Optical Reflectance and Fluorescence for Detecting Nitrogen Needs in *Zea mays* L.

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***Abstract*- Nitrogen (N) status in field grown corn (*Zea mays* L.) was assessed using spectral techniques. Passive airborne hyperspectral reflectance remote sensing, passive leaf level reflectance, and both passive and active leaf level fluorescence sensing methods were tested. Reflectance of leaves could track total Ch*a* levels in the red dip of the spectrum and auxiliary plant pigments of Ch*b* and carotenoids in the yellow/orange/red edge reflectance. Based on leaf level reflectance behavior, a modified chlorophyll absorption reflectance index (MCARI) method was tested with narrow bands from the Airborne Imaging Spectroradiometer for Applications. MCARI indices could detect variations in N levels across field plots. At the leaf level, ratios of fluorescence emissions in the blue, green, red and far-red wavelengths sensed responses that were associated with the plant pigments, and were indicative of energy transfer in the photosynthetic process. Fluorescence emissions of leaves could distinguish N stressed corn from those with optimally applied N. Reflectance and fluorescence methods are sensitive in detecting corn N needs and may be especially powerful in monitoring crop conditions if both types of information can be combined.**

I. Introduction

The N cycle is driving force for sequestration of carbon and the use of other nutrients by vegetation in the environment. The development of spectrally based indices for detecting nitrogen (N) needs in corn (*Zea mays* L.) crops is a major objective of our studies. Currently remotely sensed spectral techniques use solely reflectance to monitor the status of terrestrial vegetation. Reflectance measures at the leaf and canopy level were tested on spectral data from a N level field corn experiment. There are two types of Ch in most higher plants, Ch***a*** and Ch***b***. Ch***a*** usually exists in about a 3 to 1 ratio with Ch***b*** in the plant. When plants are under stress Ch***b*** concentration is affected before Ch***a*** is affected. Kim proposed a CARI reflectance index to normalize away the effects of soil background from Ch canopy information [1]. Daughtry et al, developed an MCARI reflectance index to track total Ch levels in the canopy [2]. This index is in the prime area of Ch***a*** absorption. McMurtrey, et al, modified the technique by developing a second MCARI index to subtract from the 1st to track yellow-orange-red reflectance changes in the plant canopy [3]. Reflectance observations alone may not be able to directly assess vegetations photosynthetic function and the plant canopies physiological dynamics. The amount of fluorescence in apparent reflectance could be useful. Red fluorescence spectral emissions have been shown to be due to chlorophylls associated with Photosystem II. Fluorescence ratios with bands at 440, 525, 685, and 740nm can relate to changes in the distribution of plant energy transfer as governed by chlorophyll content, bio-physical efficiency and a-biotic stress conditions in plants. The plant adjusts physiologically, and levels of chlorophyll change in response to the environment. The methods were tested in rain-fed field experiments at Beltsville, MD. The relationship between leaf chlorophyll concentration, absorption, transmittance, reflectance and ratios of plant fluorescence has been described in other papers concerning these experiments [4,5,6,7,8,9].

II. Procedures

A. Experimental Site

The site is located at the USDA, Beltsville, Md. It contains a multi-disciplinary project, Optimizing Production Inputs for Economic and Environmental Enhancement (OPE[3]), and is a NASA, MODIS validation site. One focus at OPE[3] is to study N management and movement from field into near ecological zones. Four hydrologically bounded 4 ha watersheds flow to a wooded riparian wetland, and first-order stream. Corn N treatment plots, large enough to capture crop and soil spatial variability are established within the site. Four N fertilizer rates at 210, 140, 70, and 28 kg N/ha, provide rates at 150%, 100%, 50% and 20% of the optimal recommended N level. The experimental design is a randomized complete block with three blocks

B. Bio-Physical Plant Measures

Canopy and leaf level data were collected at a field site. Plant leaf samples were taken for pigment extractions (Ch***a***, Ch***b*** and total carotenoid content, µg/cm2); specific leaf mass (g/m2); and, C and N tissue analysis during the growing season. Photosynthetic capacity (µmol CO2/m2/s) were calculated on individual fully expanded leaves near the top of the canopy with a Li-Cor 6400 Photosynthetic System (LI-Cor, Inc., Lincoln. NE).

C. Reflectance Measurements

The same leaf samples that were excised for bio-physical plant measures during the growing season were immediately measured for spectral attributes. Leaf level reflectance from 350-2500nm was acquired by using an integrating sphere (Li-Cor1800, Li-Cor, Inc.) attached to an ASD spectroradiometer (ASD-FR FieldSpec®Pro), with a 1nm sampling interval.

Canopy reflectance was acquired in 22 selected 10nm narrow bands of interest with an airborne Imaging spectroradiometer for Applications (ASIA) flown by 3DI of Easton Maryland.

D. Fluorescence Measurements

Percent fluorescence at 685nm in the apparent reflectance was measured using producers similar to Kim [10] and Zarco-Tejada [11], by subtracting reflectance from the leaf with and with and without a Schott RG 665 long pass filter in front of the illumination source. Freshly excised corn leaves were measured for fluorescence before they were measured for reflectance or other bio-physical data. Total fluorescence emissions were run with a SPEX fluorescence spectrophotometer from 400-800nm at specific spectral (280, 355 & 532nm) excitation wavelengths.

III. Results and Discussion

Agronomic ground truth data indicated that the N fertilizer treatments (20, 50, 100, 150% of optimal) imposed were effective in producing changes in the corn crop. Trends in these measures indicated a ranking consistent with that expected according to the N applied. In later growth stages, concentration trends developed in Ch a, Ch b, Ch a/b, total Ch, photosynthesis, tissue N content and grain yield. However, only % leaf N produced statistically significant differences between all treatment levels (Table 1.).

Table 1. ANOVA Physiological Attributes

N Level	Ch a	Ch b	Photo. µmol co2	% leaf N	Yield kg/ha
150%	43.5 a	10.6 a	35.29 a	3.2 b	10175 a
100%	44.0 a	11.2 a	36.48 a	3.4 a	10784 a
50%	32.2 a	10.5 a	34.68 a	3.0 c	9727 a
20%	17.9 b	5.5 b	30.58 a	2.4 d	6211 b

Reflectance in the 670- 685nm region from individual corn leaves decreased with increasing N treatment level. The upper two N levels could not be separated from each other, to allow for determination of the optimal plant performance level of N fertilization (Figure 1.).

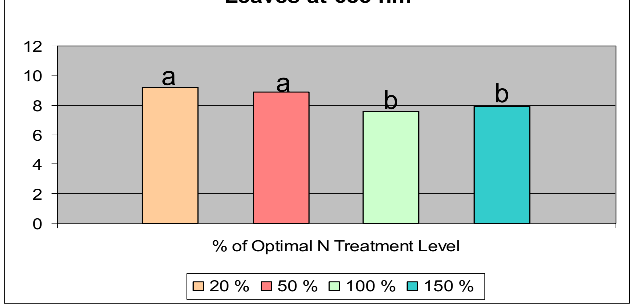
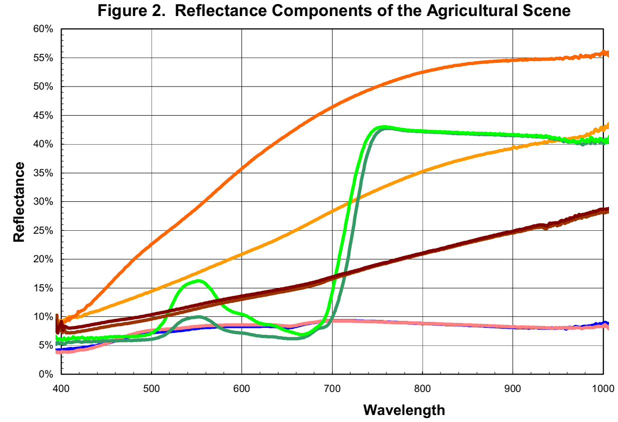


Figure 1. % Reflectance (R+F) of Corn Leaves at 685 nm

Individual components of soil background, crop residues from the last crop, dead leaves in the lower canopy and dead spots on leaves, as well as differences in live vegetation health all integrate to contribute to the total spectral attributes of the agricultural scene that will be captured by airborne ASIA image data (Figure 2.).

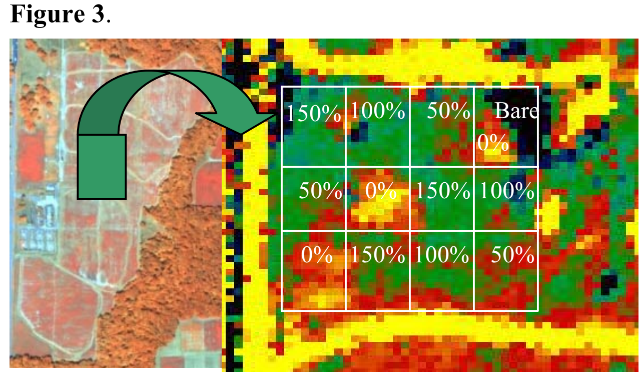
Figure 2. Reflectance Components of the Agricultural Scene



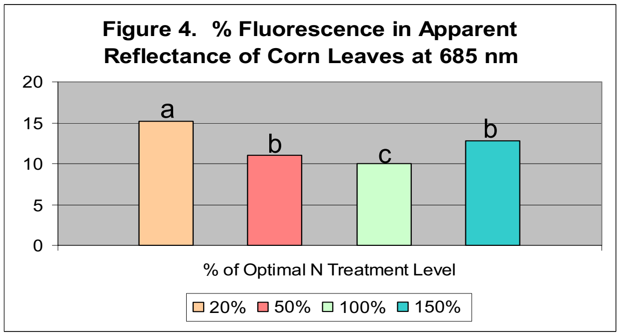
Chlorophyll a Primary plant molecule accepting photons from the sun and transferring energy to photosynthesis
Chlorophyll b - One third to one fourth the concentration of chlorophyll a, transfers all energy through chlorophyll a.
Cartenoids - Protective pigments increase during plant stress and filter some of the sun's rays.
Dead Leaf Material - Dead leaves at the bottom of the canopy dead spots and edges of leaves, all indicators of plant stress.
Crop Residue - Dead crop material on the soil surface from last season's growth.
Bare Soil - Depending on the soil type can be brighter or darker than crop residue material.
Extracted Leaf – Leaf material after plant pigment extraction with DMSO.

Specific reflectance indices (MCARI) derived from ASIA aircraft data in narrow banded wavelength regions are able to distinguish N treatment levels produced in plant canopies. Therefore the mosaic of plant canopy with like spectral attributes may be fertilized according to their N need (Figure3.).

Figure 3.



Note: Total reflectance from leaves was unable to distinguish the optimal N from the 150% level (Figure 1). However, Fluorescence in the apparent reflectance at 685nm produced significant differences between the optimal N level and, over and under fertilization in corn leaves (Figure 4).



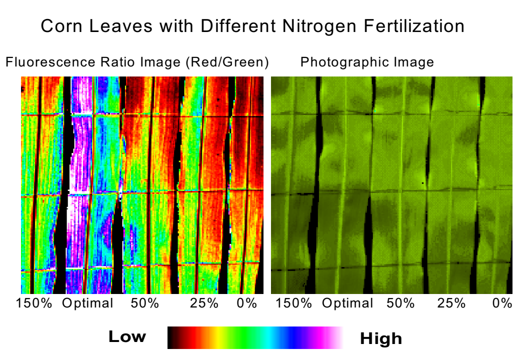
Individual leaf Fluorescence emissions taken from the corn canopy produced information that could be correlated with important physiological plant canopy attributes (Table 2.).

Table 2.
Comparison of correlation coefficients (r) of fluorescence imaging band ratios. Dates V, T, and G correspond to vegetative (n=32), tasseling (n=32), and grain fill (n=16) respectively.

Band Ratio	Growth Stage	Total Chlorophyll	N:C	LAI	Yield
Blue / Green	V	0.55**	0.50**	0.52**	ns
	T	0.43*	0.56*	0.52**	0.51**
	G	ns	ns	ns	0.60*
Red / Far-Red	V	0.79**	0.85**	0.73**	0.75**
	T	0.49**	ns	0.63**	0.44*
	G	0.53*	ns	0.71*	0.69**
Red / Blue	V	0.75**	0.82**	0.71**	0.71**
	T	0.73**	0.65**	0.74**	0.62**
	G	0.94**	0.91**	0.80*	0.87**
Red / Green	V	0.89**	0.88**	0.86**	0.72**
	T	0.77**	0.83**	0.87**	0.75**
	G	0.96**	0.94**	0.82*	0.92**
Far-Red / Blue	V	0.69**	0.75**	0.66**	0.64**
	T	0.75**	0.67**	0.71**	0.63**
	G	0.66*	0.64*	ns	ns
Far-Red / Green	V	0.89**	0.87**	0.86**	0.70**
	T	0.81**	0.87**	0.88**	0.79**
	G	0.73**	0.70*	0.78*	0.81**
Red * Far-Red / Blue * Green	V	0.84**	0.86**	0.81**	0.72**
	T	0.82**	0.82**	0.84**	0.74**
	G	0.87**	0.84**	0.85**	0.90**

Fluorescence images from leaves of different N levels could not be distinguished by the human eye (Figure 5.).

Figure 5.



IV. Summary

Researchers at the USDA in Beltsville, Md. and NASA Goddard Space Flight Center, in Greenbelt, Md. have been cooperating in joint experiments to remotely determine nitrogen levels in vegetation. Corn grown under different levels of N fertilization produced physiological plant material that effected: 1) chlorophyll a, b and, total chlorophyll and caroteniod concentration; 2) %N accumulation; 3) carbon/nitrogen ratios; 4) rates of photosynthesis; 5) biomass accumulation; and 6) final grain yields. Trends in the measures indicated a ranking consistent with those expected according to N applied. Leaf and canopy level reflectance measures were able to separate the lowest from the highest levels of N fertilization. A new airborne spectral reflectance index was able to classify several levels of N need in the field corn canopy. The amount of fluorescence in the apparent reflectance from leaves was determined. Fluorescence from leaves appeared to be better able to separate significant differences between spectral attributes of over-fertilized from the attributes of the optimal N fertilization for plant growth. Plant fluorescence has been described as a way of more closely detecting plant physiological activity and may be most useful when combined with reflectance measures that detect changes in pigment concentration.

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