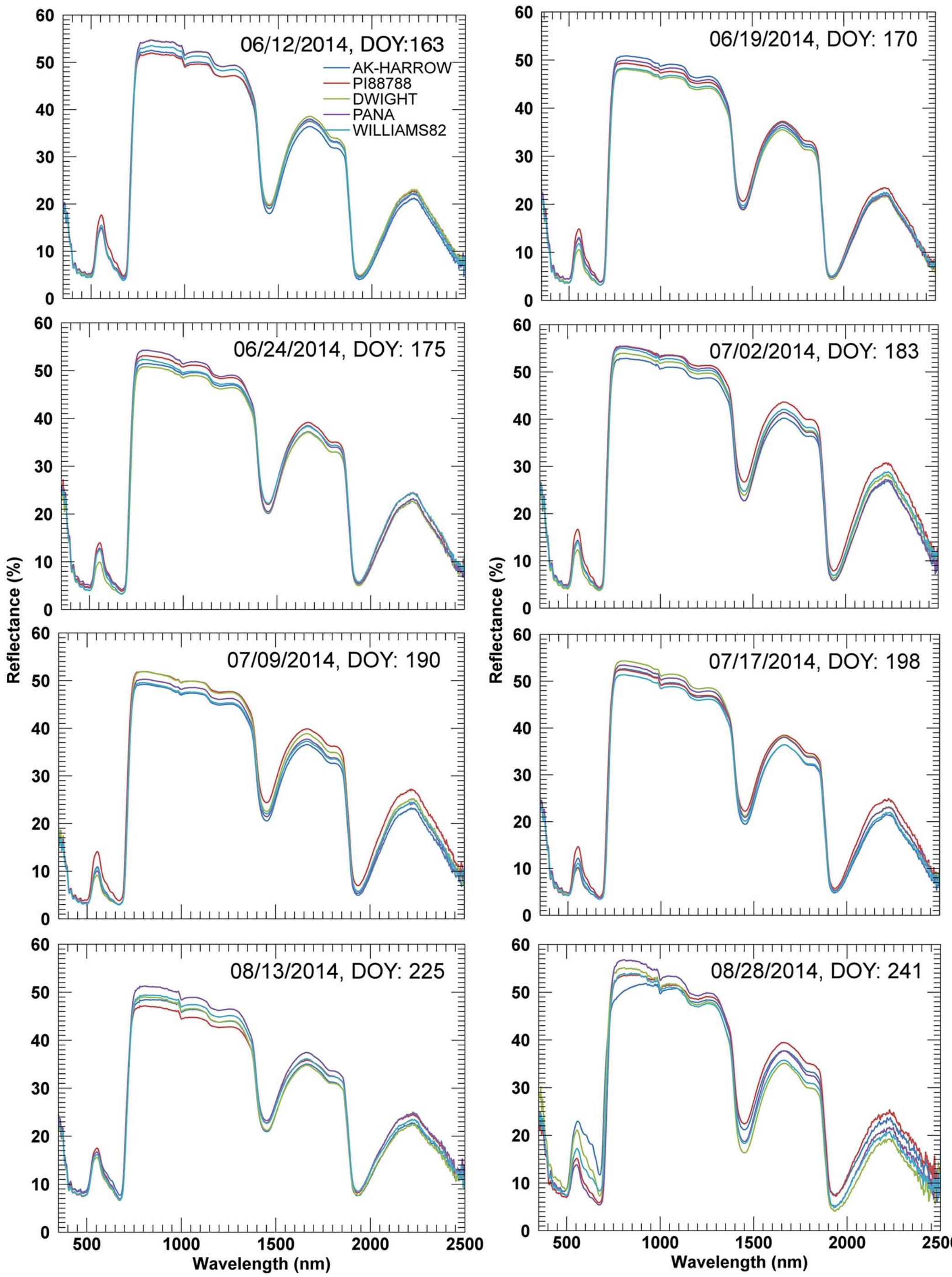

Fig. 2. Genotype average spectral of soybean at each growth stage. For each genotype, 12 spectra per cultivar were measured and averaged from fully expanded third mature leaf trifold from the top of the canopy during each growth stage. DWIGHT, PANA, and WILLIAMS82 demonstrated relatively higher tolerance to O_3 stress compared to AK-HARROW and PI88788 based on species average spectral profiles.

B. Spectral Separability Analysis

Higher near-infrared (NIR: 750–950 nm) and lower blue (450 nm) and red (650 nm) reflectance values are an indication of whether a plant is healthy. This is due to the fact that energy at red and blue wavelength regions is absorbed by chlorophyll pigments for photosynthesis. In comparison, NIR energy is reflected by healthy and turgid mesophyll cell walls in dense canopies.

Across all soybean genotypes, there was a significant effect of background O_3 concentrations on temporal profiles of leaf spectra, as shown in Fig. 2. The small differences in blue and red reflectance values during the early growth stage indicate that the soybean genotypes were yet to experience significant damage. The differences in NIR and shortwave infrared portion of the spectra may be associated with leaf internal structure, dry matter, and water content. During this stage through June 24, 2014 (DOY 175), AK-HARROW and PI88788 reflectance

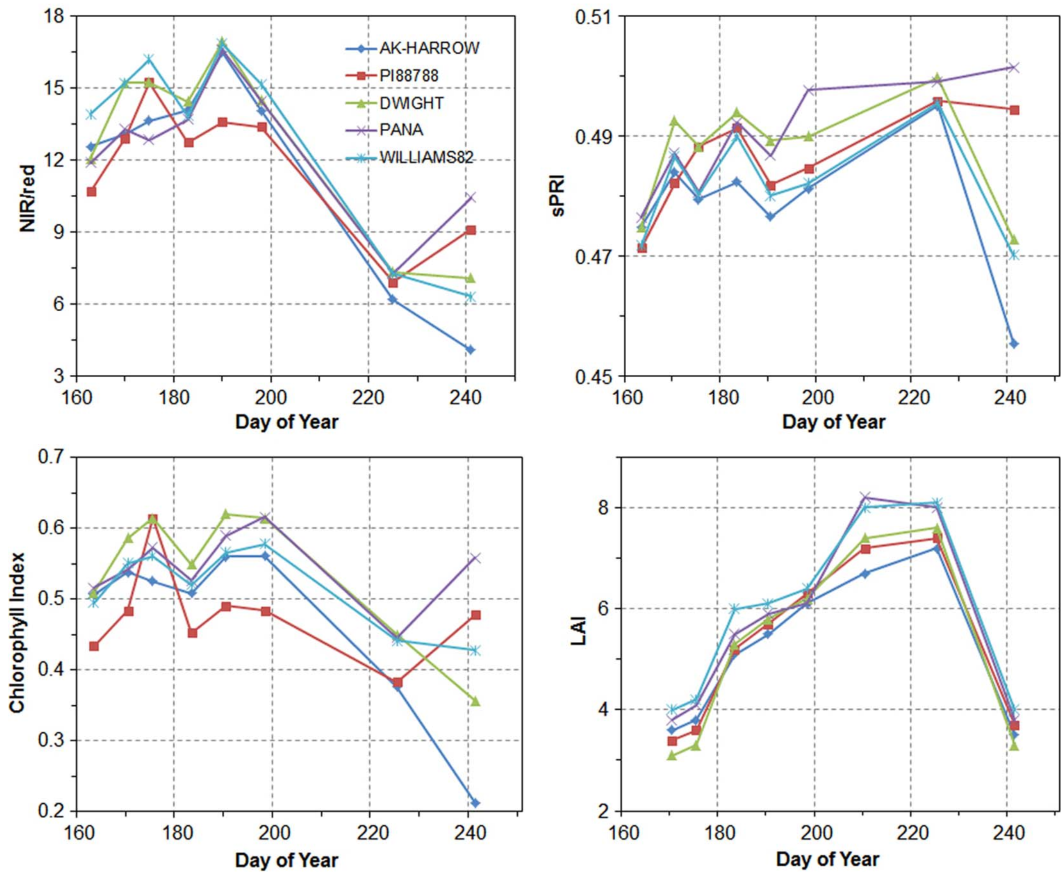

Fig. 3. Temporal profiles of the spectral indices and LAI. There was an obvious increase in the indices during the early growth stage, and then, a sharp decline of the indices on less tolerant species were observed during the late growth period possibly attributed to elevated background O_3 in later summer. Changes in LAI indicate that O_3 sensitive plants entered in its senescence as early as two weeks than the more tolerant species.

in green and NIR increased and become highest, which is particularly true for AK-HARROW. However, the green and NIR reflectance values for these two cultivars continue to decline for the rest of the growth period, which was culminated to the highest red and lowest NIR reflectance toward the end of the growing season. Increase in red and decrease in NIR reflectance is caused by decreased chlorophyll pigments and photosynthesis and, therefore, a reduction in dry matter content due to O_3 stress. These changes may become visible to our eyes only after the damage to plants become permanent. The variation of visible and NIR spectra during the early stage of growth without visible traits of change is consistent with previous findings that foliar damage from lower concentrations of O_3 may not be visible but can negatively impact photosynthetic carbon gain through light-harvesting processes [20], [21] and metabolism [22]. As reflected by increased red and decreased NIR reflectance on August 28, 2014 (DOY 241), O_3 damage on AK-HARROW and PI88788 toward the end of the growing season was evident. In early September 2014, O_3 injury was visual as these two genotypes entered to senescence, and there was no more absorption in the red portion of the spectrum that removes these colors from the amount of light that is reflected, causing the predominant red color that reaches the human eye. It is worth noting that there may be differences among the genotypes in their time to maturity irrespective of O_3, which is beyond the scope of this letter.

C. Spectral Reflectance Indices and LAI During the Growing Season

Overall, NIR/red index, CI, and sPRI show a similar trend during the growing season of the soybean cultivars (see Fig. 3). However, there were several interesting findings found from the results. 1) PI88788 demonstrated a rapid increase in the spectral indices and reached the highest value on June 24, 2014 (DOY 175). In particular, both the CI and the sPRI were higher than all other cultivars. There were drastic decreases in NIR/red and CI values on July 2, 2014 (DOY 183), but sPRI was still high, suggesting temporary closure of PI88788 stomata possibly in response to elevated background O_3. 2) All of the spectral indices of PANA were smaller than the other cultivars until after July 9, 2014 (DOY 190) and then were consistently higher for the rest of the growing season. This may indicate that PANA is more tolerant to O_3 than the other cultivars, which was consistent with the findings of Betzelberger *et al.* [13]. 3) Except for LAI values, PANA was closely followed by DWIGHT with respect to spectral reflectance indices, particularly in the late growing season when O_3 concentrations were high, suggesting DWIGHT may be as tolerant as PANA if not the most tolerant genotype among the study organisms. These findings were also supported by our field observations that AK-HARROW and PI88788 entered to senescence a couple of weeks earlier than the other three cultivars.

Exposure to elevated O_3 alters metabolism, which has the potential to alter the efficiency by which plants capture light energy, convert that energy into carbon, and partition the carbon into biomass and yield [14]. On the other hand, the plant's capacity for light interception and productivity can be determined by LAI, which is defined as the amount of leaf per unit ground area. Our results are consistent with previous studies that LAI decreases when plants are exposed to O_3 damage [23]. LAI of PANA and WILLIAMS82 was highest throughout the growing season. DWIGHT LAI was smallest at the early stage of growth but grew larger than AK-HARROW and PI88788 after two weeks. Starting from approximately around June 24, 2014 (DOY 175), the LAI for DWIGHT steadily increased and, overall, was between the LAI of more and less tolerant cultivar. A senescence-induced reduction in LAI occurred approximately two weeks earlier (DOY 224) in more sensitive AK-HARROW and PI88788 toward the end of the growing season.

IV. Conclusion

We utilized an open-air agricultural field to investigate the biochemical and physiological responses of five soybean cultivars to background O_3. Three spectral indices (NIR/red, CI, and sPRI) plus LAI were examined throughout the growing season. We demonstrated that current concentrations of background O_3 are sufficient to cause reductions in LAI and photosynthetic processes/efficiencies, which can negatively impact crop yields. Based on our preliminary data, we come to a cautious conclusion that cultivars AK-HARROW and PI88788 were the least tolerant to O_3 and that DWIGHT and PANA were the most tolerant among the cultivars we tested. The result of this letter should provide insights on the harmful effects of O_3 pollution on soybeans, which is critical in developing ozone-tolerant crops, as well as O_3 mitigation strategies for global food security. To better understand the relationship between spectral indices and cultivar ozone tolerance, further study should include plant biophysical variables, including photosynthesis, fluorescence, and yield.

Acknowledgment

The authors would like to thank F. Dohleman, J. Kinser from Monsanto, and Dr. E. Ainsworth from the University of Illinois at Urbana-Champaign.

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