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Use of a chlorophyll meter to assess nitrogen nutrition index during the growth cycle in winter wheat

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ABSTRACT

We investigated the feasibility of predicting crop nitrogen nutrition index (NNI) from chlorophyll meter readings (CMRs) during the crop cycle, for the development of a fertilization method for winter wheat (*Triticum aestivum L.*) based on the regular monitoring of crop N status. The relationship between NNI and CMR has been studied before, but only for CMRs obtained late in the season. A literature review revealed an absence of consensus concerning the most accurate equation for predicting NNI from CMR. It remains unclear which variables are the most influential and the extent to which it might be possible to overcome these uncertainties by using a normalized chlorophyll meter reading. We therefore carried out multimodel selection, comparing goodness-of-fit, prediction accuracy and likelihood, for linear, quadratic and exponential models, taking into account only biomass, cultivar, biomass plus cultivar and growth stage effects. Models were fitted with absolute and normalized measurements. We also considered the possibility of predicting NNI with a single model or with different models for each growth stage. We found that normalized measurements limited the biomass and cultivar effects, but not the growth stage effect. Furthermore, the use of normalized measurements increased prediction accuracy. However, the prediction error remained very high if the well-fertilized strip was N-deficient ($NNI < 0.9$). Finally, the best compromise was found to be a model for each growth stage, using absolute measurements, but taking into account the effects of biomass and cultivar.

1. Introduction

Competitiveness, environmental issues and market requirements for product quality are constraints that farmers must take into account when applying nitrogen (N) fertilizer. These constraints have tightened in recent years, necessitating more accurate management and the adaptation of N fertilization during the growing season (Jeuffroy et al., 2002). Efforts have focused on strategies based on in-season N applications taking soil and crop N status during the vegetative period into account, to improve the synchronization of soil N availability and crop N demand (Shanahan et al., 2008; Olfs et al., 2005). Fertilizer applications should be based on precise estimates of crop N requirements, so that the amount of nitrogen required can be applied, without excess, in conditions in which the N uptake capacity of the plant is high (Samborski et al., 2009). This precise management of fertilizer applications necessitates regular monitoring of crop N status during the growing season.

Crop N status is a function of both crop biomass and N concentration, and can be quantified by calculating the nitrogen nutrition index (NNI), a highly reliable indicator (Justes et al., 1994). NNI is calculated relative to the critical nitrogen concentration, defined as the minimal crop N concentration required for maximal aerial dry matter production (Justes et al., 1994). This critical nitrogen concentration corresponds to a NNI value of 1, and the value of NNI obtained for a particular crop therefore indicates whether the N nutrition status of the crop is limiting for maximal crop biomass production (Mistele and Schmidhalter, 2008). NNI can also be used to identify situations in which limiting N nutrition leads to yield loss relative to the local weather-dependent potential yield (Lemaire and Meynard, 1997; Jeuffroy and Bouchard, 1999). However, NNI determination requires destructive time-consuming measurements of plant N content and crop biomass. Various types of diagnostic tool have been developed for assessing the N status of a crop within the growing season, to provide farmers with decision support based on different measurements (Confalonieri et al., 2015). Plant-sap nitrate concentration, leaf chlorophyll content and crop transmittance and reflectance are the most commonly used indicators on which N fertilizer recommendations are based (Olfs et al., 2005; Samborski et al., 2008).

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Hand-held chlorophyll meters are convenient and can provide rapid results for diagnostic purposes. The relationship between chlorophyll meter reading (CMR) and crop N status has been extensively studied for various crops, including maize (Schepers et al., 1992), apple trees (Neilsen et al., 1995), cotton (Wood et al., 1992), and wheat (Follett et al., 1992; Reeves et al., 1993; Fox et al., 1994). Most studies on wheat have tried to find a relationship between CMR and grain yield that could be used to identify fields likely to display a grain yield response to additional N fertilizer and, therefore, for recommendations concerning fertilizer management (Follett et al., 1992; Reeves et al., 1993; Fox et al., 1994; Bavec and Bavec, 2001; Arregui et al., 2006). The possible use of late-season CMRs to achieve high grain quality has also been investigated (Matsunaka et al., 1997; Le Bail et al., 2005; Ortuzar-Iragorri et al., 2005). It has generally been concluded that CMR is a relevant indicator of grain protein content, grain yield or the need for additional fertilizer only for measurements taken late in the season. It is generally agreed that CMR may be affected by many external factors that can affect sensor performance, such as pests and diseases, leaf thickness, solar irradiance and water status (Samborski et al., 2009). Many studies have, therefore, focused on the use of a normalized CMR measurement (nCMR) to improve the accuracy of these diagnosis tools by limiting prediction error due to other factors than N, such as growth stage or cultivar (Arregui et al., 2006; Debaeck et al., 2006; Prost and Jeuffroy, 2007; Ziadi et al., 2010; Yuan et al., 2016). However, too little is known about the relationship between NNI and CMR to make it possible to use CMR to monitor crop N status at any time in the growing season, from the beginning of stem elongation to flowering.

We inspired from previously published mathematical models linking CMR to crop variables on C3 cereals crops with the objective to test and compare various models and variables to: i) identify the best models for predicting NNI from CMR at any time in the growing season ii) determine whether the use of nCMR rather than absolute readings improved the predictive value of models over the study period, and iii) determine whether a single model could predict NNI from CMR or nCMR at all growth stages, from the beginning of stem elongation to flowering.

2. Materials and methods

Based on a review of relevant past literature, we identified models for predicting NNI from CMR. This analysis revealed several variables of interest for inclusion in models and the need to compare the accuracy of models, for both CMR and nCMR. We fitted a set of models to a set of experimental measurements of NNI and CMR at different growth stages, from the beginning of stem elongation to flowering, recorded in a database. We used the Akaike Information Criterion (AIC) (Akaike, 1973) to identify the best model of those tested (Burnham, 2004). Furthermore, we also compared the goodness-of-fit and prediction accuracy of the various models, using mean square error and prediction error to evaluate the predictive performances of the models.

2.1. Experimental data

We compiled a dataset from measurements obtained in previous experiments with winter wheat (*Triticum aestivum*). Experimental plots were established in six years (1994–1995, 1995–1996, 1996–1997, 1997–1998, 1998–1999, 2001–2002), at 20 different sites in France and the Navarra region of Spain (Table 1). Each experiment included three or four replicates. All experiments were irrigated or well-watered to avoid drought stress. We gathered 757 treatments, each characterized by site, year, cultivar, total N rate, growth stage, measured CMR, calculated nCMR, and measured NNI (Table 1). Aerial crop biomass, the nitrogen concentration of aerial parts, and values from N-Tester® (in France) or SPAD-502® (in Spain) were obtained for each situation. Both N-Tester® and SPAD-502® measure leaf transmittance. The readings they supply are different, but highly correlated (Arregui et al., 2006). We used the correlation described by Arregui et al. (2006) to standardize the values (N-Tester® reading = 15.2 SPAD® reading − 123.6; $R^2 = 0.98$). Measurements were carried out at different growth stages: beginning of stem elongation (GS_6 on the Feekes scale, corresponding to Z_{31} on Zadoks' scale), two-node stage (GS_7, Z_{32}), ligule of the flag leaf visible (GS_9, Z_{39}), and flowering (GS_{10}, Z_{60}). Crop nitrogen concentrations were determined by the Dumas method (Horwitz et al., 1975). In addition to CMR, a normalized CMR (nCMR) of treatment *i* in experiment *j* was calculated as follows:

$$nCMR_{i,j} = \frac{CMR_{i,j}}{CMRwellfertilizedcrop_j} \tag{1}$$

We thus divided each $CMR_{i,j}$ by the CMR of the treatment receiving the largest amount of fertilizer in the same conditions (site, year, cultivar), measured at the same stage (Wang et al., 2014). This method of calculating nCMR is convenient, but there seems to be a bias when $nCMR \geq 1$ (Prost and Jeuffroy, 2007), and when the most heavily fertilized treatment does not result in a $NNI \geq 1$ (Ziadi et al., 2010).

2.2. Set of models to be fitted to our database

Based on the various models and equations investigated in previous studies, we identified a number of models for testing to determine which best predicted NNI from a CMR. Follett et al. (1992) concluded that the relationship between CMR and plant N status differed from site to site. We therefore chose mixed models, such as lme() with a random site-year effect. As such, we considered a random site-year effect to reflect the lack of independence of treatments from the same experiment, and we took into account the variability attributable to the site-year. We also compared all models fitted to normalized and absolute CMR data, to determine whether nCMR resulted in improved precision. We compared three types of relationship:

2.2.1. The linear model (L)

It has been used to improve the prediction of NNI from CMR by dynamic crop models (Naud et al., 2009) and it fits early growth stage measurements well (Arregui et al., 2002). This model assumes that NNI increases steadily with CMR and is defined as follows:

$$Y_t = a + b \times CMR_t + e_t \tag{2}$$

where Y_t is the NNI for a treatment t, CMR_t is the CMR values measured for treatment t, a and b are the two parameters of the linear trend estimated by fitting the model to the data from experimental plots, and e_t is the residual error, equal to the difference between Y_t and the linear trend.

2.2.2. The quadratic model (Q)

It has been less investigated in fewer studies. Reeves et al. (1993) found that the relationship between leaf N concentration and SPAD® readings, even at early growth stages, could be either linear or quadratic, depending on crop management. Ziadi et al. (2010) found a significant quadratic relationship between NNI and CMR, and Yang et al. (2014) suggested that the relationship between yield and NNI was quadratic at the booting stage. Unlike the linear model, the quadratic model does not assume that NNI increases steadily. It is defined as follows:

$$Y_t = a + b \times CMR_t + c \times CMR_t^2 + e_t \tag{3}$$

where *a, b* and *c* are three parameters estimated by fitting the model to the data.

2.2.3. The exponential model (E)

It has been used to analyze the relationship between nCMR and NNI for the detection of N deficiency or as an alternative to the measurement of NNI at flowering (Debaeke et al., 2006; Prost and Jeuffroy, 2007). The use of an exponential relationship between CMR and NNI