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Original article

Early detection of N deficiency in a wheat crop using physiological and radiometric methods

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Abstract – A winter wheat (cv Soissons) crop was made N deficient during stem elongation. The effects of the N stress were quantified by measuring the nitrogen nutrition index (NNI) (used as reference), proteolytic activity in the leaves, the rate of carbon dioxide (CO_2) assimilation by the leaves, green leaf area, chlorophyll concentration of the leaves and crop reflectance. A reduction in available soil N 1 week after the onset of N stress resulted in decreases in NNI and CO_2 assimilation rate, a change in leaf chlorophyll concentration affecting the lower leaves most severely, and in changes in crop reflectance. Green leaf area and leaf proteolytic activity were affected only after 2 weeks of N stress. At the onset of N deficiency, NNI was correlated with the relative leaf CO_2 assimilation rate, green leaf area, the near infrared to red reflectance ratio (XS3/XS2) and the chlorophyll concentration in the lower leaves, estimated by a SPAD value. The CO_2 assimilation rate was assessed by measuring chlorophyll concentration irrespective of N status. The non-destructive measurements of crop reflectance and leaf chlorophyll concentration appear to be appropriate indicators of the current N status of the crop. (© Inra/Elsevier, Paris.)

winter wheat / nitrogen / radiometry / leaf area / carbon assimilation

Résumé – Diagnostic précoce de déficit azoté sur une culture de blé d'hiver par l'utilisation de méthodes physiologiques et radiométriques. Une culture de blé d'hiver (cv Soissons) est soumise à des déficits en azote en cours de montaison. Les effets de l'installation de la carence azotée sur les plantes sont quantifiés à partir des mesures d'accumulation d'azote dans les parties aériennes, utilisée comme référence, de l'activité protéolitique des feuilles, de la vitesse d'assimilation du dioxide de carbone (CO_2), de la surface foliaire verte, de la teneur en chlorophylle des feuilles et de la réflectance du couvert. Une réduction de la disponibilité en azote du sol se traduit après une semaine de carence par une diminution de l'indice de nutrition azoté (INN) et de la vitesse d'assimilation du CO_2, un changement de teneur en chlorophylle des feuilles affectant en priorité les feuilles du bas, et une modification de la réflectance du couvert. La surface foliaire verte et l'activité protéolitique des feuilles ne sont affectées qu'après deux semaines de carence. L'INN apparaît corrélé à la vitesse relative d'assimilation du CO_2, à la surface foliaire, à l'indice de réflectance XS3/XS2, rapport entre la reflectance dans le proche infrarouge et dans le rouge, et à la teneur en chlorophylle des feuilles les plus basses, esti-

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mées par l'indice SPAD. De plus, la vitesse d'assimilation du CO_2 peut être estimée, indépendamment du niveau de nutrition azotée, à partir de la teneur en chlorophylle des feuilles. Les variables de réflectance du couvert et de teneur en chlorophylle des feuilles, dont la mesure est non destructrice, pourraient être utilisés comme indicateurs instantanés de l'état azoté du peuplement végétal. (© Inra/Elsevier, Paris.)

blé d'hiver / azote / radiométrie / surface foliaire / assimilation du carbone

1. INTRODUCTION

Nitrogen is often considered as the primary determinant of crop productivity [35]. Nitrogen stresses cause qualitative depreciation of crops [1] and affect plant growth by altering both leaf area and photosynthetic capacity [31]. Economic constraints and concerns about N pollution of ground water have stimulated interest in fertilizer strategies that minimize N application. Such strategies are only possible if the N status of the crop can be assessed at any time during stem elongation. Two kinds of measurements can be made:

– destructive measurements, which are usually made in the laboratory;

– non-destructive measurements, usually made in the field.

1) Many N-dependent variables have been assessed for their ability to indicate the N status of a wheat crop, with the aim of relating them with fertilizer requirements. The variables tested include growth rate [12], chlorophyll content [9], plant N content [11, 19] and nitrate reductase activity [7, 36]. The nitrogen nutrition index (NNI) [18] can be used to detect N deficiency in a crop, but often only after the event. The capacity of the nitrate reductase activity to act as an early indicator of the N status of a crop is based on the fact that N uptake by roots could be one of the factors controlling nitrogen assimilation in plants [28], since the flux coming from vacuoles or xylem regulates nitrate reduction [29].

2) The plant status can be rapidly assessed using a chlorophyll meter for the leaves, and the status of the whole canopy can be assessed using multiband reflectance measurements. Remote sensing techniques such as field radiometric measurements provide a rapid estimate of the whole canopy characteristics (leaf area index, optical properties). These measurements and the derived vegetation indices can facilitate the data acquisition and the sampling for monitoring crop evolution [3, 4].

This study was carried out during stem elongation of winter wheat to quantify the effects of N deficiency on plant physiological processes (photosynthesis, leaf area development) and, hence, to determine the physiological significance of NNI. We also assessed the effects of N deficiency on the geometrical structure and optical properties of winter wheat canopies and evaluated the value of these variables as early, sensitive and readily measurable indicators of N deficiency.

2. MATERIALS AND METHODS

2.1. Experimental conditions

Two field experiments were conducted in 1993 and 1995 on winter wheat grown in a loamy soil at Grignon, northern France (latitude 48°51' N; longitude 1°58' E). The pH of the soil was 7.7 and the organic matter content was 2.6 %. The preceding crop was maize, the residues of which were buried. The soil was ploughed and harrowed just before sowing. The wheat crops, cultivar 'Soissons', were sown on 19 October 1992 and 20 October 1994, at 350 seeds m^{-2} which resulted in a density after winter of 315 plants m^{-2} in 1993 and 310 plants m^{-2} in 1995. A complete crop protection treatment was applied: herbicide (bifidox + ioxynil + mecoprop-p + fluroxypyr) in March and fungicide treatments in April (flusilazole + fenpropimorphe + prochloraze + carbendazine) and May (tebuconazole). Thus, all weeds, pests and diseases were controlled. The climatic conditions during the 3-week period of the study for each of the two experiments are described in *table I*. The experimental design was a fully randomized block design with three replicates. Each experimental plot measured 15 m × 6 m.

The dates and amounts of fertilizer applied for the N-limiting treatment (Nl) were selected to cause N deficiency during stem elongation at stage 32 in 1993 and at stage 30 in 1995 [37]. A total of 120 kg N ha^{-1} was applied to the plants before flowering. The N fertilization regime for the control treatment (T) was determined by the predictive balance sheet method for a target grain yield of 10 t ha^{-1} [27]. The N fertilizer (220 kg N ha^{-1}) was applied in three dressings at stages 21, 30 and 32 as ammonium nitrate in a solid form *(table II)*.

The experiments were conducted over 3 weeks (T1, T2 and T3) from the beginning of N deficiency (T0) corresponding to stage 32 in 1993 and to stage 30 in 1995. Sampling dates are expressed as cumulative degree-days after sowing with a base of 0 °C.

2.2. Analytical methods

2.2.1. N measurement

Vegetation (0.170 m^2) was sampled weekly. The corresponding fresh aerial biomass varied from 75 g per sampling at TO to 190 g per sampling at T3. The stems were separated from the leaves and the leaves were then separated into stages. The upper leaves, still being formed, were labelled F1 and the leaves below were successively F2, F3, F4 and F5. All the leaves below stage F5 were classified as FB. All fresh organs were dried at 80 °C for 48 h, weighed and reduced to fine powder. The total N content of each sample was evaluated according to the Dumas principle, using a flash combustion method with an automatic N analyser (Carlo-Erba, ANA 1500, Milan, Italy). Atropine (4.84 % N) was used as the standard. Justes et al. [15] determined a critical N dilution curve for winter wheat described by the equation: N (%) = 5.35.(dry matter)$^{-0.442}$. The critical N concentration calculated by this model is the minimum concentration of total N in shoots required to produce maximum aerial dry matter at a given time and field situation. Total N concentrations in aerial dry matter (leaves + stems) from each individual plot were compared with the critical dilution curve to assess the crop N status. The nitrogen nutrition index (NNI) was calculated as the total N concentration divided by the critical N concentration. Periods of N deficiency occurred when NNI was less than 1 [18].

Table I. Climatic conditions during the three-week period of the study in 1993 and 1995.

Experiment	1993	1995
Total rainfall (mm)	95.4	65
Mean daily temperature (°C)	14.2	9.1
Mean daily global radiant exposure ($MJ.m^{-2}$)	16.6	13.3

2.2.2. Green leaf area measurement

Each week, one other microplot 0.170 m^2 was sampled and five plants selected. The leaves were separated into stages as described above and the green leaf area was measured for each group of leaves, by scanning colour images in three bands, red, green and blue (central wavebands: 450–550–645 nm – Scanner Colorgraph SC6000-C – Resolution: 48 dpi). Green parts were then distinguished from non-green parts using a digital image processing unit (Micropéricolor 3400, MATRA,

Table II. Dates and amounts of N fertiliser applied (kg N ha^{-1}).

Stages Zadoks' scale		21	30	32	Total amount N supplied before flowering
Date	1993	18/02	24/03	27/04	
	1995	21/02	22/03	21/04	
Degree-days since sowing	1993	744	941	1 219 (T0)	
	1995	899	1 076 (T0)	1 122 (T1)	
1993	Nl	60	60	0	120
	T	60	60	100	220
1995	Nl	60	0	60	120
	T	60	100	60	220

France). The methodology involved a semi-automated procedure: a threshold value was selected to separate the data into two classes, based upon a two-dimensional histogram built from red and green channels. The output variables were the total leaf area, the green leaf area and the green fraction (%).

2.2.3. Radiometric data

Radiometric measurements were performed with a CIMEL field radiometer equiped with filters simulating SPOT spectral bands [13]. This radiometer has two heads: one measures the global irradiance in each band and the other the directional radiance of the canopy. After calibration the radiometer gives directly the reflectance factor of the viewed target. The spectral bands of the radiometer are:

XS_1: (500–590) nm (green)

XS_2: (610–680) nm (red)

XS_3: (790–890) nm.(near infrared)

The radiometer was calibrated using a white panel painted with Nextel 3M white paint. The reflectance of the panel was determined with a laboratory spectrophotometer.

In 1995, two microplots, consisting of two parallel rows 17 cm apart and 50 cm long, were marked out in each experimental plot. The spectral reflectance of each sample area was measured with the radiometer placed 2 m above the ground surface. Seven series of measurements were performed between 1 076 degree-days (T0) and 1 460 degree-days from sowing.

Radiometric measurements were generally performed at solar noon ± 2 h. We used the same bands as for the SPAD chlorophyllmeter to calculate vegetation indices. Two classical vegetation indices were calculated:

– the normalized difference, NDVI = $(XS_3 - XS_2)/(XS_3 + XS_2)$;

– the ratio XS_3/XS_2.

These indices are widely used because they minimize the effects of irradiance and variations in soil optical properties [4], and are related to biophysical canopy variables, especially to leaf area index (LAI).

2.2.4. Proteolytic activity

In 1993, the proteolytic activity of leaves from the four uppermost stages was determined for both N treatments. Fresh leaves were weighed and frozen in liquid N. Proteins were extracted by the method of Rao and Croy [25]. Plant tissue (1 g) was grounded in 8 mL 1 mM EDTA (Ethylene Diamine Tetra Acetate), 5 mM cysteine in 0.1 M potassium phosphate buffer (pH 7.4). The resulting suspension was centrifuged at 30 000 *g* for 20 min. Proteolytic activity was determined as described by Peterson and Huffaker [23] by incubating 0.2 mL of the supernatant and 0.3 mL azocaseine (previously heated at 100 °C for 2 min to prevent autohydrolysis) with 0.5 mL 0.1 M citrate–potassium–phosphate buffer (pH 5.6) at 40 °C for 2 h. The reaction was stopped by adding 2 mL perchloric acid (12 %) and the precipitate was collected by centrifugation at 12 000 *g* for 5 min. Absorbance at 340 nm was used to measure the amount of diazoted amino acids liberated by proteolysis of azocaseine. A unit of proteolytic activity was defined as an increase in absorbance of 0.6 units of optical density per hour, per gram fresh weight.

2.2.5. SPAD measurement

In 1995, a SPAD chlorophyllmeter (Model 502, Minolta Co, Ltd, Japan) was used to estimate leaf chlorophyll concentration from leaf transmittance, measured at two wavelengths (650 and 940 nm) [21]. Three plants were selected per plot and per N treatment in field conditions. Measurements were taken at four positions along the lamina of each leaf to obtain a representative mean value for the leaf.

2.2.6. Carbon dioxide assimilation

Leaf CO_2 assimilation was measured in field conditions between 1000 and 1500 hours on the five uppermost leaves of three plants per N treatment and per plot using a portable infrared carbon dioxide analyser (LCA-2. ADC Co. Ltd., Hertz, UK). The analyser was configured for differential CO_2 measurement using a 6 mL leaf chamber. Photon flux density normalized at the leaf surface during measurement was from 1 500 to 1 700 $\mu mol\ m^{-2}\ s^{-1}$. The decrease in CO_2 concentration in the leaf chamber was measured over about 60 s and once equilibrated, with one measurement of CO_2 concentration every 8 s. Photon flux density, relative humidity and temperature were measured at the same time. The rate of CO_2 assimilation was calculated from the mean of these measurements. Individual leaves were detached from the plant after CO_2 assimilation had been measured and the leaf area was determined using a leaf area meter (Delta T devices Ltd, Cambridge, UK). The net assimilation rate is expressed in $\mu mol\ CO_2\ m^{-2}\ s^{-1}$.

3. RESULTS

3.1. Definition of temporary N deficiency

The amount of fertilizer applied to the control treatment in both experiments was considered non-limiting since NNI was not significantly different than 1. The other experimental treatment resulted in N deficiencies at stage 32 of stem elongation (Zadoks' scale) in 1993 and stage 30 in 1995 with NNI less than 1 *(figure 1)*. Consistent with this, there were also significant decreases in the mineral N (nitrate + ammonium) concentration in the soil (0–90 cm) (data not shown).

3.2. Carbon dioxide assimilation and leaf chlorophyll content

As for NNI, leaf CO_2 assimilation rates were affected by the N-limiting treatment as early as stage T1, which occurred approximately 120 degree-days after T0 in 1993 and 50 degree-days after T0 in 1995 *(figure 2)*. The lower leaves (F4) were the most affected by N deficiency after the first week. The upper leaves (F1 and F2) of plants in the N-limiting treatment had a CO_2 assimilation rate 80 % that of the control treatment. After 3 weeks of N deficiency, the CO_2 assimilation rate with the N-limiting treatment was 67 % that of the control treatment in 1993 (NNI = 0.57) and 75 % that of the control treatment in 1995 (NNI = 0.60).

Similarly, leaf chlorophyll A concentration as measured by SPAD in 1995 was affected by N availability within 1 week of N deficiency. The lower leaves were the most affected *(figure 3)* and after 3 weeks of N deficiency, the SPAD value of leaf number 4 in the Nl treatment was close to 50 % that of the T treatment. F2 leaves were the least affected by the N-limiting treatment. Their SPAD values were about 90 % those of the control treatment.

The CO_2 assimilation rate of entire plants was calculated from the total CO_2 assimilated by individual leaves during the experimental period. In 1995, the CO_2 assimilation rate of entire N-restricted plants, expressed as a percent of the CO_2 assimilation rate of N non-limited plants was correlated with NNI ($r^2 = 0.93$, $n = 8$) *(figure 4A)*. The SPAD index of leaves 1 and 2 was relatively constant irrespective of the N treatment, whereas the SPAD

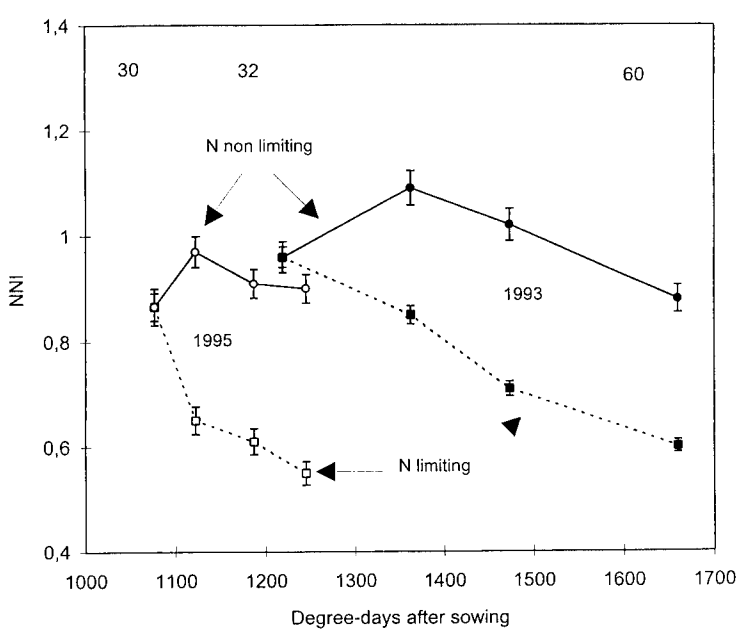

Figure 1. Changes in N nutrition index (NNI) between Zadoks' stages 30 and 60 of plants subjected to N-limiting (□) or N non-limiting (O) treatments in 1993 (closed symbols) and in 1995 (open symbols). The bars represent standard deviations ($n = 3$).

index of leaves 3, 4 and 5 decreased with decreasing NNI values *(figure 4B)*.

The rate of CO_2 assimilation of individual leaves was correlated with the SPAD value of the leaves *(figure 5)*. The experimental data from 1995 generates a curve with the following equation:

$$y = 0.0117 \,.\, (\text{SPAD})^{1.81} \quad (r^2 = 0.89 \quad n = 67)$$

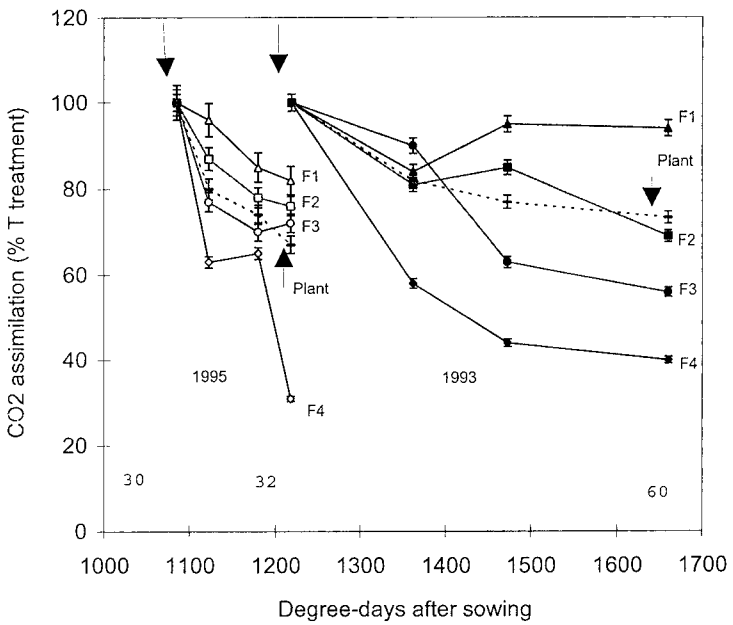

Figure 2. CO_2 assimilation rate of various leaves of plants subjected to N-limiting treatment, expressed as a percentage of that of control plants (treatment T) in 1993 (closed symbols) and in 1995 (open symbols). F1 = uppermost leaf: △-▲; F2: □-■; F3: ○-●; F4: ◇-◆; plant: ------. The bars represent standard deviations (n=3). The arrows indicate the start of N deficiency.

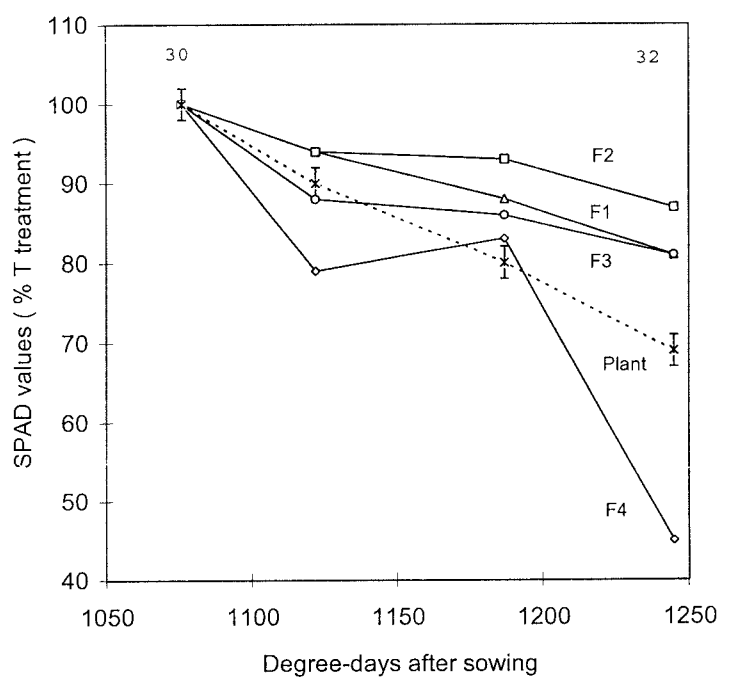

Figure 3. Chlorophyll meter (SPAD) readings for leaves F1 (△), F2 (□), F3 (○), F4 (◇) and entire shoots (---) of plants subjected to N-limiting treatment, expressed as a percentage of that of the control treatment (T) in 1995. The bars represent standard deviations ($n = 3$).

3.3. Green leaf area index

The N deficiency led to a decrease in the green leaf area index (GLAI). In both experiments, the relative GLAI of whole N-restricted plants significantly decreased only 2 weeks after T0, although in 1995 (N deprivation at stage 30) there was a significant decrease in the GLAI of individual leaves 3 and 4, at T1 *(figure 6)*. All leaves were simultanously and progressively affected by N availability by 2 weeks after T0. The entire N-restricted plant GLAI was 62 % that of controls 3 weeks after N stress at stage 30 (NNI = 0.57) in 1993 and 80 % that of controls, 3 weeks after N stress at stage 32 (NNI = 0.60) in 1995.

The relative GLAI of N-deficient plants was significantly correlated with NNI (r^2 = 0.75, n = 8) *(figure 4A)*.

The proteolytic activity in leaves of plants made N deficient at stage 32 was not significantly affected 1 week after T0, except in leaf number 4. Two weeks after the beginning of N deficiency, the proteolytic activity of the four uppermost leaves of the Nl plants was 1.2–1.7 uap h^{-1} g^{-1} fresh weight *(figure 7)* while this proteolytic activity in T plants was about 1 uap h^{-1} g^{-1} fresh weight.

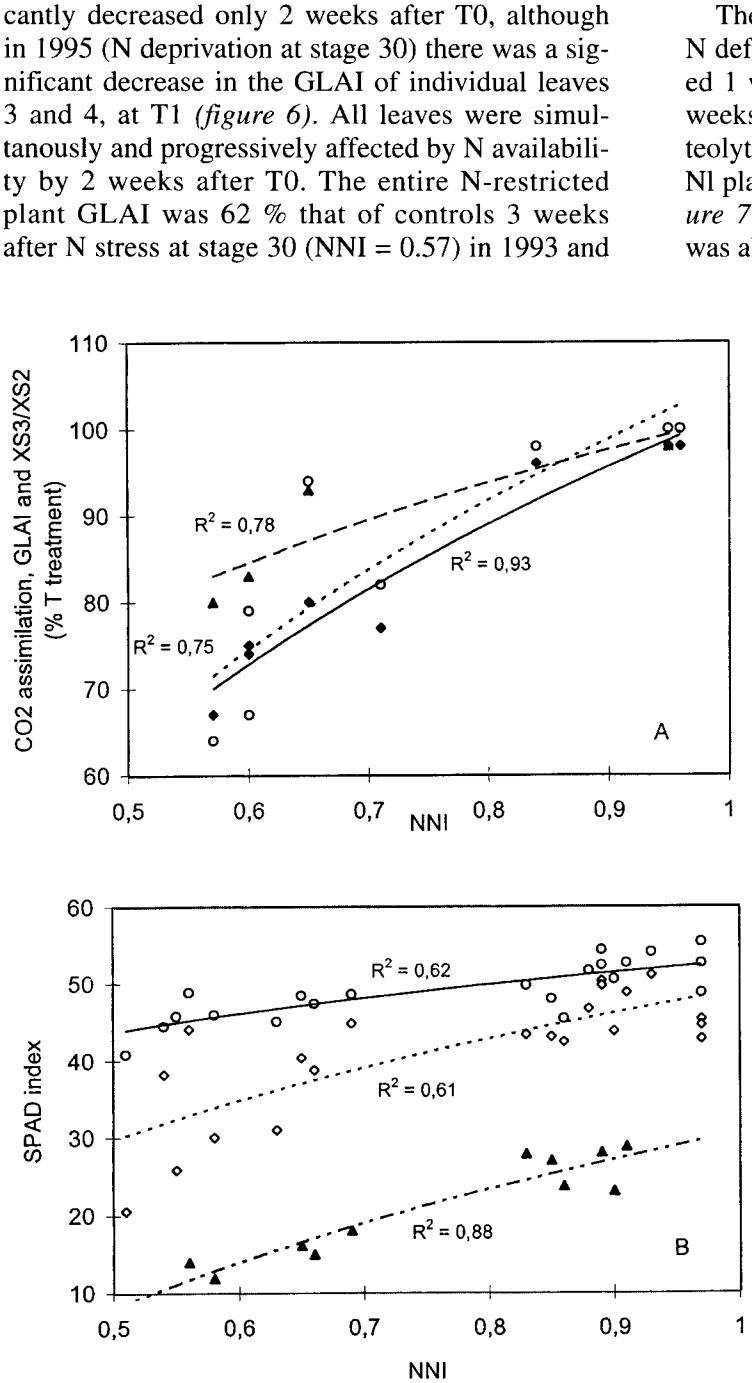

Figure 4: **(A)** Relationships between CO_2 assimilation rate (◆—◆), GLAI (○...○) and XS3/XS2 ratio (▲---▲) and the nitrogen nutrition index (NNI) for entire plants subjected to N-limiting treatment, expressed as a percentage of that of control plants (treatment T). **(B)** Relationships between the SPAD values of leaves F3 (○), F4: (◇) and F5 (▲) and NNI of plants subjected to N-limiting or non-limiting treatment.

3.4. Change in radiometric signal during stem elongation in 1995

The spectral responses to treatments in the green (XS1) and red (XS2) wavebands were both closely correlated. Reflectance decreased and reached a minimum only at about 95 % soil coverage *(figure 8)*. The increase in reflectance for all treatments at 1249 degree-days was due to measurements being made later in the afternoon. The reflectance of N-deficient plants in these two wavebands was greater than that of the control plants. The difference was significant for the green and

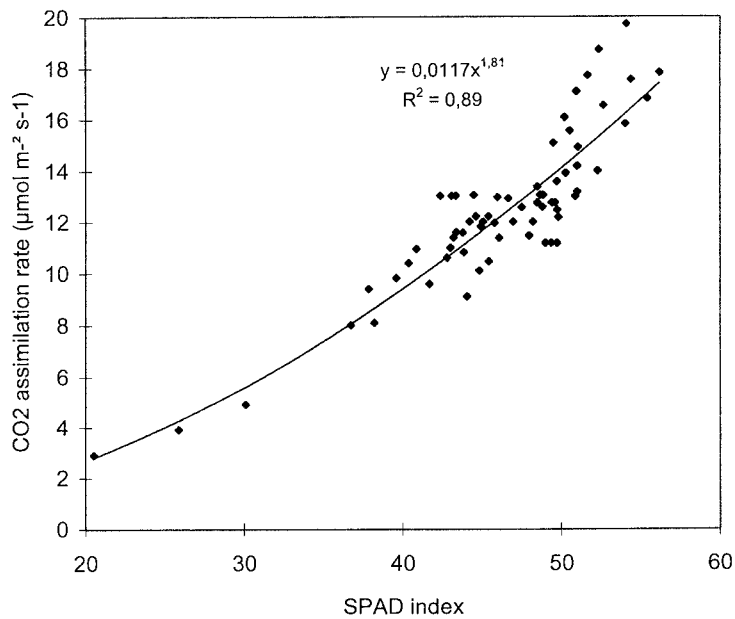

Figure 5. Relationship between CO_2 assimilation rate (µmoles . m^{-2} . s^{-1}) and chlorophyll content (SPAD index) of the leaves of plants subjected to N-limiting or non-limiting treatments in 1995.

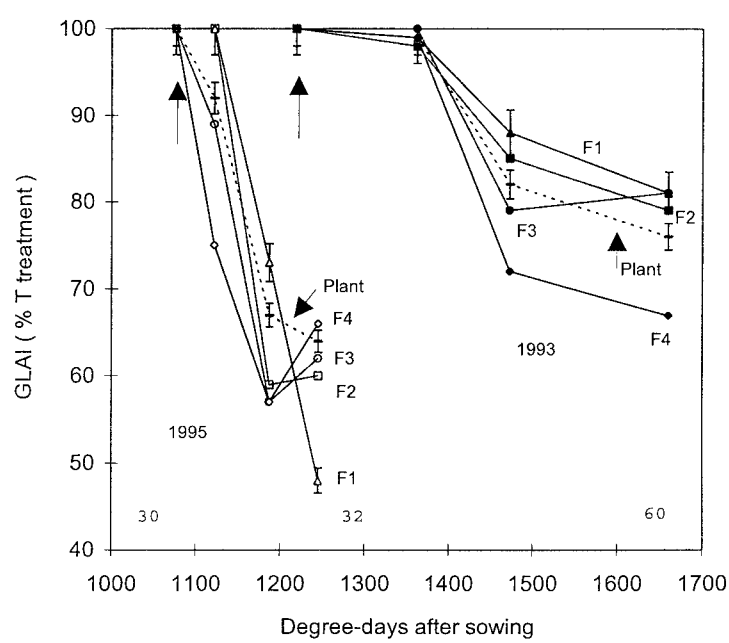

Figure 6. Green leaf area index (GLAI) of plants subjected to N-limiting treatment in 1993 (closed symbols) and in 1995 (open symbols) expressed as a percentage of the GLAI of control plants (treatment T). F1 = uppermost leaf: △-▲; F2: □-■; F3: ○-●; F4: ◇-◆; plant: ------. The bars represent standard deviations (n = 3). The arrows indicate the start of N deficiency.

red bands at stage T2 (1 171 degree-days), approximately 100 degree-days after the start of the N deficiency. After T2, the difference between the reflectances in the red band of T and Nl treatments was significant at all stages, whereas the green response was not.

The near infrared (XS3) reflectance of control plants between stages 30 and 37 increased with time from 25 to 40 % *(figure 9)*. The N deficiency resulted in a smaller increase. The difference between the T and Nl treatments was significant only after stage T3 which occured 180 degree-days after T0.

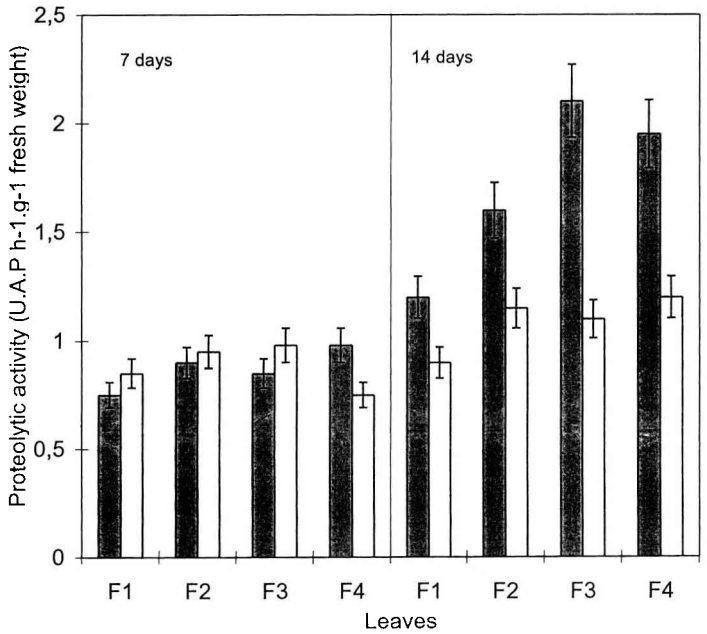

Figure 7. Proteolytic activity of leaves F1, F2, F3 and F4 of plants subjected to N-limiting treatment (grey area) and N non-limiting treatment (white area) 7 and 14 days after the start of N deficiency. The bars represent standard deviations ($n = 3$).

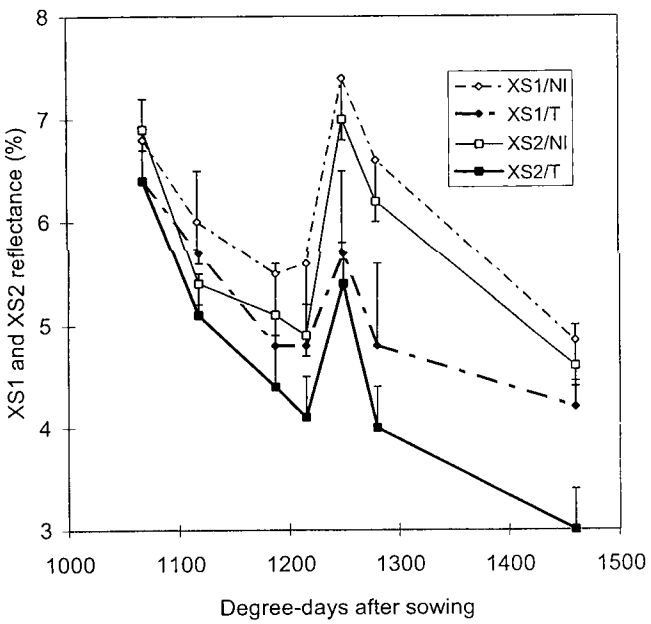

Figure 8. Reflectance in the green (XS1: ◇-◆) and red wavebands (XS2: □-■) of crops subjected to N-limiting treatment (open symbols) or N non-limiting treatment (closed symbols) in 1995. Bars represent LSD ($P = 0.05$).

The reflectance ratio in the three channels and the two vegetation indices were plotted against time *(figure 10)* and the ratio between the values for the N-limited and control plants was calculated using values averaged for each replicate at each date. The N deficiency was detected earliest by the XS2 band or the XS3/XS2 ratio, which was more sensitive than NDVI. XS1 was correlated with XS2. XS3 and NDVI were not effective as early indicators of N deficiency.

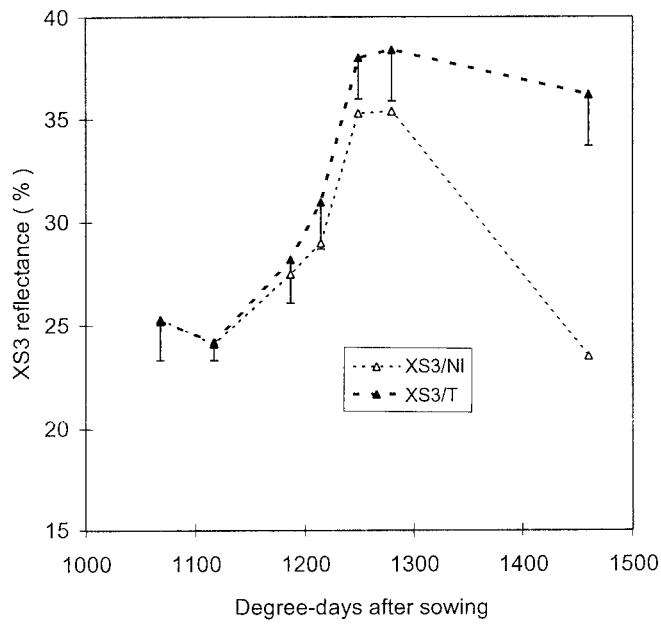

Figure 9. Reflectance in the near infra-red wavelength (XS3) of crops subjected to N-limiting treatment (△) and N non-limiting treatment (▲) in 1995. Bars represent LSD ($P = 0.05$).

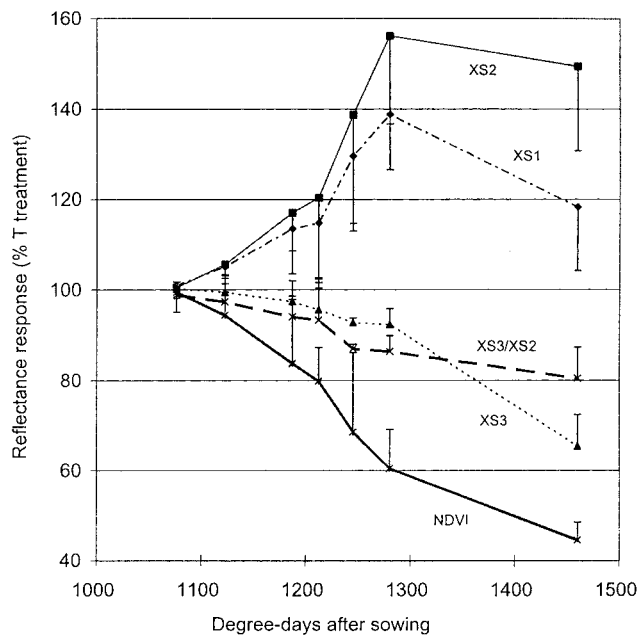

Figure 10. Changes in the reflectance ratio for N limiting and non-limiting treatments. XS1: ◆; XS2: ■; XS3: ▲; NDVI: ✖; XS3/XS2: ✳. Bars represent LSD ($P = 0.05$).

3.5. Relationships between radiometric and canopy characteristics

Various radiometric indices were tested as indicators of N status. The XS3/XS2 ratio was affected by temporary N deficiency at the same time as NNI and indicated the N status of the crop. This radiometric index was related to NNI (r^2 = 0.78, n = 4) *(figure 4A)*.

The foliar N index (FNI) (gN m^{-2} soil), defined as the leaf N content per m^2 of leaf multiplied by LAI, (the N accumulated in leaves per unit of soil) was used to characterize the canopy. The data set was used to check that foliar N index was a good indicator of the N status of the canopy (N accumulated by the entire plant = 1.53 (FNI)–0.48; r^2 = 0.97) (data not shown) and that the XS3/XS2 ratio was closely correlated with foliar nitrogen index *(figure 11)*. A straight line fitted the experimental values for the two nitrogen treatments:

$$\text{foliar N index} = 7.87 \,.\, (XS3/XS2) - 10.49$$
$$(r^2 = 0.93,\ n = 8)$$

4. DISCUSSION

The N status of wheat plants was altered by splitting N fertilizer applications to cause a temporary N deficiency, defined by an NNI less than 1. Thus, during the 3-week experiments, N absorption was limited to the available soil inorganic N. The NNI of the control plants was relatively constant and close to 1 throughout both experiments. The values of the indicators studied, in response to N deficiency, are therefore expressed as a percentage of the values for the control plants.

The primary effect of a temporary N deficiency was to cause simultaneous decreases, after 1 week, in NNI, CO_2 assimilation rate and leaf chlorophyll concentration. Several studies have shown that stomatal opening in rice [14] and wheat [30] and root conductivity in sunflower [24] are less in N-deficient plants than in controls. Under these conditions, lower transpiration rates would reduce the ionic concentration of the leaves, especially of nitrate and potassium ions, thereby favouring proteolytic degradation [33]. Indeed, there was more proteolysis 2 weeks after the beginning of N deficiency. The reduced CO_2 assimilation rate affects the synthesis of energetic substrates for the absorption, translocation and reduction of nitrate. Thus, leaf area expansion is affected by changes in the amount of assimilates flowing to the leaves. We found that N deficiency resulted in smaller final areas of individual leaves which is consistent with previous work [6, 12, 34]. Sinclair [31] suggested that the availability of N is an important determi-

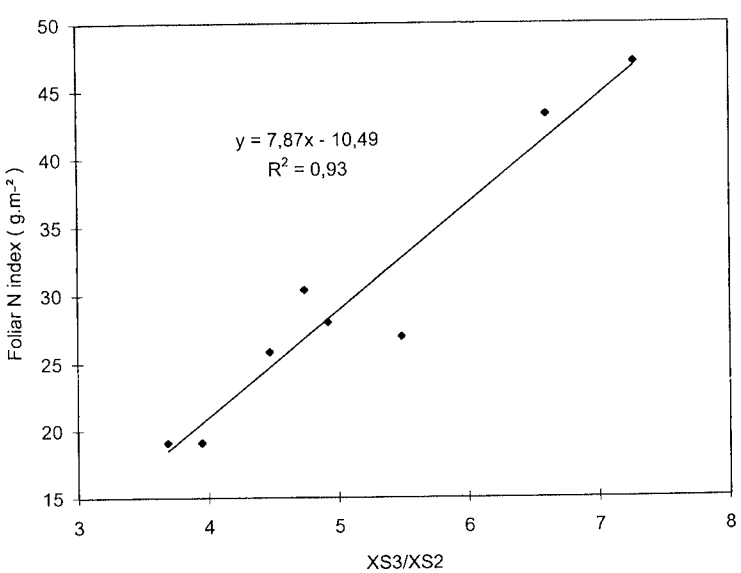

Figure 11. Relationship between the foliar N index (leaf N content per m^2 × LAI) and the reflectance ratio of the crop at near infra-red and red wavelengths (XS3/XS2).

nant of leaf area expansion because the basic composition of leaves imposes a minimum N requirement per unit of leaf mass. He reported that low N availability delayed the development of the final leaf area. Our results show that the smaller GLAI after 3 weeks of N deficiency persisted with time. At flowering, GLAI was 4.20 for N-stressed plants at stage 32 (1993) and 5.26 for the control plants; it was 3.85 for N-stressed plants at stage 30 (1995) and 5.80 for the control plants.

The response of the winter wheat crop to a temporary N deficiency was smaller in 1993 when the N stress was imposed later in the life cycle. Leaf area expansion was delayed by temporary N deficiency. However, the final area of individual leaves was less affected in 1993 than 1995. The leaves, except the flag leaf, are partially developed at stage 32 and this may result in GLAI being less affected. Nitrogen stress should have a greater effect on the growing leaves. The N stress in the 1993 experiment (at stage 32) occurred when the external daily temperature and rainfall were high *(table I)* and thus mineralization of soil N may have minimized the effects of the N stress on the crop.

Our radiometric measurements are consistent with those of Filella et al. [9], and show that radiometric measurements can be used to detect N deficiency, as soon as the expansion of leaf area is affected. The equation of Fernandez et al. [8] (%N = a–bXS1–cXS2) did not fit our data satisfactorily (data not shown). We introduced the XS3 variable to take the structure of the canopy into account, because XS3 is known to be sensitive to cumulative leaf area. The XS3/XS2 ratio accounted mainly for the change in the cumulated leaf area. This ratio detected N deficiency very early (after 1 week). The change in optical properties affected the downmost leaves first *(figure 3)*, which is assumed to have little influence on the radiometric signal.

As discussed by Baret [2], the radiometric response of incomplete canopies is mostly affected by the contrast between the optical properties of the soil and the leaves. Baret et al. [5] suggested using synthetic canopy variables, such as canopy chlorophyll content (C_{ab} . LAI in mg cm^{-2}, where C_{ab} is the chlorophyll concentration per unit leaf area) or canopy water content (C_w . LAI, in cm, where C_w is the equivalent water thickness). We find that another variable, the foliar nitrogen index (foliar N × LAI) can also be used. This index accounts for the N status of the canopy and for N stress. Wide spectral band radiometry can be used to assess foliar N index for wheat *(figure 11)*. Similarly, Baret et al. [5] showed that C_{ab} LAI is strongly correlated with reflectance in sugar beet.

NNI is a widely used indicator of N status in crops [16, 17]. This index is necessary to verify the N nutrition conditions a posteriori. The major problem with the direct use of NNI as an indicator of crop N status is the need to determine actual crop biomass to calculate NNI [20]. It was used as a reference for the other indicators tested.

Three N-dependent agronomic characteristics of the canopy (CO_2 assimilation rate, GLAI and the vegetation index XS3/XS2) were closely correlated with the N status of the crop at the start of N deficiency and their change with N availability were correlated with NNI *(figure 4A)*. The N status of the crop was sub-optimal (NNI < 1) in all cases and these relationships describe the relevance of the physiological and radiative effects of the N stress of the crop. Plant N concentration determines not only the amount of CO_2 assimilation per unit leaf area [22, 32], but is also closely correlated with the rate at which carbon is used for leaf expansion [10] and hence with the structure of the canopy. The onset of N deficiency, involving a progressive decrease in NNI from 1, is accompanied by a simultaneous decrease in relative CO_2 assimilation rate and in the relative XS3/XS2 ratio. In our experiments, CO_2 assimilation was 80 % that of the control treatment and XS3/XS2 was 83 % that of the control treatment for an NNI of 0.70. The decrease in GLAI was slightly delayed relative to these two indicators. The time between the decrease in the CO_2 assimilation rate and that of GLAI varied from 65 degree-days when the N deficiency occurred at stage 30 to 110 degree-days when it occurred at stage 32. These indices make it possible to detect N deficiency in a crop at an early stage and to evaluate the size of this deficiency.

The CO_2 assimilation rate of the leaves could be estimated independently of leaf stage and N fertilization, using their SPAD index *(figure 5)*. The photosynthetic function of leaves is determined by their chlorophyll concentration, the estimation of which by SPAD index is significantly correlated with the concentration of N in leaf tissue dry matter [26]. We found *(figure 4A)* that N deficiency resulted in a decrease in the chlorophyll concentration in lower leaves (especially leaves 4 and 5), whereas the upper leaves retained enough N for optimal photosynthetic function. This reflects the ability of plants to adapt to N-deficient conditions. Thus, simple and non-destructive leaf chlorophyll meter readings on the basal leaves would facilitate determination of the N status of plants in the field.

Wheat crops adapt well to split N fertilization. Assessment of the N status of the wheat crop by absorbance measurements of the leaves, using a chlorophyll meter or reflectance measurements of the canopy (radiometry) is practical and enables N fertilization to be adjusted to the soil N supply. However, as these results were obtained with only one cultivar of winter wheat grown at one experimental site, over two years, further studies are required to confirm them.

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