NUTRIENT STRESS OF CORN PLANTS: EARLY DETECTION AND DISCRIMINATION USING A COMPACT MULTIWAVELENGTH FLUORESCENT LIDAR

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ABSTRACT

The objectives of this study were to assess the capability of laser-induced fluorescence (LIF) to discriminate between nitrogen and sulfur deficiencies in corn that result in similar effects on leaf colour, and to determine the relationship between changes in LIF emission spectra and plant growth inhibition. Corn plants were grown in the greenhouse and fertilised for 35 days with nutrient solutions of different nitrogen (N) and sulfur (S) concentrations. Every 6 to 8 days during the experiment, fluorescence data were remotely obtained using an FLS-PL compact multiwavelength fluorescence lidar developed by our group. Excitation wavelengths varied from the UV to the VIS spectral range over the measurement cycle. The corresponding fluorescence spectra were recorded at each laser pulse with a CCD-based multi-channel detector. On the same day as the fluorescence measurements were made, the leaf reflectance was measured and leaf samples were collected for the N, S and chlorophyll {$a+b$} (Chl{$a+b$}) content analyses.

N deficiency caused a significant decrease in the accumulation of plant biomass and the Chl{$a+b$} leaf contents after 6 days of treatments, whereas S deficiency led to significant decreases in Chl{$a+b$} leaf contents and the accumulation of plant biomass after 6 and 18 days of treatments, respectively. The losses of Chl{$a+b$} in N- and S-deficient plants were similarly correlated to the changes in the R740/R540 reflectance ratio and in the F690/F740 fluorescence ratio (the numbers shown are the corresponding wavelengths at which fluorescence intensities were measured). The R740/R540 and F690/F740 ratios proved useful for the detection of decreases in the leaf Chl{a+b} contents but they could not discriminate between N and S deficiencies. On the other hand, throughout the experiment we observed large differences between the effects of N and S deficiencies on LIF emission spectra. Only the N deficiency induced changes, which could be related to a decreased transmittance of the UV radiation through the leaf epidermis. The value of this transmittance was estimated using ratios of the far-red fluorescence intensities induced by 360 nm- and 440 nm-laser pulses (FRF_{ex360}/FRF_{ex440}). The FRF_{ex360}/FRF_{ex440} ratio correlated linearly (R^2=0.93) with the leaf nitrogen content. These results demonstrate that LIF offers potential for early nutrient stress discrimination.

INTRODUCTION

Much interest in the early assessment of nutrient stresses of agricultural crops has been generated recently by the increasing acceptance and use of precision agriculture technologies. Aerial and satellite remote

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sensing of vegetation colour (spectra of the reflected solar radiation) can be useful in assessing the general characteristics of vegetation, such as total biomass and water content (1). However, reflectance from vegetation colour sensing lacks the specificity and the selectivity necessary to discriminate plant stresses because different stress conditions may cause similar pigment losses. In addition, changes in colour in most cases represent a late response of plants to stress (1,2). Incorrect diagnosis due to an inability to discriminate between stresses may lead to wrongly targeted management interventions with the consequence of losses in resources and time.

Over the past decade, laser-induced fluorescence (LIF) of plants has been explored as a tool in land vegetation studies (3, 4, 5). Compared with reflectance, LIF, and particularly UV-induced fluorescence, may be a more accurate indicator of the physiological state of plants and may be able to detect the impacts of environmental stresses on them at earlier growth stages. The UV excitation of green leaves induces two distinct types of fluorescence: a blue-green fluorescence (BGF) in the 400-600 nm range, and Chl fluorescence (ChlF) in the red to far-red region (650-800 nm) of the spectrum. The relative intensities of these two fluorescent bands are highly sensitive to intrinsic leaf properties and environmental factors. It is well established that ChlF represents an intrinsic probe of photosynthesis that can act as a quantitative indicator of its photochemical efficiency (6,7). BGF has not yet been associated with any physiological function despite its high sensitivity to environmental factors (4). Ferulic acid, a hydroxycinnamic acid that is covalently esterified to the polysaccharides of cell walls in the leaf epidermis and veins, represents the main emitter of BGF (8, 9, 10), although other substances can also contribute to the BGF emission (3).

Several studies have already indicated the potential of LIF to detect nutrient stresses in plants, particularly nitrogen deficiency. In general, N-deficient plants show substantial increases in the BGF/ChlF intensity ratio when compared with unstressed plants (4, 11, 12, 13). Even so, two important questions remain to be answered in order to assess the real usefulness of LIF in detecting nutrient stresses in plants. Firstly, can UV-induced fluorescence distinguish between different nutrient stresses such as N and S deficiency? These two deficiencies cause a similar loss of leaf chlorophyll content and are therefore very difficult to visually distinguished from each other (14). Secondly, how are changes in the LIF emission spectra related to plant growth inhibition? This relationship has, to our knowledge, never been demonstrated. In this report, we present the results of experiments designed to answer these two questions.

MATERIALS AND METHODS

Corn (*Zea mays* L. cv. DK 389 BtY) plants (originally, three per pot) were grown in the greenhouse from May to June 1999 with supplemental lighting. Day and night temperatures were maintained at 22°C and 19°C respectively. The photoperiod was 16 hours. The corn seedlings were watered with a full strength nutrient solution for two weeks. Then they were thinned to one plant per pot and randomly assigned to eight treatments. Each treatment used the same basic nutrient solution and one of four nitrogen levels (0, 20, 40 or 66 mM of NO_3^- marked as N0, N1.5, N3 and N5 levels, respectively), or one of four levels of sulfur (0, 1.86, 3.72 or 6.20 mM SO_4^{2-} marked as S0, S1.5, S3, S5 levels, respectively). The four N treatments contained the optimal S concentration (S5) while the four S treatments contained an optimal N concentration (N3).

Every six to eight days during the experiment, four plants from each treatment were sampled for various measurements. LIF spectra obtained by exposing the most recently expanded leaf of each plant to laser pulses of 360 nm to 871 nm were recorded using a compact multiwavelength Fluorescent Lidar System model PL (FLS-PL). The distance between the leaf and the FLS-PL was five meters. The laser source was an excimer (XeCl) laser emitting nanosecond pulses (308 nm) fired directly at the leaf or through an automatic system of four successive fluorescent dyes in order to provide additional excitation pulses at 360, 440, 480 and 630 nm. One measurement cycle was completed in less than two seconds and consisted of

five series of 29 pulses (one series per wavelength). Measurements were made in a shady area of the greenhouse on dark-adapted leaves in order to minimise variations caused by non-photochemical quenching of ChlF (7). The data were stored in a computer for later analysis. Following the LIF measurements, the reflectance spectrum from the same leaves was recorded with a LI-1800 portable spectroradiometer (LI-COR, Lincoln, NE USA) equipped with an integrating sphere. Then, the leaves were weighed and the area of each was measured with a LI-3100 Area Meter (LI-COR, Lincoln, NE USA). Finally, three leaf disks (10 mm^2 each) were cut from each leaf to be used in the analysis of chlorophyll $\{a+b\}$ content.

The disks were stored at -20°C until the chlorophyll analysis could be carried out. The three disks from a given plant were homogenized and the chlorophylls were extracted in two successive portions of cold methanol (-20°C). The material was completely colourless by the end of this process. The two portions were mixed and the concentration was measured according to Porra et al. (15).

Leaf area and fresh weight of the remaining shoot material were measured. The material was dried at 70°C for 48 hours and then weighed again. After it was ground into powder, the plant material was digested according to the protocol of Issac and Johnson (16), and the N content was determined with a TRAACS 800 auto-analyser (Bran & Luebbe, Elmsford NY, USA). Each plant's S content was measured according to the method of Havlin and Soltanpour (17) using a plasma spectroscopic technique (ICP-AES) with an OPTIMA 3200 DV spectrometer (Perkin Elmer Corp., Norwalk CT, USA). Data analysis was performed using the SuperANOVA statistical program (Abacus Concept Inc., Berkeley, CA USA).

RESULTS AND DISCUSSION

Effects of N and S deficiencies on plant growth and on N, S and Chl{a+b} contents in leaves

The dry biomass accumulation over time by corn plants fertilised with different levels of N and S is shown in Figure 1. By the sixth day of treatment, comparisons of means indicated that dry weights of the N0 plants were significantly lower than those of N1.5, N3 and N5 plants (Figure 1A). After 18 days of treatment, growth was significantly inhibited in the S0 and N1.5 plants (Figure 1B). By the end of the experiment, the dry weights of N0, N1.5 and S0 plants were 31%, 87% and 52%, respectively, of that of plants fertilised at the highest level of N. Similar inhibitory effects on growth of N and S deficiencies were also observed on fresh biomass and total leaf area (results not shown).

As expected, the N and S leaf contents were significantly lower in the N0 and S0 plants than in plants fertilised at higher levels of N and S. Significantly lower N contents in N1.5 plants relative to N3 and N5 plants appeared after 18 days of treatment (Figures 1C and 1D). In addition to these treatment differences, a general decline of both N and S contents over the course of the experiment occurred, even in optimally fertilised plants. Only in N0 plants did the N content level off during the second half of the experiment.

The changes in the plant N and S contents were accompanied by decreases in leaf Chl{a+b} contents (Figures 1E and 1F). In fact, the Chl{a+b} and N contents were linearly correlated (R2= 0.81), as were Chl{a+b} and S contents (R2= 0.81) (results not shown). These changes of Chl{a+b} contents were closely related to changes in the leaf reflectance spectra, particularly in the R740/R540 reflectance ratio. By pooling the data from the different N and S treatments, we obtained a good linear correlation (R2= 0.84) between leaf Chl$\{a+b\}$ contents and the R740/R540 reflectance ratio (Figure 2). This correlation showed that this reflectance ratio can provide useful information about the decrease in the leaf Chl$\{a+b\}$ content of corn plants grown under nutrient deficiency. However, the ratio cannot determine whether the changes in the Chl$\{a+b\}$ contents are caused by an N deficiency or an S deficiency.

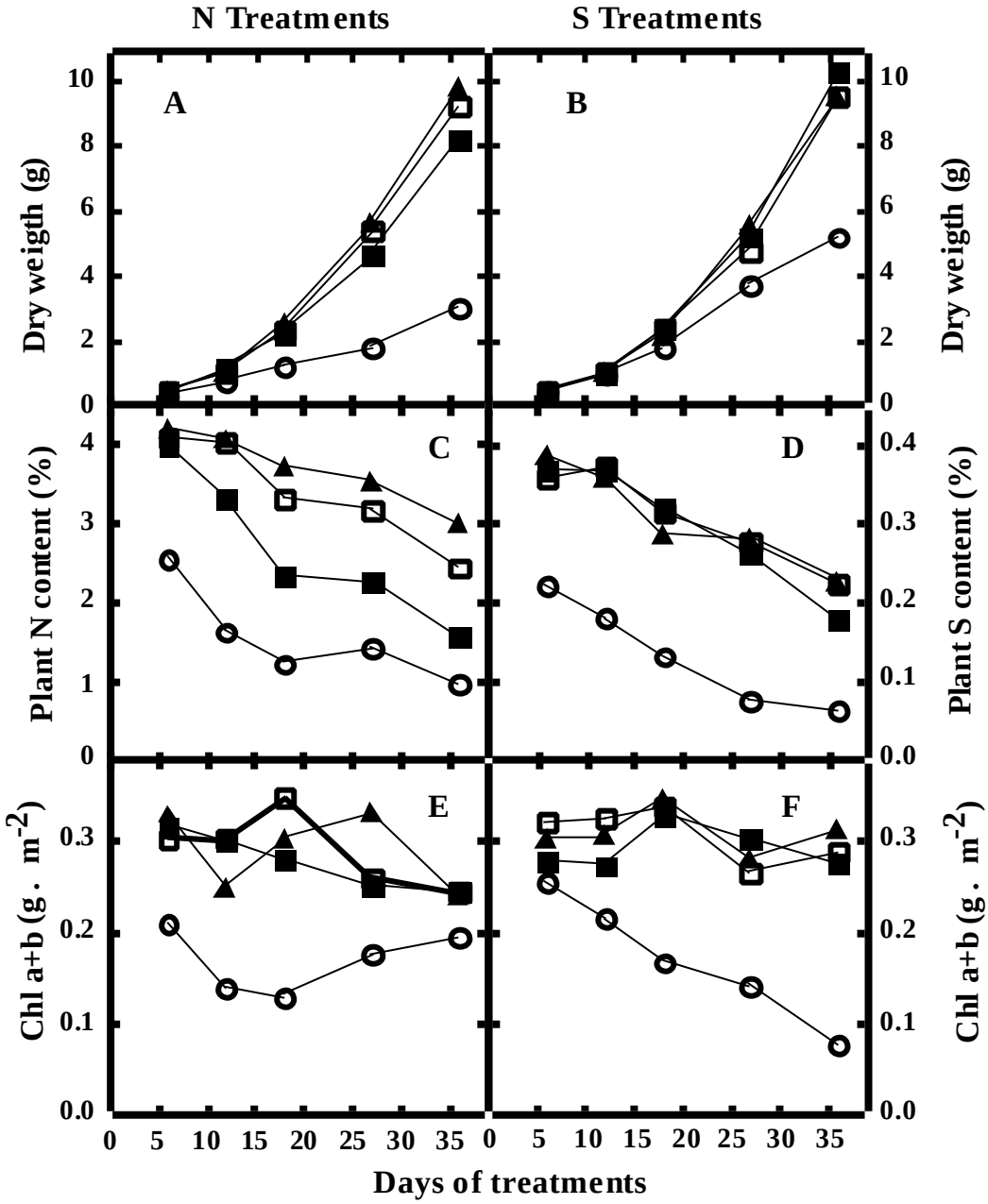

Figure 1: Plant dry weights (A, B), shoot nitrogen content (C), shoot sulfur content (D), and leaf chlorophyll {*a*+*b*} content (E, F) throughout the experiment, of greenhouse corn plants grown with various levels of nitrogen (A, C, E) or sulfur (B, D, F). The relative fertilisation levels were: 0 (open circles), 1.5 (closed squares), 3 (open squares), and 5 (closed triangles). See Materials and Methods for details.

Effects of N and S deficiencies on UV- and VIS-induced fluorescence emission spectra

To illustrate the effects of N and S deficiencies on LIF, the emission spectra induced by laser pulses of four different excitation wavelengths are presented in Figures 3 and 4. These spectra each represent the average of four independent measurements made on the most recently expanded leaves of corn plants in treatments N0 and N5 (Figure 3), and S0 and S5 (Figure 4) after 18 days. The following observations were made: A) Chlorophyll fluorescence of negligible intensity was induced by the 308 nm-laser pulses; B) the intensity of the BGF (F460) induced by 308 nm and 360 nm laser pulses on N-deficient (N0) or S-deficient leaves was not statistically different from those on optimally fertilised, control leaves (N3 and S5); C) when using

the 360 nm-laser pulses, ChlF intensity emitted by N0 plants was very low relative to that of N5 plants, whereas similar ChlF intensities were emitted by S0 and S5 plants; D) in contrast, the intensity of the ChlF induced by VIS-laser pulses (440nm and 630nm) was similar or slightly lower from N0 and S0 plants than from control plants; E) for all excitation wavelengths, the intensities of the red ChlF (F690) relative to the far-red ChlF (F740) were higher in N0 and S0 plants than in N5 and S5 plants. The high F690/F740 ratio of N- and S-deficient leaves was related to their lower leaf Chl{$a+b$} contents (18). By pooling the F690/F740 and Chl{$a+b$} data from different N and S treatments, we obtained a linear correlation (R^2=0.86) between these two parameters (results not shown) similar to the correlation between the Chl{$a+b$} content and the R740/R540 reflectance ratio (Figure 2). It is noteworthy that the linearity of the relationship is due to the rather limited range of the Chl{$a+b$} content measured in this experiment. For a broader range of Chl{$a+b$} contents, the relationship between F690/F740 and Chl{$a+b$} content was shown to be curvilinear (18).

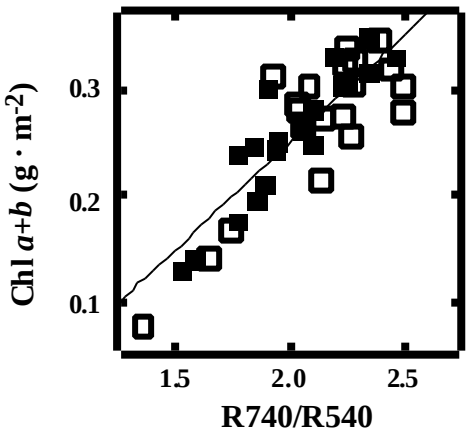

Figure 2. Linear relationship (R^2=0.84) between the leaf chlorophyll content and the R740/R540 reflectance ratio measured in corn leaves grown at various levels of N (open squares) and various levels of S (closed squares) over the course of the experiment. The values of chlorophyll content are the same as in Figures 1E and 1F.

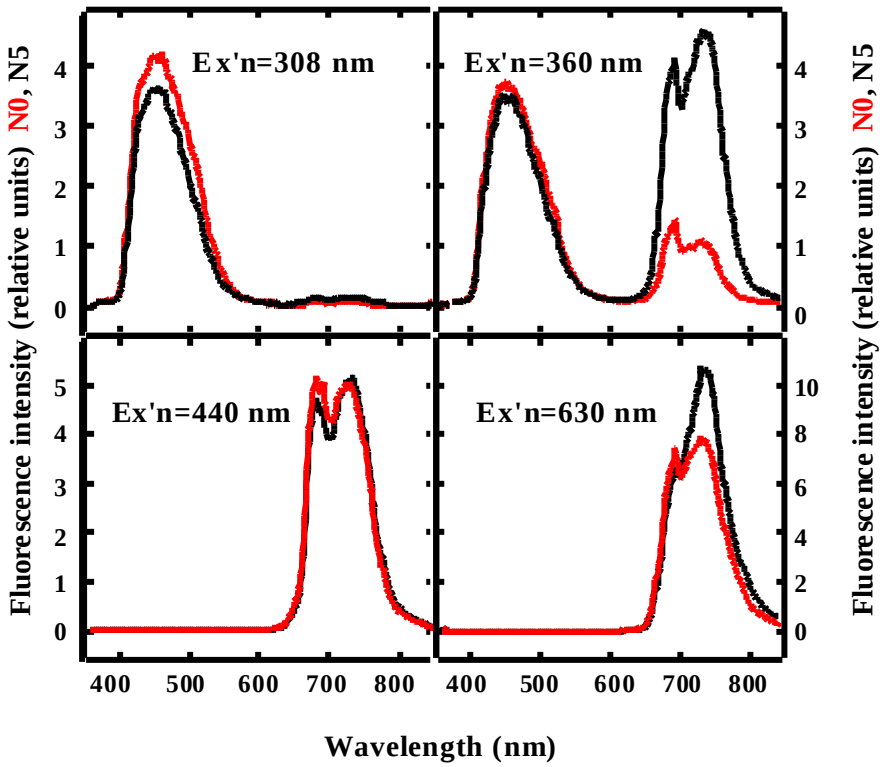

Figure 3. Fluorescence emission spectra induced by laser pulses of different wavelengths in leaves of corn plants grown for 18 days in the absence (N0, red line) or in the presence (N5, black line) of nitrogen.

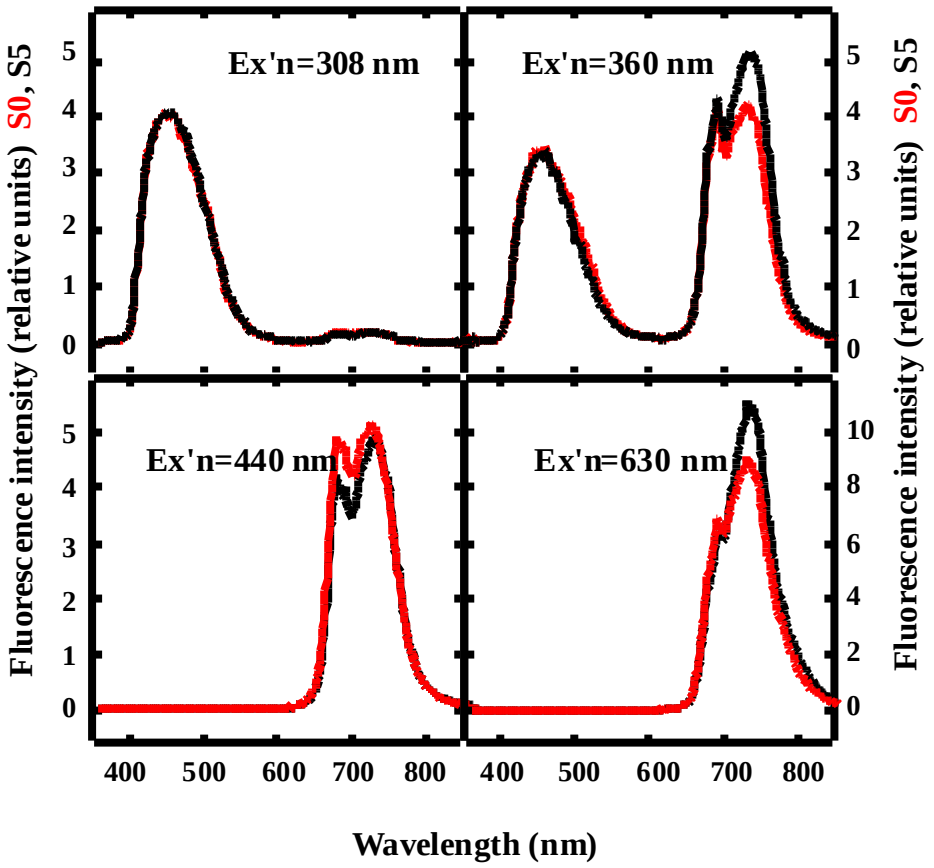

Figure 4. Fluorescence emission spectra induced by laser pulses of different wavelengths in leaves of corn plants grown for 18 days in the absence (S0, red line) or in the presence (S5, black line) of sulfur.

Effects of N and S deficiencies on BGF/ChlF and on UV transmittance by the leaf epidermis

Figures 3 and 4 clearly show that N and S deficiencies have different effects on the LIF spectral signatures, particularly for the excitation wavelength of 360 nm, at which ChlF is much lower in leaves of N0 plants relative to the leaves of plants in other treatments. These changes can be quantitatively estimated using the ratio of the blue (F460) to the far-red (F740) fluorescence intensities induced by the 360 nm-laser pulses (Figure 5). This ratio is considered the most sensitive parameter of LIF in detecting plant stress (4). The changes in the F460/F740 ratio over the course of the experiment are presented in Figure 5 for leaves of corn plants grown at different levels of N and S.

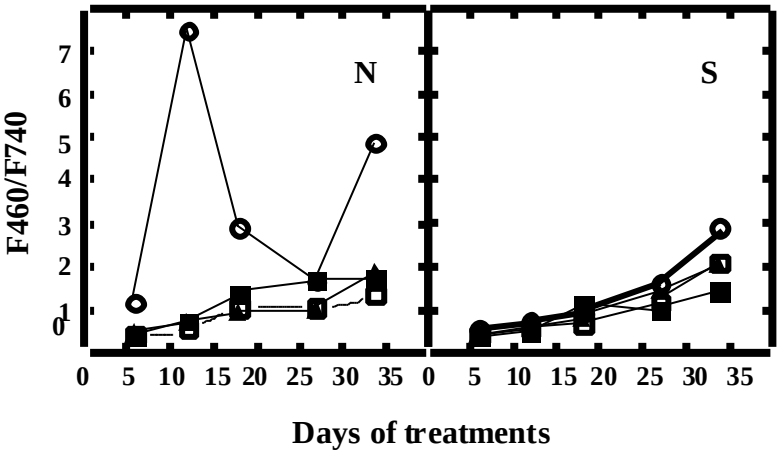

Figure 5. Temporal variations of the BGF/ChlF ratio (F460/F740) induced by laser pulses of 360 nm in leaves of corn plants grown at different levels of N and S. The relative fertilisation levels were: 0 (open circles), 1.5 (closed squares), 3 (triangles), and 5 (open squares).

From day six to day 35 of the experiment, the F460/F740 ratio was significantly higher in N0 plants than in plants grown at other N levels, except for the N0 and N1.5 measurements made on day 27. In contrast, there was no significant effect of S deficiency on the F460/F740 ratios (except for the S0 and S1.5 measurements made on day 35). This indicates that the F460/F740 fluorescence ratio can be used to discrimi-

nate between the N and S deficiencies in corn leaves. The F460/F740 ratios measured on leaves of plants in the N0 treatment varied greatly during the experiment, while the values from plants in other treatments increased steadily as the plants aged. The origin of the great variation of F460/F740 of N0 leaves is unknown. However, the increase in this ratio for plants in other treatments is consistent with the results obtained by Lichtenthaler *et al.* (13), which showed an increase in the F460/F740 ratio with age, from young to mature corn leaves.

Large variations of F460/F740 in the leaves of N0 plants prompted us to seek an alternative parameter that could detect and estimate N deficiency of corn more specifically. As mentioned above, the effects of N deficiency on the fluorescence emission spectra were characterised by large decreases in the ChlF intensities induced by UV (360 nm) laser pulses but not in those induced by VIS (440 nm and 630 nm) laser pulses (Figure 3). This indicated that transmittance of the UV radiation through the leaf epidermis was lower in N-deficient corn leaves than in N-fertilized corn leaves. As less UV excitation radiation reached the chlorophyll molecules in the leaf mesophyll -- due to accumulation of phenolic compounds in the leaf epidermis that absorb UV radiation -- the lower the UV-induced ChlF intensity was. Conversely, the intensity of ChlF induced by VIS light was not affected because VIS light (440nm and 630 nm) was not absorbed by the leaf epidermis (4). The UV transmittance by the leaf epidermis can therefore be estimated using the ratio of the far-red ChlF intensities (FRF, or F740) induced with laser pulses of 360 nm and 440 nm. This approach has been successfully used in a recent study (19) to estimate epidermal transmittance of UV radiation in leaves of various habitats.

Figure 6 presents the ratio of FRF intensities excited by 360 nm- and 440 nm-laser pulses (FRFex360/FRFex440) measured on leaves of plants grown under different N and S treatments. Throughout the experiment, the FRFex360/FRFex440 ratio was consistently and significantly lower for leaves of N0 plants than for leaves of plants at other N levels, whereas no significant effect of S deficiency on the FRFex360/FRFex440 ratio could be detected. As the experiment progressed, a general decrease in the epidermal transmittance of the UV radiation occurred, which, by the end of the experiment, diminished the differences in FRFex360/FRFex440 between plants with different contents of N. Due to a technical difficulty during the measurements, the excitation intensities in the FRFex360/FRFex440 ratio of the fourth set of measurements (day 27) were systematically lower than during other sets of measurements. Figure 6 does not include the corresponding FRF_{ex360}/FRF_{ex440} data. However, the analysis of relative variations of the FRF_{ex360}/FRF_{ex440} ratio measured on that day also indicated statistical differences between the N0 and N1.5 versus N3 and N5 treatments.

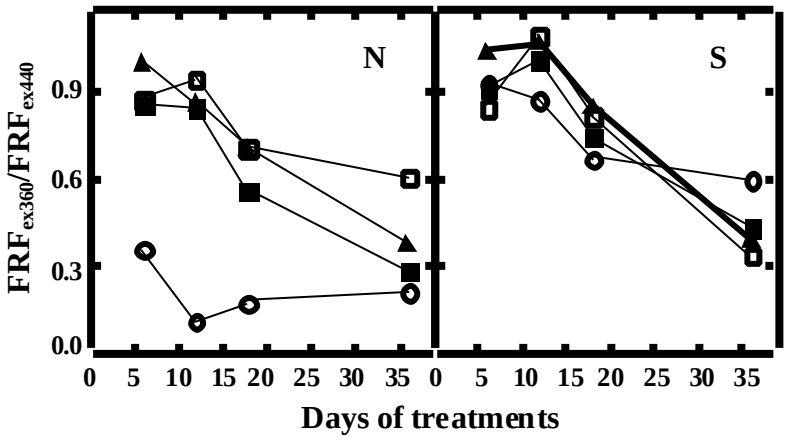

Figure 6. Variations of the epidermal transmittance of UV radiation estimated using the ratio of the far-red fluorescence intensities induced by 360 nm- and 440 nm-laser pulses (FRF_{ex360}/FRF_{ex440}) on leaves of corn plants grown with different levels of N and S. Relative fertilisation levels were: 0 (open circles), 1.5 (closed squares), 3 (triangles), and 5 (open squares).

In our preliminary experiments on sunflower plants, we observed, using the FRF_{ex360}/FRF_{ex440} ratio, that among several mineral deficiencies tested, only N-deficient plants showed significant decreases in the UV

transmittance through the leaf epidermis (results are not presented here). This fluorescence ratio appeared to be a specific indicator of a plant N content, or N status. In this context, it was particularly interesting to see that the FRF_{ex360}/FRF_{ex440} ratio was highly correlated (R^2= 0.93) with the N content in corn plants grown at various N levels and with different periods of treatments (Figure 7). This indicates that the FRF_{ex360}/FRF_{ex440} ratio can be used to detect and quantitatively estimate decreases in N contents due to either N deficiency or to plant aging (Figure 1C).

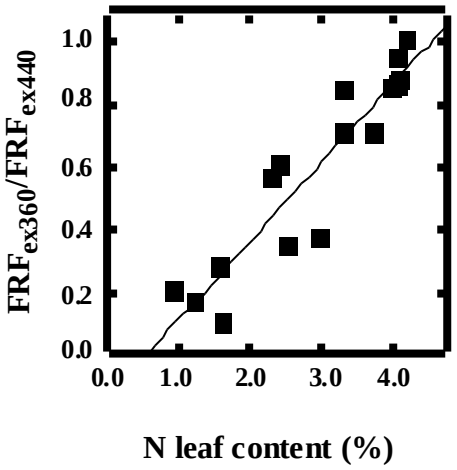

Figure 7: Linear relationship (R^2= 0.93) between the FRF_{ex360}/FRF_{ex440} ratios and N contents in corn (measured at different times during its growth). The values of N content are the same as those in Figure 1C.

In a parallel experiment, the linear relationship between N content and the FRF_{ex360}/FRF_{ex440} ratio was also observed for corn plants grown in the field under different levels of N fertilisation (R^2=0.84, n=6, data not shown), despite the fact that plants grown outdoor exhibit much lower UV transmittance through the leaf epidermis than that of greenhouse-grown plants. Plant exposure to natural UV radiation increases the concentration of phenolic compounds and flavonoids in the vacuoles of epidermal cells (20, 21). It is well known that ferulic acid is the main contributor to BGF of these compounds (9, 10). However, two major flavanols that accumulate in the vacuoles of epidermal cells absorb UV-radiation but do not emit BGF (3). The presence of these compounds in the epidermis attenuates the UV excitation of Chl molecules in deeper mesophyll cells and, consequently, decreases the intensity of the UV-induced ChlF (4). The magnitude of this decrease in ChlF is great compared with the increase in BGF due to ferulic acid accumulation (22). Therefore the increase in BGF/ChlF after adaptation to natural UV radiation may primarily originate from UV-absorbing but non-fluorescing phenolic compounds rather than ferulic acid. Our results therefore indicate that the effects of N deficiency and of natural UV radiation on the UV transmittance by leaf epidermis are additive.

CONCLUSION

- Using UV-induced fluorescence allowed the detection and discrimination of N and S deficiencies in corn plants.
- Such a distinction was not possible using VIS-FR leaf reflectance or VIS-induced fluorescence.
- Changes in the laser-induced fluorescence intensity spectra occur prior to or simultaneously with plant growth inhibition.
- N-deficient but not S-deficient leaves show a significant increase in the F460/F740 ratio. The most likely origin of this effect is the lowered UV transmittance by the leaf epidermis, which can be estimated using the FRF_{ex360}/FRF_{ex440} ratio.
- The FRF_{ex360}/FRF_{ex440} ratio can be used as a specific N indicator that is linearly correlated with the plant N content.

ACKNOWLEDGMENTS

The authors thank Marcel Tétreault for his technical assistance, and Josée Owen for her assistance in editing the manuscript.

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