Stafford, J., Werner, A. (eds): Precision Agriculture.
Wageningen Academic Publishers, ECPA Berlin, 15.-19.6.03
S. 103-108

Non-contacting chlorophyll fluorescence sensing for site-specific nitrogen fertilization in wheat and maize

C. Bredemeier and U. Schmidhalter
Technical University of Munich, Department of Plant Nutrition. Freising, Germany
bredemei@wzw.tum.de

Abstract

The relationship between laser-induced chlorophyll fluorescence intensity (690 nm and 730 nm), ratio F690/F730 and nitrogen content in wheat and maize was characterized using two different sensors (growth chamber and field sensor). In the field, laser-induced fluorescence was measured at a distance of approximately 3.3 m from the canopy and the sensed area was approximately 6-7 m^2. The fluorescence ratio F690/F730 was inversely correlated with N content and uptake, dry biomass and SPAD value. The results indicate that nitrogen uptake and biomass can be reliably detected through chlorophyll fluorescence measurements under field conditions.

Keywords: biomass detection, laser chlorophyll fluorescence, nitrogen content, sensor

Introduction

Map- and sensor-based approaches are basic methods of implementing site-specific management (SSM) for the variable-rate application of crop inputs. The majority of available technologies in SSM utilizes the mapping approach based on grid sampling, soil analysis, map generation and variable-rate application. On the other hand, sensor-based methods of plant analyses could detect nutrient requirements on-the-go (real-time sensing) and simultaneously apply fertilizer rates based on those needs, avoiding costs for soil sampling or destructive plant sampling, analysis and data management.
One technique to observe the N-status of plants by non-contacting method is the detection of chlorophyll fluorescence. Laser-induced chlorophyll fluorescence is the optical emission from chlorophyll molecules in the plant that have been excited to a higher energy level by absorption of electromagnetic radiation from an active source. The chlorophyll fluorescence spectra of the upper leaf side exhibit two fluorescence maxima: one near 690 nm and a second one around 735 nm (Lichtenthaler & Rinderle, 1988). The role played by N in chlorophyll synthesis suggests that N-deficiency can be detected based on changes in the plant's fluorescence spectra. In this sense, it is expected that the fluorescence spectra of the plant can be used for fast determination of the leaf chlorophyll content and as an indicator of the relative concentration of N (McMurtrey et al., 1994). The main advantage of active laser-based fluorescence sensors compared to passive sensors is the possibility of measurement almost independent of light conditions, perhaps even during the night. In addition, the fluorescence signal has a very low background, since the signal comes mainly from the green parts of the plant (Lichtenthaler & Rinderle, 1988).

Materials and methods

Experiments with wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were carried out under controlled environmental (two years) and field conditions (one year). The experiments were carried out between 2000 and 2002. The treatments consisted of different N-fertilization levels. The amount of nitrogen applied varied between 90 and 210 kg N/ha in wheat and between 70 and 220 kg N/ha in maize.

Different measurement systems were used in our experiments. For the growth chamber measurements, we used a self-constructed portable fluorescence sensor. This sensor detects the fluorescence at 680 nm and 740 nm at a distance of around 15 cm from the plants. For the field evaluations, we used a tractor-mounted fluorescence sensor developed by Planto GmbH company (Leipzig, Germany). This sensor detects the fluorescence emitted at 690 nm and 730 nm. The sensor was mounted at the rear of the tractor at a height of around 3.0 m above the plant canopy (Figure 1). A laser beam stimulates the emission of fluorescence, which is detected at a distance of approximately 3.3 m between canopy and sensor. The canopy is scanned in a 0.5 m wide strip. Strips of approximately 15 m in length were measured and the total area sensed was around 6-7 m^2. The chlorophyll fluorescence ratio F680/F740 (growth chamber sensor) or F690/F730 (field sensor) was then calculated.

The field experiments were carried out with the objective to spatially match destructive ground-truth measurements of biomass and nitrogen content with chlorophyll fluorescence measurements. After the measurements were done, fresh and dry biomass weight, shoot nitrogen content and chlorophyll content (using the chlorophyll meter Minolta SPAD-502®) were determined on the sensed area. Fresh biomass was determined on the integral sensed area, dry weight and nitrogen content were determined on representative subsamples from each plot. SPAD measurements were done thirty times on each plot on the youngest fully developed leaves. All measurements were at least four times replicated on independent plots. The SPAD meter is used to measure the relative greenness of leaves, which is directly related to the chlorophyll content.

The SPAD meter determines the relative amount of chlorophyll in leaves by measuring transmittance at red (650 nm) and near infrared (940 nm). On the basis of these two transmittances the instrument calculates a number (called "SPAD value", unit less) that was strongly positively correlated with the chlorophyll content (Bredemeier & SchmidhalterThe nitrogen uptake was also determined. Destructive harvests were done with a green forage chopper with 1.5 m cutting width equipped with a weighing unit.

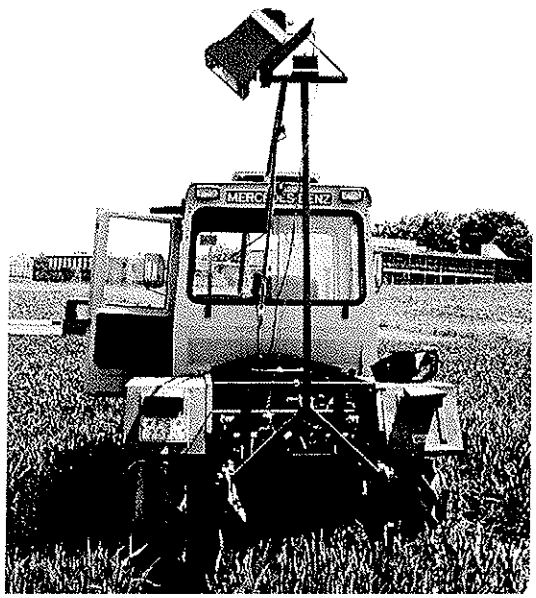

Figure 1. Prototype tractor-mounted chlorophyll fluorescence sensor used for field evaluations. The laser-induced chlorophyll fluorescence is excited and detected at a distance of approximately 3.3 m between canopy and sensor.

The effect of light intensity and air temperature on the ratio F680/F740 was studied under controlled environmental conditions, using a self-constructed portable fluorescence sensor. For the measurement, the leaf was kept at an angle of 90° with respect to the laser excitation beam and at a distance of 14 cm from the laser device. The fluorescence intensity was measured under different light intensities (from 5 to 840 (mol m^{-2} s^{-1}) and air temperatures (from 5 °C to 25 °C) by exposing plants grown under previously comparable conditions for several hours to varying environmental conditions. All measurements were replicated and subjected to statistical analysis.

Results and discussion

Growth chamber measurements

The chlorophyll fluorescence ratio F680/F740 was little affected by the light intensity between 5 and 840 µmol m^{-2} s^{-1} under controlled environmental conditions, in spite of the great differences in the fluorescence yield between plants grown under different light intensities. The fluorescence yield at 680 nm was about 44 % higher and at 740 nm about 46 % higher at 5 µmol m^{-2} s^{-1} than at 840 µmol m^{-2} s^{-1} (data not shown). On the other hand, the ratio F680/F740 was little affected by light intensity (Figure 2A). The ratio F680/F740 varied around 1.0 % between 5 and 840 µmol m^{-2} s^{-1}. The laser-induced chlorophyll fluorescence system operates thus under different light conditions with the same accuracy, even at low light intensities. This feature of the ratio F680/F740 represents a great advantage compared with reflectance measurements, since reflectance varies greatly depending on light intensity.
The fluorescence emission was affected by air temperature. The fluorescence intensity of both bands increased as the air temperature decreased (data not shown). The increase in fluorescence yield with decreasing air temperature from 25 °C to 5 °C was larger in the 740 nm fluorescence band than in the 680 nm one, leading to a decrease in the ratio F680/F740 from approximately 2.05 to 1.75 with a decrease in the air temperature from 25 °C to 5 °C (Figure 2B).
The chlorophyll fluorescence intensity in wheat at 680 nm and 740 nm increased (r^2=0.75 and 0.89, respectively) with increasing dry biomass under controlled environmental conditions (Figure 3A). This increase was relatively larger at 680 nm than at 740 nm. The fluorescence ratio F680/F740 decreased from around 2.0 to 1.8 with an increase in the dry biomass from approximately 5 to 9 g/pot (r^2=0.78) (Figure 3B). The ratio F680/F740 was inversely correlated with chlorophyll meter

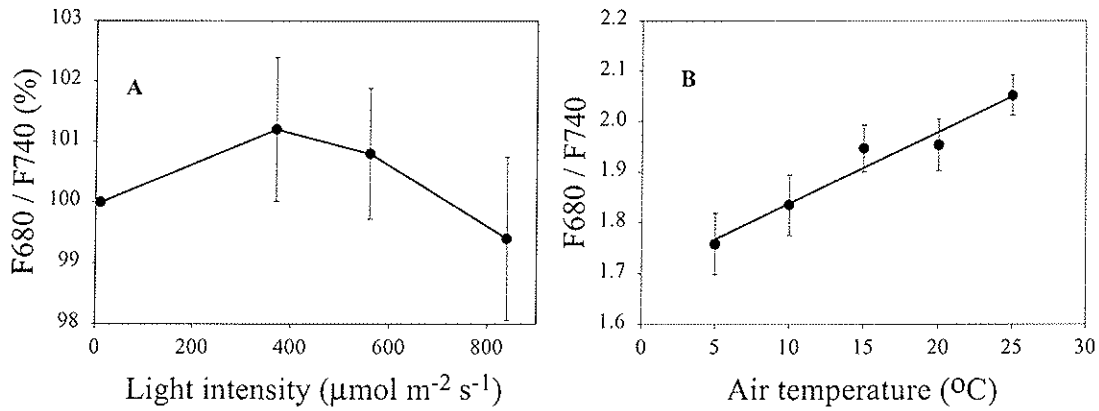

Figure 2. Effect of light intensity (A) and air temperature (B) on the chlorophyll fluorescence ratio F680/F740 under controlled environmental conditions. Bars indicate the standard deviation of the mean. In Figure 2A, the ratio F680/F740 at 5 µmol m^{-2} s^{-1} was fixed as 100%.a

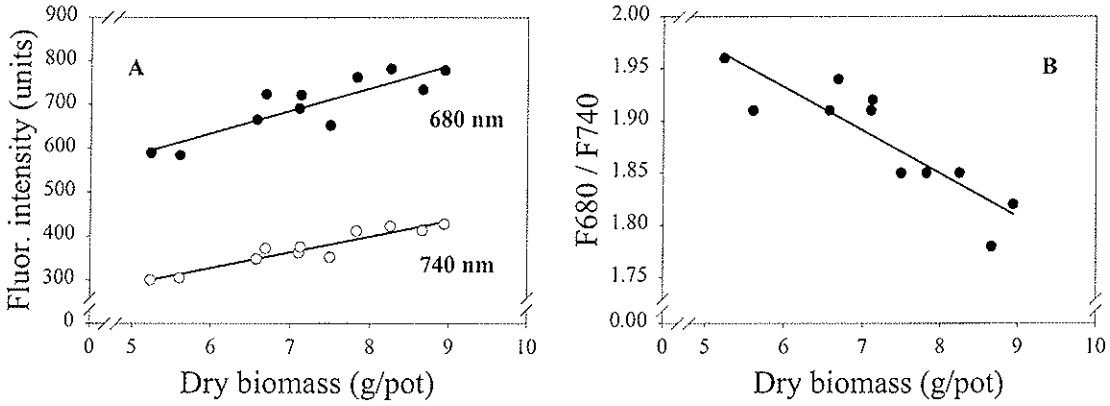

Figure 3. Relationship between chlorophyll fluorescence intensity at 680 nm and 740 nm (A), fluorescence ratio F680/F740 (B) and dry biomass under controlled environmental conditions.

(SPAD-502) measurements (r^2 between 0.33 and 0.44 - data not shown). This result indicates that chlorophyll fluorescence measurements can be influenced by both chlorophyll content and biomass quantity.

Field experiments

Under field conditions the fluorescence ratio F690/F730 was well correlated with the different parameters evaluated (SPAD value, biomass, N content and uptake) in the sensed area of winter wheat. The ratio F690/F730 at BBCH 32 (BBCH Monograph, 1997) varied approximately from 0.89 (in plants without N-fertilization) to 0.80 (in plants receiving 140 kg N/ha), while the SPAD values varied from 38.5 to 49.4 (Table 1). The goodness of linear fits between nitrogen content, nitrogen uptake, biomass and SPAD value to fluorescence ratio mean was as follows: 0.78, 0.87, 0.87, 0.88.

At BBCH 55 the chlorophyll fluorescence was also measured and correlated with dry biomass, N content and SPAD value. All parameters were affected by the amount of N-fertilizer (Table 2). The ratio F690/F730 decreased from 0.839 (in plants without N-fertilization) to 0.751 (in plants receiving 210 kg N/ha up to this moment) and was well correlated with dry biomass, nitrogen content and nitrogen uptake.

The chlorophyll fluorescence of maize was also evaluated under field conditions. The fluorescence intensity at 690 nm and 730 nm evaluated 60 days after sowing increased with increasing chlorophyll content (SPAD value) (Figure 4A). As a result of higher N-fertilization, more

Table 1. SPAD value, dry biomass, N content and uptake and chlorophyll fluorescence ratio F690/F730 of winter wheat in BBCH 32 as affected by the amount of N fertilizer.

N fertilizer applied kg N ha^{-1}	SPAD value	Dry biomass kg ha^{-1}	N content mg g^{-1}	N Uptake kg N ha^{-1}	F690/F730
No N	38.5	3823.4	14.3	55.5	0.89
50	42.7	4491.9	18.4	83.4	0.87
90	45.8	5712.5	20.4	119.3	0.81
110	45.2	5843.5	18.8	110.0	0.85
140	49.4	6404.3	26.5	169.9	0.81

Table 2. SPAD value, dry biomass, N content and uptake and chlorophyll fluorescence ratio F690/F730 of winter wheat in BBCH 55 as affected by the amount of N fertilizer.

N fertilizer applied kg N ha^{-1}	SPAD value	Dry biomass kg ha^{-1}	N content mg g^{-1}	N uptake kg N ha^{-1}	F690/F730
No N	39.2	4647.9	14.5	68.4	0.84
90	45.5	5696.5	19.7	124.5	0.81
130	46.5	6663.8	18.6	124.4	0.80
170	46.9	7135.3	20.0	143.1	0.76
210	49.3	7776.0	23.3	181.4	0.75

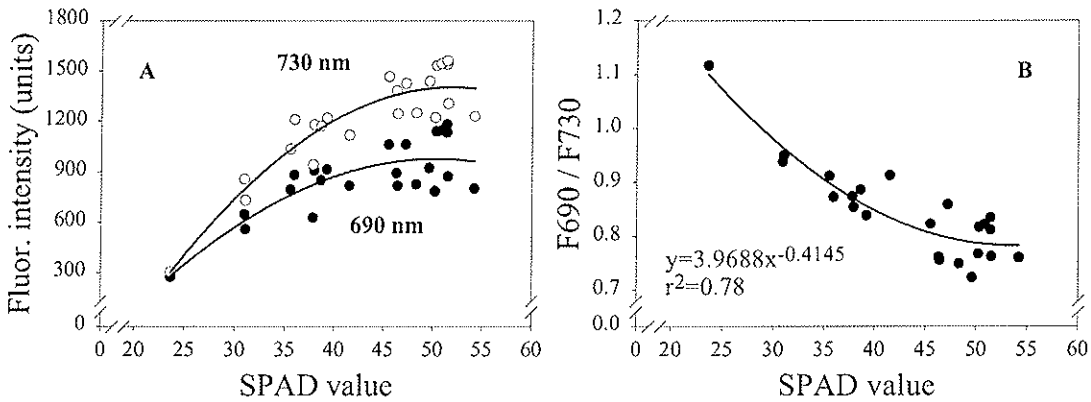

Figure 4. Relationship between chlorophyll fluorescence intensity at 690 nm and 730 nm (A), fluorescence ratio F690/F730 (B) and SPAD value in maize under field conditions.

chlorophyll was produced (higher SPAD value). In consequence, more fluorescence was emitted and detected in the sensed area.

The increase in fluorescence intensity with a higher chlorophyll content was more pronounced at the 730 nm fluorescence band than at the 690 nm one. With increasing chlorophyll content, the 690 nm fluorescence band is decreased by a preferential reabsorption of the emitted fluorescence at 690 nm by the chlorophylls, due to the partial overlapping of the absorption spectrum of the chlorophylls with the fluorescence emission spectrum between 640 nm to around 710 nm (Lichtenthaler & Rinderle, 1988). For that reason, the ratio F690/F730 decreased with an increase in the chlorophyll content (SPAD value) (Figure 4B).

Conclusions

The fluorescence ratio F690/F730 under field conditions was well correlated with chlorophyll content and N supply in both wheat and maize. The fluorescence intensity at 690 nm and 730 nm increased as the SPAD value increased, while the ratio F690/F730 was inversely correlated with N uptake and SPAD value. The results showed that N-fertilization levels in the field could be differentiated by means of fluorescence ratio measurements. Under controlled environmental conditions (portable sensor), the chlorophyll fluorescence ratio F680/F740 was affected by air temperature, but little influenced by light intensity. Under field conditions, using the sensor developed by Planto GmbH, the temperature effect on the fluorescence measurement has to be

further tested. Temperature effects, however, can easily be accounted for by appropriate sensor technologies already available within the field sensor. Chlorophyll fluorescence does not react sensitively do mild or even moderate drought (unpublished data). Using sulfur containing fertilizers for the first nitrogen dressing allows to avoid disturbing effects on sites where such deficiencies might occur. Other nutrient limitations have to be considered if deficiencies are likely to occur. The same principles for nitrogen content measurements apply to laboratory and field measurements with measurements done on individual leaf positions, whereas biomass could more precisely be detected under field conditions representing whole canopies in contrast to laboratory conditions where only leaves or plants at best were detected in pot-grown plants.

The results indicate that nitrogen uptake and biomass can be detected by means of chlorophyll fluorescence measurements. Based on these values, it should be possible to monitor on-line the spatial variation of the N-status of plants. The information obtained on-the-go can be combined with a fertilizing algorithm to control the amount of N fertilizer being applied (variable rate technology).

References

BBCH-Monograph (1997). Growth stages of mono- and dicotyldenous plants. Ed. by U. Meier et al. Berlin; Wien. Blackwell Wissenschafts-Verlag.

Bredemeier, C. and Schmidhalter, U. 2001. Laser-induced chlorophyll fluorescence as a tool to determine the nitrogen status of wheat. In: Proceedings of the 3rd European Conference on Precision Agriculture, edited by G. Grenier and S. Blackmore, agro Montpellier 899-904.

Lichtenthaler, H.K. and Rinderle, U. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytical Chemistry 19 29-85.

McMurtrey, J.E., Chappelle, E.W., Kim, M.S., Meisinger, J.J. et al. 1994. Distinguishing nitrogen fertilization levels in field corn (*Zea mays* L.) with actively induced fluorescence and passive reflectance measurements. Remote Sensing of Environment 47 36-44.