LABORATORY MEASUREMENTS ON HERBACEUS SPECIES GROWN IN CONTROLLED ATMOSPHERE BY MEANS OF THE ENEA LIDAR FLUOROSENSOR

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ABSTRACT

The lidar fluorosensor apparatus built at the ENEA Research Centre in Frascati, has been proven to be a versatile instrument for analyzing the Laser Induced Fluorescence (LIF) of chlorophyll both in the spectral and in the time domains. The system, has been applied in the spectral domain to study the effects of gaseous pollutants on herbaceus species. Some preliminary results are hereafter presented and discussed.

KEYWORDS: Fluorosensor, Remote Sensing, Pollutant, SO_2, O_3.

1. INTRODUCTION

LIF lidar systems have been recently proven to be very useful instruments in remote monitoring of vegetation health, since they are capable to identify and measure the stress status of plants [1]. The lidar fluorosensor apparatus built at the ENEA Centre in Frascati, has been used to monitor some herbaceus species grown under fully controlled conditions in the glasshouse of the Pisa University. Laboratory experiments have been carried out to check the effects of plant on poisoning with the most common pollutant gases, which can be found with high concentrations in urban and industrial areas. The purpose was to ascertain the remote sensing apparatus sensitivity to the effects of these pollutant gases and to assess the validity of the present analysis of LIF data. The plant samples have been divided in two groups for each species, the former containing the control samples, the latter containing samples polluted with SO_2 and O_3 at concentration typically expected in the field. Effects of the pollutant gases have been monitored on the vegetation pigments emission bands through the whole visible region. In particular, the F690/F735 ratio (red/red ratio) of chlorophyll fluorescence has been considered. Differences among the various groups of plants were found in several spectral bands and are here discussed.

[1] Student

2 - MATERIALS AND METHODS

2.1. The apparatus

The ENEA lidar fluorosensor [2] is based on an excimer laser source exciting the fluorescence of chlorophyll pigments contained in the chloroplasts of terrestrial plants as well as algae. The emitted fluorescence is optically collected by a Newtonian telescope and analyzed both in spectral and time domains by means of an Optical Multichannels Analyzer (OMA III EG&G) and a Streak Camera (C2830 Hamamatsu), respectively.

The laser source is emitting @ 308 nm with a maximum prr of 200 Hz, and a low divergence (0.4 mrad) beam. Each laser pulse has a duration af 16 ns (HWFM) and an energy of 100 mJ. The choice of the excitation wavelength (wl) is the result of a trade-off among different requirements, such as a high chlorophyll excitation efficiency, high power pulse, robustness, reliability of the laser source and eye-safe wl operation.

The use of a Raman cell, filled with CH4 and optically pumped by the excimer laser, allows to obtain additional exciting laser lines for exploring the excitation efficiency vs wl. However, frequency shifting towards the visibile region has resulted in a bit more complex experimental arrangement, due to the eye-safe operation requirements.

In the laboratory, the sample under test was placed at a distance of about 15 m from the receiving telescope and irradiated top-down by steering both the transmitter and the receiver lines of sight. The laser spot at this distance was about 20 cm^2 and a complete covering of the target has been accomplished by slowly rotating the sample (5-10 turns/min).

2.2. Sample preparation

All the herbaceus samples of Spinach and Lolium have been sown and grown in pots inside the glasshouse of the Dip. Coltivazione e Difesa Specie Legnose, Univ. Pisa, in suitable pots (20*30*5 cm^3) under fully controlled conditions. The pots have been placed into ermetically closed perspex boxes (1x1x1 m^3) allowing for immission of the pollutant gas without affecting the environment of the other boxes or of the glasshouse.

A group of pots in the first box has been exposed for a period of 10 hours to air mixed with 120 p.p.b. of SO_2, while a comparable number of pots in a second box has been used as the reference samples group. A different group of boxes with pots has been placed under artificial actinic light in a special chamber into the glasshouse. In the artificially illuminated chamber the pots of the first box have been exposed to O_3 for a period of 4 hours at concentration levels of 100 ppb, the second box has been exposed twice, and the last unpolluted group has been used as reference.

At the end of the exposure periods, all the polluted plants look like their corresponding reference samples making impossible any easy visual screening. The samples have been quickly carried from Pisa to our laboratory and analyzed in a few hours. Table I shows the kind of treatment and the exposure times for each group.

TABLE I - Exposition pollutants of various plants groups.

N. of pots	Plant	Pollutant gas	Time [hours]	Concentration [ppb]	Group
3	Spinach	None			I
3	"	SO_2	10	120	II
3	Lolium	None			III
3	"	SO_2	10	120	IV
3	Spinach	None			V
3	"	O_3	4	100	VI
3	"	O_3	4 + 4	100	VII
3	Lolium	None			VIII
3	"	O_3	4	100	IX
3	"	O_3	4 + 4	100	X

In order to properly compare spectra arising from different measurements, normalization of the raw data acquired by the OMA have been performed. The spectra summed over a number of 100 or 200 laser shots have been normalized for an equivalent number of laser shots (100), and correction for the spectral response of the photosensitive array detector has been taken into account. Due to the high reproducibility of the pulse temporal shape and energy emitted by our laser, different averaged spectra are assumed to be normalized with respect to the laser energy without any further processing.

The features of the spectra considered and analyzed in the present work are the F690/F740 spectral ratio, the F450/F690 ratio and the total blue and total red fluorescence, which carry most of the information about the plant physiological status and photosyntetic activity . The content of each spectral band, needed for red/red and blue/red ratios, results from integrating the LIF spectrum with a 20 nm bandwidth around the band centre wavelength.

3 - RESULTS & DISCUSSION

3.1. General results

Some interesting observations arise from the analysis of the experimental results. In figs. 1a-b we report the LIF spectra (excitation @ 308 nm), of Lolium plants exposed to SO_2 and to O_3, respectively, together with the corresponding control samples spectra. Variations due to gaseous pollution are found both in the red/red ratio and in the total blue and red fluorescence intensity. The blue fluorescence provides additional informations in the definition of stress sensitive regions in the space of spectral ratios.

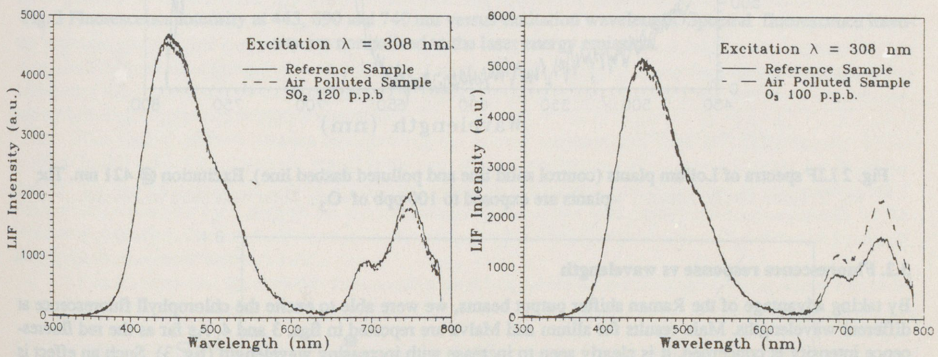

Fig. 1 LIF spectra of Lolium plants (control: solid line; polluted: dashed line). Excitation @ 308 nm. a) exposed to 120 ppb of SO_2; b) exposed to 100 ppb of O_3

By comparison, LIF spectra resulting from excitation @ 421 nm exhibit only the chlorophyll-a (Chl-a) red fluorescence , while the blue fluorescence around 450 nm is almost absent (fig. 2).

After normalization over the laser pulse energy and spot area, the excitation efficiency vs wl have been measured for different herbaceous species, including also allium and malva. Data for lolium and spinach show that the red fluorescence following the 421 nm excitation is much larger (about a factor 60) than the corresponding emission at 308 nm excitation.

By comparing the corresponding LIF spectra and spectral ratios, it turns out that O_3 is by far more effective than SO_2 as plant pollutant, also taking into account the shorter exposition time and the lower concentrations used for the former gas. This fact may be due to the decreased concentration of CO_2 in an ozone polluted atmosphere.

Another important feature, already noticed in field campaigns [4] and confirmed in this experiment, is that in general a plant stress status is reflected both in an increased red/red spectral ratio and in an in-

creased blue fluorescence, although this is not true for the group of plants treated twice with O_3. This seem to indicate that the validity of F690/F740 ratio as a stress indicator is limited to only very little stresses. When highly polluted samples are examined, more complex physiological phenomena involving leaf structure permanent damages may be involved.

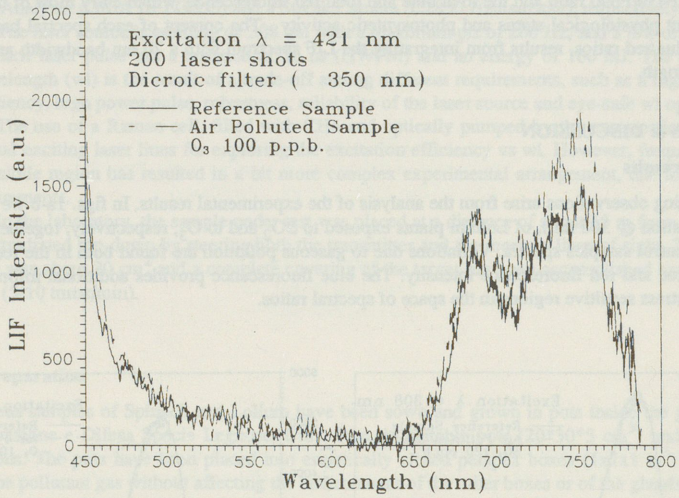

Fig. 2 LIF spectra of Lolium plants (control solid line and polluted dashed line). Excitation @ 421 nm. The plants are exposed to 100 ppb of O_3.

3.2. Fluorescence response vs wavelength

By taking advantage of the Raman shifter output beams, we were able to excite the chlorophyll fluorescence at different wavelengths. Main results for allium and Malva are reported in figs. 3 and 4. As far as the red fluorescence intensity is concerned, it is clearly seen to increase with increasing wavelength (fig. 3). Such an effect is probably due not only to an increasing chlorophyll pigments absorption cross section, which is a maximun around 450 nm, but also to a larger penetration depth of the laser light into the illuminated leaves, so that a greater number of chlorophyll molecules can fluoresce. If the red fluorescence vs wl seems to be easily understood, explanation of the R_1/R_2 ratio behaviour, shown in fig. 4 for allium and Malva is more difficult. Actually and at least for the samples considered, the dependence of the R_1/R_2 ratio vs wl seems typical for each investigated herbaceous species. Therefore it is of great importance for the analysis of fluorescence experimental data is the exciting laser wl which strongly affects the R_1/R_2 ratio, so that it is mandatory to associate to each determination of R_1/R_2 ratio the excitation wl before attempting any comparison with different lidar systems.

3.3. LIF spectra vs laser energy

Another important parameter to take into account in designing an efficient lidar fluorosensor is laser the pulse energy, since the LIF SNR increase may saturate with increasing pulse energy and signal distortion may appear due to the occurrence of annealing effects. A complete analysis of this topic is not performed in the present work, where we were interested only to operate out of the saturation region. In order to check this point, extensive measurements on different herbaceous species vs the laser pulse energy have been carried out, and typical results obtained for basil are reported in fig. 5. Observation of these data seems to indicate that for all the energy range used there is no signal saturation.

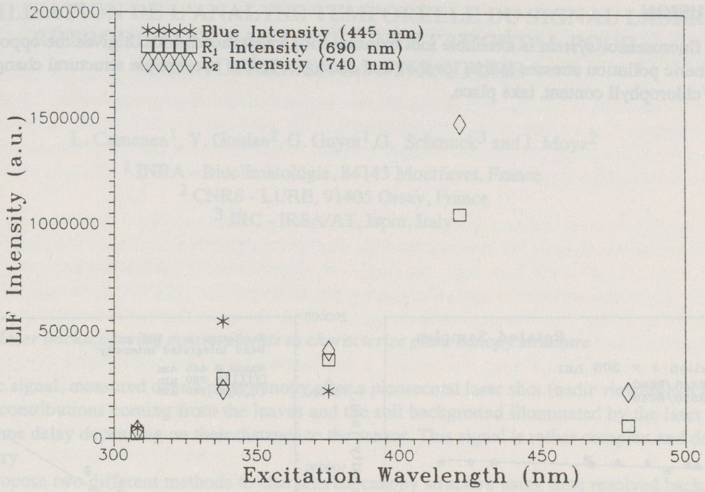

Fig. 3 Fluorescence intensity at 445, 690 and 740 nm versus excitation wavelength. Spectral fluorescence intensity are normalized to the laser energy emission.

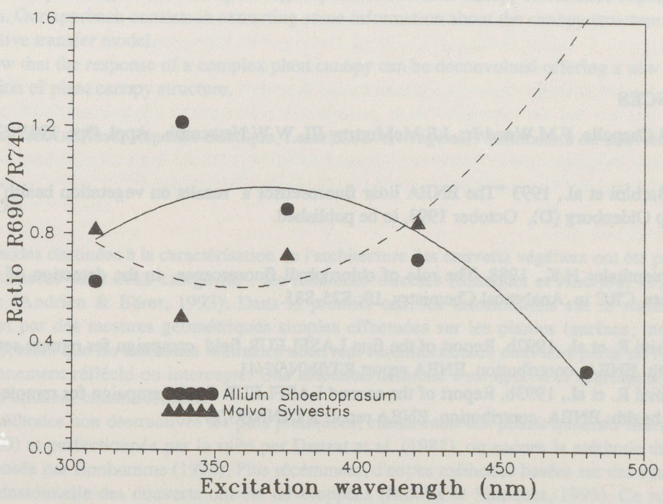

Fig. 4 R_1/R_2 ratio vs excitation wl for Allium and Malva plants.

4. CONCLUSION

The ENEA fluorosensor system is a reliable tool in monitoring vegetation health. It gives the opportunity to detect atmospheric pollution stresses at low level of leaf damages, before irreversible structural changes, such as a decrease of chlorophyll content, take place.

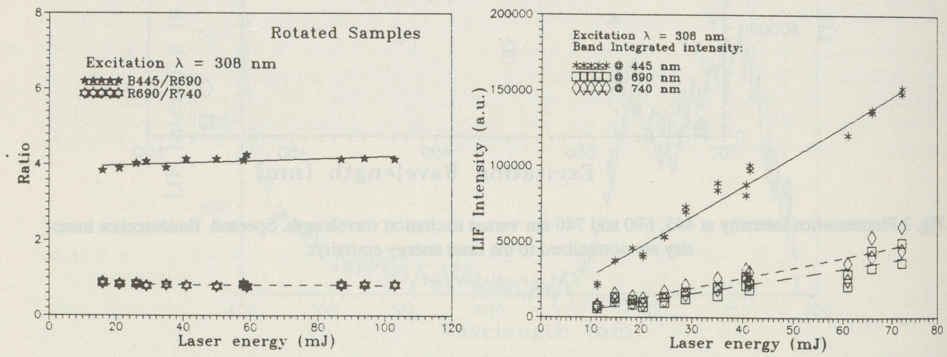

Fig. 5 Fluorescence intensity at 445, 690 and 740 nm vs laser energy. Laser excitation wl 308 nm.

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