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EFFECTIVENESS OF CHLOROPHYLL METER MEASUREMENT IN WINTER WHEAT AT FIELD SCALE LEVEL

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This paper examines the relationship among chlorophyll meter Yara N-Tester readings, nutrition status and growth parameters (leaf area index (LAI), plant height) of the winter wheat plants. Data used in this study were collected in 2010 from two fields located in the Czech Republic (area 52 and 38 ha) from different farms, both with uniform and conventional crop management. The monitoring of crop stands was done at growth stage BBCH 30 in a regular sampling grid with 150 m distance between points (27 and 18 points). At each sampling point, the plant height, LAI (Delta-T SunScan) and the chlorophyll concentration (Yara N-Tester) were recorded. Plant samples were taken to analyse the content of main nutrients (N, P, K, Mg, Ca and S). The results of plant analysis showed that both fields were in different nutrition status: one in a correct status and another had a complex nutritional deficit (K, Ca and N). Linear regressions and ANOVA proved that under a multiple nutritional deficit, N-Tester readings responded to the growth of the crop, while in the adequate nutritional conditions the sensitivity of N-Tester to the variation in the nitrogen concentration is lower. The relationships between crop parameters and chlorophyll meter readings are not generalisable and thus the interpretation of N-Tester results has to be done separately for each field.

Key words: plant diagnosis, nitrogen status, leaf transmittance, crop fertilisation, precision agriculture

The management of nitrogen fertiliser in cereals is crucial for obtaining high yields and for sustainable development (Lichtfouse *et al*. 2011; Miao *et al*. 2011; Srinivasan 2006), which is the reason why there are various research groups working for optimising it. The approaches to the problem come from different angles: leaf analysis, simulation models and spectral measurements (proximal or remote) related to the plant nitrogen concentration.

The biggest obstacle in the approximation with leaf analysis (Beaufils 1973; Marschner 1995), the most common of all, is the sampling of the crop and the cost of laboratory analysis. There are different strategies for the interpretation of the analysis (range of sufficiency and relationships between nutrients), which have proven their effectiveness when they are within the operating conditions. The use of simulation models (Basso *et al*. 2011; Behera & Panda 2009; Rahn *et al*. 2010) implies computer skills and carrying out the agronomic testing for calibration, but they can become effective in the management of nitrogen in crops. The strategy most economical and simple, and so the more easily transferred to farmers, is the use of the spectral vegetation indices (Feng *et al*. 2008; Raz *et al*. 2003; Zhu *et al*. 2006) by proximal or remote measurements, which are related to the plant nitrogen concentration (some of them are designed specifically for this purpose). There are many instruments currently available in the market, such as Yara N-Test-

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er, which recommend nitrogen rates without leaf analysis and complex computer simulations based on the spectral plant characteristics.

All the strategies mentioned above have studies that show their effectiveness in certain cases (aforementioned). There are also studies that show their weaknesses in generalising the results to different conditions (Amundson & Koehler 1987; Basso *et al*. 2010; Li *et al*. 2010; Patel *et al*. 2010; Rodriguez-Moreno & Llera-Cid 2011). Particularly, when trying to simulate their use in real farms, where interactions are complex, the large area requires a reduction in sampling and the methodology has to be marked as unreliable. The implementation of strategies in these real and complex scenarios needs to be improved.

This study examines the relationship among Yara N-Tester, the concentrations of nutrients in plants (N, P, K, Mg, Ca and S) and crop growth parameters (plant height and leaf area index (LAI)) of winter wheat in two fields in central Europe (Czech Republic). The objectives of the study are three. The first is to determine if the N-Tester can estimate the nitrogen concentration when the ranges of the nutrients are at appropriate levels. Under these conditions, the variance of nitrogen does not determine the leaf parameters, which may affect the effectiveness of N-Tester. The second is to check whether under a complex nutritional deficit that affects severely the crop development, the N-Tester responds to changes in other nutrients and, if so, in what way. The third is to test whether the relationship between N-Tester value and plant nitrogen concentration is the same in both fields thereby evaluating its generalisability. This study will increase the knowledge about the use of N-Tester for improvement of the recommendations and determination of the uncertainty associated with it.

MATERIALS AND METHODS

Field experiments and data collection

The experiment was carried out at two fields in the South Moravia region of the Czech Republic. The field “Pachty” (48°59’ N, 16°38’ E) has an area of 52.5 ha and is located in a flat region (elevation 176–182 m) with an average annual temperature of 9.2°C and precipitation of 483 mm per year; its predominant soil type is Chernozem. The field “Haj” (49°15’ N, 17°06’ E) has an area of 37.8 ha and is located in a hilly terrain (elevation 280– 342 m) with an average annual temperature of 9.25°C and precipitation of 542 mm per year; its predominant soil type is haplic Luvisol.

In 2010, the winter wheat (*Triticumaestivum* L., cultivar Eureka and Baletka) was planted at both localities (fore-crop: sunflower at Pachty, spring barley at Haj) under uniform crop management within the fields following the common agricultural standards in the Czech Republic. The crop had a multiple nutritional deficit with large spatial variability in the field “Pachty”, while in the field “Haj” the crop was grown correctly and uniformly (more details are given in the Results and discussion section).

The plant samples were taken at BBCH 30 growth stage (Zadoks *et al*. 1974) for analysis of nutrients in the above-ground dry matter (concentration of N, P, K, Ca, Mg and S) according to the methodology valid in the Czech Republic (Zbíral 2005). Simultaneously, the height of plants, the LAI (measured by SunScan, Delta-T Devices Ltd., UK) and chlorophyll content of leaves (using an N-Tester, YARA International ASA, Germany) were recorded. Sampling was carried out on a regular grid of 150 × 150 m (27 points in Pachty, 18 in Haj) within a circle of 5 m diameter at each sampling point (Figure 1).

Yara N-Tester is based on the Minolta SPAD 502, which measures light transmitted by the plant leaf at two different wavelengths, 650 and 940 nm (Arregui *et al*. 2006). Thirty random measurements are recorded to get the representative value in each sampling point, always following the recommendations of the manufacturer of the device.

Regarding final production, yield sampling was made in the same grid at both localities two weeks before the harvest. Spikes were cut from an area of 0.2 m^2 at two places within a circle of 5 m of diameter on each point of the sampling grid. Spikes were threshed in the lab to get the yield (15% grain moisture, standard).

Information about soil parameters was obtained from the soil survey described in recent studies (Lukas *et al*. 2009a; Lukas *et al*. 2009b). Soil samples were taken from a depth of 0–0.3 m in a 50 m regular grid (214 soil samples in Pachty and 152 samples in Haj). Samples were analysed for pH value and content of base nutrients (P, K, Mg and Ca) according to the Mehlich III methodology (Zbiral & Nemec 2000).

Data analysis

As a first step, descriptive statistics and normality tests (Kolmogorov–Smirnov´s test) were calculated for the observed variables. The nitrogen concentration in both fields was studied to determine if their dispersion were comparable in magnitude, and so it would be equally appropriate to look for the relationship between N-Tester and the nitrogen concentration in both fields.

The study of the relationship between N-Tester and nitrogen concentration at both localities was based on tests of significance (p-value), measures of the magnitude (coefficient of determination) and sign of the relationship (correlation coefficient). This same procedure was used to study the relationships between the N-Tester and concentrations of other nutrients (P, K, Ca, Mg and S) and crop growth parameters (plant height and LAI). The latter identifies factors that may influence the nitrogen fertiliser recommendation by N-Tester.

The last step was to compare the relationship between N-Tester and nitrogen concentration in both locations. It determines whether it is possible to extrapolate the relationship from one field to other fields in similar conditions (same crop and geographic area).

RESULTS AND DISCUSSION

Tables 1 and 2 show the descriptive statistics of the concentrations of nutrients (N, K, P, Ca, Mg and S), the crop growth parameters (plant height and LAI), N-Tester records and yield data. The Kolmogorov–Smirnov´s test proved the normal distribution of all examined variables at a significance level of 0.05. The values of all variables, except LAI, show that the plant nutritional status in the field Pachty is better than in the field Haj (lower mean values and greater variances), which was later confirmed by the crop yield.

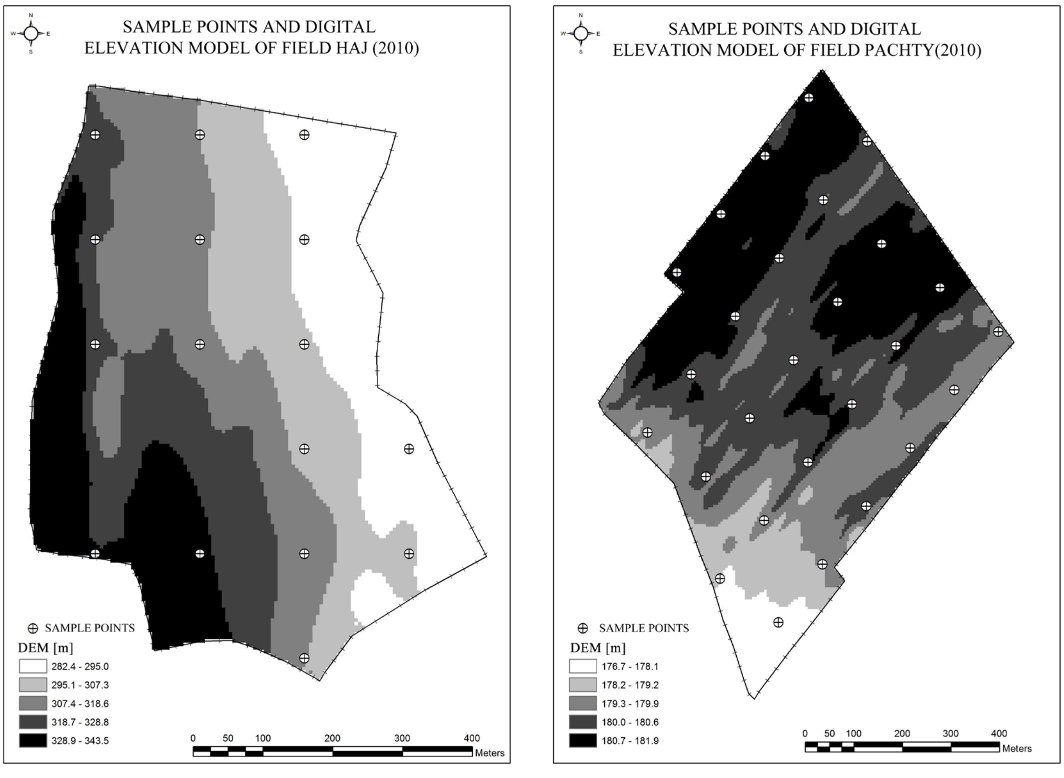

Figure 1. Sample points and digital elevation model for the fields Haj (left) and Pachty (right)

The standard deviation of nitrogen concentration in the field Haj (0.15) represents around 40% of deviation in the field Pachty (0.38). If the estimate of the nitrogen concentration using the function calculated with the data from the field Pachty has an uncertainty close to the deviation of the nitrogen concentration in the field Haj, then it is likely that the relationship cannot be recognised in the field Haj.

Nutritional diagnosis

Interpretation of the soil sampling, based on the Mehlich III methodology (Zbíral 2005), recommended correction of the K and pH levels in the field Pachty. There were no variable rate application of fertilisers carried out, and so the leaf analysis endorses the K, Ca (related to the problem with the pH) and N.

T a b l e 1

Statistical summary of results of plant measurement and analysis obtained at BBCH 30 and harvest sampling in the field Pachty (27 samples)

	Height [cm]	LAI	N-Tester	N [%]	P [%]	K [%]	Ca [%]	Mg [%]	S [%]	Yield [t/ha]
Mean	28.82	1.68	441.00	3.65	0.35	3.35	0.28	0.11	0.20	7.96
Median	28.10	1.64	435.00	3.64	0.34	3.35	0.28	0.11	0.20	7.67
Standard deviation	3.90	0.51	52.64	0.38	0.05	0.35	0.05	0.02	0.02	1.56
Coefficient of variability [%]	13.53	30.36	11.94	10.41	14.29	10.45	17.86	18.18	10.00	19.60
Variance	15.21	0.26	2770.62	0.15	0.00	0.12	0.00	0.00	0.00	2.44
Skewness	0.42	0.78	-0.27	0.96	0.34	0.51	0.13	0.80	0.20	0.54
Kurtosis	-0.30	0.78	-0.66	1.29	-0.93	0.31	-0.69	0.06	0.69	-0.61
Minimum	21.30	0.92	336.00	3.12	0.27	2.78	0.20	0.08	0.16	5.60
Maximum	37.00	3.01	517.00	4.75	0.45	4.24	0.38	0.15	0.26	11.06
Kolmogorov–Smirinov's p-value	0.91	0.87	0.75	0.90	0.85	0.98	0.77	0.48	0.68	0.62

T a b l e 2

Statistical summary of results of plant measurement and analysis obtained at BBCH 30 and harvest sampling in the field Haj (18 samples)

	Height [cm]	LAI	N-Tester	N [%]	P [%]	K [%]	Ca [%]	Mg [%]	S [%]	Yield [t/ha]
Mean	29.43	1.36	584.78	5.00	0.49	3.64	0.49	0.11	0.33	9.83
Median	29.55	1.36	575.50	5.00	0.49	3.63	0.49	0.11	0.33	9.42
Standard deviation	2.24	0.49	29.39	0.15	0.03	0.21	0.03	0.01	0.03	1.23
Coefficient of variability [%]	7.61	36.03	5.03	3.00	6.12	5.77	6.12	9.09	9.09	12.51
Variance	5.03	0.24	863.71	0.02	0.00	0.04	0.00	0.00	0.00	1.52
Skewness	0.00	0.38	0.63	-0.69	-0.66	-0.45	0.06	-0.66	1.16	0.76
Kurtosis	1.45	1.09	0.18	0.19	0.09	0.28	-0.69	-0.44	3.65	-0.10
Minimum	24.40	0.40	537.00	4.68	0.42	3.16	0.43	0.10	0.28	7.97
Maximum	34.50	2.54	646.00	5.24	0.54	3.95	0.55	0.12	0.41	12.32
Kolmogorov–Smirinov's p-value	0.82	0.95	0.75	0.76	0.85	0.96	0.96	0.62	0.56	0.34

The results of soil and plant analysis did not identify nutritional deficiencies in the field Haj. The spatial variability recorded in crop parameters was attributed to the gradient in the soil moisture, a result of the relief. Figures 1 and 2 show the crop yield and the digital elevation model, respectively; the coincidence of spatial patterns is noticeable.

Study of the N-Tester reading in the field Pachty

Table 3 shows the significance and the correlation coefficients between all variables. There is a significant and positive relationship between N-Tester and nitrogen concentration. The coefficient of correlation is equal to 0.58 and the root mean square error (RMSE) is 0.31%. The value of RMSE corresponds to 80% of the deviation of the nitrogen concentration in the field Pachty.

If the RMSE is representative of the potential of the N-Tester to estimate the nitrogen concentration, the relationship between the N-Tester and nitrogen concentration will not be recognised in the field Haj because the RMSE is more than twice the standard deviation recorded there.

There is a significant relationship between N-Tester values and plant height (correlation coefficient equal to 0.43). This relation is important because it is not redundant; there is no relationship between the nitrogen concentration and the plant height. A multiple regression analysis showed that 42% of the variance of N-Tester values could be explained by the nitrogen concentration and plant height. However, the plant height could be considered as an indicator of crop biomass density (Ehlert

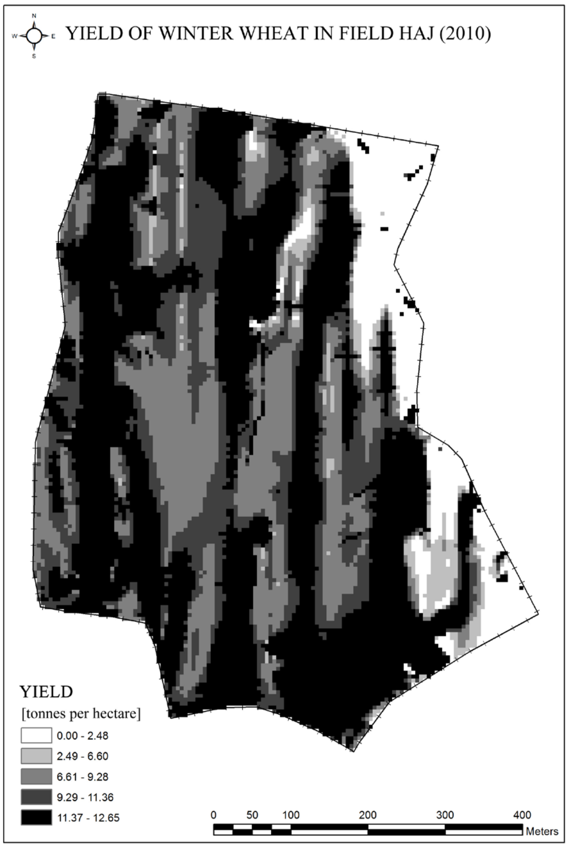

Figure 2. Map of winter wheat yield in the field Haj calculated from the sample data and interpolated by digital elevation model

T a b l e 3

Correlation coefficients for relationships between variables obtained during sampling of the crop in the field Pachty

Coefficient ofcorrelation	Height [cm]	LAI	N-Tester	N [%]	P [%]	K [%]	Ca [%]	Mg [%]	S [%]
Height [cm]		0.34	0.43^{+}	0.24	-0.02	0.74^{+++}	0.40^{+}	0.27	0.31
LAI	0.34		0.02	0.15	0.05	0.34	0.12	0.08	0.15
N-Tester	0.43^{+}	0.02		0.58^{++}	-0.30	0.48^{+}	0.52^{++}	0.30	0.47^{+}
N [%]	0.24	0.15	0.58^{++}		-0.34	0.49^{++}	0.60^{+++}	0.66^{+++}	0.83^{+++}
P [%]	-0.02	0.05	-0.30	-0.34		-0.10	-0.54^{++}	-0.60^{+++}	-0.04
K [%]	0.74^{+++}	0.34	0.48^{+}	0.49^{++}	-0.10		0.58^{++}	0.31	0.54^{++}
Ca [%]	0.40^{+}	0.12	0.52^{++}	0.60^{+++}	-0.54^{++}	0.58^{++}		0.59^{++}	0.42^{+}
Mg [%]	0.27	0.08	0.30	0.66^{+++}	-0.60^{+++}	0.31	0.59^{++}		0.46^{+}
S [%]	0.31	0.15	0.47^{+}	0.83^{+++}	-0.04	0.54^{++}	0.42^{+}	0.46^{+}	

$^{+}$, $^{++}$ and $^{+++}$ stand for 0.05, 0.01 and 0.001 significance levels *et al*. 2008). A new proof of this is that the height is significantly related to the concentration of K and Ca, two nutrients that act as limiting factors in the field. Although N-Tester was developed to support the decision for nitrogen fertilising, the results showed that this measurement is influenced by the deficiencies of other nutrients too. In this case, the positive relationship with other nutrients is an obstacle since higher nitrogen fertilisation doses would not solve the problem with other nutrients.

An alternative strategy would be to set a threshold for the N-Tester: those plants that do not reach this threshold have deficiencies, which should be studied using more advanced tools that an N-Tester. The fertilisation plan would be directed to plants that could offer a higher yield. From this perspec-

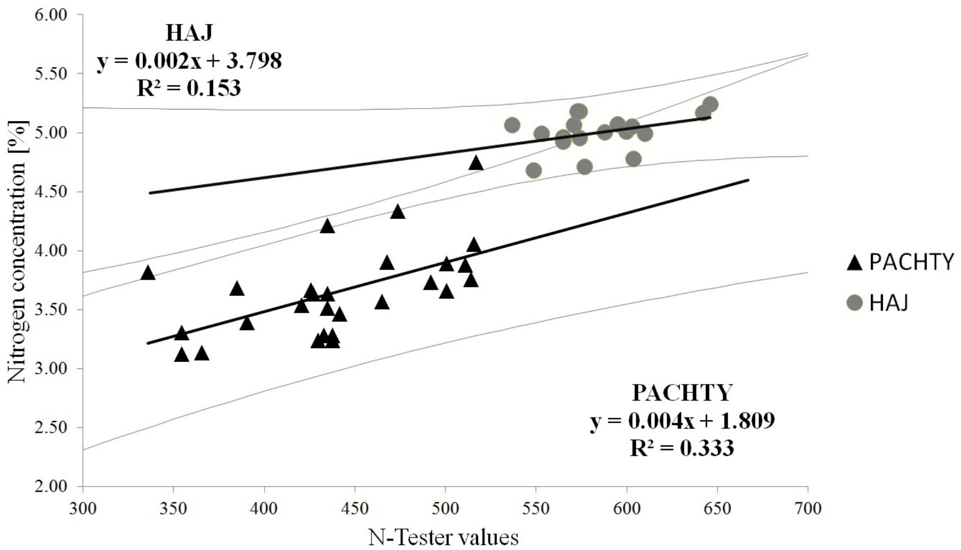

Figure 3. Scatter plot of N-Tester values versus the plant nitrogen concentration in the fields Pachty and Haj. Equation, goodness of fit and confidence interval (95%). Linear regression with confidence intervals for the prediction 95%.

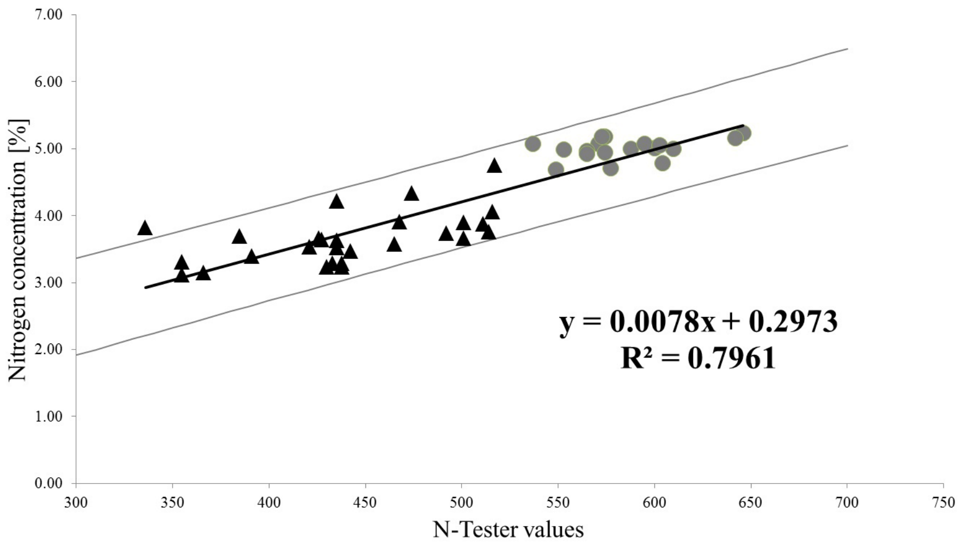

Figure 4. Scatter plot of N-Tester values versus the plant nitrogen concentration with the data from both fields. Equation, goodness of fit and confidence interval (95%). Joint linear regression with confidence intervals for the prediction at 95%.

T a b l e 4

Correlation coefficients of relationships between variables obtained during sampling of the crop in the field Haj

Coefficient of correlation	Height [cm]	LAI	N-Tester	N [%]	P [%]	K [%]	Ca [%]	Mg [%]	S [%]
Height [cm]		0.43	0.09	-0.26	-0.32	-0.44	-0.37	-0.46	0.05
LAI	0.43		-0.37	-0.72^{+++}	-0.13	-0.05	-0.24	-0.32	-0.53^{+}
N-Tester	0.09	-0.37		0.39	0.01	0.08	0.03	-0.03	0.20
N [%]	-0.26	-0.72^{+++}	0.39		0.01	0.02	0.31	0.44	0.61^{++}
P [%]	-0.32	-0.13	0.01	0.01		0.57^{+}	0.17	0.11	-0.11
K [%]	-0.44	-0.05	0.08	0.02	0.57^{+}		0.03	0.19	0.08
Ca [%]	-0.37	-0.24	0.03	0.31	0.17	0.03		0.19	0.03
Mg [%]	-0.46	-0.32	-0.03	0.44	0.11	0.19	0.19		0.17
S [%]	0.05	-0.53^{+}	0.20	0.61^{++}	-0.11	0.08	0.03	0.17	

$^{+}$, $^{++}$ and $^{+++}$ stand for 0.05, 0.01 and 0.001 significance levels

tive, the relationship with other nutrients is positive. This is because the plants with a complex nutritional deficit would not waste nitrogen fertilisers, unable to solve any nutrient deficiencies.

Study of the N-Tester reading in the field Haj

No significant relationships were found between N-Tester and nutrients concentration (Table 4). The same result was obtained between N-Tester readings and crop parameters (height and leaf area). Based on the statistical summary of data (Table 2), the crop was in a good nutritional status throughout the field Haj, something that had not happened in the field Pachty.

The noise associated with the N-Tester signal readings was higher than effects of the differences in the nitrogen concentration and other parameters. This is understandable given the order of magnitude of the error in the estimate of the nitrogen concentration by N-Tester in the field Pachty. As the result, a uniform treatment would be recommended for this field.

The generalisation of the regression model obtained in the field Pachty to the field Haj is impossible. The 50% of the confidence intervals (at 95%) of the estimates do not include the true values of nitrogen concentration (Figure 3). These estimates were obtained by applying the function found in the field Pachty to the N-Tester readings from the field Haj.

Further studies are necessary, but the result of this study is sufficient evidence to conclude that the fertiliser recommendations by N-Tester require more than a simple and universal conversion table. The interpretation of the N-Tester readings depends on the nutritional status of the crop. Zhang *et al*. (2008) remark that small and moderate deficiencies of N could not be diagnosed with reasonable certainty until it was too late to make in-season fertilisation.

The results contradict, at least apparently, the studies supporting the use of Minolta SPAD 502-like devices. Since these studies were carried out on a data set taken from different fields, it was decided to test the combination of the data sets from both localities to determine the relationship between N-Tester values and plant nitrogen concentration. The foregoing studies show a poor result, but the reason for the discrepancy between the results is expected to be found.

Figure 4 shows the regression model obtained with the combined data set. The coefficient of determination is equal to 0.797, a higher value than those obtained in the separate analysis of the data from the fields Pachty (0.33) and Haj (0.15), which is what the studies supporting the use of Minolta SPAD 502-like devices found. A detailed analysis revealed that this regression model is not a solution because the RMSE increased, exceeding the levels obtained in the separate analyses of each field. In all cases, the errors represent a high percentage of nutrient variability in the field, making them ineffective. This analysis explains the discrepancy and helps in understanding the results of other authors and studies where the effects of using N-Tester were identified, but not in the expected magnitude (Han *et al*. 2005; Liang *et al*. 2004).

CONCLUSIONS

The results of this study showed that the interpretation of N-Tester readings strongly depends on the nutritional status of the crop. Most of the variance in N-Tester signal is unrelated to any of the observed variables in both localities, even when there was a severe nutritional deficiency (variation in crop yield was equal to 20%), and the information gathered has been enough for crop monitoring and yield estimation in both fields.

The N-Tester responds significantly to the nutrient status and development of the crop. This fact negatively influences the recommendation for nitrogen fertilising by N-Tester because higher doses of nitrogen would not solve the deficiency of other nutrients. In contrast, the results of a survey of a crop with a balanced nutritional status did not show any relation between N-Tester and nutrient concentrations. The effects of variations in the concentrations were below the threshold of sensitivity of N-Tester.

A data set composed of samples from different fields is ideal for evaluating the strength of the relationship between the Minolta SPAD 502-like devices and the nitrogen concentration. The principle of the device could be fully tested, and it is not enough to determine that it is effective within a field. If the range of the nitrogen concentration in a field is lower, the device cannot reach the necessary precision. This study shows this explains the different results obtained in previous assessments of the potential of N-Tester.

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