#### **WHAT DO YOU DO WHEN YOUR N-RICH REFERENCE FAILS?**

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#### **Abstract**

In recent years, canopy reflectance sensing has been investigated for in-season assessment of crop N health and fertilization. Typically, the procedure followed compares the crop in an area known to be non-limiting in N (the N-rich area) to the crop in a target area, which may be inadequately fertilized. Measurements from the two areas are used to calculate a relative reflectance to represent the potential need for additional N fertilizer. Establishing N rich areas or strips is often inconvenient for farmers, since this coincides with other demanding spring operations. The objective of this study was to answer the question of what do you do when the N-rich reference fails. Two studies were conducted. In the first, a total of 16 field-scale experiments were conducted over four growing seasons (2004-2007) in three major soil areas of Missouri. Multiple blocks of randomized N rate response plots traversed the length of each field. Each block consisted of 8 N treatments from 0 to 235 kg N ha<sup>-1</sup> on 34 kg N ha<sup>-1</sup> increments, topdressed between vegetative growth stages V7 and V11. Adjacent to the response blocks, N-rich  $(235 \text{ kg N} \text{ ha}^{-1})$  reference strips were applied at or just after planting. Crop canopy reflectance sensor measurements in the format of inverse simple ratio values (Vis/NIR) were obtained from the N response blocks and adjacent treatment strips at the time of top-dress N application. Viewed in frequency distribution diagrams, canopy sensor ISR values for target corn were almost always higher than those for N-rich corn, had a greater range of values, and were more positively skewed. A model was developed successfully predicted 75% of the variation in average N-rich reference sensor measurements. In the second study the effect of hybrid on canopy sensor readings was explored. Canopy sensing (Crop Circle) readings were taken through most growth stages from V3 to V10 on 11 (2008) and 8 (2009) hybrids. Variability within and between hybrids was most noted for corn less than 60 cm in height. Results showed that soil type and soil surface wetness impacted canopy readings more than hybrid. The results could prove useful in determining reasonable ranges for N-rich reflectance values in variable rate N applications.

#### **Introduction**

Crop-canopy reflectance measurements and associated algorithm derivation for N fertilizer recommendations are under development or are currently in use for wheat, corn, cotton, rice, and other crops. Typically the algorithms used to determine how much N to apply are derived by comparing sensor measurements of the crop in an area known to be non-limiting in N (also called N-rich or N-rich reference) to sensor measurements of the crop where N fertilization is likely needed (sometimes called the target area) (Teal et al., 2006; Solari et al., 2008; Sripada et al., 2008; Kitchen et al., 2010). Measurements from the two areas are used to calculate a relative reflectance to represent the potential need for additional N fertilizer. In principle, the greater the difference in sensor measurements between sufficient-N reference crop and un-fertilized or deficiently-fertilized crop, the more N fertilizer is needed. The approach somewhat normalizes the confounding effects numerous management and environmental factors will have on understanding N need for the specific location in question.

An aspect of this technology that has discouraged some farmers from considering adoption is the requirement to have an N-rich reference area. Many farmers are unwilling to consider this approach once they realize the time commitment of the extra tasks coincides with other timedemanding spring operations. The challenge amplifies when, if on the day of canopy sensing and N application, the N-rich reference areas require revisiting because of spatial or diurnal effects on the measurements (Scharf et al., 2007). In some cases, farmers who use the technology have not ensured an area of well-fertilized corn in a field, or they did not adequately mark the area for reconnaissance. Since reflectance characteristics of well-fertilized corn are necessary in calculating the sufficiency index, having a means of estimating these values based on growth stage and/or corn height would be helpful as a contingency. Some have indicated an N-rich reference value could be predicted (Holland and Schepers, 2010). This might be based upon crop growth stage, cumulative growing degree units, or even on sensor measurements taken from the crop that is yet to be fertilized. If a proven procedure could be developed where an N-rich reference value could be predicted, adoption of this technology for N management would be greatly accelerated.

#### **Objective**

The objective of this research was to assess from a range of corn production fields whether Nrich reference values could be determined in ways other than by N-rich reference plots/strips.

#### **Materials and Methods**

Two independent studies were conducted to address unique ways to achieve this objective.

#### **Study 1.**

A total of 16 field-scale (400 to 800 m in length) experiments were conducted over four growing seasons (2004-07) in three major soil areas of Missouri: river alluvium, deep loess, and claypan. In general, these fields were representative of other cropped fields in their locale, with some within-field variability in landscape and soil. Cooperating producers selected the planting date, hybrid, planting population, and prepared and planted each field with their own equipment.

Multiple blocks of N rate response plots were arranged in a randomized complete block design traversing the length of the field. Each block consisted of 8 N treatments from 0 to 235 kg N  $\text{ha}^{-1}$ on 34 kg N  $\text{ha}^{-1}$  increments, top-dressed sometime between vegetative growth stages V7 and V11. Because of plot dimensions differed over the four year period. Details of the design of each year are described in Kitchen et al. (2010). For 2006 and 2007, a complete second set of field-length blocks was also established where either 34 or 67 kg N ha<sup>-1</sup> was uniformly applied over the whole set of blocks shortly after corn emergence. The  $34$  kg N ha<sup>-1</sup> rate was used when the producer had applied  $\sim$  33 kg N ha<sup>-1</sup> during pre-plant operations.

The number of treatment sets varied from 3 to 28 per field, depending on the plot length, length of the field, and whether the study included the second set of blocks with early N fertilization. In all, a total of 223 sets of response plots were obtained from the 16 field experiments. Adjacent to and usually on both sides of the response blocks, N-rich  $(235 \text{ kg N} \text{ ha}^{-1})$  reference strips were also established. These ran the full length of the field and were treated shortly after corn emergence. An AGCO Spra-Coupe (AGCO Corp., Duluth, GA) high-clearance applicator equipped with an AGCO FieldStar Controller was used to top-dress solution UAN (28 or 32% N) fertilizer shortly after emergence in order to establish the N-rich reference strips.

Previous work reported on the relationships of canopy sensor readings and yield response to added N at the time of sensing was given for these fields (Kitchen et al., 2010). Using information from that investigation, we focus here on differences in canopy sensor measurements from N-rich corn and target corn that had only received 0 to 67 kg N ha<sup>-1</sup> near planting time. Crop canopy reflectance sensor (model ACS-210, Holland Scientific, Inc., Lincoln, NE) measurements were obtained within the same day from the corn canopy of N-rich reference strips and the corn where little or no N had been applied at planting (target area). Two sensors were mounted on the front of the applicator at  $\sim 60$  cm above rows 2 and 5 of the 6-row corn strip. As the Spra-Coupe drove through the field, reflectance data and GPS coordinates were recorded on the tablet PC in the Spra-Coupe cab. Canopy sensor data from the N-rich reference area and the target area were collected, and the inverse simple ratio (ISR) (Gong et al., 2003), which is the ratio of the visible to near-infrared (Vis/NIR) measurements was the canopy measurement selected for use in this study. With the ISR, greener healthier corn has a lower value than corn showing an N deficiency.

Frequency distribution figures were created for each site to visually observe the population of sensor readings associated with N-Rich and Target areas. Next, multiple linear regression was used to model the field-average ISR values of N-rich reference corn. Variables considered were field-average ISR values of target corn, standard deviation of target ISR values, crop growth stage, and N amount applied at or near planting.

#### **Study 2.**

Three Missouri sites were chosen for analysis from the 2008 growing season (Columbia, Henrietta, and Marshall) and two sites were chosen from the 2009 growing season (Columbia and Henrietta). Each site was rented ground that was planted and maintained by the University of Missouri Agricultural Extension Service Variety Trial Testing Program. There were 110 corn hybrids used at each site, submitted for trial from different seed companies. For this study, a subset of similar maturing hybrids (~114 day maturity) was selected. Eleven hybrids were used in 2008, and 8 hybrids in 2009. Sites were planted in a randomized block design.

Starting when the corn was about 10 cm tall and on 3-5 day intervals, each site was revisited and a set of measurements obtained. A handheld Crop Circle canopy sensor (Model ACS-210, Holland Scientific, Inc., Lincoln, NE) was used in this study. Readings were obtained by holding the sensor approximately 45 cm over the top of the plants and taking measurements down each of the center two rows of each plot. The data from the Crop Circle were averaged to get an overall inverse simple ratio (ISR) of each plot.

Statistical software (SAS, SAS Institute Inc., Cary, North Carolina, USA), was used to determine important relationships between data sets. After viewing scatter graphs of ISR versus height, it was determined that when corn was ~60 cm there was notable change in ISR values. After this height, it was clear that either plant physiology had changed or that soil color was no longer a contributing factor in the canopy measurements. Therefore the data was split into two growth stage datasets: one less than 60 cm and the other greater than or equal to 60 cm. Proc REG was used to model yield against ISR by year and by growth phase. Proc GLM was used to assess site and hybrid effects. The model was examined by year and growth phase.

#### **Results and Discussion**

#### **Study 1.**

The number of sensor observations collected for each site varied, but ranged from about 2,000 to 13,000/site for N-rich corn and 4,000 to 39,000/site for target corn. Fig. 1 provides relative frequency distribution of ISR canopy sensor readings from 16 site-years. The legend of each siteyear shows the amount of N applied near planting. Sites from 2006 and 2007 include two target



**Figure 1. Relative frequency of inverse simple ratio (ISR) canopy sensor readings for 16 fields over 4 growing seasons.** 

areas, as described in the methods. A quick glance at these frequency figures shows great variance from site to site when comparing values from the N-rich reference to the target corn.

As a population, canopy sensor ISR values for target corn were almost always higher than for Nrich corn, had a greater range in values, and were more positively skewed. Higher values of ISR represent corn under greater N stress, because of less biomass and/or less leaf chlorophyll. Relative differences between the target corn and the N-rich corn can be partially explained by three factors. First, the more mature the crop at the time of sensing, the closer to canopy closure, such that less soil is in view of the sensor with it in the nadir position. As this happens, both the target corn and the N-rich corn tend to converge to values in the 0.10 to 0.25 range. Second, the amount of N fertilizer applied either before planting or shortly after emergence helps explain differences in sensor values for some sites. From this study we have target corn receiving a range of N from 0 to 67 kg N ha<sup>-1</sup>. Greatest differences between N-rich corn and target corn generally occurred when the target corn had no early N (compare frequency distributions of COP04, HAC07, and SAN07 sites). Third, differences in N-rich and target corn sensor readings were associated with within- and between-field variability of soil N-supplying capacity. For example, the GEB07 field was on a soil that showed great potential for mineralizing N, and therefore only subtle differences are seen when comparing target and N-rich corn. The greatest within-field soil variation occurs with alluvial soils from along riverways.

With most sites the distribution of readings for N-rich reference corn values is lower (meaning greater biomass and/or greener plants) and is less positively skewed when compared to the population of readings from the target corn. This result showing different populations of readings is consistent evidence of the sensor's ability to detect differences in N status of the crop. The distribution shift can generally be seen as a two-step shift to lower values for 2006 and 2007 sites, where two target N areas were included. For the N-rich corn in this study, the lowest ISR values obtained were typically in the 0.10 to 0.15 range.

As indicated earlier, the primary rationale for this analysis was that many farmers see the process of creating and managing for N-rich reference areas as time-consuming, and therefore a barrier to adoption. Regression models were developed for predicting the field-average N-rich reference obtained from this 16-field dataset. One model illustrated in Fig. 2 shows that the average ISR value of the target corn and growth stage alone could be used to model 75% of the variation in ISR of N-rich reference corn.

This analysis provides promising evidence that an N-rich reference could be predicted directly from corn that is being sensed for fertilization. Operationally, when starting on a new field, the applicator vehicle equipped with sensors could quickly obtain enough values to calculate a value



Figure 2 A model developed to predict N-rich reference ISR values.

to represent the N-rich reference. Then as the vehicle continued through the field, that N-rich value could be adjusted to include the additional measurements being collected. Concepts of this approach are similarly advocated in another study (Holland and Schepers, 2010).

A real advantage of this procedure is that the N-rich reference is being derived from readings over all areas of the field being sensed, not just a small reference area (often near the field entrance) that may not be representative of the field as a whole. Thus, the procedure automatically captures the effects that within-field soil and landscape variations have on the sensor values. Similarly, any daily temporal factors that might affect sensor readings [e.g., leaf wetness, sun angle, sensor drift, clouds (Scharf et al., 2007)] could be easily accounted for by including a time filter which uses only recently collected sensor measurements when estimating the N-rich reference value.

#### **Study 2.**

The graphs of corn ISR versus plant height (Fig. 3) for all hybrids show a very distinct trend similar to an exponential decay model. In the early stages of growth when corn was  $\sim$ 20 cm tall, ISR values were in the 0.3 to 0.5 range. This was due to the small amount of corn biomass, with the sensor detecting mostly soil. Once plants attained a height of 60 cm, there was a noticeable shift in the ISR readings, with ISR values decreasing much more slowly as height increased beyond that point.



Fig. 3. Reflectance as ISR values for 11 hybrids in 2008 (left column) and 8 hybrids in 2009 (right column) in relation to crop height.

From AOV, site significantly impacted response variables in both growth stages in 2008, but was less of a factor in 2009 (AOV results not shown). Part of this may be due to the fact there was only 2 sites in 2009, and therefore less statistical power in analysis for the second year. The soils of these study sites are quite contrasting, with the Columbia site being on a claypan soil, the Henrietta site being on an alluvial soil, and the Marshall site on a loess soil. With these differences came differences in surface soil color, soil depth and overall soil productivity. Therefore, the fact that site was found to be significantly different was not surprising.

Hybrid only significantly affected ISR during growth stage 2 of 2008 (data not shown). Both biomass and color of the soil and crop are contributing factors to the reflectance measurements obtained by the canopy sensor. By examining the significance values for height (an indicator of biomass) and SPAD (an indicator of color), one might gain a better understanding of the relative contribution to ISR. Hybrid had no significant effect on height, whereas it consistently affected SPAD readings as a function of plant color.

To determine the norm of what one would expect to see in a typical growing season, boundary lines were drawn with both years of ISR data combined (Fig. 4). This provides better



Fig. 4. Canopy reflectance as ISR values combined over both years, relative to crop height. Drawn on the graph are upper and lower ~98% boundary lines.

understanding of limits of ISR for healthily corn as a function of crop height. The boundary line concept uses a subset from the "best and worst performing points" from a graph and by using either an equation to create a curve or drawing in a curve, the upper and lower boundary is defined (Kitchen et al., 1999, Webb 1972). It is assumed that points between the lines represent plants that were grown under typical growing conditions and performed normally; whereas, points not between the boundary lines represent unusual conditions (Kitchen et al., 1999). Thus, for corn that is 60 cm tall, one would expect ISR values to range from about 0.15 to 0.24. If sensor readings exceeded 0.24, some other factor (e.g. crop stand) was likely compromised. If ISR values were less than 0.15 at this corn height, other issues (e.g. poor weed control, sensor malfunction) might be considered.

#### **Summary**

This analysis of active crop-canopy reflectance sensing was conducted with two studies. In study one, on field-average values of N-rich reference and target area corn were compared. While our results look promising for not having N-rich reference areas, further evaluation is needed before advocating such a strategy to farmers. For example, this analysis was obtained from the whole-field populations of sensor readings and should be compared to a similar analysis done only from portions of the field. Further, verification of N-rich reference prediction models using independent datasets obtained from other fields is needed. If this N-rich reference strategy proves consistent and reliable for making in-season N fertilization recommendations, more farmers will likely be interested in adopting this technology.

In the second study examining well N fertilized hybrids, seasonal changes in canopy sensor reflectance were well documented. This data quantifies the potential range of sensor readings one might expect for well fertilized corn. The data could be used to set boundaries to the variation that users might expect to see in canopy measurements of well fertilized corn over the growing season. Early in the season, ISR was predominantly affected by soil variations, but as the season progressed, these variations became less of a factor. Little significant effect by hybrid was found on ISR. When no N reference is available, these findings could be used by farmers to form a contingency N reference estimate for variable rate N applications.

#### **Disclaimer**

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