Evaluating spectral vegetation indices for a practical estimation of nitrogen concentration in dual-purpose (forage and grain) triticale

F. Rodriguez-Moreno* and F. Llera-Cid

*Centro de Investigación "La Orden-Valdesequera". Junta de Extremadura. Finca "La Orden".
Ctra. N-V, km 372. 06187 Guadajira (Badajoz). Spain*

Abstract

There is an ample literature on spectral indices as estimators of the crop's chlorophyll concentration, and, by extension, of the nitrogen concentration. In this line, the suitability of 21 of these indices was evaluated as nitrogen concentration indicators for the dual-purpose (fodder and grain) triticale (X *Triticosecale* Wittmack). The interval of interest was the one in that it would be possible to intervene to correct the deficiency of nitrogen (defined according to practical criteria); one peculiarity of this study is that it only develops a model for that period; more developments complicate the profitability, because the annual stability is not guaranteed and calibration studies are expensive. The results showed that, although there are significant correlations between the greenness indices and the crop's nitrogen concentration, for none of the spectral indices the relationship can reach acceptable values that encourage their use in the new techniques of precision agriculture of low cost. One solution for improving the effectiveness and reduce costs could be to use the information contained in the spectral signature beyond what is easily explicable by biochemistry and biophysics, in other words, using data mining in the search for new spectral indices directly related to the concentration of nitrogen in plant and stable throughout crop development. At present, the squared correlation coefficient (R^2) of the best fits reach 0.5 for the later phenological stages, this mark is reduced to 0.3 with an approach of low cost.

Additional key words: cereals; leaf reflectance; nutritional status; precision agriculture; radiometry; remote sensing.

Resumen

Evaluación de índices de vegetación espectrales para la estimación de la concentración de nitrógeno en triticale de doble aptitud (forraje y grano)

Existe una extensa literatura que describe el potencial de los índices espectrales como indicadores de la concentración de clorofila en el cultivo y, por extensión, de la concentración de nitrógeno. En esta línea se encuentra este trabajo, donde se evalúa la idoneidad de los 21 índices espectrales más usados para realizar estimaciones en triticale (X *Triticosecale* Wittmack) de doble propósito (forraje-grano). El intervalo fenológico de interés se define siguiendo criterios prácticos, es aquel durante el cual se puede actuar para corregir una deficiencia de nitrógeno. Una peculiaridad es que sólo se desarrolla un modelo para todo ese periodo; más desarrollos complicarían la rentabilidad, ya que la estabilidad de los modelos no está garantizada y las calibraciones son costosas. Los resultados mostraron que, aunque existe correlación significativa entre los índices de verdor y la concentración de nitrógeno, para ninguno de los índices espectrales la relación alcanza valores que animen a su uso en las metodologías de bajo coste. Para mejorar la efectividad y reducir costes se podría usar la información contenida en la firma espectral más allá de lo que es fácilmente explicable por la bioquímica-biofísica, en otras palabras, usar la minería de datos en la búsqueda de índices espectrales directamente relacionados con la concentración de nitrógeno y estables a lo largo del desarrollo del cultivo. El coeficiente de correlación al cuadrado (R^2) del mejor de los ajustes existentes alcanza un valor de 0,5 para los últimos estadios fenológicos, marca que se reduce a 0,3 al emplear una metodología de bajo coste.

Palabras clave adicionales: agricultura de precisión; cereales; estado nutricional; radiometría; reflectancia de la hoja; teledetección.

* Corresponding author: fernando.rodriguez@juntaextremadura.net

Abbreviations used: aR^2 (adjusted correlation coefficient); NDVI (normalized difference vegetation index); RMSE (square root of the mean square error).

Introduction

Reflectance measurements can be used to obtain the values of the most widely used spectral indices (reviewed in Ustin *et al.*, 2004) as indicators of chlorophyll concentration. These indices, together with the canopy radiative transfer models, allow one to estimate the state of the vegetation from satellite and aerial images. Our ultimate goal is to make this approach a reality in agriculture and the action plan begins with identifying the most appropriate spectral index and estimating the magnitude of its relationship with nitrogen concentration. This study assesses the potential of the methodology in easily reproducible conditions on farms. The immediate objective is to determine if the spectral indices keep the effectiveness reported by other authors when implementation costs are reduced (only a model for relating reflectance and nutritional status by campaigning on a stage with high variability), which is necessary to facilitate the transfer.

Some of the spectral indices commonly used as indicators of chlorophyll concentrations include corrections for the effect of the soil on the measurements of the canopy reflectance (Huete, 1988; Rondeaux *et al.*, 1996; Zarco-Tejada *et al.*, 2004), unnecessary precautions in this study because it works with reflectance measurements of the leaf.

This work is in line with that described by Heege *et al.* (2008). They related the measurements of greenness, obtained by sensors mounted on farm equipment, with the dose of nitrogen fertilizer. They used fluorescence and reflectance measurements, in the case of reflectance; they tested the determination of the red-edge inflexion point both numerically and empirically.

The work of Li *et al.* (2010), although similar to this one, differs in that in this study is limited to one the number of models that relate the spectral index and the nitrogen concentration in the plant throughout the development, instead of looking for relationships for specific growth stages, that is what has been done so far because the plant changes during its development (Marschner, 1995; Azcón-Bieto and Talón, 2003) complicate another approach.

With the dual-purpose triticale, one has the possibility of allowing livestock to graze more than once without ruining the harvest. After each grazing by livestock the plant has to regenerate the above-ground part and with it the ability to synthesize chlorophyll, but the below-ground part is unaffected so that the plant's nitrogen absorbing capacity remains intact. The result

is an imbalance in the first weeks after each cutting which is manifest in a yellowing of the plant. This is not a symptom of nitrogen deficiency, since there has been neither a decrease in the concentration of this element nor an interruption in plant growth. This characteristic is a major additional obstacle to the transference of radiometric technology to the dual-purpose triticale case.

Material and methods

As part of a study at the «La Orden-Valdesequera» Research Centre aimed at determining the optimal combination of seeding density, number of grazing and doses of nitrogenous fertilizers for growing triticale (X *Triticosecale* Wittmack), the reflectance of the leaves throughout the growth of the crop was measured.

The experimental design was a split-split-plot with four replicates. The first factor was the seeding density (400, 500 and 600 plants m^{-2}), the second the number of times the crop was cut to simulate grazing (0, 1 and 2 grazing by livestock) and the third the dose of nitrogenous fertilizer (0, 75 and 125 kg ha^{-1}). Each factor had three levels, so that there were 108 experimental plots in total, each of 30 m^2. The leaf reflectance measurements were made at 80, 117, 132, and 164 days after seeding (campaign 2009). Together with these measurements, crop samples were taken and sent to the laboratory for the determination of the concentration of total nitrogen by the Kjeldahl method.

The weather is a key factor in plant growth and climate variability is sufficient to cause changes in the evolution of the crop year after year; for this reason, more important than the number of days from sowing is to indicate the phenological stage in which was the plant. Table 1 shows the correspondence between the number of days after seeding, the crop's phenological stage and the description of growth stage.

The growth stages were determined using the Zadoks (Zadoks *et al.,* 1974) and Feekes scales (Feekes, 1941; Large, 1954). On this matter illustrative charts can be found in the book of Rawson and Gómez Macpherson (2000).

The influence of each factor on the nitrogen concentration measured for each of the 108 plots on each sampling day was analyzed (it is unknown whether all the factors at all levels have an effect on the concentration of nitrogen in plant). The analysis of variance for factorial designs (statistical analysis that corresponds

Table 1. Correspondence between the number of days after seeding and the crop's phenological stage (triticale)

Days after seeding	Phenological stage		Description
	Zadoks scale	Feekes scale	
80	35	7-8	Stem elongation (5th node detectable)
117	40	9	Booting
132	46	10	Booting (flag leaf sheath opening)
164	65	10.5.2	Anthesis half-way

to the split-split-plot design) determines a grouping of the elementary experimental plots according to the nitrogen content on each of the four sampling dates. All calculations were done using R 2.9 (R Development Core Team, 2004).

The crop's spectral signature is sensitive to phenological changes; this is a good reason to test the relation between the spectral index and the nitrogen concentration for each sampling date. This approach suffers from a serious handicap, a commercial application is difficult since in that case calibration studies would be needed for each growth stage, which compromises the economic viability of this technique. This is the reason because in this work, the restriction that only one model should cover the entire period of interest was established.

On each sampling date, 20 leaves at random were collected in each of the 108 elementary plots. Ten estimates of the reflectance (each averaging 50 readings) were made of these samples, using the ASD FieldSpec 3 spectroradiometer for this. This device has a spectral range of 350-2,500 nm, a sampling interval of 1.4 nm for the range 350-1,000 nm and 2 nm for the range 1,000-2,500 nm, and a spectral resolution of 3 nm at 700 nm and 10 nm from 1400 nm to 2100 nm. Readings were performed using a plant probe plus leaf clip. The light source of the plant probe is a halogen bulb with a colour temperature of $2,901 \pm 10$ K.

For each of the different groups of elementary plots defined according to their concentration of nitrogen on each sampling date, the mean reflectance was cal-

Table 2. Spectral indexes related to the content in chlorophyll used

Spectral index	Formulation[1]	Author
Green NDVI[2]	$(R780 - R550) / (R780 + R550)$	Gitelson and Merzlyak (1996)
Green reflectance	R550	Biochemical deduction
Logarithm of reciprocal reflectance	$\log(1/R737)$	Yoder and Pettigrew-Crosby (1995)
Modified chlorophyll absorption in reflectance index	$[(R700 - R670) - 0.2 * (R700 - R550)] * (R700 / R670)$	Daughtry et al. (2000)
Modified red-edge normalized difference vegetation index	$(R750 - R705) / (R750 + R705 - 2 * R445)$	Sims and Gamon (2002)
Modified red-edge ratio	$(R750 - R445) / (R705 - R445)$	Sims and Gamon (2002)
Near infrared reflection	R800	Biochemical deduction
Normalized difference vegetation index	$(R800 - R670) / (R800 + R670)$	Deering (1978)
Pigment specific normalized difference a	$(R800 - R675) / (R800 + R675)$	Blackburn (1998)
Pigment specific normalized difference b	$(R800 - R650) / (R800 + R650)$	Blackburn (1998)
Pigment specific simple ratio a	$R800 / R675$	Sims and Gamon (2002)
Pigment specific simple ratio b	$R800 / R650$	Sims and Gamon (2002)
Ratio analysis of reflectance spectra a	$R675 / R700$	Blackburn (1999)
Ratio analysis of reflectance spectra b	$R675 / (R650 * R700)$	Blackburn (1999)
Ratio of near infrared to green	$R800 / R550$	Biochemical deduction
Ratio of near infrared to red	$R800 / R670$	Biochemical deduction
Reciprocal reflectance	$1 / R700$	Gitelson et al. (1999)
Red edge inflect point	$(700 + 40) * \{[(R670 + R780) / 2] - R700\} / (R740 - R700)$	Guyot et al. (1988)
Red reflectance	R670	Biochemical deduction
Red-edge NDVI	$(R750 - R705) / (R750 + R705)$	Sims and Gamon (2002)
Zarco-Tejada & Miller	$R750 / R710$	Zarco-Tejada et al. (2001)

[1] R + number: reflectance at number nm.   [2] NDVI: normalized difference vegetation index.
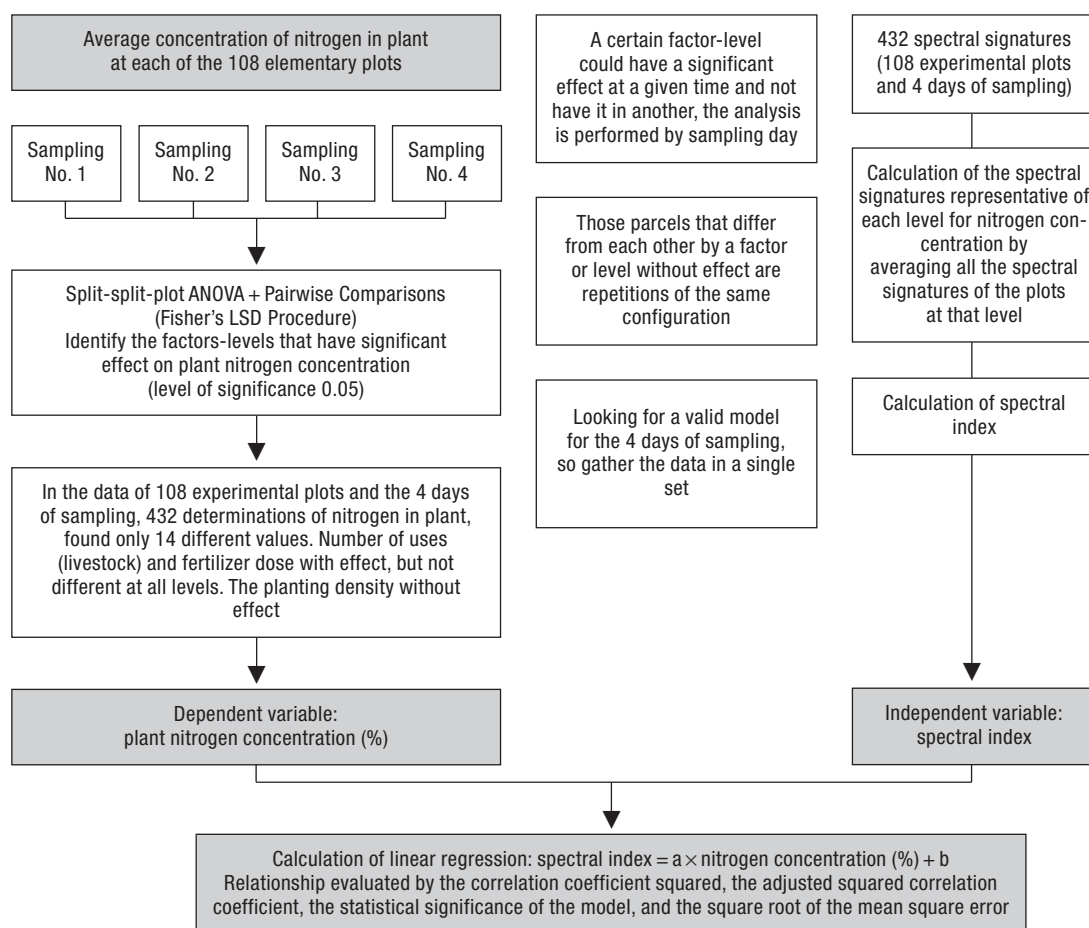
Figure 1. Flow chart that summarizes the experiment design and data processing methods.

culated by averaging the readings taken in their respective elementary plots. The average spectral signature for each of the groups identified during the growth of the crop was then used to determine the value of each of the selected spectral indices given in Table 2.

The relationship between the different spectral indices and the nitrogen concentrations was evaluated by calculating the correlation coefficient squared (R^2), the adjusted squared correlation coefficient (aR^2), the square root of the mean square error (RMSE) and the statistical significance (p-value) of the model (analysis of the variance explained as against the residual). Figure 1 is a flowchart that summarizes the whole process.

Results and discussion

Table 3 shows that all the models, except the one developed for the modified chlorophyll absorption in reflec-

tance index, explained a significant portion of the variance of the plant's nitrogen concentration, but in none of the cases the magnitude of the relationship was enough for developing a profitable methodology. The highest R^2 was 0.31, and corresponded to the green reflectance index.

This study determines the reduction in the effectiveness if one tries to estimate the concentration of nitrogen in the plant by a low-cost approach. The reduction is 38% (the value of R^2 is reduced from 0.5 to 0.31), a significant reduction received with a positive evaluation because of the complexity involved in developing a single model for different phenological stages and the enormous handicap which entails the double duality of the crop. It is understood that this reduction in effectiveness is the highest possible and that's not enough to discard the approach of a precision agriculture of low cost, at least remains to evaluate the potential of data mining in the exploitation of the information contained in spectral signature.

Table 3. Goodness of the fit between spectral indices and the concentration of nitrogen

Spectral index	R^2	Adjusted R^2	*p*-values for model	RMSE[1]
Green NDVI[2]	0.235	0.233	0.000	0.691
Green reflectance	0.315	0.314	0.000	0.653
Logarithm of reciprocal reflectance	0.179	0.177	0.000	0.715
Modified chlorophyll absorption in reflectance index	0.008	0.006	0.065	0.786
Modified red-edge normalized difference vegetation index	0.058	0.056	0.000	0.766
Modified red-edge ratio	0.047	0.045	0.000	0.771
Near infrared reflection	0.122	0.120	0.000	0.740
Normalized difference vegetation index	0.139	0.137	0.000	0.732
Pigment specific normalized difference a	0.123	0.121	0.000	0.739
Pigment specific normalized difference b	0.231	0.229	0.000	0.692
Pigment specific simple ratio a	0.115	0.113	0.000	0.743
Pigment specific simple ratio b	0.208	0.206	0.000	0.703
Ratio analysis of reflectance spectra a	0.027	0.025	0.001	0.779
Ratio analysis of reflectance spectra b	0.252	0.250	0.000	0.683
Ratio of near infrared to green	0.213	0.211	0.000	0.700
Ratio of near infrared to red	0.131	0.128	0.000	0.736
Reciprocal reflectance	0.226	0.224	0.000	0.695
Red edge inflect point	0.032	0.030	0.000	0.777
Red reflectance	0.173	0.171	0.000	0.718
Red-edge NDVI	0.156	0.154	0.000	0.725
Zarco-Tejada & Miller	0.134	0.132	0.000	0.735

[1] RMSE: square root of the mean square error. [2] NDVI: normalized difference vegetation index.

The spectral signature of a leaf is extremely sensitive to the conditions affecting the leaf itself and to the conditions under which the measurements are made. These conditions vary considerably throughout the period during which it would be possible to act to correct a possible nitrogen deficiency in the crop, for this reason the models were specific to a growth stage, something that complicates the profitability and thus the transfer of technology, this study explores this field and its conclusion suggests that the cost of using a single model is not as high as could be expected.

In light of the results, exactly of the results of F test and associated *p*-value for significance of the model, one can say that the independent variables, spectral indices, are important in explaining the observed variation in dependent variable, concentration of nitrogen in plant. These indices have a biochemical and biophysical basis, the existence of that relationship was expected, different is the magnitude of it, so small that makes one think about the weakness of using non-specific index for the concentration of nitrogen in plant.

It is confirmed that, in cultivating dual-purpose triticale, calculations based solely on a greenness measurement, such as the green reflectance index (the index which gave the best correlation in this study), would lead to obtaining erroneous estimates of the nitrogen concentration.

The single model developed for the entire period of interest —from stem elongation (when tillering has ended) through booting and heading to flowering— gave lower correlations between the vegetation spectral indices and the crop's nitrogen concentration than those reported in similar studies in which different phenological intervals were modeled separately (Heege *et al.*, 2008; Li *et al.*, 2010). In particular, the value of R^2 was reduced by about 40%, from 0.5 for the final growth stages (Li *et al.*, 2010) to the value of 0.3 for the entire period of crop growth.

The techniques of precision agriculture have to reach to the cultivation of the dual-purpose triticale. Since nitrogen's role in a plant is not restricted to be a component of chlorophyll and the spectral signature of the leaf is function of its composition and configuration, it has to be possible to derive new spectral indices which estimate nitrogen concentrations based on other variables besides of the greenness of the plant.

The results do not provide reliable indications of spectral features of those indices which best correlate with the crop's nitrogen concentration. Some authors, among others, the already cited Heege *et al.* (2008)

and Li *et al.* (2010), have attempted to find relationships between the various spectral indices to explain their rank in terms of suitability (greater R^2). The lack of correspondence between the different rankings might be considered a further reason for not addressing this issue.

References

AZCÓN-BIETO J., TALÓN M., 2003. Fundamentos de Fisiología Vegetal. McGraw-Hill-Interamericana de España. [In Spanish].

BLACKBURN G.A., 1998. Quantifying chlorophylls and carotenoids at leaf and canopy scales: an evaluation of some hyperspectral approaches. Remote Sens Environ 66, 273-285.

BLACKBURN G.A., 1999. Relationships between spectral reflectance and pigment concentrations in stacks of deciduous broadleaves. Remote Sens Environ 70, 224-237.

DAUGHTRY C.S.T., WALTHALL C.L., KIM M.S., COLSTOUN E.B., MCMURTREY J.E., 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sens Environ 74, 229-239.

DEERING D.W., 1978. Rangeland reflectance characteristics measured by aircraft and spacecraft sensors. Ph. D. diss. Texas A&M Univ, College Station. 338 pp.

FEEKES W., 1941. De Tarwe en haar milieu [The wheat and its environment]. Vers. XVII Tech. Tarwe Comm. Groningen, 560-561. [In Dutch].

GITELSON A., MERZLYAK M., 1996. Signature analysis of leaf reflectance spectra: algorithm development for remote sensing of chlorophyll. J Plant Physiol 148, 495-500.

GITELSON A.A., BUSCHMANN C., LICHTENTHALER H.K., 1999. The chlorophyll fluorescence ratio R735/F700 as an accurate measure of the chlorophyll content in plants. Remote Sens Environ 69, 296-302.

GUYOT G., BARET F., MAJOR D.J., 1988. High spectral resolution: determination of spectral shifts between the red and infrared. Int Arch Photogram Remote Sens 11, 750-760.

HEEGE H.J., REUSCH S., THIESSEN E., 2008. Prospects and results for optical systems for site-specific on-the-go control of nitrogen-top-dressing in Germany. Precis Agric 9, 115-131.

HUETE A.R., 1988. A soil-adjusted vegetation index (SAVI). Remote Sens Environ 25, 295-309.

LARGE E.G., 1954. Growth stages in cereals: illustration of the Feeke's scale. Plant Pathol 3, 128-129.

LI F., MIAO Y., HENNIG S., GNYP M., CHEN X., JIA L., BARETH G., 2010. Evaluating hyperspectral vegetation indices for estimating nitrogen concentration of winter wheat at different growth stages. Precis Agric 11, 335-357.

MARSCHNER H., 1995. Mineral nutrition of higher plants, 2nd ed. Academic Press.

R DEVELOPMENT CORE TEAM, 2004. R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna. Available in http://www.R-project.org. [1 May, 2010].

RAWSON H.M., GÓMEZ MACPHERSON H., 2000. Irrigated wheat. FAO, Roma.

RONDEAUX G., STEVEN M., BARET F., 1996. Optimization of soil-adjusted vegetation indices. Remote Sens Environ 55, 95-107.

SIMS D.A., GAMON J.A., 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures, and developmental stages. Remote Sens Environ 81, 337-354.

USTIN S.L., JACQUEMOUD S., ZARCO-TEJADA P., ASNER G., 2004. Remote sensing of environmental processes: State of the science and new directions. In: Manual of remote sensing, vol. 4. (Ustin S.L., vol. ed). Remote sensing for natural resource management and environmental monitoring. ASPRS. John Wiley and Sons, NY. pp. 679-730.

YODER B.J., PETTIGREW-CROSBY R.E., 1995. Predicting nitrogen and chlorophyll content and concentrations from reflectance spectra (400-2,500 nm) at leaf and canopy scales. Remote Sens Environ 53, 199-211.

ZADOKS J.C., CHANG T.T., KONZAK C.F., 1974. A decimal code for the growth stages of cereals. Weed Res 14, 415-421.

ZARCO-TEJADA P.J., MILLER J., NOLAND T.L., MOHAMMED G.H., SAMPSON P.H., 2001. Scaling-up and model inversion methods with narrowband optical indices for chlorophyll content estimation in closed forest canopies with hyperspectral data. IEEE T Geosci Remote Sens 39, 1491-1507.

ZARCO-TEJADA P.J., MILLER J.R., MORALES A., BERJON A., AGUERA J., 2004. Hyperspectral indices and model simulation for chlorophyll estimation in open-canopy tree crops. Remote Sens Environ 90, 463-476.