Early detection of nitrogen deficiency in corn using fluorescence

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Abstract

Soil scientists have made significant progress in quantifying and characterizing spatial variability in soils using proximal sensing technologies. However, such soil sensing efforts must be coupled with crop sensing to make better and most efficient nutrient management decisions. A new type of sensor using induced fluorescence offers the possibility to detect nitrogen deficiency in corn. This study attempts to determine if fluorosensing allow detection of nitrogen deficiency at early growth stages of corn. Our data shown that fluorescence sensor can detect nitrogen deficiency from growth stage V6 for most fluorescence based parameters. With blue light induction, the nitrogen deficiency can even detect nitrogen deficiency as early as V5 growth stage of corn. At close proximity soil covered with residues can contribute soil background noise in the fluorescence signal, however, above 15 cm this noise is not significant.

Keywords: nitrogen use efficiency, chlorophyll fluorescence, corn

Introduction

Spatial and temporal variability in soils is well documented. In the last decade, soil scientists have made significant progress in quantifying and characterizing spatial variability in soils using proximal sensing technologies. However, such soil sensing efforts must be coupled with crop sensing to make better and most efficient nutrient management decisions. The current commercially available crop canopy sensing tools such as Green Seeker™ provide normalized difference vegetation index (NDVI) that present a poor correlation with yield at early crop growth stages. For example in corn (*Zea Mays* L.) best correlations are attained at crop growth stages of V10 or higher which is too late for farmers to make in-season nitrogen (N) management decisions. (Elwadie et al., 2005; Teal et al., 2006; Martin et al., 2007, Shaver, 2010). Another emerging approach for the detection of N variability and crop need in corn is the use of proximal fluorescence sensor.
The hypothesis of this study was that fluorosensing has potential to detect N-deficiency at early crop growth stage in corn. The specific objectives were (1) to determine if fluorescence sensing can detect differences in corn plants treated with four different N rates and (2) to determine which parameter could consistently and more reliably detect N-deficiency in corn plants and (3) to verify if soil background emits fluorescence that may add noise to the data.

Material and methods

Study site
This study was conducted in a greenhouse at Colorado State University from October 2010 to February 2011. Corn (*Zea maize* L.) plants (variety DKC45-79) were grown in 11 liters plastic pots of 25 cm in diameter by 23 cm high. Each pot contained 8 kg of soil. Four nitrogen treatments were used for this study: 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 by removing 3 cm of top soil and adding the liquid fertilizers in order to have the products near the root zone. Three seeds were laid in place in a row and top soil was put back. Water was supplied by drip irrigation at the

rate of 80 ml per day until crop growth stage V6, when it was increased to 160 ml per day. Because of the discrepancy with the sensor during the V2 to V4 growth stages of corn, the experiment was repeated with only 6 pots per treatment. All materials and procedures were the same.

Fluorescence Sensor

The sensor used for this study was the Mutiplex®3 hand-held multi-parameter optical sensor (FORCE-A, Orsay, France). This sensor exploits a signal emitted by plant fluorescent pigments (fluorophores) after excitation. The four excitation channels are UV (375 nm), blue (450 nm), green (510 nm) and red (630 nm) and the three detection channels are yellow, red and far-red. For the purpose of this study, four ratios out of the ten that are automatically computed by the Multiplex®3 plus one ratio computed from fluorescence signals were used (Table 1). The sensing area is about 10 cm diameter. The Multiplex®3 was set to make an average over 500 induction/detection cycles for each reading. More details about the sensor can be found in Cerovic et al. (2009).

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

Parameter	Description	Formula*
NBI_R	Nitrogen balance index (red)	FRF_{UV}/RF_{Red}
NBI_G	Nitrogen balance index (green)	FRF_{UV}/RF_{Green}
NBI_B **	Ratio of UV induced far-red fluorescence on blue light induced red fluorescence	FRF_{UV}/RF_{Blue}
SFR_R	Simple fluorescence ratio (red)	FRF_{Red}/RF_{Red}
SFR_G	Simple fluorescence ratio (green)	FRF_{Green}/RF_{Green}

*Excitation waveband is in subscript **This parameter was not automatically computed by the sensor and was calculated with signal average.

Sensor Readings

Readings were taken twice a week from V4 to V9 crop growth stage. The pots were laid out in rows that were 75 cm apart from each other's. There were five pots in each row. Five readings were taken on the center row by holding the sensor static, 10 cm above the canopy at five random spots. This process was repeated for each of the four set of pots (different N treatment). At tasseling, plants were cut, dried and weighted. For the assessment of soil background noise, five readings were taken on the two soil covers at eleven different heights from 10 cm to 60 cm in increments of 5 cm.

Statistical analysis

For each selected parameters and for each growth stage, an ANOVA was performed to detect significant difference among fluorescence readings (a=0.05). In the case of significant difference, a Tukey's HSD test was used to compare treatments. All statistical analyses were done using the statistical software R with the functions "aov" and "TukeyHSD" (R Development Core Team 2010).

Results

Nitrogen treatment effect

The different nitrogen treatments had a significant effect on dry weight (Figure 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.

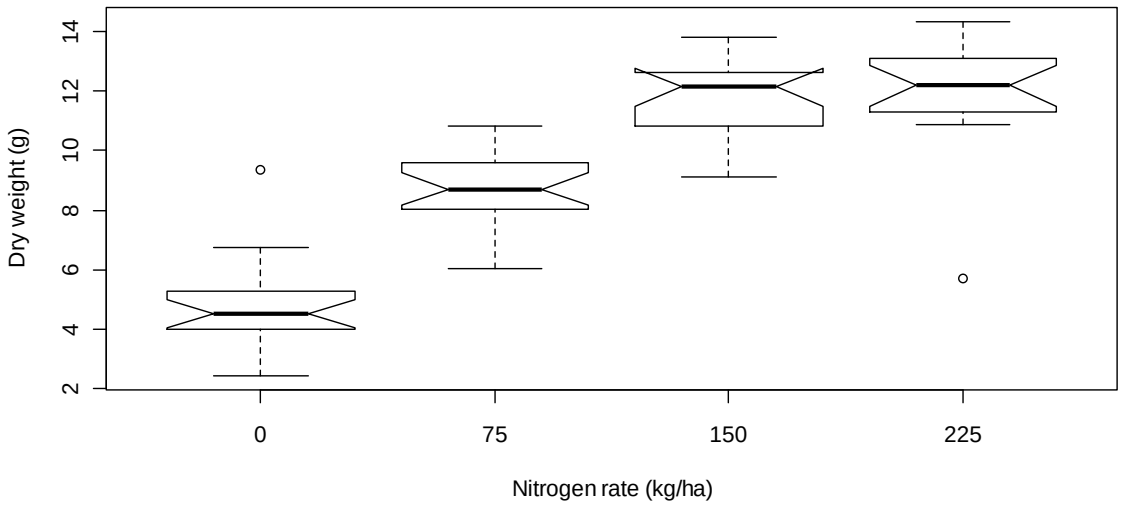

Figure 1. Boxplots of the difference in dry weights for the four N rate treatments. Boxplots with notches that do not overlap are significantly different (α=0.05).

Fluorescence

Five fluorescence based parameters were investigated to detect corn N-deficiency. Both simple fluorescence ratio (SFR_R and SFR_G) presented good potential for N-deficiency detection from V6 growth stage of corn (Fig. 2). The nitrogen balance index measured with red excitation (NBI_R) and it presented good potential for N-deficiency detection from V6 growth stage of corn (Fig. 2). From V7, all four N-rate treatments were significantly different.

Soil background effect

Fluorescence emitted from soil was detected at close distance above ground (Fig. 3). For the interpretation, ground emitted fluorescence was compared with the fluorescence emitted by a corn plant at V6 growth stage with 75 kg/ha supply of nitrogen. At 10 cm above ground, the fluorescence emitted was sometimes higher than the fluorescence from corn (RF_UV on crop residues in Fig. 3). At more than 15 cm above ground, no signal was significantly different from fluorescence recorded at 60 cm above the ground. Crop residues had a significant impact on fluorescence emission for all signals.

Discussion

The methodology used to produce corn plants with different levels of nitrogen deficiency was proven efficient by the significant dry weight differences that existed among N-treatments. From V7 growth stage of corn, all parameters used in this study were able to detect N-deficiency in corn. This was consistent with the findings from previous studies by Chappelle et al., 1984; Cartelat et al., 2005; Zhang & Tremblay, 2010). Prior to V7 however, not all parameters performed equally. The patented nitrogen balance indexes (NBI_G and NBI_R) were more powerful than the simple fluorescence ratios (SFR_G and SFR_R) to detect differences among treatments from V7 growth stage of corn. However, at V6 the detection potential was comparable and even worse in the case of NBI_G. The inferior potential of NBI_G to detect differences at V6 may potentially be explained by the fact that chlorophyll and carotenoids do not absorb green light and thus the light at this waveband penetrates deeper in the mesophyll (Buschmann, 2007).

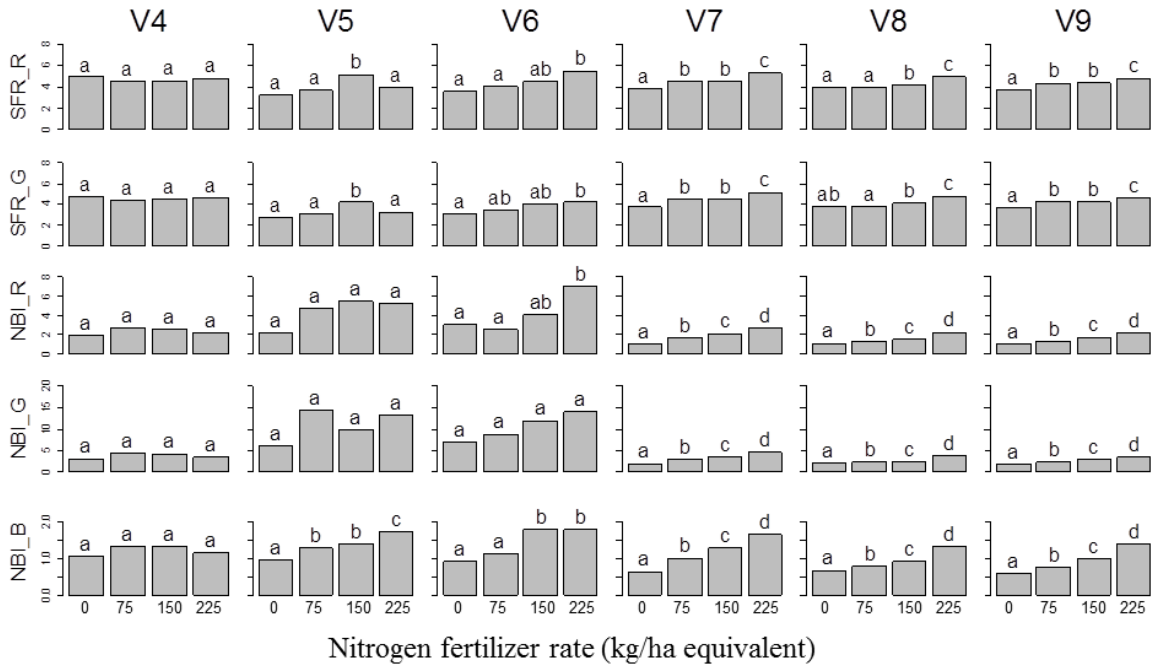

Figure 2. Bar graphs of the average value of each parameters (mentioned on the left axis), for each growth stages from V4 to V9 (mentioned on the top of the figure) and for each nitrogen rate (mentioned at the bottom of the figure). Different letters means significant difference (α=0.05) within the same growth stage and the same fluorescence parameter value.

At 10 cm above ground, the fluorescence emitted by soil cover with crop residues was significantly different from zero. Daughtry et al. 1995 observed that fluorescence could be used to detect the presence of crop residues on the ground. It is was therefore concluded that the ratio of UV induced far-red fluorescence on blue light induced red fluorescence offers a very good potential to detect N deficiency as early as V5 growth stage of corn and that soil background has no effect on the readings at this growth stage.

Acknowledgments

We want to acknowledge Force-A company for lending us the Multiplex®3 sensor. Thanks also to the employees of the greenhouse and to Sam Streeter for technical help.

References

Complete reference list is available upon request