Proximal Sensing for Early Detection of Nitrogen Deficiency in Corn for In-season Precision Nitrogen Management

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ABSTRACT

Early detection of nitrogen deficiency is essential to site-specific nitrogen management for practical and physiological reasons. Current proximal sensing techniques based on reflectance do not allow reliable detection of nitrogen deficiency prior to V8 growth stage of corn. Another technique based on fluorescence also offers the possibility to detect nitrogen deficiency of plants. The objective of this project was to assess the possibility to detect nitrogen deficiency prior to V8 growth stage of corn based on fluorescence readings. Our results acquired from greenhouse grown plants indicate that fluorescence sensing provide a good indication of corn nitrogen deficiency from V6 growth stage of corn.

Keywords: site-specific nitrogen management, chlorophyll fluorescence, corn

INTRODUCTION

The global nitrogen use efficiency (NUE) is less than 40 % globally (Cassmann, 2002). The growing amount of nutrients from agriculture activities that leaches in the environment and the higher price of nitrogen (N) fertilizers constitute serious concerns for public and farmers (Roberts, 2008). One way of improving NUE is by targeting N-fertilizer when and where the plant will absorb it and turn it into yield (Shanahan et al., 2008). Corn plants do not need the same quantity of N across the season. The maximum N uptake period starts around V6 to V8 growth stages and last up to V16 to V18 growth stage and it is at the beginning of this period that N should be available for the plant in sufficient quantity (Scharf et al., 2006). Nitrogen under the form of nitrates (best form for plant absorption) is very soluble and mobile and N fertilizer applied before the maximum N uptake period have greater chances to leach in the environment, especially in spring conditions when precipitation events are more frequent. On the other hand, if the N fertilizers are applied after the beginning of the maximum N uptake period, the plant will absorb sub-optimal quantity of N and yield will be lower. Thus, to better manage N fertilizer and increase NUE, temporal heterogeneity in N needs should be taken into account and N should be applied around V6 to V8 growth stages of corn. Accordingly, spatial heterogeneity of N-needs should also be taken into account for optimal N use.

The soil fertility varies from one location of the field to the other and N-fertilizer application can be spatially modulated according to soil fertility. Based on the maximum N

uptake period, the appropriate N rate should be decided between V6 and V8 growth stage of corn. However, current proximal canopy sensing tools using normalized difference vegetation index (NDVI) provide a poor correlation with yield prior to V8 growth stage of corn (Elwadie et al., 2005; Teal et al., 2006; Martin et al., 2007). Using leaf reflectance sensors, plants with N deficiency seems to be detected too late for practical implementation of site-specific N management. Another emerging approach for the detection of N deficiency in corn is the use of fluorescence sensor.

Fluorescence is a property of certain pigments, the fluoreophores, which re-emit light after being exposed to light. Chlorophyll is a fluorescent pigment that emits fluorescence in the red to far-red (690 nm to 740 nm) regions of the light spectrum after light excitation (Buschmann et al., 2000). Fluorescence emitted in the red to far-red region of the spectrum is often referred to as chlorophyll fluorescence (ChlF) and can be used to assess plant chlorophyll content (Lorenzen, 1966). Chappelle et al. (1984) observed significantly different ChlF emission between corn plants with complete nutrient supply and corn plants with N-deficiency at both 690 nm and 740 nm. A fluorescence based index, called the nitrogen balance index (NBI), exploit the ratio of far-red fluorescence excited by UV light to red fluorescence excited by either green or red light to detect nitrogen deficiency pf green plants (Cartelat et al., 2005).

The hypothesis of this study was that the sensing of fluorescence has the potential to detect N-deficiency in corn earlier than V8 growth stage. The specific objective was to determine if fluorescence sensing can detect differences in corn plants treated with four different N rates before V8 growth stage of corn.

MATERIALS AND METHODS

This experiment was conducted in a greenhouse at Colorado State University from October 2010 to February 2011. The soil used for this experiment was collected at Colorado State University's Agricultural Research Development and Education Center, located in Fort Collins, Colorado ($40^{\circ} 40' 38.24'' N$, $104^{\circ} 58' 44.76'' W$). In this field, soil was sampled in five locations and was sent for nitrogen content analysis. Among the five locations, the one with the lowest residual nitrogen content was chosen to take the soil used for the greenhouse study. The soil was sieved at 5 mm. A composite sample from the soil collected at this location was sent for analysis. Soil texture was classified as a sandy clay loam and residual NO₃-N content was 1.7 mg/kg.

Corn (*Zea maize* L.) plants (variety DKC45-79) were grown in 11 liters plastic pots. Each pot contained 8 kg of soil. Four nitrogen treatments were used: control (0 kg/ha), low (75 kg/ha), intermediate (150 (kg/ha) and high (225 kg/ha). For each nitrogen rate, 20 pots were prepared, giving a total of 80 pots. Prior to planting, reagent grade fertilizer was added to each pot. Nitrogen was added under the form of ammonium nitrate (NH₄NO₃) at the rate of 0 mg/pot for control pots, 583 mg/pot for low N pots, 1167 mg/pot for intermediate N pots and 1750 mg/pot for high N pots. For each of the eighty pots, 2899 mg of potassium phosphate (KH₂PO₄) and 53 mg of zinc sulfate (ZnSO₄) were added. There were three corn

plants per pots. Weeds were hand removed every other day. Water was supplied by drip irrigation every day.

The sensor used for this study was the Mutiplex®3 hand-held multi-parameter optical sensor (FORCE-A, Orsay, France). The sensing area is about 10 cm in diameter. The Multiplex®3 was set to make an average over 500 induction/detection cycles for each reading (Table1). The four induction channels are UV, blue, green and red and the three detection channels are yellow (YF), red (RF) and far-red (FRF). The flash induces the emission of fluorescence and filters allow the selection of the wavebands of interest. The Multiplex®3 automatically computes two nitrogen balance indexes (NBI), the green NBI (NBI G) and the red NBI (NBI R; Table 1).

Readings were taken twice a week from V4 to V8 growth stage of corn by holding the sensor 10 cm above the top leaves of each pot. This process was repeated for each of the four sets of pot (different N treatment). At tasseling, plants were cut, dried and weighted.

For each selected parameter and for each growth stage, an ANOVA was used to detect significant difference among fluorescence reading ($\alpha = 0.05$). In the case of significant difference, a Tukey's HSD test was used to compare treatments. The same analysis was done for corn plants dry weights. All statistical analysis was done using the statistical software R with the functions "aov" and "TukeyHSD" (R Development Core Team 2010).

Parameter	Description	Formula*
NBI_R	Nitrogen balance index (red)	$\frac{1}{500}\sum_{i=1}^{500}\frac{FRF_{UV_i}}{RF_{R_i}}$
NBI_G	Nitrogen balance index (green)	$\frac{1}{500}\sum_{t=1}^{500}\frac{FRF_{UV_t}}{RF_{G_t}}$

Table 1. Decemptors used for this study along with their description and formula

*Fluorescence waveband is indicated as FRF for far-red fluorescence and RF for red fluorescence and induction waveband is in subscript. UV=Ultra-violet; G=Green; R=Red.

RESULTS

Nitrogen treatment effect: The different nitrogen treatments had a significant effect on dry weight (Fig. 1). Dry weight resulting from 150 kg/ha N rate and 225 kg/ha N rate were not significantly different from each other's. All other treatments resulted in significantly different dry weights.



Figure 1. Boxplots of the difference in dry weights for the four N rate treatments. Boxplots with notches that do not overlap are significantly different ($\alpha = 0.05$).

<u>Fluorescence:</u> Two parameters were investigated to detect corn N-deficiency. The first parameter was the nitrogen balance index measured with red excitation (NBI_R) and it presented good potential for N-deficiency detection from V5 growth stage of corn (Fig. 2). From V7, all four N-rate treatments were significantly different. The second parameter was the nitrogen balance index measured with green excitation (NBI_G) and it presented good potential for N-deficiency detection from V6 growth stage of corn (Fig. 2). From V7, all four N-deficiency detection from V6 growth stage of corn (Fig. 2). From V7, all four N-deficiency detection from V6 growth stage of corn (Fig. 2). From V7, all four N treatments were significantly different.

DISCUSSION

The main outcome of these results is the fact that induced fluorescence, as measured by Mutiplex®3, enabled the detection of N deficiency prior to V8 growth stage of corn (Fig. 2). Both NBI_R and NBI_G enabled the distinction between the lowest N rate (0 kg/ha) and the highest N rate (225 kg/ha) from V4 growth stage of corn. Previous studies have observed the potential of induced fluorescence to detect N deficiency (Chappelle et al., 1984; Cartelat et al., 2005; Zhang & Tremblay, 2010). However, no paper in the literature has mentioned the potential for induced fluorescence to detect N deficiency at such early growth stages.

Our results indicate that induced fluorescence is a promising approach to detect nitrogen deficiency in corn at early growth stages opening new possibilities for the practical implementation of site-specific nitrogen management. These results were obtained in a greenhouse experiment and field experiment should be implemented to evaluate the potential of this technology in a real corn field.



Figure 2. Bar graphs of the average value of each parameter (mentioned on the left axis), for each growth stages from V4 to V8 (mentioned on the top of the figure) and for each nitrogen rate (legend at the bottom of the figure). Different letters indicate significant difference (α =0.05) within the same growth stage and the same fluorescence parameter.

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REFERENCES

- Buschmann, C., Langsdorf, G. and Lichtenthaler, H. K. 2000. Imaging of the blue, green, and red fluorescence emission of plants: An overview. Photosynthetica 38(4) 483-491.
- Cartelat, A., Cerovic, Z. G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J. L., Jeuffroy, M. H., Gate, P., Agati and G., Moya, I. 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (Triticum aestivum L.). Field Crop. Res. 91(1): 35-49.
- Cassman, K. G., Dobermann, A. and Walters, D. T. 2002. Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. AMBIO: A Journal of the Human Environment 31(2) 132-140.

- Chappelle, E. W., McMurtrey, J. E., Wood, F. M. and Newcomb, W. W. 1984. Laserinduced fluorescence of green plants. 2: LIF caused by nutrient deficiencies in corn. Appl. Opt. 23(1): 139–142.
- Elwadie, M. E., Pierce, F. J. and Qi, J. 2005. Remote Sensing of Canopy Dynamics and Biophysical Variables Estimation of Corn in Michigan. Agron. J. 97(1): 99-105.
- Lorenzen, C. J. 1966. A method for the continuous measurement of in vivo chlorophyll concentration. Deep Sea Research and Oceanographic Abstracts 13(2) 223-227.
- Martin, K. L., Girma, K., Freeman, K. W., Teal, R. K., Tubańa, B., Arnall, D. B., Chung, B., Walsh, O., Solie, J. B., Stone M. L. and Raun, W. R. 2007. Expression of variability in corn as influenced by growth stage using optical sensor measurements. Agron. J. 99(2): 384-389.
- R Development Core Team (2010). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
- Roberts, T. L. 2008. Improving nutrient use efficiency. Turkish Journal of Agriculture and Forestry 32(3) 177-182.
- Scharf, P., Lory, J. A. and Grundler, J. A. 2006. Best Management Practices for Nitrogen Fertilizer in Missouri, MU Extension, University of Missouri-Columbia. IPM1027 http://extension.missouri.edu/explorepdf/agguides/pests/ipm1027.pdf, p.3 (Last visit 03/26/2012)
- Shanahan, J. F., Kitchen, N. R., Raun, W. R. and Schepers, J. S. 2007. Responsive inseason nitrogen management for cereals. Computers and Electronics in Agriculture 61(1) 51-62.
- Teal, R. K., Tubana, B., Girma, K. Freeman, K. W., Arnall, D. B., Walsh O. and Raun, W. R. 2006. In-season prediction of corn grain yield potential using normalized difference vegetation index. Agron. J. 98(6): 1488-1494.
- Zhang, Y. P. and Tremblay, N. 2010. Evaluation of the Multiplex® fluorescence sensor for the assessment of corn nitrogen status. Proceedings of the 10th conference on precision agriculture, July 18-21, 2010, Denver, CO, USA.