A COMPARISON OF SPECTRAL REFLECTANCE AND LASER-INDUCED CHOLORPHYLL FLUORESCENCE MEASUREMENTS TO DETECT DIFFERENCES IN AERIAL DRY WEIGHT AND NITROGEN UPDATE OF WHEAT

B. Mistele & U. Schmidhalter

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

ABSTRACT

Chlorophyll fluorescence and spectral reflectance analysis are both powerful tools to study the spatial and temporal heterogeneity of plants` biomass and nitrogen status. Whereas reflectance techniques have intensively been tested for their use in precision fertilizer application, laser-induced chlorophyll fluorescence has been tested to a lesser degree, and there are hardly any comparative studies. Therefore, we used both techniques in field and laboratory experiments since more than eight years and compared their performance in detecting nitrogen uptake and aerial dry biomass of wheat with a particular focus on detecting possible disturbances by environmental factors. We tested both sensors in field experiments with tractor-based systems, mounted about 2 m above the canopy and measuring besides the tractor with an oblique angle of sensor view. Validation measurements were performed on large calibration areas of 25 m² in size. Fertilizer applications varied between 0-220 kg N ha^{-1} and grain yields between 3-12 t ha^{-1}. The results obtained show that strong relationships exist between reflectance indices and total aerial N (R^2 = 0.90 to 0.99) and aerial dry biomass (0.77 to 0.92), from the end of tillering at growth stage GS 30 to flowering at GS 65, whereas laser-induced two-wavelength chlorophyll fluorescence detected total aerial N with R^2 of 0.78 to 0.86 and aerial dry biomass with R^2 of 0.77 to 0.85. Spectral reflectance measurements were influenced by both sensor geometry and solar angle. A strong influence of direct sunlight and temperature on the fluorescence ratio F_{690}/F_{730} was evidenced. Therefore, a model was developed to describe the light-matter interaction in plant leaves. Both sensors were well suited to describe relative differences in aerial dry weight biomass and nitrogen uptake at relevant fertilizer application stages, but the spectral values were not always depicting a continuous evolution.

Keywords: Light intensity model, active light sensor, LIF, temperature model

INTRODUCTION

It is well known that the nitrogen demand of a crop varies within single fields due to spatial differences in soil conditions (McBratney et al., 1999; LaRuffa et al., 2001). Especially, knowledge of the in-field variability of nitrogen uptake is seen as important information in order to derive site specific N fertilizer recommendations. Proximal spectral measurements and laser induced chlorophyll fluorescence are both powerful tools to study the spatial and temporal heterogeneity of plants` biomass and nitrogen status. Reflectance techniques have intensively been tested for their use in precision fertilizer application. Previous research has shown that spectral measurements can indirectly describe biomass dry weight (Serrano et al., 2000; Hansen et al., 2003) and total aerial nitrogen (Vouillot et al., 1998; Mistele et al., 2004; Li et al., 2008; Mistele et al., 2010) of plants. Biomass and nitrogen uptake are recommended parameters as directive for nitrogen application in high yielding areas (Link et al., 2005; Heege et al., 2008; Thoren and Schmidhalter, 2009).

Spectral measurements in the nadir are frequently influenced by the zenith angle (Major et al., 2003). To reduce the bi-directional influence in field measurements quadrilateral oblique view optics have been used (Reusch, 2003; Mistele and Schmidhalter, 2010). Another approach to eliminate the bi-directional influence are active reflectance measurements with it own light source. This active light measurements promise operating at dawn or in the night also (Raun et al., 2002; Holland et al., 2004; Jasper et al., 2009). But spectral reflectance of a canopy in the field is always a mixed signal of soil reflectance and plant reflectance (Major et al., 2003). Laser induced chlorophyll fluorescence provides information about the actual state of the photosynthesis apparatus of plants. It gets signal from green leaves only. The intensity ratio F_{690}/F_{730} of the two peaks of chlorophyll fluorescence at 690 nm (red) and 730 nm (far red) is well correlated with the (active) chlorophyll content of the leaves and can therefore be used to detect the chlorophyll content of the plants (Krause et al., 1991; Günther et al., 1994; Buschmann, 2007; Thoren and Schmidhalter, 2009).

Laser induced chlorophyll fluorescence has been tested less than reflectance measurements, and there are hardly any comparative studies. Therefore, we tested both techniques in field and laboratory experiments since more than eight years and compared their performance in detecting nitrogen uptake and aerial dry biomass of wheat with a particular focus on detecting possible disturbances by environmental factors.

MATERIAL AND METHODS

Experimental fields

The experiments were conducted at the research station of the Technische Universität München in Bavaria in the South West of Germany at longitude 11.70E and latitude 48.40N in 2002 to 2004 and 2006 to 2009. Winter wheat (*Triticum aestivum*) was used as the experimental plant.

Table 1: Field experiments in 2002 to 2009

	Year						
	2002	2003	2004	2006	2007	2008	2009
Replications	5	5	11	4	4	4	4
Cultivars	1	1	2	9	7	7	7
Samplings	2	3	4	5	5	5	5
Fertilizer Rates	0, 90, 130, 170, 210	0, 90, 130, 170, 210	0, 90, 130, 170, 210, 250	0, 100, 160, 220	0, 100, 160, 220	0, 100, 160, 220	0, 100, 160, 220
	---------- kg ha^{-1} ----------						
Plot Size	900	900	900	45	45	45	45
	---------- m^{-2} ----------						
Reflectance Sensor	Sensor 1	Sensor 1	Sensor 1, Sensor 2	Sensor 2	Sensor 2	Sensor 2 ALS	Sensor 2 ALS
Fluorescence Sensor	n.a.	Planto	Planto	Fritzmeier	Fritzmeier	Fritzmeier	Fritzmeier

The experimental design was a randomised block design. The research included different nitrogen treatments and partly different cultivars as indicated in Table 1. The experiments were carried out together with the Chair of Plant Production Systems in the years 2006-2009.

One or two days after the sensor measurements, the plants were harvested with a green forage chopper with a weighing unit to determine the above-ground biomass dry weight. Spectral data and sampling area were linked by GPS. Small plots were harvested, 1.5 m in width and around 8 to 3 m in length, decreasing with biomass accumulation. The biomass sampling was done on a subsequent portion, separated by 1.5 m distance (Mistele and Schmidhalter, 2010) covering exactly the FOV of spectral measurements. A representative sub sample was collected and dried after weighing to estimate the total dry matter yield (kg ha^{-1}). The dried samples were milled and analysed for nitrogen content (g N g^{-1} dry matter) with a Dumas elementary analyser (Macro-N, Foss Heraeus, Hanau, Germany). The total aerial nitrogen (aboveground N-uptake) was calculated as above-ground biomass dry weight x total nitrogen content.

Reflectance measurements

Two experimental tractor-based radiometers with an optical configuration comparable to the Yara sensor (Yara GmbH & Co. KG, Dülmen, Germany) were used to measure the sun induced reflectance. In the years 2002 and 2003 a five-wavelength scan mode was used (sensor 1), whereas modified electronics (tec5, Oberursel, Germany) enabled hyperspectral readings (sensor 2) for the measurements in 2004 to 2009. Both spectrometers contained two units of a Zeiss MMS1 silicon diode array spectrometer with a spectral detection range from 400 to 1000 nm and a bandwidth of 3.3 nm. One unit was linked with a diffuser and measured the sun radiation as a reference signal. Simultaneously the second unit measured the canopy reflectance with quadrilateral view optics (Lammel et al., 2001; Mistele and Schmidhalter, 2010)

Active light canopy reflectance measurements were made with a sensor comparable to the N-Sensor® ALS (Yara International, ASA) but with a single sensor and USB interface. The active light sensor comprises a transmitter with a xenon flashlight, providing multi-spectral light (650-1100 nm) of high intensity and a receiver with 4 photodiodes and interference filters in front of them. Filters with 730, 760, 900 and 970 nm centre wavelength and a half band width of 10 nm were used in order to retrieve relative reflectance. The reflectance intensities were used to calculate the indices R_{760}/R_{730} and R_{970}/R_{900}. The unit was mounted 1.3 m above ground and covered approximately 0.15 m² FOV (Jasper et al., 2009).

Laser induced chlorophyll fluorescence measurements

The measurements were performed with the Laser-N-Detector, a fluorescence measurement system developed by Planto GmbH (Leipzig, Germany). Laser light with a wavelength of 630 nm was emitted by a laser diode inside the sensor system and excited chlorophyll molecules in the plant leaves over and above that caused by sunlight, thereby inducing additional fluorescence radiation. This radiation was detected inside the sensor system by a specialized telescope optic with beam splitters and filters at the wavelengths of 690 nm and 730 nm. The separation of the laser-induced fluorescence from the sunlight-induced fluorescence was done using a pulsed laser beam at 2 kHz.

The measurement system was mounted on a tractor roof with the sensors oriented at an angle of 45◦ to the horizontal plane towards the plant stands on both sides of the tractor. The measurement distance between sensor and plants was 3–4 m with a FOV of 16 mm². This sensor used a scan function providing areal-scanning of a strip (width about 0.75 m and located about 2 m away from the wheel track). This areal scanning allowed simultaneous determination of the nitrogen content and of biomass via the parameter biomass density index (BDI_{LICF}). The nitrogen content and total aerial nitrogen of the plants was determined by calculating the fluorescence intensity ratio of the wavelengths 690 nm and 730 nm, whereas the calculation of BDI_{LICF} was performed by statistical evaluation of the fluorescence signal counts. Thus, F_{690}/F_{730} and BDI_{LICF} represent independent measurement values because of the different evaluation algorithms (Thoren and Schmidhalter, 2009).

Another laser induced chlorophyll fluorescence senor was used by the Chair of Plant Production Systems. It was a hand-held fluorescence sensor MiniVeg N (Fritzmeier GmbH & Co, Großhelfendorf, Germany), a single unit with the same technology as the tractor mounted system. A pulsed laser at 660 nm was used as exciting light. A photomultiplier detected the fluorescence light at 690 and 730 nm. The ratio between F_{690}/F_{730} was calculated as vegetation index (Limbrunner and Maidl, 2007). This system measures in contact to the canopy with a FOV of 10 mm². The optics was set 0.1 m below the top of the canopy to make sure that the leaves were always in direct contact to the optics during measurements.

RESULTS

Destructively measured canopy parameters total aerial nitrogen and total biomass were related to sun induced reflectance for all years. The relationship was constant on a high level for total aerial nitrogen. Total biomass was also related to sun based spectral measurements.
In Figure 1 the quadratic relationships between reflectance indices and total aerial nitrogen and total biomass are indicated for the year 2003 as an example.

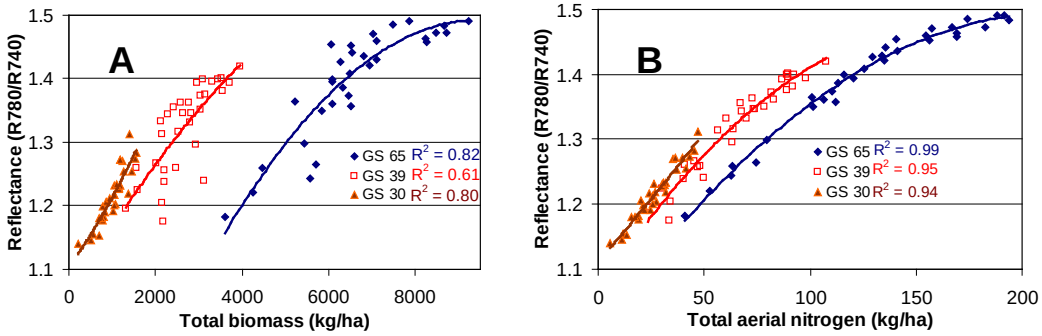

Figure 1: Relationship between sun induced reflectance (R_{780}/R_{740}) and total biomass (A) and total aerial nitrogen (B) in 2003 at growth stage (GS) 30, 39 and 65.

For all measurement dates the coefficient of determination for the relationship between total aerial nitrogen and spectral measurements was higher than for total biomass. The regression fit between total biomass and reflectance changed with the development of crop stand resulting in a shift of the regression curve along the x-axis. This was less pronounced for the relationship between total aerial nitrogen and canopy reflectance.

These measurements stemmed from optimum measurement conditions with regard to sky and zenith angle conditions. But sun induced spectral measurements are not fully stable at different sun intensities and change in zenith angle as depicted in Figure 2.

Measurements at completely diffuse radiation compared to measurements at totally direct radiation as depicted in Figure 2a resulted in a shift of the regression curve along the y-axis. Whereas for measurements at different daytime and different zenith angle no difference could be seen at week crop stands. But as more nitrogen was taken up by the canopy the lower was the increase in the index value measured by the spectrometer. This resulted in a saturation effect for the spectral index beginning at 120 kg ha^{-1} nitrogen for the measurements at low zenith angle at 6:00 pm.

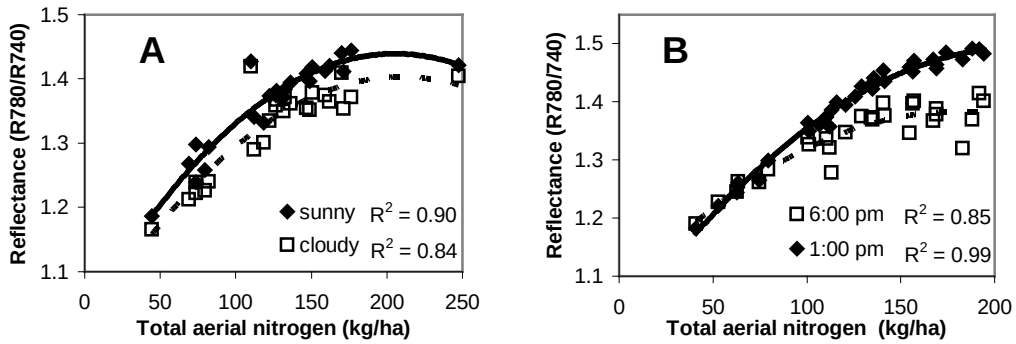

Figure 2: Relationship between total aerial nitrogen and sun induced reflection (A) at different radiation conditions (cloudy sky and full sun at midday) in 2002 at growth stage 53 and (B) at different daytime (1 pm and 6 pm) in 2003 at growth stage 65.

The active light reflectance sensor measured total aerial nitrogen with coefficients of determination similar to sun induced reflectance as depicted in Figure 3. Earlier measurements reflected closer relationships than measurements at later growth stages. Compared to measurements with sun as light source the regression fits differed. The slope and the intersection on the y-axis changed clearly between growth stages for the relation between total aerial nitrogen and active light reflection. The measurements with the active light reflectance sensor showed no influence of daytime as depicted in Figure 4.

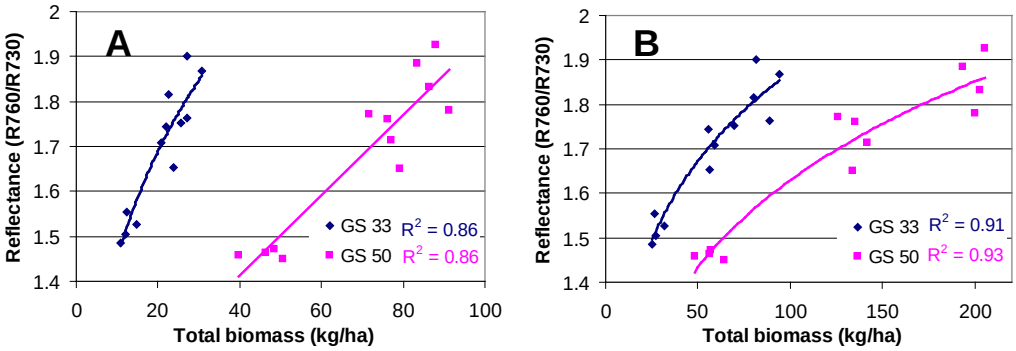

Figure 3: Active light source reflectance measurements with the index R_{760}/R_{730} as a function of (A) shoot dry biomass and (B) N uptake for spring wheat with 3 nitrogen level (0, 80, 160 kg ha^{-1}) at different growth stages in 2009.

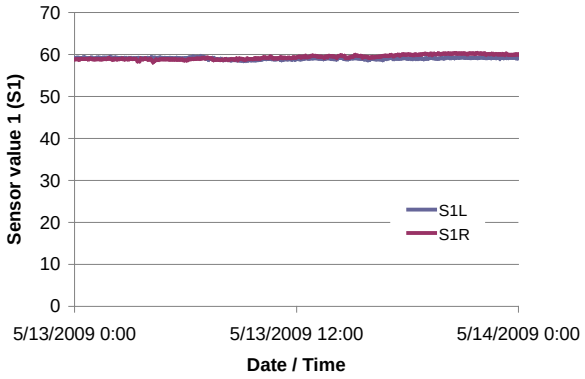

Figure 4: Diurnal measurements with the active light reflectance sensor for 24 hours at a low nitrogen plot (S1R) and a high nitrogen plot (S1L) (personal communication with permission, Reusch 2010).

Results from laser-induced chlorophyll fluorescence ratio measured with the Planto device were similar to the results from sun induced reflectance measurements as depicted in Figure 5 and 6. The relationship between total biomass and fluorescence ratio was slightly closer compared to reflectance measurement, whereas the relationship between total aerial nitrogen and fluorescence was somewhat weaker. The distance between the regression fits of the single measurements was much larger compared to the spectral measurements while the fits between the BDI_{LICF} and both, total biomass and total aerial nitrogen, were close at growth stage 30 and 39. A decreasing coefficient of determination was observed throughout plant development.

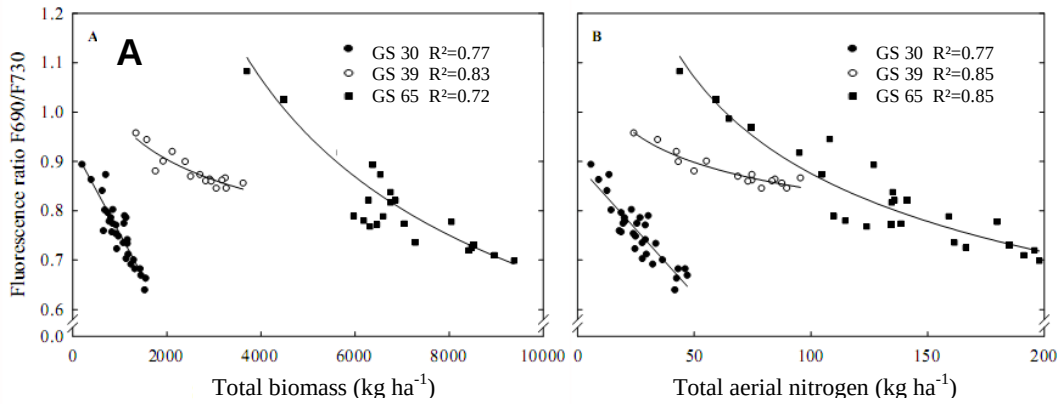

Figure 5: Laser-induced chlorophyll fluorescence ratio F_{690}/F_{730} measured with the Planto device as a function of (A) shoot dry biomass and (B) N uptake for winter wheat at different growth stages in 2003. Lines represent fitting regression curves (Bredemeier and Schmidhalter, 2005).

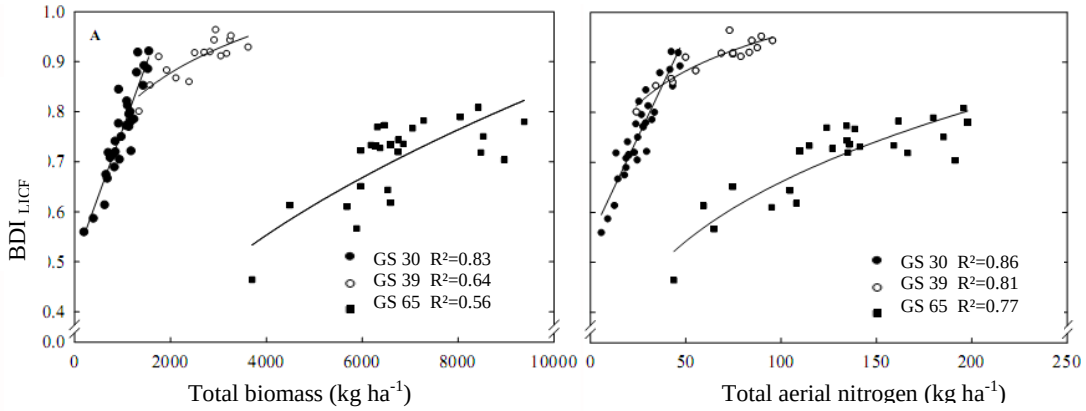

Figure 6: Biomass diversity index (BDI_{LICF}) measured with the Planto device as a function of (A) shoot dry biomass and (B) N uptake for winter wheat at different growth stages in 2003. Lines represent fitting regression curves (Bredemeier and Schmidhalter, 2005).

For the fluorescence ratio measured with the Fritzmeier device comparable coefficients of determination could be found for the relationship to total aerial nitrogen as for the Planto device. These results are depicted in Table 2.

Table 2: Coefficient of determination (adj. R^2) for exponential regressions between total aerial nitrogen and fluorescence ratio measured with the Fritzmeier device for different growth stages (EC) and five cultivars for the years 2004 to 2006. Significance levels are indicated. Data taken with permission from the authors (Limbrunner and Maidl, 2007).

Growth stage	Cortez		Flair		Orestis		Pegassos		Xanthos	
	R	Sig.	R	Sig.	R	Sig.	R	Sig.	R	Sig.
EC 30	0.80	***	0.60	***	0.66	***	0.51	***	0.73	***
EC 32	0.75	***	0.75	***	0.78	***	0.84	***	0.89	***
EC 37	0.84	***	0.81	***	0.84	***	0.91	***	0.94	***
EC 45	0.81	***	0.89	***	0.93	***	0.87	***	0.84	***
EC 65	0.93	***	0.89	***	0.72	***	0.67	***	0.80	***

But laser induced chlorophyll fluorescence measurements are highly depending on environmental conditions, too. To quantify the impact of temperature, experiments have been conducted under stable condition in a growth chamber with the Planto device, where only temperature was changed. Figure 7 depicts a strong linear change in the fluorescence ratio between 7 °C to 23 °C, but for warmer leaf temperatures up to 33 °C no influence was observed.

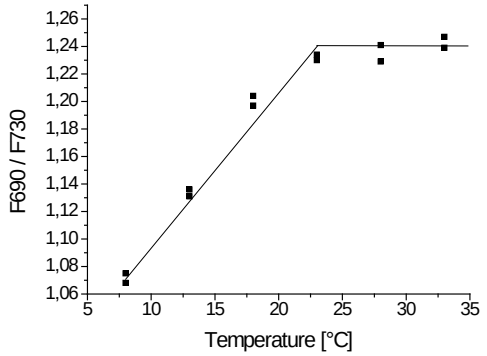

Figure 7: Relationship betweeen leaf temperature and laser induced chlorophll fluorescence measured with the Planto device in a growth chamber experiment (Thoren et al., 2010).

However not only temperature but also light intensity was obsered to disturb the fluorescence ratio as depicted in Figure 8. Typical values of the F_{690}/F_{730} ratio of a wheat crop stand are shown in Figure 8a. The x-axis presents values according to the N application level of the measurement area. The upper curve is that obtained under shady, not direct sunlit conditions, whereas the lower curve shows the same stand under sunlit conditions. The amount that the curve shifts is linearly dependent on the intensity of the incident sunlight, as shown by the results for winter wheat (Figure 8b). Whereas measurements on the shady sides do not show a dependency on light intensity (vertical line), the sunlit sides show a linearly increasing deviation from the "shady ratio". The deviations of the single measurements from the straight lines represent the different N contents of the measured crops.

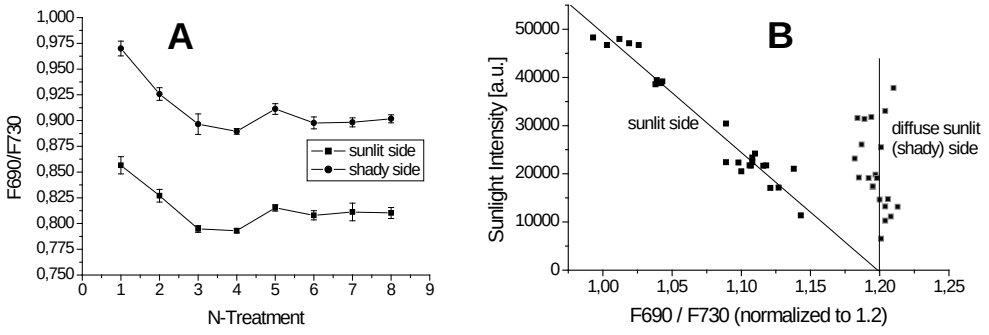

Figure 8: Dependence of the F_{690}/F_{730} ratio on sunlight intensity with (A) downshift of the fluorescence ratio measured from the directly sunlit side of wheat under field conditions at BBCH 45 (May 31, 2002) and (B) normalized field measurements of wheat crop stands from different sites on days with direct sunlight. Whereas measurements on the direct sunlit side show a linear relation between light intensity and the F_{690}/F_{730} ratio, measurements on the diffuse lit side show no influence of light intensity (Thoren et al., 2010).

To obtain a more thorough understanding of the physical and biological mechanisms underlying our observations we looked at the light-matter interaction taking place in the illuminated leaf of the measured plant.

A growth chamber experiment was set up to measure the effect of radiation intensity independent of other environmental conditions. With the data depicted in Figure 9 an improved model was developed with new parameters (Thoren et al., 2010).

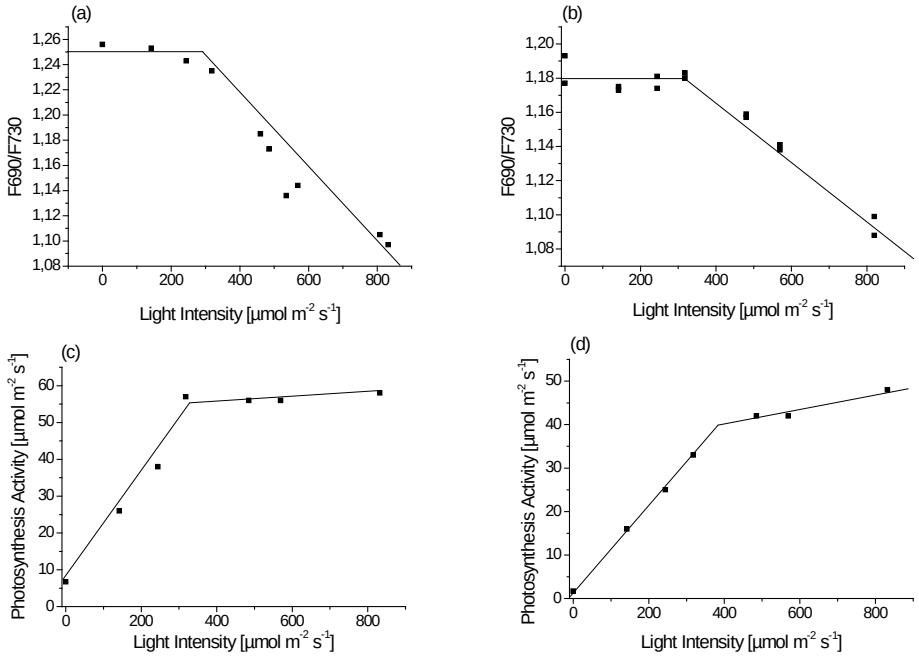

Figure 9: Relationship between light intensity and the F_{690}/F_{730} fluorescence ratio (a, b) and photosynthetic activity (c, d). Growth chamber experiments of wheat at different growth stages (a, c = BBCH 25-30; b, d = BBCH 32) (Thoren et al., 2010).

DISCUSSION

The comparison of sensor measurements to canopy parameters demonstrates that it is possible to obtain good estimates of the total aerial N in undisturbed canopies with tractor based sensor systems under field conditions. These scanning methods are rapid, easy, non-destructive, tractor based and applicable to field scaled dimensions. At optimum environmental conditions all four devices measured valuable data but the optimum conditions differ. Sun induced reflectance measurements worked best under full sun around midday. At high zenith angle the sun radiation penetrates deeply into the single leaf and the canopy. The single light beam has a short way to transmit the leaf and to pass the canopy. Whereas the single light beam at low zenith angle has a longer way to pass the leaf and the canopy, the light penetrates the canopy not as deep. As the

leaf absorption is weak for near infrared light, it is diffusively reflected, mainly by different optical densities of water filled cell walls and air-filled intercellular spaces. This could be the reason for the relatively higher reflectance in the visible area than in the near infrared wavelength because the near infrared light still penetrates the canopy deeper than the visible light. At more biomass per area the effect becomes stronger and at higher biomass and low zenith angle a saturation effect for the relationship between spectral measurements and canopy parameter as for instance total aerial nitrogen is observed.

Active light source reflectance measurements estimate the canopy parameters completely independent of sun radiation and zenith angle. This could be seen as big advantage compared to sun induced reflectance measurements but the quality of the measurements seems to be not as high as for optimal sun induced reflectance measurements. These results are not in line with Jasper et al. (2009). They reported a coefficient of determination for the relationship between the active light sensor reflectance index and the nitrogen uptake of R^2=0.97 for growth stages 31 and 39, however, for a rather not well-distributed dataset. The data without zero nitrogen plots look similar to our measurements with more scattered data.

For the Planto laser induced chlorophyll fluorescence measurement device, similar as for the active light sensor, the relationship between total aerial nitrogen and fluorescence ratio was not as good as for sun induced reflectance measurements. These results agree with Thoren and Schmidhalter (2009). They compared a handheld reflectance sensor with the Planto device in a rape experiment. They found a slightly closer relationship between reflectance measurements and nitrogen uptake than between fluorescence ratio and nitrogen uptake. With regard to the light saturation effect we assume, in accordance with Krause & Weis (1991), that the fluorescence efficiencies in the upper part of the leaf decrease as the photosynthesis system becomes saturated with respect to excitation energy with sufficient irradiation of the leaf surface. The decrease of the fluorescence efficiencies is related to a change in the fluorescence quenching mechanisms. More details about the model of the effect of light intensity on the chlorophyll fluorescence ratio are described in Thoren et al. (2010). The results differ from observations by Günther et al. (1994). They reported that there was no influence of light intensity on the fluorescence ratio whereas in our results a clear impact could be observed. These researchers could probably not find an effect of light intensity because they measured in a horizontal direction and not opposite to the direction of the sun radiation. An exclusive feature of the Planto device is the opportunity to analyse the number of measurements with fluorescence signal in relation to the number of total measurements (BDI_{LICF}). All these measurement points come from vivid plants because dead plants and soil do not produce fluorescence. This could provide a chance to estimate biomass at early growth stages before GS 30 or discriminate healthy leaves from fungi infected (Kuckenberg et al., 2008).

The Fritzmeier laser induced chlorophyll fluorescence measurement device delivers best results at growth stage 37. Measurements at earlier growth stage are possible, but do not offer the same quality. This may be an effect of the technical set up that requires the device to measure in close contact to the leaves. Crop stands at growth stage 30 have short plants with soft stems and leaves and it is

probably difficult to lead the leaves close to the device. The tractor mounted system measures also the canopy height to adjust the laser device to an optimum measuring position. This canopy height could probably also give independent information about the amount of biomass, completely independent of the fluorescent signal.

Besides the aspect of the technical quality of the measurements reflecting total aerial nitrogen the availability of suitable fertilizing algorithms is most important. The best canopy sensor gets worthless for the agriculture if the fertilizing algorithm does not fit to the crop, to the region or is just too complicated. Heege et al. (2008) overview fertilization algorithms currently adopted for passive and active systems in Germany.

CONCLUSIONS

The four sensor systems showed all high coefficients of determination for the relationship between sensor value and total aerial nitrogen. But each system has also its weak points. Sun induced reflectance measurements had the highest coefficients of determination but were most sensitive to environmental conditions like zenith angle and darkness. For all reflectance measurements the reflectance wavebands were not independent from each others. The laser induced chlorophyll fluorescence sensors depicted noisier sensor values, but for the Planto sensor the BDI_{LICF} is completely independent from the fluorescence ratio. Also the principle of chlorophyll fluorescence, where only active leaves emit signals, gives probably a wider field of applications compared to reflectance measurements.

REFERENCES

Bredemeier, C. and Schmidhalter, U. 2005. Laser-induced chlorophyll fluorescence sensing to determine biomass and nitrogen uptake of winter wheat under controlled environment and field conditions. p. 273-280. *In* Stafford, J. (ed.) Precision agriculture: Papers from the 5th European Conference on Precision Agriculture, Uppsala. Wageningen Academic Publ., Wageningen, the Netherlands.

Buschmann, C. 2007. Variability and application of the chlorophyll fluorescence emission ratio Red/Far-red of leaves. Photosynth. Res. 92: 261-271.

Günther, K. P., Dahn, H. G. and Lüdeker, W. 1994. Remote sensing vegetation status by laser-induced fluorescence. Remote Sens. Environ. 47:10-17.

Hansen, P. M. and Schjoerring, J. K. 2003. Reflectance measurement of canopy biomass and nitrogen status in wheat crops using normalized difference vegetation indices and partial least squares regression. Remote Sens. of Environ. 86:542-553.

Heege, H., Reusch, S. and Thiessen, E. 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.

Holland, K. H., Schepers, J. S., Shanahan, J. F. and Horst, G. L. 2004. Plant canopy sensor with modulated polychromatic light source. *In* Mulla, D. J. (ed.) Proceedings of the 7th International Conference on Precision Agriculture, Minneapolis, MN, USA.

Jasper, J., Reusch, S. and Link, A. 2009. Active sensing of the N status of wheat using optimized wavelength combination: impact of seed rate, variety and growth stage. p. 23-30. *In* Henten van E.J., Goense D. and Lokhorst, C. (ed.) Precision Agriculture ´09: Papers from the 7th European Conference on Precision Agriculture, Wageningen, the Netherlands.

Krause, G. H. and Weis, E. 1991. Chlorophyll Fluorescence and Photosynthesis - the Basics. Annu. Rev. Plant Phys. 42, 313-349.

Kuckenberg, J., Tartachnyk, I. and Noga, G. 2008. Evaluation of fluorescence and remission techniques for monitoring changes in peel chlorophyll and internal fruit characteristics in sunlit and shaded sides of apple fruit during shelf-life. Postharvest Biol. Tec. 48:231-241.

Lammel, J., Wollring, J. and S. Reusch. 2001. Tractor based remote sensing for variable nitrogen fertilizer application. p. 694-695. *In* Horst, W.J. (ed.) Plant nutrition: food security and sustainability of agro ecosystems through basic and applied research. 14th International Plant Nutrition Colloquium, Hannover. Kluver Academic Publ., Dordrecht, the Netherlands.

LaRuffa, J. M., Raun, W. R., Phillips, S. B., Solie, J. B., Stone, M. L. and Johnson, G. V. 2001. Optimum field element size for maximum yields in winter wheat, using variable nitrogen rates. J. Plant Nutr. 24:313-325.

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

Limbrunner, B. and Maidl, F. X. 2007. Non-contact measurement of the actual nitrogen status of winter wheat canopies by laser-induced chlorophyll fluorescence. p. 173-179. *In* Stafford, J. (ed.) Precision agriculture '07. Papers presented at the 6th European Conference on Precision Agriculture, Skiathos, Greece. Wageningen Academic Publ., Wageningen, the Netherlands.

Link, A., Jasper, J. and Reusch, S. 2005. Suitability of different crop parameters for the determination of site-specific nitrogen fertilizer demand. p. 297-303. *In* Stafford, J. (ed.) Precision agriculture ´05: Papers from the 5th European Conference on Precision Agriculture, Uppsala. Wageningen Academic Publ., Wageningen, the Netherlands.

Major, D. J., Baumeister, R., Toure´, A. and Zhao, S. 2003. Methods of measuring and characterizing the effects of stresses on leaf and canopy signatures. p. 165-175. *In* T.VanToai et al. (ed.) Digital imaging and spectral techniques: Application to precision agriculture and crop physiology. ASA Spec. Publ. 66. ASA, Madison, WI.

McBratney, A. B. and Pringle, M. J. 1999. Estimating average and proportional variograms of soil properties and their potential use in precision agriculture. Precision Agriculture 1:125-152.

Mistele, B., Gutser, R. and Schmidhalter, U. 2004. Validation of field-scaled spectral measurements of the nitrogen status in winter wheat. p. 1187-1195. *In* Mulla D. J. (ed.) Proceedings of the 7th International Conference on Precision Agriculture and other Precision Resources Management, Minneapolis, MN, USA.

Mistele, B. and Schmidhalter U. 2010. Tractor-Based Quadrilateral Spectral Reflectance Measurements to Detect Biomass and Total Aerial Nitrogen in Winter Wheat. Agron. J. 102, 499-506.

Raun, W. R., Johnson, G. V., Solie, J. B., Stone, M. L., Mullen, R. W., Freeman, K. W., Needham, D. E., Washmon, C. N. and Reed, S. 2002. *In* Robert, P.C. (ed.) Real-time sensing and N fertilization with a field scale Greenseeker applicator. p. 1546-1556. Proceedings of the 6th International Conference on Precision Agriculture, Minnesota, USA.

Reusch, S. 2003. Optimisation of oblique-view remote measurement of crop N-uptake under changing irradiance conditions. p. 573-578. *In* Stafford, J. and Werner, A. (ed.) Precision agriculture: Papers from the 4th European Conference on Precision Agriculture, Berlin. Wageningen Academic Publ., Wageningen, the Netherlands.

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

Thoren, D. and Schmidhalter U. 2009. Nitrogen Status and Biomass Determination of Oilseed Rape by Laser-Induced Chlorophyll Fluorescence. Eur. J. Agron. 30, 238-242.

Thoren, D., Thoren, P. and Schmidhalter U. 2010. Influence of Ambient Light and Temperature on Laser-Induced Chlorophyll Fluorescence Measurements. Eur. J. Agron. 32, 169-176.

Vouillot, M. O., Huet, P. and Boissard, P. 1998. Early detection of N deficiency in a wheat crop using physiological and radiometric methods. Agronomie 18:117-130.