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Fluorescence Imaging Techniques for Monitoring Vegetation

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Abstract- Fluorescence sensing systems for the remote assessment of vegetative parameters are currently being developed and tested for use from a variety of sensing platforms. Multi-spectral fluorescence imaging techniques are being applied to assess plant responses to changes in its growth environment. Fluorescence abaxial vs. adaxial spatial patterns of band emissions are discussed with respect to individual leaves excised from a field corn canopy supplied with four levels of nitrogen fertilization. Fluorescence emissions for adaxial leaf surfaces were found significantly lower in intensity and specific emission bands and band ratios were found sensitive to N fertilization level.

I. INTRODUCTION

The principle of fluorescence involves the absorption of a specific wavelength of light by a fluorophore followed by the dissipation of the absorbed energy by light emission of a longer wavelength within a very short (< 200 ns) period of time. *In vivo* fluorescence emissions from vegetation occur throughout the ultraviolet to visible regions of the spectrum, with maxima occurring in five distinguishable bands: the UV-A, blue, green, red, and far-red [1,2].

The UV-A fluorescence emission band can only be observed when plant are irradiated with UV-B (~280 nm) and has been attributed to plant proteins which contain aromatic amino acids [2]. The overlapping blue-green fluorescence emission bands are a convolution of fluorescence emissions originating from several plant constituents [1]. The majority of the static blue fluorescence (i.e., variations occur over days to weeks) has been attributed to hydroxycinnamic acids, primarily ferulic acid covalently linked to the leaf epidermis and cell walls [3]. Additional blue fluorescent compounds have a smaller but more dynamic contribution (i.e., variations occur over hours to days) to the *in vivo* blue fluorescence emission [4,5]. Prime candidates for the green band are flavins in the oxidized form [6], with the fluorescence feature becoming more pronounced with longer wavelengths of excitation. Several investigations have demonstrated relationships between these fluorescence bands and ratios of these bands to plant health and growth condition, reviews [4,5,8]. Fluorescence measurements have shown a great deal of promise in the remote assessment of the relative impact of environmental factors, including: nutrient supply in crop canopies [2,8,9], differentiation of crops and trees grown under controlled exposures of elevated O₃ [10], UV-B irradiation [11,12], and quantifying the amount of crop residue covering agricultural soil surfaces [13].

Currently, several research groups are using fluorescence sensing systems operating from a variety of platforms to receive fluorescence information and are relating this information to the physiological status of the plants in both terrestrial and aquatic ecosystems [14,15]. The present study was designed to investigate relationships between adaxial and abaxial *in vivo* fluorescence patterns in response to nitrogen (N) supply. The results from this study provide considerations for future design and development of fluorescence sensing instrumentation and measurement protocols. These fluorescence investigations are ultimately intended to provide information that can be incorporated into prescription algorithms for site specific variable applications of N containing fertilizers for crop production.

II. METHODS AND MATERIALS

A. Plant Material

The field site is located at the USDA Beltsville Agricultural Research Center and is part of an intensive test site for a multi-disciplinary project entitled Optimizing Production Inputs for Economic and Environmental Enhancement (OPE³). The primary focus of OPE³ is to develop farming strategies that conserve natural resources while maintaining or increasing long-term farm profitability. Corn (*Zea mays* L.) nitrogen treatment plots, large enough to capture the spatial variability of crop and soil parameters, were established within the OPE³ field site. The experimental design was a randomized complete block with treatment groups of 210, 140¹, 70, and 28 kg N / ha, which provided plant growth conditions ranging from classical symptoms of N deficiency to physiological conditions consistent with an excess supply of N. Fluorescence and physiological measurements from field corn were obtained from the third leaf from terminal at the grain fill (R3) reproductive stage. Refer to table 1 [16] in this proceeding for a corresponding summary of grain yield, foliar pigment and N contents.

B. Leaf Level Fluorescence Imaging

The laboratory Fluorescence Imaging System (FIS) was used to obtain the leaf level fluorescence emissions from abaxial and adaxial leaf surfaces. FIS consists of a UV excitation source, a digital image detection camera, and a computer interface for data collection and instrument control.

¹ N rate recommended by the University of Maryland Soil Testing Laboratory for corn grown on a Maryland sandy loam soil.

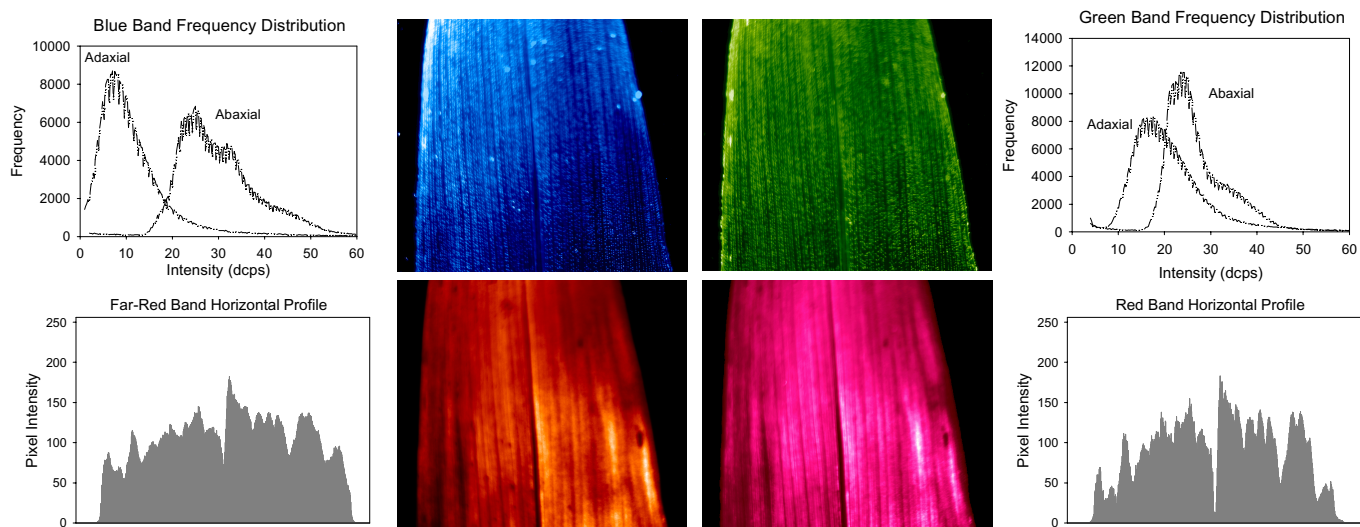


Figure 1. Four band fluorescence imagery for a field corn leaf grown under a 140 kg N / ha application rate.

The FIS illumination source is comprised of UV-A fluorescent lamps filtered through Schott UG-1 bandpass glass filters (50 nm FWHM) which provides a nearly uniform intensity of 3.3 W/m^2 centered at 365 nm. The detection system is comprised of a cooled (-40°C) intensified CCD camera coupled to an 50 mm lens which captures a 400 cm^2 area in to a 16 bit, 1036 line X 1032 sample file. The camera is attached to an automated filter wheel which contains four band pass (10 nm FWHM) interference filters centered at 450 nm, 550 nm, 680 nm, and 740 nm. The camera responsivity and variation due to non-uniform illumination are calibrated using a homogeneous liquid flat field fluorescent target and the units for FIS data are presented in digital counts per second (dcps).

III. RESULTS

Spatial patterns in abaxial vs. adaxial fluorescence emission over leaf surfaces were characterized using FIS imagery. Sample multispectral imagery for the adaxial surface from field corn leaves grown under the recommended N application rate of 140 kg/ha is shown in figure 1. Linear color intensity scales were applied to each individual image such that hues were selected as an identifying characteristic for each spectral band (i.e., blue, green, red, far-red). Each color scale was individually stretched to display the spatial distribution of fluorescence emission and not intended for intensity comparisons. The mid rib was clearly discernable in all images and has lower fluorescence emission intensity in the blue, red, and far-red bands, while the green band emission is slightly elevated in the mid rib relative to the surrounding leaf tissue. The longitudinal bundle sheaths were more clearly discernable in the well fertilized leaf and emissions were lower relative to inter-veinal areas. Leaf trichomes (hair structures) have a significant contribution to the adaxial blue-green fluorescence. Elevated abaxial emission were observed in all four bands with particularly distinct frequency distributions in the blue-green

emission bands figure 1. Similar spatial features were observed for low N leaves (data not shown). Histograms to the side of the red and far-red images indicate that there are significant changes in photosynthetic activity across the adaxial leaf surface. This phenomenon makes treatment classifications using stand alone red or far-red bands difficult. The adaxial fluorescence intensity tends to increase with N fertilization, plateau for optimal rates, and under certain super optimal N rates a slight decrease in emission can be observed (figure 2). Abaxial emission blue-green band intensities exhibit an inverse relationship to N fertilization level with emissions decreasing with increasing N application rate.

The most distinct mean separations were found for the green/blue and red/blue band ratios (table 1). An inverse relationship was observed between N fertilization rate and adaxial band ratios with two levels of separation indicating distinctions between high and low N regimes. In addition, the reported ratios indicated a clear separation of adaxial and abaxial leaf surfaces.

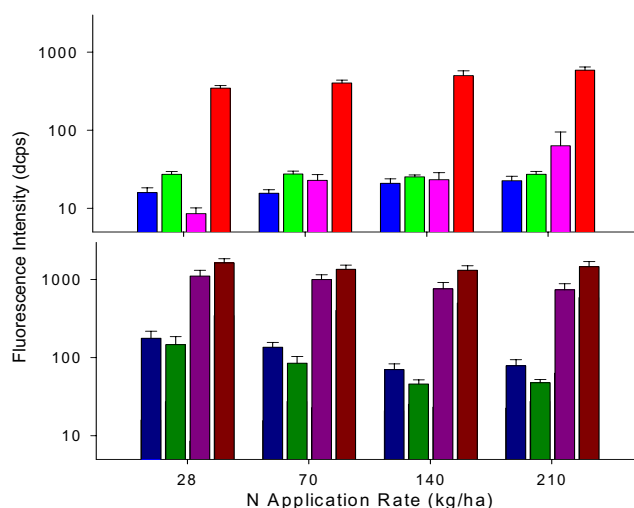


Figure 2. Means and SE (n=21) for blue, green, red, far-red respective bands for the adaxial (top chart) and abaxial (bottom chart) leaf surface at four levels of N fertilization.

Table 1. Mean comparison (LSD_{.05}) for within fluorescence band ratios.

N Rate kg/ha	Green / Blue		Blue / Red	
	Adaxial	Abaxial	Adaxial	Abaxial
210	1.46 b	0.90 c	1.16 b	0.27 c
140	1.58 b	0.88 c	1.24 b	0.13 c
70	2.29 a	0.68 c	1.18 b	0.18 c
28	2.30 a	0.85 c	1.82 a	0.18 c

IV. DISCUSSION

The differences in adaxial vs. abaxial fluorescence emissions have direct bearing on the nature of the fluorescence signal observed at canopy levels. The Laser Induced Fluorescence Imaging System (LIFIS) is capable of capturing four band fluorescence images in the presence of ambient solar radiation [9]. Such systems are capable of capturing near field four band fluorescence images from portions of the crop canopy at a variety of view angles. A blue fluorescence horizontal plant profile obtained from LIFIS is shown in figure 3. At this view angle, abaxial leaf surfaces are the predominant fluorescent scene component which, as previously, noted exhibit an inverse relationship to N fertilization level as opposed to the direct relationship observed from adaxial leaf surfaces.

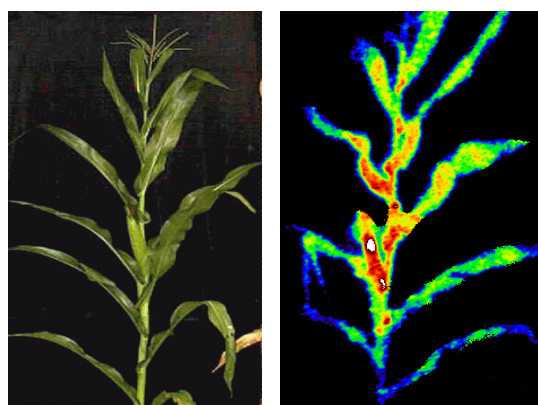


Figure 3. LIFIS blue fluorescence image of a field corn plant freshly excised from the canopy. Red hues indicate high intensity.

The leaf inclination angle distribution of corn canopies under optimal growth conditions tends toward horizontal. At view angles from NADIR to 45° off-NADIR, the uppermost adaxial leaf surfaces would be the primary fluorescent components contributing to the canopy emission. Under stress, the canopy architecture moves from planophile to erectophile with abaxial leaf surfaces facing outward to minimize light absorption and evapo-transpiration. Under these conditions one can expect an increased abaxial leaf fraction leading to increases canopy fluorescence signal as the severity of stress increases.

V. CONCLUSIONS

From the fluorescence foliar analysis, it was determined that abaxial fluorescence emissions are elevated in intensity over abaxial surfaces in the blue, green, red, and far-red emission bands. This information has pointed to the utility of canopy level fluorescence imaging systems where leaf orientation and canopy architecture can lead to changes in signal intensity that are indicative of stress. The data presented in the current study indicated that, not only are fluorescence profiles sensitive to N fertilization level, they could additionally be exploited for the detection of plant stress factors such as drought when obtained at canopy observation levels.

VI. REFERENCES

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