2013

Use of a virtual-reference concept to interpret active crop canopy sensor data

Kyle H. Holland
Holland Scientific, kholland@hollandscientific.com

James S. Schepers
Agricultural Research Service, james.schepers@gmail.com

Precision Agric (2013) 14:71–85
DOI 10.1007/s11119-012-9301-6

Use of a virtual-reference concept to interpret active crop canopy sensor data

Kyle H. Holland · James S. Schepers

Published online: 18 December 2012

Abstract Active crop canopy sensors make possible in-season fertilizer nitrogen (N) applications by using the crop as a bio-indicator of vigor and N status. However, sensor calibration is difficult early in the growing season when crops are rapidly growing. Studies were conducted in the United States and Mexico to evaluate procedures to determine the vegetation index of adequately fertilized plants in producer fields without establishing a nitrogen-rich reference area. The virtual-reference concept uses a histogram to characterize and display the sensor data from which the vegetation index of adequately fertilized plants can be identified. Corn in Mexico at the five-leaf growth stage was used to evaluate opportunities for variable rate N fertilizer application using conventional tractor-based equipment. A field in Nebraska, USA at the twelve-leaf growth stage was used to compare data interpretation strategies using: (1) the conventional virtual reference concept where the vegetation index of adequately fertilized plants was determined before N application was initiated; and (2) a drive-and-apply approach (no prior canopy sensor information for the field before initiating fertilizer application) where the fertilizer flow-rate control system continuously updates a histogram and automatically calculates the vegetation index of adequately fertilized plants. The 95-percentile value from a vegetation-index histogram was used to determine the vegetation index of adequately fertilized plants. This value was used to calculate a sufficiency index value for other plants in the fields. The vegetation index of reference plants analyzed using an N-rich approach was 3–5 % lower than derived using the virtual-reference concept.

Keywords Real-time sensors · Algorithm · Sufficiency index · Nitrogen · Vegetation index

K. H. Holland
Holland Scientific, Lincoln, NE, USA
e-mail: kholland@hollandscientific.com

J. S. Schepers (✉)
Agricultural Research Service, United States Department of Agriculture, and Agronomy and Horticulture Department, University of Nebraska, Emeriti, Lincoln, NE, USA
e-mail: james.schepers@gmail.com

Introduction

Calibration of laboratory and field instruments usually involves correlating data collected from a selected device with a concentration in some type of accepted standard material, compound, or material. This approach works well for most physical and chemical measurements; however, such procedures become problematic when dealing with plants that go through a number of phenological stages during the growing season. In addition, genetic differences between cultivars can affect the architecture of plant canopies and relative color of the leaves. While laboratory procedures can be used to quantify parameters like leaf nitrogen (N) concentration, interpreting such data for the purpose of making management decisions is difficult because of cultivar and growth stage differences. Other factors such as cropping history, previous manure applications and cultural practices can also affect crop vigor and color. For these reasons, Peterson et al. (1993) utilized the findings of Schepers et al. (1992) and proposed normalizing Minolta SPAD chlorophyll meter readings (Minolta Corporation, Ramsey, New Jersey, USA)[1] to reference a situation that was known to have received modestly excessive amounts of N fertilizer. The reference crop needs to be managed the same as the rest of the field or treatments except for having received enough N so that the crop is not N deficient. This field situation is sometimes referred to as being “N-rich”. During the normalization process, the SPAD meter readings from the plants in question are divided by the reading from the reference plants. The resulting quotient was originally termed the “Sufficiency Index” (SI) and used to make decisions regarding fertigation of corn in Nebraska, USA. Later the concept was extended to guide sensor-based in-season N fertilizer recommendations (Biggs et al. 2002). Some scientists prefer to discuss in-season crop vigor measurements in terms of the potential for a yield response so they invert the SI value and call it a “Response Index” (Raun et al. 2001). It should be noted that the reason the N-rich reference concept works to normalize SPAD data is because a little extra N availability is not harmful to corn plants. At some point along the scale of N adequacy, another nutrient becomes limiting and subsequent N uptake amounts to “luxury consumption”. At this point, leaf chlorophyll status is maximized as recorded by SPAD meters.

The SI concept was developed for research plots that were intentionally positioned on the landscape to minimize spatial variability. Extending the normalization concept to whole-field situations that have likelihood for considerable spatial variability raises questions related to determining the appropriate reference value. If one assumes that additional N fertilizer in selected areas of the field will reduce or remove the spatial variability in yield, then the task at hand would be to characterize an area with adequate N and use the SPAD value from that area as the reference for the entire field. In practice, producers following this strategy typically install one or more N-rich strips in their fields to use as the reference (Raun et al. 2010). For convenience and to simplify record keeping, producers would prefer to use the same area of the field as the N-rich reference year-after-year. However, using the same area of the field as the N-rich reference for a second year violates the premise that the reference should represent the nutrient status of the rest of the field in all respects except for having received additional N fertilizer. Therefore, it is imperative that the N-rich reference strips be moved to a new area each year.

[1] Mention of a company or trade name does not imply endorsement by the USDA-ARS or the University of Nebraska.

Raun et al. (2005) observed spatial variability in plant vigor within N-rich strips in most wheat fields so they developed sprayer equipment to establish a grouping of nine small N-rate plots (each 1 m^2) in a 3 × 3 configuration. This application device allowed them to readily place many mini-N rate plots within a field. However, they found the border effect between N-rate plots made it difficult to clearly identify the reference plot within the group of nine plots. They subsequently transformed the 3 × 3 grouping into more N rates in larger plots within a field strip with progressively higher or lower N rates (referred to as ramped calibration strips). Each sub-plot was typically ~16-m long so multiple ramps of nine N rates could be established within a field strip. They soon realized that soil properties frequently changed substantially within the distance of one complete ramp (perhaps 150 m total length). Scharf et al. (2005) modified the ramp concept by establishing a series of adjacent N-rate strips that could be harvested with a combine fitted with yield-monitoring equipment. The yield map was broken into 16-m long segments which allowed them to construct a series of N-response functions along the length of the field strip. It should be noted that the yield values were subject to the uncertainties associated with yield mapping and the series of N rates were only randomized between strips. Solari et al. (2008) enlarged the mini-N rate concept so that sub-plots were the width of the planter by 16-m long to keep the N-rate sub-plots in close proximity (using either 2 × 2 or 3 × 3 groupings). This design addressed the need for randomization within a grouping and made it possible to evaluate the effect of soil properties on the shape of individual N-response functions. Positioning the check plot (zero N) in the middle of a 3 × 3 grouping provides useful comparisons to assess the influence of N mineralization for each region of the field. Hand harvesting of such studies provides excellent data, but the approach is quite laborious. Scientists in the Midwest of the United States have begun establishing multiple N-rate groupings in selected fields according to management zones with initial findings that show yield plateau and shape of the N-response functions vary within fields and especially between fields (Solari et al. 2010; Roberts et al. 2012).

The above findings indicate that the in-season vegetation index value (integration of chlorophyll status and the amount of biomass) of "reference" plants probably needs to be determined by management zone rather than for an entire field because yield potential varies within a field. The goal of this research was to test and evaluate an analytical method developed by Holland (2009) to interpret active sensor data and thereby systematically determine the vegetation index (VI) value of reference plants without establishing an N-rich strip as suggested by other approaches (Holland and Schepers 2011). This approach is termed "virtual reference" because it statistically characterizes plants that demonstrate a level of vigor that is comparable to those commonly found within an N-rich strip, but without having to actually apply extra N fertilizer, a practice that is restricted in some countries or situations. It is anticipated that producers and commercial applicators may find it inconvenient to drive through a field to establish a reference-plant VI value using the virtual-reference concept before making variable-rate N applications. An auto-interpretation approach called "drive-and-apply" was developed to build and update a VI histogram upon entering a field while making variable-rate applications. A final goal is to compare the recommended N rates using the drive-first virtual reference approach with a modified virtual reference approach that continuously updates a histogram of VI data and calculates a progressively more inclusive reference VI value while driving through the field and making N applications.

Materials and methods

This study was conducted in maize fields in Mexico (longitude −107.479764 and latitude 24.749025) at the V5 growth stage (Ritchie et al. 1997) and in Nebraska, USA (longitude −98.764934 and latitude 40.751016) at the V12 growth stage. Both fields had been graded for furrow irrigation (0–1 % slope) but the Nebraska field was under linear-drive sprinkler irrigation at the time of the study. The soil in Nebraska is classified as a Hord silt loam (fine-silty, mixed mesic Pachic Haplustolls) while the soil in Mexico is unclassified with a silty-clay loam texture. Both fields were under 8-row wide management strips planted at populations of 100 000 plants ha^{-1} with 0.75-m row spacing in Mexico and 74 000 plants ha^{-1} with 0.91-m row spacing in Nebraska.

The field strips in Mexico were 630-m long and planted to white maize with a pre-plant N application of 80 kg N ha^{-1} as urea-ammonium nitrate (UAN). This study involved three replications but only one transect was used in the analysis because of the large amount of data involved (continuous stream of 3 250 data points). The 400-m long strips in Nebraska had been planted to continuous yellow maize since 1991 with five N rates (0, 50, 100, 150 and 200 kg N ha^{-1}) applied to the same plots at planting. Other strips were in a corn/soybean rotation or in continuous corn with a base N rate of 150 kg ha^{-1}. Individual plots were each 16-m long and separated with a 1-m wide bare-soil alley. Data are presented as treatment means. Each strip accommodated four replications of randomized treatments. Two strips were involved in the study, thus providing eight replications.

At the V5 growth stage (1 February, 2010) in Mexico, two Crop Circle ACS-210 (Holland Scientific, Inc., Lincoln, Nebraska, USA) active sensors were mounted on a bar in front of a tractor. Sensors were positioned at least 600 mm above the tallest plants in rows two and three of the 8-row plots using a 4-row variable rate liquid fertilizer applicator. These sensors were set to record canopy reflectance in the amber (595 nm) and near infrared (NIR, 880 nm) wavebands at 5 Hz to correspond with GPS data collected at the same rate. Rate of travel through the field was $\sim$5.8 km h^{-1} ($\sim$1.6 m s^{-1}) which amounted to a set of recorded sensor readings about every 320 mm (average of approximately four plants).

At the V9 (14 July, 2009) and V12 (21 July, 2009) growth stages in Nebraska, two Crop Circle ACS-470 active sensors were mounted on a John Deere high-clearance sprayer. Sensors were positioned at least 600 mm above the tallest plants in rows three and six of the 8-row plots. These sensors were set to record canopy reflectance in the red (670 nm), red-edge (730 nm) and near infrared (NIR, >760 nm) wavebands at 5 Hz to correspond with GPS data collected at the same rate. Rate of travel through the field was $\sim$4.5 km h^{-1} ($\sim$1.25 m s^{-1}) which amounts to a set of recorded sensor readings about every 250 mm (average of approximately two plants).

Nitrogen application model and calibration method

The N application model utilized in this research involved directly inserting normalized sensor data (SI values) into a generalized plant growth function. The SI is defined as the ratio of a real-time sensed crop property to the same measurement from a known or standard crop (reference) and is described mathematically as

$$SI = \frac{VI_{\text{Sensed Crop}}}{VI_{\text{Reference}}} \tag{1}$$

where, SI is the sufficiency index ($0 \leq \mathrm{SI} \leq 1$), $\mathrm{VI}_{\mathrm{Sensed\ Crop}}$ is the vegetation index (or measurement) of the sensed crop, and $\mathrm{VI}_{\mathrm{Reference}}$ is the vegetation index (or measurement) of the non-N limited crop.

The SI calculation applies to any VI or in-season crop parameter. When the expression is applied to harvest data it represents relative yield. All data were processed using the Chlorophyll Index (CI) developed by Gitelson et al. (2003, 2005) in Eq. 2. The CI term for the reference and sensed crops in Eq. 2 were substituted for the generalized VI terms in Eq. 1. The CI has the following mathematical form:

$$CI_{\mathrm{SR}} = \left[\frac{\rho_{\mathrm{NIR}}}{\rho_{\mathrm{SR}}} - 1\right] \tag{2}$$

where, ρ_{NIR} is the near infrared (NIR) waveband reflectance and ρ_{SR} is the spectral waveband reflectance (SR = 730 nm for red-edge with ACS470 sensor or SR = 595 nm for amber with ACS210 sensor).The N application model developed by Holland and Schepers (2010) was utilized for this research. The N application model, incorporating a back-off function, has the mathematical form:

$$N_{\mathrm{APP}} = \left(N_{\mathrm{OPT}} - N_{\mathrm{PreFert}} - N_{\overline{\mathrm{OM}}}\right) \cdot \sqrt{\frac{(1 - \mathrm{SI})}{\Delta SI \cdot \left(1 + 0.1 \cdot e^{m \cdot (\mathrm{SI}_{\mathrm{Threshold}} - \mathrm{SI})}\right)}} \tag{3}$$

where, N_{OPT} is the economic optimum N rate (EONR) or the maximum N rate prescribed by producers, N_{PreFert} is the sum of fertilizer N applied prior to crop sensing and/or in-season N application, $N_{\overline{\mathrm{OM}}}$ is the N credit for the average organic matter content within the field. SI is the sufficiency index, ΔSI is the sufficiency index difference parameter, m is the back-off rate variable ($0 < m < 100$) and $\mathrm{SI}_{\mathrm{Threshold}}$ is the back-off cut-on point.

The EONR can be calculated using yield data from an N-rate response study. Monetary values are assigned to the value of grain and cost of N fertilizer to generate a profit function such that EONR occurs when the cost of the last unit of N fertilizer equals the value of the increased yield associated with the last unit of fertilizer N application. The N_{OM} values are typically estimated to be 20–30 kg N ha^{-1} for each 1 % organic matter, but should be verified by local extension specialists. The ΔSI parameter is the difference between the SI of healthy plants (SI = 1.0) and those that can no longer recover to full-yield conditions with the addition of N fertilizer (i.e. relative yield <1.0). A back-off function is needed to reduce the rate of N application in situations with reduced yield potential (i.e. plant density and vigor problems). The back-off parameters (m and $\mathrm{SI}_{\mathrm{Threshold}}$) allow the user to determine how fast the N application rate declines to zero.

For the Nebraska analysis, the back-off function in Eq. 3 was incorporated to conserve N for SI values <0.65. The rate parameter m determines the rate at which the N application model decreases the N application rate and the $\mathrm{SI}_{\mathrm{Threshold}}$ determines when the back-off function starts to limit N supply. For this work, m was set to 40, $\mathrm{SI}_{\mathrm{Threshold}}$ was set to 0.65 and ΔSI was set to 0.30. The organic matter credit $N_{\overline{\mathrm{OM}}}$ was set to 40 kg ha^{-1} for this research. This value was based on the test site average soil organic matter of 2 %. The back-off feature and organic matter correction were not engaged during the analysis of the Mexico data.

Sensor calibration was performed by statistically analyzing the entire string of real-time sensor data from Mexico. In Nebraska, the data were post processed by manually discarding values from the bare-soil alleys and associated plot borders with mixed vegetation/soil reflectance (usually 2–3 sets of readings at the end of each plot). A histogram of the

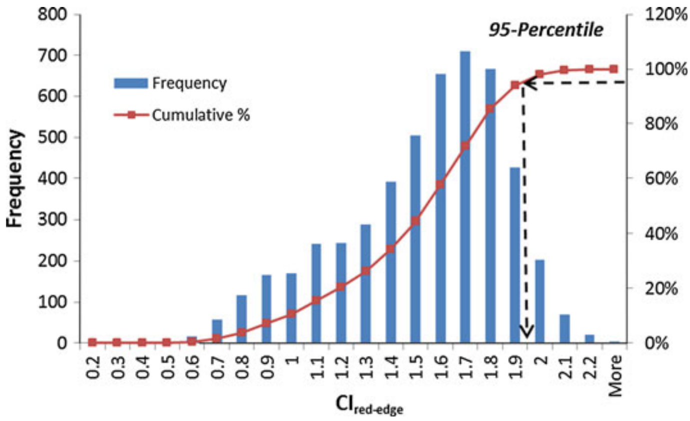

Fig. 1 Red-edge chlorophyll index distribution and cumulative percentile for maize plants grown under irrigation in Nebraska (V12 growth stage). The 95-percentile value was utilized as the reference for N-sufficient plants

amber or red-edge CI values ($CI_{red\text{-}edge}$) was constructed to examine the shape of the distribution function. The 95-percentile cumulative value from the histogram was selected as the reference for each replication from which to make SI calculations and simulate fertilizer N applications (Fig. 1). Accumulation was performed from the lowest CI bin to the highest CI bin. The 95-percentile point was determined from the histogram data via linear interpolation and used to calculate SI values. Fertilizer N recommendations were calculated using the algorithm of Holland and Schepers (2010).

Results and discussion

Figure 1 illustrates the basis of the virtual reference concept by plotting the frequency of each bin or grouping of VI values obtained within a field strip or area. Analytical routines within Microsoft Excel were used to generate this histogram having the desired range in bin values. Selecting the 95-percentile value from the histogram as the VI for reference plants was somewhat arbitrary, but coincides with the 95 % SI value proposed as the threshold to initiate fertilization when using SPAD meters (Peterson et al. 1993).

Reference vegetation index comparison

Sensor data collected while driving through a producer's maize field in Mexico was used to compare approaches when determining the VI of reference plants (Fig. 2). Many plants in this field strip would have had an adequate supply of N because these plants had received 80 kg N ha^{-1} at planting time (injected 80–100 mm from the seed-row) and plant N uptake at the V5 growth stage would have been <40 kg N ha^{-1}. The reference CI_{amber} value (using ACS-210 sensors) determined for the 3-s approach used with GreenSeeker active sensors (Trimble Navigation Limited, Sunnyvale, California, USA; formerly NTech Industries, Ukiah, California, USA) was 5.057. This approach uses the highest 3-s running Normalized Difference Vegetation Index (NDVI) value in a field strip as the reference VI value. The 95-percentile CI value for adequately fertilized maize (i.e. vegetation index of

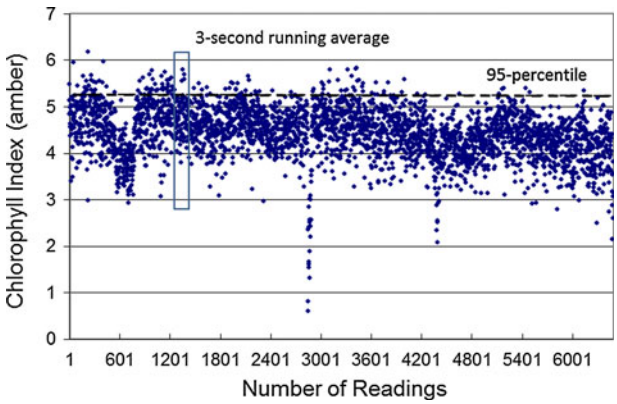

Fig. 2 Chlorophyll index values collected with pair of ACS210 amber sensors at 5 Hz in Mexico on maize at V5 growth stage while driving through field. Rectangle shows area of field with highest 3-s average value (CI = 5.057) and dashed line shows 95-percentile value (CI = 5.206) from histogram of CI values. Average CI = 4.430

reference plants) using the virtual reference approach was 5.206. The 3-s value was 2.9 % lower than the 95-percentile value, but both were considerably higher than the average CI of 4.430 that is sometimes preferred as the reference.

Effect of growth stage on vegetation index

It is important to have reliability in the information generated by crop canopy sensors, but reference crop VI values will trend over time. Growth stages that are conducive to in-season N applications depend on the crop-clearance capabilities of the implements that carry the sensor. Any trend in VI values of reference plants depends on the structure of the specific VI used. Early in the growing season, NIR reflectance values typically increase rapidly until canopy closure occurs. Thereafter, the canopy becomes denser and NIR reflectance continues to increase at a slower rate. However, at some point in time the footprint of the sensor only captures the upper part of the canopy that has a relatively constant density and so NIR reflectance tends to be stable until the crop begins to mature. In contrast, visible reflectance of plants with adequate nutrition decreases as crops grow because photosynthesis captures progressively more of the visible light. About the time of canopy closure, red reflectance reaches a near-zero level until the crop approaches maturity (Gitelson and Merzlyak 1996). Red-edge reflectance remains responsive to plant chlorophyll status over much of the growing season (Gitelson et al. 2003). In the case of a high-clearance applicator, the average $CI_{red\text{-}edge}$ reference value was 2.105 using the 95-percentile approach at the V9 growth stage for the Nebraska study, which is comparable to the 1.947 value at V12 (Table 1). The slightly lower $CI_{red\text{-}edge}$ value at the V12 growth stage is attributed to continued N uptake during this 7-day period of rapid growth. The three-leaf difference in growth stage resulted in statistically different reference VI values ($P < 0.05$ using ANOVA). Spatial variability across the eight replications was quite low (CV = 3.8 and 2.8 % at V9 and V12, respectively).

Sufficiency index calculations showed a significant increase in SI values for each date as pre-plant N rates increased from 0 to 200 kg ha^{-1} (Tables 2, 3). The average SI values were not statistically different ($P = 0.05$) between V9 and V12 sensor readings. It is worth

Table 1 Reference values for sufficiency index calculations at V9 and V12 corn growth stages in Nebraska

Sampled reference values		
Rep	CI(Ref) at 95 % threshold	
	V-9	V-12
1	2.046	1.936
2	2.051	1.910
3	1.981	1.865
4	2.064	1.891
5	2.159	2.021
6	2.213	1.982
7	2.164	1.990
8	2.162	1.980
Mean	2.105	1.947
SD	0.080	0.055
CV (%)	3.8	2.8

Reference values were obtained utilizing the 95-percentile cumulative values from a histogram of red-edge chlorophyll index values ($CI_{red-edge}$) generated for each replication

Table 2 Comparison of average sufficiency index (SI) values at five N rates for irrigated corn at the V9 growth stage in Nebraska

V-9	Sufficiency index values				
Rep	N rates (kg ha^{-1})				
	0	50	100	150	200
1	0.582	0.754	0.813	0.901	0.904
2	0.575	0.713	0.831	0.839	0.929
3	0.514	0.715	0.787	0.858	0.872
4	0.507	0.723	0.899	0.813	0.885
5	0.533	0.641	0.901	0.824	0.898
6	0.512	0.683	0.722	0.802	0.809
7	0.540	0.726	0.813	0.757	0.854
8	0.626	0.721	0.778	0.814	0.854
Mean	0.549	0.709	0.818	0.826	0.876
SD	0.042	0.034	0.060	0.042	0.037
CV (%)	7.7	4.8	7.4	5.1	4.2

Sufficiency index reference values for each replication are listed in Table 1

noting that the SI values for the highest pre-plant N rate (200 kg N ha^{-1}) was less than 1.0. Traditionally, the highest N rate would be used as the reference value to calculate the SI in plot studies (Peterson et al. 1993; Varvel et al. 2007). In the case of SPAD meter readings, only "representative plants" were selected for measurement. These meters have the capability to log and average up to 30 readings, but unless they are specially equipped to log the individual readings, it is not possible to post-evaluate the SPAD values and perform statistical analyses. It is possible to view and discard individual plant readings in the field before clearing the memory and proceeding to make more measurements. In the case of this study, all plants in the selected rows were monitored to generate and record over 70 readings per plot (average of 35 CI values for each sensor). Each reading at 5-Hz intervals was the average of ~8 000 readings per sensor. These readings included missing, diseased and injured plants (double plants were very rare). It is postulated that some plants in plots

Table 3 Comparison of average sufficiency index (SI) values at five N rates for irrigated corn at the V12 growth stage in Nebraska

V-12	Sufficiency index values				
Rep	N rates (kg ha^{-1})				
	0	50	100	150	200
1	0.514	0.695	0.827	0.887	0.872
2	0.524	0.657	0.829	0.844	0.910
3	0.462	0.686	0.779	0.830	0.869
4	0.487	0.679	0.845	0.840	0.904
5	0.479	0.628	0.828	0.843	0.925
6	0.510	0.686	0.800	0.845	0.858
7	0.486	0.734	0.859	0.793	0.881
8	0.610	0.709	0.804	0.860	0.898
Mean	0.509	0.684	0.822	0.843	0.890
SD	0.046	0.032	0.026	0.027	0.023
CV (%)	9.0	4.7	3.1	3.1	2.6

Sufficiency index reference values for each replication are listed in Table 1

with "adequate" N expressed reduced vigor that was probably associated with late emergence or affected by early-season immobilization caused by incorporated crop residues from the previous year. The CI used in this study is so named because it is sensitive to leaf chlorophyll content. In contrast, NDVI is primarily sensitive to living biomass that would be expected to increase as the crop grows. In contrast, the $CI_{red\text{-}edge}$ values might be expected to decline as the N deficiency becomes more pronounced.

The net effect of N stress on maize yield is provided in Table 4. Average relative yield for check plots was only 0.38 (4.60 Mg ha^{-1}) compared to the average SI of 0.55 and 0.51 at V9 and V12, respectively (Tables 2, 3). Relative yield at the 50 kg ha^{-1} N rate (0.71) was very comparable to the SI values of 0.71 and 0.68 at V9 and V12, respectively (Tables 2, 3, 4). Comparing relative yields with the SI values for all fertilizer N rates showed a strong relationship at both V9 and V12 growth stages (Fig. 3). Regression analysis calculations indicated SI values of 0.87 and 0.88 corresponded to a relative yield of 1.0, respectively. Limitation in the above relative yield calculation is that the highest N-rate plots were assumed to have an adequate supply of N and their yields were used as the reference. However, it is quite probable that the 200 kg ha^{-1} N rate was not adequate to satisfy crop needs because yields were continuing to increase (Table 4). This hypothesis is supported by the fact that the virtual reference approach identified ~12 % of the sensor readings between SI of 0.88 that corresponded to a relative yield of 1.0 using the regression analysis and the SI of 1.0 at the 95-percentile value. Figure 4 illustrates the distribution of sensor readings for plants in each fertilizer-rate group. The 200 kg N ha^{-1} plots contained many plants that were apparently less vigorous or had a less dense canopy than the 95-percentile level and the 150 and 100 kg N ha^{-1} plots contained a number of plants that were more vigorous than the 95-percentile value.

Reference vegetation index approaches

Establishing an N-rich strip(s) in a field near planting time is probably unnecessary for determination the N needs of a maize crop between the V6 and V15 growth stages. For example, at the V9 growth stage of corn, plants have accumulated ~20 % of the total N

Table 4 Comparison of relative yield values at five N rates for irrigated corn

Rep	Relative yield values N rates (kg ha^{-1}) 0	50	100	150	200
1	0.460	0.726	0.958	0.911	1.000
2	0.457	0.692	0.876	0.964	1.000
3	0.285	0.694	0.875	0.886	1.000
4	0.280	0.739	1.037	0.846	1.000
5	0.209	0.763	0.859	0.993	1.000
6	0.415	0.713	0.920	0.940	1.000
7	0.359	0.752	0.981	0.947	1.000
8	0.555	0.604	1.105	0.934	1.000
Mean	0.377	0.710	0.951	0.927	1.000
SD	0.115	0.050	0.087	0.046	
CV (%)	30.6	7.0	9.1	5.0	
Yield (Mg ha^{-1})	4.60	8.60	11.55	11.23	12.12

Sufficiency index reference values for each replication are listed in Tables 2 and 3

that will be in the crop at harvest, which only amounts to ~35 kg ha^{-1} for a 14 Mg ha^{-1} yield. Therefore, a planting time application of ~80 kg N ha^{-1} should be adequate to avoid N stress until the V7–V10 growth stages (Shanahan et al. 2007).

The historic economic optimum N rate (EONR) for the Nebraska field is 196 kg N ha^{-1} using data from Varvel et al. (2007). It is reasonable to assume that the $CI_{red\text{-}edge}$ values for the 150 and 200 kg ha^{-1} N rate should be non-N limiting at the V9 and V12 growth stages. However, average SI for all plots were less than 1.0 indicating that there were areas within each plot that had reduced levels of chlorophyll or where plant density was reduced due to N deficiency (Tables 2, 3) even though the N supply should have been adequate. Lowering the reference threshold criteria below 95-percentile (i.e. lowering $CI_{red\text{-}edge}$ reference) would raise the SI for all situations, but this would reduce fertilizer N application rates which might not optimize the yield potential of the crop.

The occurrence of vigorous and high chlorophyll content plants with respect to the pre-plant N treatments was evaluated by grouping the data by N rate and generating histograms for each that used a common range of categories. The histograms illustrate that nearly all of the 95-percentile plants were growing in the 150 and 200 kg N ha^{-1} plots (Fig. 4). Plants at the $CI_{red\text{-}edge}$ reference value of 2.105 (Table 1, V9) within the 100, 150 and 200 kg ha^{-1} N treatments constituted 26, 20 and 54 % of the reference pool, respectively.

The virtual-reference concept was originally proposed to circumvent the need for producers to establish N-rich strips in their fields and as a vegetation index value for adequately fertilized reference plants (Holland 2009). This concept involves driving through the field one or more times to collect sensor data from which a reference crop vegetation index value can be extracted (i.e. 95-percentile value, peak 3-s running average value or mean of N-rich strip). Commercial applicators and large producers with multiple employees are averse to the N-rich strip concept because of: (1) legal implications if the location of the N-rich strip is not properly identified and the wrong plants are used to determine the VI of reference plants, (2) it is essential that N-rich strips move to a new location in the field each year and (3) it is unrealistic to expect all operators to have the

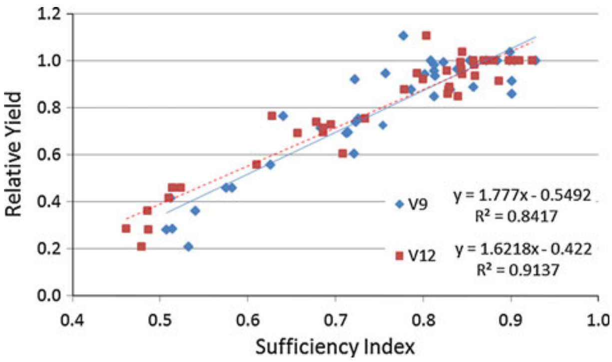

Fig. 3 Relationship between relative yield of irrigated maize in Nebraska that was fertilized at five N rates and sensor-derived $CI_{red\text{-}edge}$ based sufficiency index (SI) at the V9 (*solid blue*) and V12 (*dashed red*) growth stages (Color figure online)

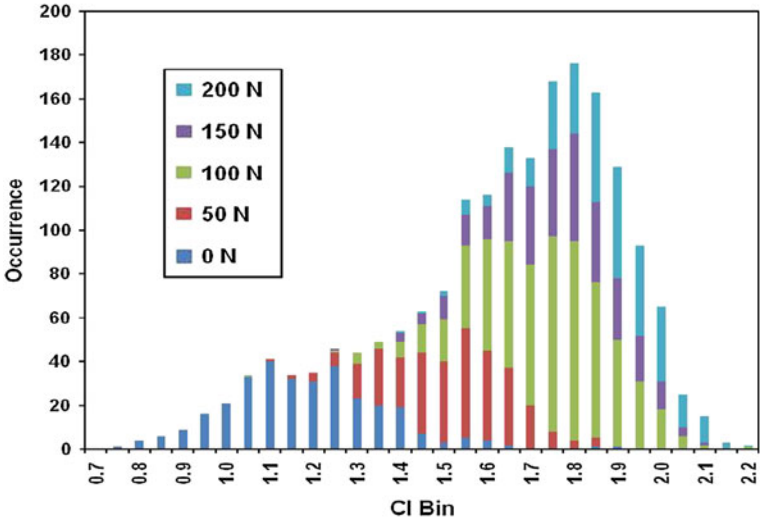

Fig. 4 Occurrence of red-edge chlorophyll index values ($CI_{red\text{-}edge}$) within irrigated corn plots receiving five pre-plant N rates (data compiled for eight replications at V9)

training and skills to understand and operate the sensor/flow control system with the confidence to over-ride unforeseen situations in fields.

Implementation

An alternative to collecting a sequence of sensor data before starting the variable-rate N applications is the "Drive-and-Apply" approach. Conceptually, it is a modification of the virtual-reference concept in that the histogram is not generated at the end of the field transect(s) but rather it is continuously updated while driving through the field and applying N fertilizer. The accuracy of the VI for the reference crop that is extracted from the histogram (95-percentile value) continues to improve as the system is exposed to more plants while it rapidly trains itself and auto-calibrates the fertilizer N recommendation.

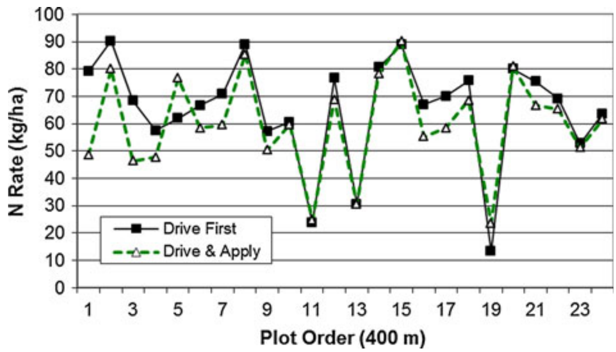

Fig. 5 Recommended N application rates within a field strip of irrigated maize in Nebraska containing 24 N-rate plots (four replications). Nitrogen recommendations compare the "Drive-First" and "Drive-and-Apply" calibration approaches at the V12 growth stage. Recommended N application rates were averaged within each plot

Two examples illustrate the functionality of the "Drive-and-Apply" approach. The first in Nebraska compares the N application rate for the "Drive-First" version of the virtual-reference concept with the "Drive-and-Apply" version for V12 plants. The in-season N application rate for each of the 24 consecutive plots (end-to-end in one long field transect) was based on the 95-percentile reference value extracted from the histogram after driving through the field (Fig. 5). The "Drive-and-Apply" N recommendations were based on the crop reference value that was continuously updated while driving through the field. In both cases, the recommended N application rates shown in Fig. 5 are the average of all in-plot values (~35 sensor readings). The order of the first six plots (one replication starting on the left side of Fig. 5) was 100, 50, 150, 200, 0 and 150 kg N ha^{-1}. After the sensors had encountered a full range of plant vigor while traveling through the first replication, the N application rate for the "Drive-and-Apply" approach mimicked the "Drive-First" N application rate better and better as the system trained itself.

The second example from the producer field in Mexico illustrates the "Drive-First" N application rate for maize at the V5 growth stage (Fig. 6). The sensor-based N-application rate gradually increased while driving through the field as plant vigor gradually decreased. The average N application rate was 96.8 kg ha^{-1} but varied by 39 kg N ha^{-1} (77–116 kg N $ha^{-1)}$ from one end of the field strip to the other based on a linear regression analysis ($n = 3\ 000$ pairs of sensor readings in 600 s). This field strip was used to evaluate how the "Drive-and-Apply" application rates would be affected if producers started in an area of high plant vigor compared to a lower vigor area. The "Drive-and-Apply" recommended N rates were compared to the "Drive-First" rates in Fig. 7. Nitrogen rates were not recommended for the first 60 s. Sensor data collected during the first 60 s were used to generate a histogram from which a 95-percentile value was determined and used to calculate the SI values from sensor data collected during the next 60 s. After 120 s, the histogram was updated to include all sensor data thus far from which the SI was calculated for the next 60 s. The histograms, SI values, and N recommendations were updated accordingly throughout the length of the field strip (10 min). The same procedure was followed when starting at the less vigorous end of the field. Under commercial applications conditions, the software could be programmed to continuously update the histogram and fine-tune the 95-percentile value. The simulated 60-s histogram update procedure used in Fig. 7 (maize at V5 in Mexico) showed that when starting at the most vigorous end of the

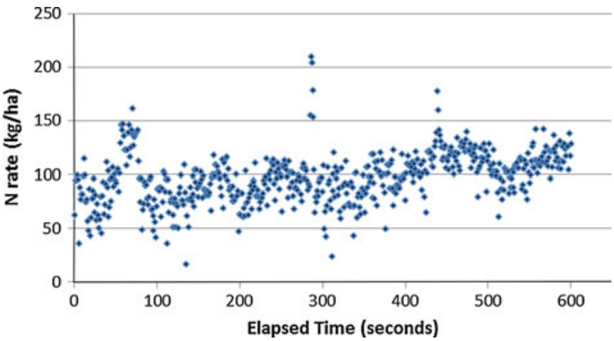

Fig. 6 Recommended N application rates for irrigated maize in Mexico using the "Drive-First" calibration approach at the V5 growth stage. Nitrogen rates were updated at 1 s intervals

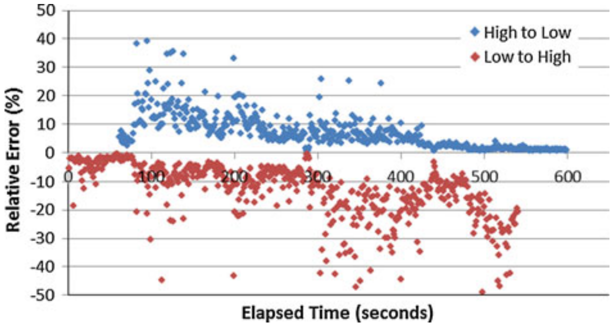

Fig. 7 Relative error in "Drive-and-Apply" N application rates compared to "Drive-First" N application rates for irrigated maize in Mexico at the V5 growth stage. Crop vigor (high to low) and (low to high) represents the direction of travel. Nitrogen rates were not predicted for the initial 60 s in each direction while the sensors and flow-rate controller were in the training mode

field the "Drive-and-Apply" approach over-applied N by ~15 %. When starting at the least vigorous end of the field strip the "Drive-and-Apply" approach under-applied N by ~25 %. In both situations, the system had auto-calibrated itself in less than 10 min. In practice, producers using the "Drive-and-Apply" approach are advised to start in an area that is among the most vigorous and would ideally include the least vigorous plants in the field within the first few minutes.

Producers or implement drivers that have no knowledge of the field would seemingly be at a disadvantage when using the "Drive-and-Apply" approach based on the results demonstrated in Fig. 7. However, on a field basis, Fig. 7 illustrates that in a worst-case scenario (driving from low to high vigor crop) the system auto-calibrated itself within the first 10 min. Starting with a high-vigor crop reduced the auto-calibration time to about 7 min in this study. In practice, operators should strive to start in an area that seemingly offers a range in crop vigor within the first few minutes. For example, the arrangement of plots receiving a range in N rates in Fig. 5 achieved a reasonable degree of auto-calibration within the first 2 min when traveling at 6 km h^{-1}. An alternative would be to apply a uniform rate determined by the producer for the first 10 0min or so, or until a modest number of data points have been collected (perhaps 4 000). In a situation where the entire

crop is obviously N deficient and there is no N-rich area to establish the VI for reference plants, producers should be consulted to establish the desired N application rate.

The ultimate test of any sensor calibration procedure is how well the calculated in-season SI (or response index) values are correlated with crop N status and yield. This is because the SI values are used to calculate the in-season fertilizer N recommendations. In the case of the Nebraska study, the pre-plant N application rates were used to generate situations within the field where plants would be exposed to a range in soil N availability. The ensuing plants had a range in crop N status at the time of sampling (V12) that was highly correlated with yield (Fig. 3). This strong relationship is the key to making in-season fertilizer N recommendations. Data presented in Fig. 3 illustrates that SI values generated using the virtual reference concept are strongly correlated with yield and should be useful when making in-season fertilizer N recommendations. However, common sense and an understanding of the virtual reference concept need to prevail when using the "Drive-and-Apply" approach when making in-season N recommendations as illustrated in Fig. 7.

Limitations and cautions associated with remote sensing also apply to the virtual reference approach for interpreting data. For example, crops growing under water deficit conditions exhibit short-term changes and then longer-term effects on canopy reflectance if the stress persists. When possible, it is advisable to avoid sensor-based in-season variable-rate N applications to crops under stressed conditions because reflectance values may not be indicative of crop N status and even if N is applied to the soil surface it might not become available to the crop.

Conclusion

The virtual reference concept proposed and discussed in this paper offers a reliable and convenient way for producers and commercial operators to establish the vegetation index of vigorous plants that are assumed to have an adequate supply of N. Quantifying the vegetation index of reference plants is essential to calibrate active canopy sensor algorithms. Drive-First and Drive-and-Apply virtual reference approaches move beyond the limitations and problems of establishing an N-rich strip in each field. The examples presented illustrate the practicality and ease of use of the virtual reference concept. However, cautions are appropriate when interpreting any and all active crop sensor data derived under water-stress conditions or when N-stress is apparent throughout a field. Similarly, fields with multiple hybrids should be treated as a new field because of potential differences in reflectance characteristics. Analyzing sensor data displayed in histograms can provide new insights into factors that influence crop vigor and spatial variability.

Acknowledgments Special appreciation is extended to Mr. Adalberto Mustieles, Musol LLC, Culiacan, Mexico for working with producers to establish field studies.

References

Biggs, G. L., Blackmer, T. M., Demetriades-Shah, T. H., Holland, K. H., Schepers, J. S., & Wurm, J. H. (2002). Method and apparatus for real-time determination and application of nitrogen fertilizer using rapid, non-destructive crop canopy measurements. U.S. Patent #6,393,927. Issued May 28, 2002.

Gitelson, A. A., Gritz, U., & Merzlyak, M. N. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology, 160*, 271–282.

Gitelson, A., & Merzlyak, M. N. (1996). Signature analysis of leaf reflectance spectra: Algorithm development for remote sensing of chlorophyll. *Journal of Plant Physiology, 148*, 494–500.

Gitelson, A. A., Viña, A., Rundquist, D. C., Ciganda, V., & Arkebauer, T. J. (2005). Remote estimation of canopy chlorophyll content in crops. *Geophysical Research Letters, 32*, L08403. doi:10.1029/2005Gl022688.

Holland, K. H. (2009). Sensor-based chemical management for agricultural landscapes. U.S. Patent #7,723,660. Issued May 25, 2010.

Holland, K. H., & Schepers, J. S. (2010). Derivation of a variable rate nitrogen application model for in-season fertilization of corn. *Agronomy Journal, 102*, 1415–1424.

Holland, K. H., & Schepers, J. S. (2011). Active-crop sensor calibration using the virtual reference concept. In J. V. Stafford (Ed.), *Precision Agriculture 2011, Proceedings of the 8th European Conference on Precision Agriculture* (pp. 469–479). Prague: Czech Republic, Czech Centre for Science and Society.

Peterson, T. A., Blackmer, T. M, Francis, D. D., & Schepers, J. S. (1993). Using a chlorophyll meter to improve N management, NebGuide G93-1171A. Lincoln: University of Nebraska-Lincoln Cooperative Extension Service.

Raun, W., Solie, J., May, J., Zhang, H., Kelly, J., Taylor, R., et al. (2010). Nitrogen Rich strips for wheat, corn and other crops. Publication E-1022. Stillwater: Oklahoma State University Extension.

Raun, W. R., Solie, J. B., Stone, M. L., Johnson, G. V., Lukina, E. V., Thomason, W. E., et al. (2001). In-season prediction of yield potential using wheat canopy reflectance. *Agronomy Journal, 93*, 131–138.

Raun, W. R., Solie, J. B., Stone, M. L., Martin, K. L., Freeman, K. W., & Zavodny, D. L. (2005). Automated calibration stamp technology for improved in-season nitrogen fertilization. *Agronomy Journal, 97*, 338–342.

Ritchie, S. W., Hanway, J. J., Thompson, H. E., & Benson, G. O. (1997). How a corn plant develops. Special reprint 48 (revised edition). Ames: Iowa State University of Cooperative Extension Service.

Roberts, D. F., Ferguson, R. B., Kitchen, N. R., Adamchuk, V. I., & Shanahan, J. F. (2012). Relationships between soil-based management zones and canopy sensing for corn nitrogen management. *Agronomy Journal, 104*, 119–129.

Scharf, P. C., Kitchen, N. R., Sudduth, K. A., Davis, J. G., Hubbard, V. C., & Lory, J. A. (2005). Field-scale variability in optimal N fertilizer rate for corn. *Agronomy Journal, 97*, 452–461.

Schepers, J. S., Francis, D. D., Vigil, M., & Below, F. E. (1992). Comparison of corn leaf nitrogen and chlorophyll meter readings. *Communications in Soil Science and Plant Analysis, 23*(17–20), 2173–2187.

Shanahan, J. F., Kitchen, N. R., Raun, W. R., & Schepers, J. S. (2007). Responsive in-season nitrogen management for cereals. *Computer & Electronics in Agriculture, 61*, 51–62.

Solari, F., Shanahan, J. F., Ferguson, R. B., & Adamchuk, V. I. (2010). An active sensor algorithm for corn N applications based on a chlorophyll meter sufficiency index framework. *Agronomy Journal, 102*, 1090–1098.

Solari, F., Shanahan, J., Ferguson, R., & Schepers, J. (2008). Active sensor reflectance measurements of corn nitrogen status and yield potential. *Agronomy Journal, 100*, 571–579.

Varvel, G. E., Wilhelm, W. W., Shanahan, J. F., & Schepers, J. S. (2007). Nitrogen fertilizer applications for corn based on sufficiency index calculations. *Agronomy Journal, 99*, 701–706.