SPECTRAL HIGH-THROUGHPUT ASSESSMENTS OF PHENOTYPIC DIFFERENCES IN SPIKE DEVELOPMENT, BIOMASS AND NITROGEN PARTITIONING DURING GRAIN FILLING OF WHEAT UNDER HIGH YIELDING WESTERN EUROPEAN CONDITIONS

K. Erdle and Urs Schmidhalter

Department of Plant Sciences, Chair of Plant Nutrition,
Technische Universität München,
Emil-Ramann-Str. 2, 85350 Freising-Weihenstephan, Germany

ABSTRACT

Single plant traits such as green biomass, spike dry weight, biomass and nitrogen (N) transfer to grains are important traits for final grain yield. However, methods to assess these traits are laborious and expensive. Spectral reflectance measurements allow researchers to assess cultivar differences of yield-related plant traits and translocation parameters that are affected by different genetic material and varying amounts of available N. In a field experiment, six high-yielding wheat cultivars were grown with N supplies of 0, 100, 160 and 220 kg N ha^{-1}. Wheat canopies were observed spectrally throughout the grain-filling period and three spectral parameters were calculated. To describe the development of the vegetative plant parts (leaves+culms) and the spikes, plants were sampled four times during grain filling. Dry weights and the relative dry matter content were recorded for leaves+culms and spikes. The N status of the plants was assessed by measuring the total N concentration and by calculating the above-ground N uptake. In case of an equal and sufficient N supply of 160 kg ha^{-1}, the final grain dry matter was best assessed by spectral indices offering information about physiological maturity. Observing effects of various N supply, good correlations were found between spectral indices and single plant traits throughout grain filling but varied with N supply and development stage. The normalized difference vegetation index, NDVI, was strongly affected by the saturation effects of increased N concentration. The red edge inflection point, REIP, predicted plant traits with r^2 values up to 0.98. However, in plants with advanced senescence, the REIP was less efficient in describing plant traits. The NIR-based index R_{760}/R_{730} was closely related to yield-related plant traits at early grain filling. Compared to the REIP, the R_{760}/R_{730} index was resistant to strong chlorophyll decays being able to predict plant traits at late grain filling, with r^2 values of up to 0.92. Spectral reflectance measurements may represent a promising tool to assess phenotypic differences in yield-related plant traits during grain filling.

Keywords: spike development; biomass translocation; nitrogen translocation; nitrogen uptake; phenotyping; remote sensing; source-sink relations; high yielding

INTRODUCTION

The early vigor and nutritional status of crops strongly influences the actual vegetative development and the final yield. Spectral remote sensing is used to assess the vigor, nutritional status, and growth characteristics of wheat in the vegetative phase (Raun et al., 2001; Aparicio et al., 2002; Prasad et al., 2007; Kipp et al., 2014; Li et al., 2014). With sensors, light reflected by plants can be measured and is converted into an electrical output. Near-infrared reflected light (NIR, app. 700-2500 nm) is most sensitive to the structural components (Campbell, 2002), while the visible light (VIS, app. 400-700 nm) mainly depends on the chlorophyll concentration of the leaves. The combination of red and NIR based indices such as the normalized difference vegetation index (NDVI; Rouse et al., 1974) were found to be an indirect measurement of crop characteristics (Aparicio et al., 2000; Serrano et al., 2000; Aparicio et al., 2002; Bort et al., 2005; Li et al., 2008; Li et al., 2014). In regions of high-yielding cereal crops like Europe, vegetation indices were generated that depend on NIR reflectance being more resistant to saturation effects. Compared to NDVI, the red edge inflection point (REIP) lying between the NIR and the red-light bands (Guyot et al., 1988) and NIR/NIR-based indices offer more reliable signals with dense crop stands (Mistele et al., 2004; Heege et al., 2008; Mistele and Schmidhalter, 2010a; Reusch et al., 2010; Erdle et al., 2011; Kipp et al., 2014).

Only few studies based on pot experiments or on broad genetic variability were done to observe the reproductive phase of wheat spectrally (Aparicio et al., 2002; Babar et al., 2006a; Babar et al., 2006b; Prasad et al., 2007; Kipp et al., 2013). However, modern wheat cultivars are closely related genetically and therefore spectral differentiation is more complicated (Ferrio et al., 2005). Processes during the time of grain filling affect grain yield (Reynolds et al., 2009). Leaves and culms as well as the source-sink relationship of spikes and grains, are strongly related to final grain yield (Brooking and Kirby, 1981; Fischer, 2011). Next to genetic variation, nutrient supply has a strong influence on this relationship. Assessments of specific morphological and physiological crop characteristics have been frequently done and were shown to be related to the final yield (Fischer and Stockman, 1980; Stockman et al., 1983; Abbate et al., 1997). However, the methods used are highly laborious and expensive. Spectral remote sensing, which is already well established in crop nutrient management, may offer an efficient approach to observe relevant plant traits interconnected to cultivar specific grain yield.

As varied N supply is expected to trigger larger cultivar differences the focus of this study was to include N deficiency and surplus next to a sufficient N supply to test the potential of spectral sensing to characterize post-anthesis yield development. Therefore, the objectives of this study were i) to assess genetic differences in yield-related plant traits using spectral remote sensing post-anthesis and ii) to estimate the influence of varying N supply on the relationship between these plant traits and vegetation indices of high-yielding wheat cultivars.

MATERIAL AND METHODS

Study site and biomass sampling

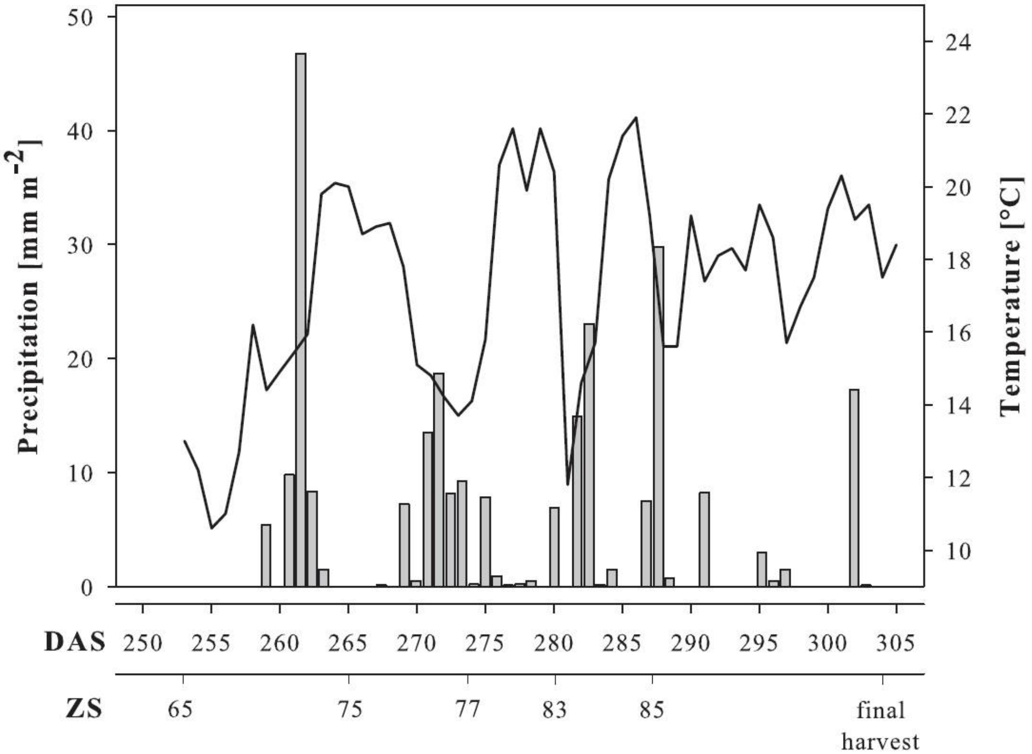

Fig. 1: Daily sums of precipitation and course of average temperatures during grain filling from 253 to 303 days after anthesis (DAS). Dates of anthesis at Zadoks stage (ZS) 65, the four sampling dates ZS 75, 77, 83 and 85, and the final harvest at 303 DAS are indicated (adopted from Erdle et al., 2013b).

The trial was carried out on a Cambisol with homogenous characteristics in southwestern Germany (11°41´60” E, 48°23´60” N). Average yearly precipitation is approximately 800 mm with an average temperature of 7.5 °C.

In 2009, rainfall events summed up to 262 mm m^{-2}, with a mean temperature of 17.3 °C (Fig. 1). Grain yields in this area vary between 6 and 10 t ha^{-1}. The field was managed conventionally and adopted local standards. The residual soil nitrate of a soil NO_3-N level of 45 kg ha^{-1} was determined using a simplified soil nitrate quick-test method (Schmidhalter, 2005). Plots of six locally adapted winter wheat (*Triticum aestivum* L.) cultivars were grown in a randomized block design with four replicates. Four N treatments (as calcium ammonium nitrate) were applied at rates of 0 (N_0), 100 (N_{100}), 160 (N_{160}), and 220 (N_{220}) kg N ha^{-1}. No fertilizer was applied prior to planting. Following local standards, fertilizer application was done at Zadoks growth stages (ZS; (Zadoks et al., 1974) 30 (stem-elongation) and 40 (booting) with 30, 60, and 90 kg N ha^{-1} for the N treatments of 100, 160, and 220 kg N ha^{-1}, respectively. At ZS 50 (heading) 40 kg ha^{-1} were given, aimed to increase the protein quality.

Plant sampling was done at ZS 75 (medium milk), 77 (late milk), 83 (early dough) and 85 (soft dough) referring to 265, 273, 280, and 287 days after sowing (DAS), respectively (Fig. 1). At each time, 0.12 m^2 of the plots´ center were cut at ground level, resulting in approximately 70 spiked wheat culms plus leaves. The biomass sample was packed, immediately weighed and afterwards partitioned into spikes and the remaining shoot parts (leaves+culms). The partitions were weighed again and then dried. Dry matter content (DM in %) was calculated and total N concentration (%) was detected with mass spectrometry using an Isotope Radio Mass Spectrometer with an ANCA SL 20-20 preparation unit (Europe Scientific, Crewe, UK). The above-ground N uptake (N_{up} in kg ha^{-1}) of spikes and leaves+culms was calculated as a product of the dry weight and the total N concentration. The relationships of both DW development and N uptake between spikes and leaves+culms were calculated as:

$$\text{DW index} = \frac{\text{spike DW}}{\text{leaf DW} + \text{culm DW}}$$

$$\text{N}_{\text{up}}\ \text{index} = \frac{\text{spike N}_{\text{up}}}{\text{leaf N}_{\text{up}} + \text{culm N}_{\text{up}}}$$

At 303 DAS, a combine was used to harvest the crop of an equally treated plot next to the sampled one for final grain yield. Especially for the N level of 160 kg ha^{-1} which goes conform to practical standards of the region, grain dry matter content (grain DM) was observed additionally to show potential genetic differences on a farm-level N treatment.

Spectral reflectance measurements

Previous to biomass sampling, spectral reflectance of the canopy was recorded. A passive bi-directional spectrometer (tec5, Oberursel, Germany) was used in order to enable hyperspectral readings. The spectrometer contained two Zeiss MMS1 silicon diode array spectrometers detecting a range of 400 to 1000 nm at a bandwidth of 3.3 nm (Mistele and Schmidhalter, 2010a). One unit was linked to a diffuser that detected solar radiation as a reference. The second unit simultaneously measured the plant canopy reflectance with a 12 ° field of view (FOV) at the fixed height of 1.40 m above the ground (circular FOV of 0.28 m^2). To provide the best possible conditions for passive recording, the spectral measurements were conducted under clear sky conditions shortly before or at noon. Sensor outputs were co-recorded along with the GPS coordinates from an RTK-GPS unit (real-time kinematic global positioning system; Trimble, Sunnyvale, CA, USA). Up to 40 readings per plot central were averaged. The sensor system was mounted on a frame attached to the tractor´s front. Measurement direction was done from west to east in order to avoid any shading of the measuring unit. The tractor had a ground clearance of 0.90 m, which allowed it to pass over the plots without touching the canopy. Four spectral parameters (Tab. 1) were tested to characterize the post-anthesis development of wheat cultivars. The indices proved to provide a thorough estimation of the nutritional status and biomass development of wheat (Peñuelas et al., 1993; Schmidhalter et al., 2003; Mistele et al., 2004; Reusch, 2005; Heege et al., 2008; Mistele and Schmidhalter, 2008; Mistele and Schmidhalter, 2010b; Reusch et al., 2010; Erdle et al., 2011).

Table 1: Spectral indices selected for post-anthesis characterization of wheat.

Abbreviation	Formula	Reference
NDVI	$(R_{780} - R_{670})/(R_{780} + R_{670})$	(Rouse et al., 1974)
REIP	$700 + 40((R_{670} + R_{780})/2 - R_{700})/(R_{740} - R_{700})$	(Guyot et al., 1988)
R_{760}/R_{730}	R_{760}/R_{730}	(Erdle et al., 2011)

Thus, these indices were chosen in this study as they bear also a high potential to identify traits of wheat after anthesis. The NDVI (Rouse et al., 1979) uses spectral patterns of red and NIR spectral regions. The REIP (Guyot et al., 1988) is calculated based on a mathematical model including VIS and NIR spectral information. One additional NIR/NIR based indices was calculated as R_{760}/R_{730} (Mistele and Schmidhalter, 2010b; Erdle et al., 2011).

Data processing and statistical analysis

With SPSS 11 (SPSS Inc., Chicago, USA) the main effects and interactions were tested using a generalized linear model. An ANOVA was used to differentiate the means of the cultivars. Duncan’s multiple comparison procedure was applied based on the studentized range test with a P value of 5 %. Linear relationships were used for each N level a) between yield and plant parameters destructively sampled during grain filling and b) between spectral indices and the plant parameters as potential yield components.

RESULTS

Relationships between grain yield and plant parameters

As grain yield also depends on the development of spikes and grains, the four plant parameters spike DW, spike DM content, DW index and N_{up} index were set in relation to the final grain yield. These plant traits are closely connected to the single cultivars, therefore simultaneously mirroring phenotypic effects. The relationships between plant traits and final grain yield considering both, development stages and N application rates are shown in Tab. 3. The relationships strongly varied depending on the development stages and the N treatments. Compared to the N levels of N_0, N_{100} and N_{160}, the relationships within the N level N_{220} were less close. The majority of the relationships were less close at the development stage ZS 85.

The relationships of grain yield to spike DW were best within N_0 at ZS 75 reaching r^2 values of up to 0.95 decreasing with increasing N supply up to N level N_{160}. Similarly, the DW index was correlated to grain yield as the spike DW: Good relationships were found at the N level N_{100}, low predictive accuracies were found at ZS 85 in N_{160} and N_{220}. Strongly inconsistent relationships were found between the final grain yield and the N_{up} index. R^2 values varied between 0.17 to 0.80 not showing a specific pattern related to dates or N supply.

Table 3: Coefficients of determination for the relationships between four yield components and the final grain yield. Spike dry weight (DW), spike dry matter content (DM), dry weight index (DW index) and N uptake index (N_{up} index) were assessed at the four N application levels 0 (N_0), 100 (N_{100}), 160 (N_{160}) and 220 kg ha^{-1} (N_{220}) at Zadoks stages (ZS) 75, 77, 83 and 85. The models follow linear courses. The significant coefficients of determination are highlighted as $^{*}P \leq 5\%$, $^{**}P \leq 1$ and $^{***}P \leq 0.1\%$ for n = 6 (adopted from Erdle et al., 2013a).

Yield components	ZS	Coefficient of determination for grain yield			
		N_0	N_{100}	N_{160}	N_{220}
Spike DW	75	0.95***	0.92**	0.68*	0.47
	77	0.61	0.75*	0.75*	0.39
	83	0.08	0.64	0.71*	0.79*
	85	0.29	0.20	0.74*	0.36
Spike DM	75	0.26	0.82*	0.85**	0.34
	77	0.74*	0.88**	0.79*	0.64
	83	0.70*	0.80*	0.71*	0.56
	85	0.56	0.59	0.41	0.06
DW index	75	0.68*	0.81*	0.73*	0.58
	77	0.28	0.85**	0.66*	0.67*
	83	0.70*	0.84**	0.84**	0.74*
	85	0.76*	0.59	0.36	0.37
N_{up} index	75	0.47	0.80*	0.59	0.43
	77	0.32	0.63	0.67*	0.26
	83	0.77*	0.58	0.62	0.23
	85	0.70*	0.50	0.17	0.21

Relationships between plant parameters and spectral indices

The spectral parameters used in this study were related to the plant parameters during the period of post anthesis at varying N supply. The NDVI and the R_{760}/R_{730} index were mainly influenced by the effect of the development stage, closely followed by the effect of nitrogen supply (Tab. 4). The REIP was nearly equally influenced by the N treatment and development stage. The cultivars' effect on spectral parameters was quite strong as well, showing F-values higher than the interactions with the exception of R_{760}/R_{730} for development stages x N treatment. For all indices used in this study, the magnitude of the reflectance indices decreased chronologically but increased with the amount of N applied (Fig. 2, Erdle et al., 2013b).

Relationships between the spikes DW and the NDVI were poor compared to those of the REIP and the R_{760}/R_{730} index (Tab. 5). The REIP described the spike DW well with r^2 values up to 0.85. However, in contrast to the relationships to other spectral indices, at low N supply, the relationships' slopes of the REIP became positive at ZS 85 which changed within N_{220} (Fig. 2, Tab. 5).

Table 4: F-values of the general linear model (GLM) for three vegetation indices across six wheat cultivars, four development stages (Dev. stage) and four N treatments. All main effects and interactions are significant at $P \leq 0.1\%$ (adopted from Erdle et al., 2013b).

Source of variation	df	NDVI	REIP	R_{760}/R_{730}
Model	50	387.7	207.1	468.0
Cultivar	5	229.2	60.2	211.2
Dev. stage	3	4419.4	1466.3	4437.3
N treatment	3	1444.2	1676.0	2091.0
Cultivar × Dev. stage	15	21.2	12.6	9.6
Cultivar × N treatment	15	5.7	5.6	15.7
Dev. Stage × N treatment	9	27.2	39.3	264.2
Residual	335			
Total	384			

The NDVI better predicted spike DM content with progressive grain filling (Tab. 5). The best NDVI − spike DM relationships were found at ZS 85, with r^2 values ranging between 0.81 and 0.93. The REIP within N_{100}, N_{160} and N_{220} increased its predictive accuracy of the spike DM content to up to r^2 0.94. However, there was a strong decrease in the relationship quality at ZS 85 due to the effect mentioned above. Similar to that, the R_{760}/R_{730} index increased predictive qualities of spike DM content to r^2 values of up to 0.92, however, slightly decreasing at ZS 85. The REIP−DW index relationship resulted in high r^2 values of up to 0.87. Results at ZS 85 were repeatedly affected by the inverted direction of the regressions. The best relationships between the DW index and the R_{760}/R_{730} index were found within N_{100} with r^2 values of up to 0.95 at ZS 83.

Compared to the previous plant parameters, the best relationships were found between the N_{up} index and the three calculated vegetation indices (Fig. 2) especially at early grain filling or low N supply with r^2 values getting up to 0.97.
Grain DM, which was only evaluated for N_{160} due to practical relevance was the crop trait which was consistently and highly related to the spectral indices with r^2 values between 0,67 and 0,85 throughout grain filling with the exception of REIP at ZS 85.

DISCUSSION

Grain filling is one of the most crucial periods in yield development of cereal crops. Wheat cultivars differ in terms of the spike development during post anthesis and are affected by varying nitrogen supply. In this study, post anthesis phenotypic variations in spike development were assessed spectrally under varying N supply.

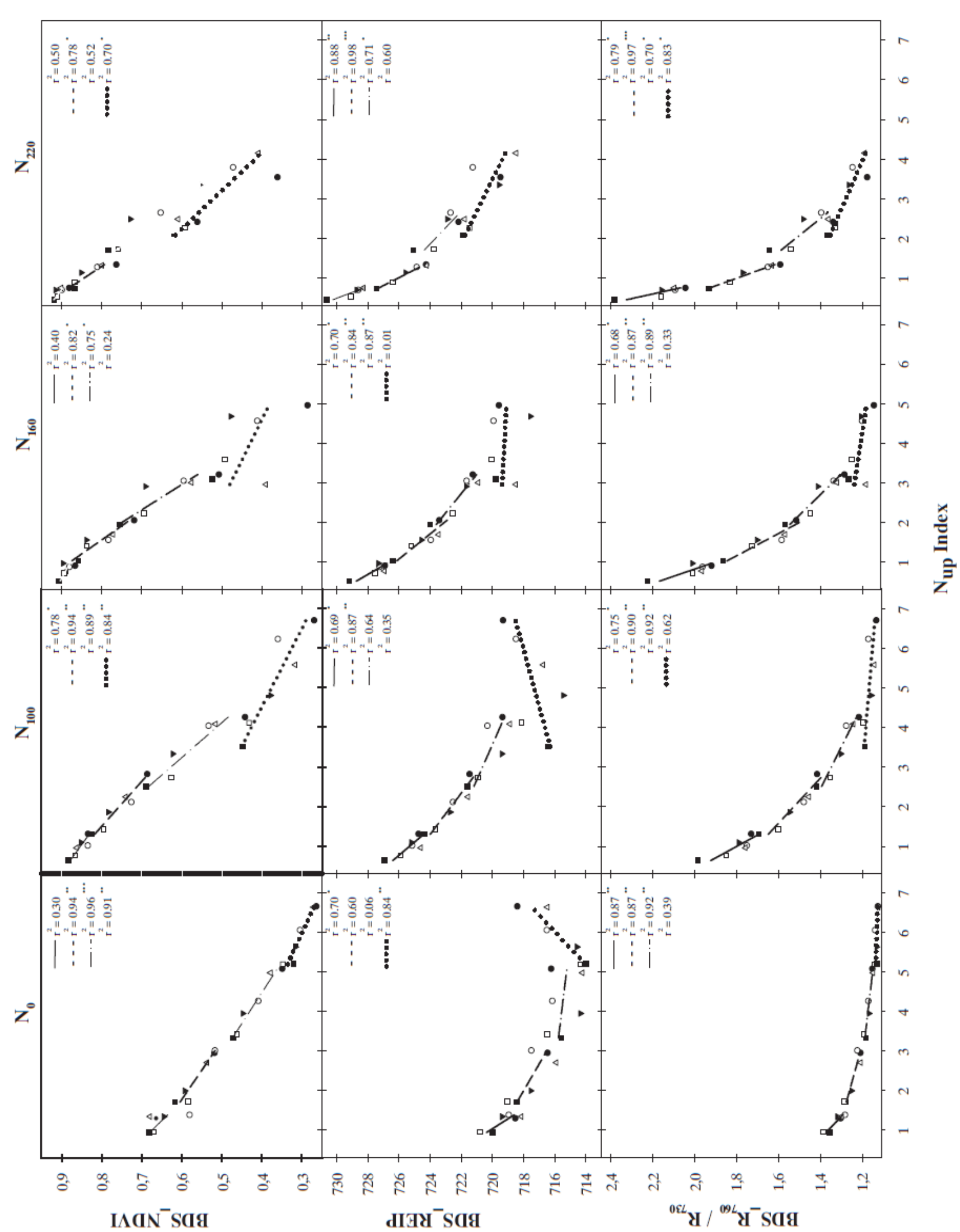

Fig. 2: Linear relationships between the mean values (n = 4) of the normalized difference vegetation index (NDVI), the red edge inflection point (REIP) and the R_{760}/R_{730} index and the yield component of N uptake index (N_{up} index) are shown

for the nitrogen application levels 0 (N_0), 100 (N_{100}), 160 (N_{160}) and 220 kg ha^{-1} (N_{220}). Cultivars are indicated as Cubus (●), Ellvis (○), Impression (▼), Pegassos (Δ), Solitär (■) and Tommi (□). The regression lines for the single sampling dates are indicated chronologically as Zadoks stages 75, 77, 83 and 85 and their respective coefficients of determination (r^2). Significance levels were chosen at $^{*}P \leq 5\%$, $^{**}P \leq 1\%$ and $^{***}P \leq 0.1\%$ for n = 6 (adopted from Erdle et al., 2013a).

The NDVI was mainly affected by saturation effects, which is well known at high crop densities (Aparicio et al., 2000; Serrano et al., 2000; Li et al., 2008; Erdle et al., 2011). With progressing senescence, the quality of differentiating cultivars increased since saturation is also reduced by decreasing chlorophyll concentrations (Fig. 2). However, insufficient N supply accelerates the senescence of wheat (Spiertz and Ellen, 1978; Spiertz and De Vos, 1983; Dreccer et al., 2000; Ercoli et al., 2008) and thus, decreases plants light absorbance. This triggers a saturation effect inverse to the one taking place with very high chlorophyll densities as the NDVI is dependent on sufficient red light absorbance by the chlorophyll. With progressive grain filling, the relationship of the REIP to the plant traits changed from strongly negative to a positive orientation at low N levels (Fig. 2). As the leaf area index (LAI) and the chlorophyll concentration decrease with grain filling, the REIP moves towards shorter wavelengths until a minimum is reached and begins increasing again, although the LAI continues to decrease (Guyot et al., 1992; Railyan and Korobov, 1993). The change of interrelation is therefore based on this dissymmetric relationship, buffered by increasing N supply. Comparisons between REIP and R_{760}/R_{730} index showed that NIR-based indices were less sensitive to saturation effects close to anthesis (Heege et al., 2008; Mistele and Schmidhalter, 2010b; Erdle et al., 2011). This was also apparent for very low chlorophyll concentrations at late grain filling. The R_{760}/R_{730} index was more insensitive to low chlorophyll concentrations than the NDVI and the REIP and hence, superior in distinguishing cultivar differences (Erdle et al., 2013). The close relationships observed between spike DW and final grain yield (Tab. 3) can be related to the assumption that changes in spike DW mirror grain filling, and thus, yield development (Ehdaie et al., 2008). However, the decrease of the relationships quality between spike DW and grain yield, especially at low N levels, indicates an influence of nutrient supply. Earlier reports have shown an imbalance between the development of chaff and grains when assimilates were limited (Fischer and Stockman, 1980; Stockman et al., 1983; Abbate et al., 1997). Variable N supply influences spike size and chaff portion parallel to the amount of chaffs dry weight mobilized to the grains (Dreccer et al., 2000; Ercoli et al., 2008). If the plant is running out of nutrient supply at low N levels and grain filling is progressing, these variable effects might have initiated the loss of relationship between spike DW and final grain yield. In combination with the low chlorophyll concentration of the spikes (Zhou and Wang, 2003) and the acceleration of senescence, spectral reflection was not able to satisfactorily detect cultivar differences in spike DW during late grain filling (Fig. 2). The R_{760}/R_{730} index was less affected by saturation and thus, was able to predict spike DW adequately. Final grain yield was more closely related to spike DM content (Tab. 3) which can be due to the relationship of structural components and kernel number (Brooking and Kirby, 1981; Fischer, 2011), as these components are the principle base for final grain yield (Reynolds et al., 2009). The structure´s spectral information must have a strong relationship with the NIR wavebands (Campbell, 2002). The NDVI and the R_{760}/R_{730} index, both linked with NIR wavebands, describe the spike DM content well. However, the R_{760}/R_{730} index independence of saturation effects, was able to predict spike DM content throughout grain filling for any N supply (Erdle et al., 2013).

Table 5: Coefficients of determination for the relationships between the normalized difference vegetation index (NDVI), the red edge inflection point (REIP) and the R_{760}/R_{730} index and five yield components: Spike dry weight (DW), spike dry matter content (DM), dry weight index (DW index), N uptake index (N_{up} index) and grain dry matter content (Grain DM, only at N_{160}) were assessed at the four N application levels 0 kg ha^{-1} (N_0), 100 kg ha^{-1} (N_{100}), 160 kg ha^{-1} (N_{160}) and 220 kg ha^{-1} (N_{220}) at Zadoks stages (ZS) 75, 77, 83 and 85. The models follow linear courses. The significant coefficients of determination are highlighted as $^{*}P \leq 5\%$, $^{**}P \leq 1$ and $^{***}P \leq 0.1\%$ for n = 6. Positive relationships are written in bold letters (changed from Erdle et al., 2013a, 2013b).

Spectral Indices	ZS	Spikes DW				Spikes DM				DW index				N_{up} index				Grain DM
		N_0	N_{100}	N_{160}	N_{220}	N_0	N_{100}	N_{160}	N_{220}	N_0	N_{100}	N_{160}	N_{220}	N_0	N_{100}	N_{160}	N_{220}	N_{160}
NDVI	75	0,02	0,46	0,68*	0,49	0,00	0,57	0,43	0,36	0,07	0,76*	0,49	0,47	0,03	0,78*	0,40	0,50	0,79*
	77	0,49	0,51	0,07	0,42	0,63	0,46	0,42	0,29	0,83*	0,77*	0,64	0,55	0,94**	0,94**	0,82*	0,78*	0,69*
	83	0,51	0,51	0,23	0,46	0,65	0,68*	0,76*	0,67*	0,68*	0,74*	0,39	0,44	0,96***	0,89**	0,75*	0,52	0,75*
	85	0,33	0,33	0,52	0,17	0,83**	0,93**	0,84	0,81*	0,25	0,54	0,59	0,74*	0,91**	0,84**	0,24	0,70*	0,72*
REIP	75	0,42	0,77*	0,66	0,84**	0,50	0,71*	0,71*	0,64	0,43	0,71*	0,73*	0,81*	0,70	0,69*	0,70*	0,88**	0,67*
	77	0,83*	0,83*	0,43	0,85**	0,78*	0,71*	0,82*	0,68*	0,40	0,87**	0,65	0,76*	0,60	0,87**	0,84**	0,98***	0,79*
	83	0,11	0,82*	0,55	0,51	0,12	0,74*	0,92**	0,94**	0,04	0,86**	0,56	0,49	0,06	0,64	0,87**	0,71*	0,70*
	85	**0,72***	**0,07**	0,31	0,56	**0,87***	**0,29**	0,03	0,27	**0,39**	**0,22**	0,00	0,45	**0,84****	**0,35**	0,01	0,60	0,04
R_{760}/R_{730}	75	0,71*	0,68*	0,66	0,74*	0,32	0,68*	0,61	0,50	0,44	0,78*	0,72*	0,71*	0,87**	0,75*	0,68*	0,79*	0,73*
	77	0,81*	0,74*	0,25	0,76*	0,84*	0,67*	0,69*	0,53	0,70*	0,87**	0,68*	0,71*	0,87**	0,90**	0,87**	0,97***	0,81*
	83	0,00	0,66*	0,42	0,46	0,73*	0,87**	0,92**	0,86**	0,65	0,95***	0,49	0,47	0,92**	0,92**	0,89**	0,70*	0,83*
	85	0,16	0,21	0,73*	0,34	0,23	0,77*	0,91**	0,78*	0,03	0,39	0,59	0,79*	0,32	0,62	0,33	0,83*	0,85**

The grain DM content was one plant parameter most stabile described by any spectral information assessed. The grain DM content was shown by Calderini et al. (2000) and Slafer et al. (2009) to be apromising trait to assess the physiological maturity of grains. With physiological maturity, the deposition of assimilates into the grain has been completed, and only the water content has to be decreased by natural or artificial ripening (Slafer et al., 2009). Early maturing cultivars were found to be best adapted to high-yielding conditions (Stapper and Fischer, 1990). With spectral detection of the grain DM content which allows the observation of the physiological maturity of wheat, favoured crossbreds can be found and advanced harvests are possible by applying desiccants without losing yield during natural ripening.

Fischer (2011) described the rate of biomass transfer from leaves and culm to the spike to be strongly genetically dependent. The increasing range of cultivar DW index with progressive grain filling supports this finding (Tab. 5). Due to the small change in the spike DW and the DW index at high N supply at ZS 85, a maximal grain filling must be reached. In combination with the still changing DW indices at lower N levels, a sink limitation might be postulated at high N levels. Due to its resistance to saturation effects, the R_{760}/R_{730} index best explained the DW index throughout grain filling.

The N_{up} index was not consistently related to the final grain yield (Tab. 3). In contrast, the N_{up} index was strongly related to any spectral index of this study (Fig. 2). This relationship has been previously established in the literature (Schmidhalter et al., 2003; Moges et al., 2004; Reusch, 2005; Mistele and Schmidhalter, 2008). However, these experiments were done during the vegetative growth period of the crops. The effect of low-N status on senescence post anthesis was not observed yet. The R_{760}/R_{730} N_{up} index relationship was similarly affected by the decay of chlorophyll like the NDVI and the REIP but was less prone to the effect of missing differentiation with advanced senescence.

CONCLUSIONS

With a nitrogen supply of 100 and 160 kg ha^{-1}, wheat cultivars showed good relationships between single yield components and final grain yield. Spectral reflectance measurements were able to describe yield-related plant traits during grain filling. The grain DM content is known to be an indicator of physiological maturity in wheat and could best be predicted by the spectral indices. The best relationships were found for spike DM content and the N_{up} index. The NDVI was strongly affected by saturation effects with high chlorophyll concentrations shortly after anthesis. In contrast, the REIP lost its predictive quality with advanced chlorophyll decay at the end of grain filling. Compared to the REIP, the R_{760}/R_{730} index was less prone to effects of varying chlorophyll concentrations and best described cultivar plant traits, in spite of varying senescence.

ACKNOWLEDGMENTS

This research was funded by the BMBF project CROP.SENSE.net Nr. 0315530C

REFERENCES

Abbate, P.E., F.H. Andrade, J.P. Culot, and P.S. Bindraban. 1997. Grain yield in wheat: Effects of radiation during spike growth period. Field Crops Research 54:245-257.

Aparicio, N., D. Villegas, J. Casadesus, J.L. Araus, and C. Royo. 2000. Spectral vegetation indices as nondestructive tools for determining durum wheat yield. Agronomy Journal 92:83-91.

Aparicio, N., D. Villegas, J.L. Araus, J. Casadesus, and C. Royo. 2002. Relationship between growth traits and spectral vegetation indices in durum wheat. Crop Science 42:1547-1555.

Babar, M.A., M.P. Reynolds, M. van Ginkel, A.R. Klatt, W.R. Raun, and M.L. Stone. 2006a. Spectral reflectance indices as a potential indirect selection criteria for wheat yield under irrigation. Crop Science 46:578-588.

Babar, M.A., M.P. Reynolds, M. Van Ginkel, A.R. Klatt, W.R. Raun, and M.L. Stone. 2006b. Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. Crop Science 46:1046-1057.

Bort, J., Casadesus, J., Nachit, M. M., Araus, J. L. (2005): Factors affecting the grain yield predicting attributes of spectral reflectance indices in durum wheat: growing conditions, genotype variability and date of measurement. Int. J. Remote Sens. 26, 2337–2358.

Brooking, I.R., and E.J.M. Kirby. 1981. Interrelations between stem and ear development in winter wheat: the effects of a Norin 10 dwarfing gene, Gai/Rht_2. Journal of Agricultural Science 97:373-381.

Calderini, D.F., Abeledo, L.G., Slafer, G.A., 2000. Physiological maturity in wheat based on kernel water and dry matter. Agron. J. 92, 895–901.

Campbell, J.B. 2002. Introduction to remote sensing. 3rd ed. The Guilford Press, New York.

Dreccer, M., A. Schapendonk, G. Slafer, and R. Rabbinge. 2000. Comparative response of wheat and oilseed rape to nitrogen supply: absorption and utilisation efficiency of radiation and nitrogen during the reproductive stages determining yield. Plant and Soil 220:189-205.

Ehdaie, B., G.A. Alloush, and J.G. Waines. 2008. Genotypic variation in linear rate of grain growth and contribution of stem reserves to grain yield in wheat. Field Crops Research 106:34-43.

Ercoli, L., L. Lulli, M. Mariotti, A. Masoni, and I. Arduini. 2008. Post-anthesis dry matter and nitrogen dynamics in durum wheat as affected by nitrogen supply and soil water availability. European Journal of Agronomy 28:138-147.

Erdle, K., B. Mistele, and U. Schmidhalter. 2011. Comparison of active and passive spectral sensors in discriminating biomass parameters and nitrogen status in wheat cultivars. Field Crops Research 124:74-84.

Erdle, K., B. Mistele, and U. Schmidhalter. 2013a. Spectral high-throughput assessments of phenotypic differences in biomass and nitrogen partitioning during grain filling of wheat under high yielding Western European conditions. Field Crops Research 141:16-26.

Erdle, K., B. Mistele, and U. Schmidhalter. 2013b. Spectral assessments of phenotypic differences in spike development during grain filling affected by varying N supply in wheat. J. Plant Nutr. Soil Sci. 2013, 000, 1–12.

Ferrio, J.P., D. Villegas, J. Zarco, N. Aparicio, J.L. Araus, and C. Royo. 2005. Assessment of durum wheat yield using visible and near-infrared reflectance spectra of canopies. Field Crops Research 94:126-148.

Fischer, R.A. 2011. Wheat physiology: a review of recent developments. Crop & Pasture Science 62:95-114.

Fischer, R.A., and Y.M. Stockman. 1980. Kernel number per spike in Wheat (*Triticum aestivum* L.): Responses to preanthesis shading. Functional Plant Biology 7:169-180.

Guyot, G., F. Baret, and D.J. Major. 1988. High spectral resolution: Determination of spectral shifts between the red and the near infrared. International Archives of Photogrammetry and Remote Sensing 11:750-760.

Guyot, G., F. Baret, and S. Jacquemoud. 1992. Imaging spectroscopy for vegetation studies. Kluwer Academics, Dordrecht.

Heege, H.J., S. Reusch, and E. Thiessen. 2008. Prospects and results for optical systems for site-specific on-the-go control of nitrogen-top-dressing in Germany. Precision Agriculture 9:115-131.

Kipp, S., B. Mistele, and U. Schmidhalter. 2013. Identification of stay-green and early senescence phenotypes in high-yielding winter wheat, and their relationship to grain yield and grain protein concentration using high-throughput phenotyping techniques. Functional Plant Biology 41:227-235.

Kipp, S., B. Mistele, P. Baresel, and U. Schmidhalter. 2014. High-throughput phenotyping early plant vigour of winter wheat. European Journal of Agronomy 52, Part B:271-278.

Li, F., B. Mistele, Y. Hu, X. Chen, and U. Schmidhalter. 2014. Reflectance estimation of canopy nitrogen content in winter wheat using optimised hyperspectral spectral indices and partial least squares regression. European Journal of Agronomy 52, Part B:198-209.

Li, F., M.L. Gnyp, L. Jia, Y. Miao, Z. Yu, W. Koppe, G. Bareth, X. Chen, and F. Zhang. 2008. Estimating N status of winter wheat using a handheld spectrometer in the North China Plain. Field Crops Research 106:77-85.

Mistele, B., Gutser, R., Schmidhalter, U. (2004): Validation of fieldscaled spectral measurements of nitrogen status in winter wheat, in D. Mulla: 7th International conference on Precision Agriculture and other precision resources management. July 25–28, Minneapolis, Minnesota, USA, pp. 1187–1195.

Mistele, B., and U. Schmidhalter. 2008. Estimating the nitrogen nutrition index using spectral canopy reflectance measurements. European Journal of Agronomy 29:184-190.

Mistele, B., and U. Schmidhalter (2010a): Tractor-based quadrilateral spectral reflectance measurements to detect biomass and total aerial nitrogen in winter wheat. Agronomy Journal 102:499-506.

Mistele, B., Schmidhalter, U. (2010b): A comparison of spectral reflectance and laser-induced chlorophyll fluorescence measurements to detect differences in aerial dry weight and nitrogen uptake of wheat, in R. Khosla: 10th International Conference of Precision Agriculture. July 18–21, Denver, Colorado,

Moges, S.M., W.R. Raun, R.W. Mullen, K.W. Freeman, G.V. Johnson, and J.B. Solie. 2004. Evaluation of green, red, and near infrared bands for predicting winter wheat biomass, nitrogen uptake, and final grain yield. Journal of Plant Nutrition 27:1431-1441.

Prasad, B., B.F. Carver, M.L. Stone, M.A. Babar, W.R. Raun, and A.R. Klatt. 2007. Potential use of spectral reflectance indices as a selection tool for grain yield in winter wheat under great plains conditions. Crop Science 47:1426-1440.

Railyan, V.Y., and R.M. Korobov. 1993. Red edge structure of canopy reflectance spectra of triticale. Remote Sensing of Environment 46:173-182.

Raun, W.R., J.B. Solie, G.V. Johnson, M.L. Stone, E.V. Lukina, W.E. Thomason, and J.S. Schepers. 2001. In-season prediction of potential grain yield in winter wheat using canopy reflectance. Agronomy Journal 93:131-138.

Reusch, S. (2005): Optimum waveband selection for determining the nitrogen uptake in winter wheat by active remote sensing, in J. Stafford: Proceedings of the 5th European Conference on Precision Agriculture. Wageningen Academic Publishers, Wageningen, the Netherlands. Uppsala, Sweden, pp. 261–266.

Reusch, S., Jasper, J., Link, A. (2010): Estimating crop biomass and nitrogen uptake using CropSpec TM, a newly developed active crop-canopy reflectance sensor. 10th International Conference on Precision Agriculture. July 18–21, Denver, Colorado.

Reynolds, M., M.J. Foulkes, G.A. Slafer, P. Berry, M.A.J. Parry, J.W. Snape, and W.J. Angus. 2009. Raising yield potential in wheat. Journal of Experimental Botany 60:1899-1918.

Rouse, J. W., Haas, Jr. R. H., Schell, J. A., Deering, D. W. (1974): Monitoring vegetation systems in the Great Plains with ERTS, NASA SP-351. Third ERTS-1 Symposium. NASA, Washington, DC, pp. 309–317.

Schmidhalter, U., Jungert, S., Bredemeier, C., Gutser, R., Manhart, R., Mistele, B., Gerl, G. (2003): Field-scale validation of a tractor based multispectral crop scanner to determine biomass and nitrogen uptake of winter wheat. In J. Stafford and A. Werner: 4th European Conference on Precision Agriculture. Wageningen Academic Publishers, pp. 615–619.

Schmidhalter, U. 2005. Development of a quick on-farm test to determine nitrate levels in soil. Journal of Plant Nutrition and Soil Science 168:432-438.

Serrano, L., I. Filella, and J. Penuelas. 2000. Remote sensing of biomass and yield of winter wheat under different nitrogen supplies. Crop Science 40:723-731.

Slafer, G.A., Kantolic, A.G., Appendino, M.L., Miralles, D.J., Savin, R., 2009. Crop development: genetic control, environmental modulation and relevance to genetic improvement of crop yield. In: Sadras, V.O., Calderini, D.F. (Eds.), Crop Physiology: Applications for Genetic Improvement and Agronomy. Academic Press, London, pp. 277–308.

Spiertz, J., and N. De Vos. 1983. Agronomical and physiological aspects of the role of nitrogen in yield formation of cereals. Plant and Soil 75:379-391.

Spiertz, J.H.J., and J. Ellen. 1978. Effects of nitrogen on crop development and grain growth of winter wheat in relation to assimilation and utilization of assimilates and nutrients. Neth. J. Agric. Sci. 26 (1978) 210-231.

Stapper, M., Fischer, R.A., 1990. Genotype, sowing date and plant spacing influence on high-yielding irrigated wheat in southern New South Wales. II: growth, yield and nitrogen use. Aust. J. Agric. Res. 41, 1043–1056.

Stockman, Y.M., R.A. Fischer, and E.G. Brittain. 1983. Assimilate supply and floret development within the spike of wheat (triticum aestivum L.). Functional Plant Biology 10:585-594.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research 14:415-421.

Zhou, Q., and J. Wang. 2003. Leaf and spike reflectance spectra of rice with contrasting nitrogen supplemental levels. International Journal of Remote Sensing 24:1587 - 1593.