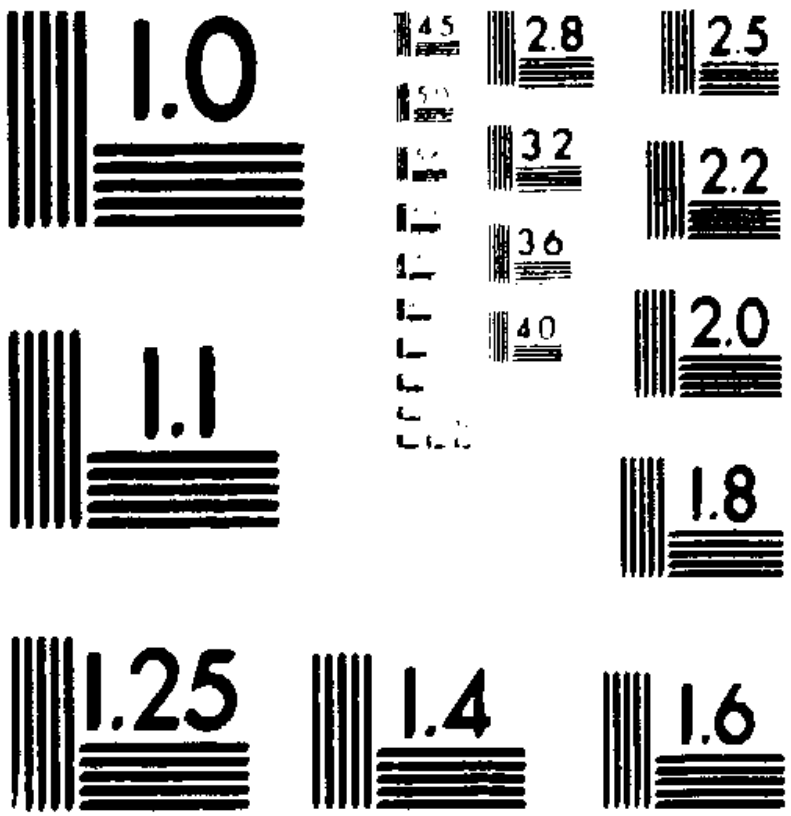

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Abstract

The first European joint field campaign for remote sensing of vegetation health has been held in Viterbo (October 6th to 18th, 1991) in the frame of EUREKA/LASFLEUR project. Italian groups, from University, ENEA and CNR, participated to this campaign together with several German groups from different Institutes. The lidar fluorosensor system built at ENEA Frascati for remote sensing of water and territory has been modified to detect fluorescence from trees in a field experiment. The new version of the set-up is presented together with the spectral and time resolved measurements performed. Results are discussed in view of correlating present data with the plant photosynthetic activity under different weather conditions and water stresses.

Riassunto

Nell'ambito del progetto EUREKA/LASFLEUR si è svolta a Viterbo dal 6 al 18 Ottobre 1991 la prima campagna europea congiunta di misure sul campo volte ad accertare la salute di piante di alto fusto con tecniche di telerilevamento. Alla campagna hanno partecipato gruppi italiani universitari, ENEA e CNR e vari gruppi tedeschi. L'apparato lidar fluorosensore, già realizzato all'ENEA INN/SVIL per misure sul territorio e sulle acque, è stato modificato per effettuare diagnostiche su piante di alto fusto in esperimenti sul campo. In questo rapporto sono presentate sia la nuova versione dell'apparato che le misure, risolte spettralmente e temporalmente, effettuate durante la campagna. Nella discussione dei risultati si è cercato di correlare i dati ottenuti con l'attività fotosintetica delle piante e con l'eventuale stress idrico.

I. INTRODUCTION

In 1990 a lidar fluorosensor system has been completed at ENEA Frascati and used in laboratory tests and reference measurements [1]. The system, originally designed for remote sensing of water surface pollution [2], turned out to be sensitive also to visible fluorescence emission from vegetable samples analyzed in the laboratory.

In November 1990, the ENEA group responsible for this fluorosensor system has been admitted to the EUREKA/LASFLEUR Project, aimed to ascertain the feasibilitity of remote monitoring the health of forests by fluorescence lidar measurements. ENEA participation was addressed mainly to task IV (lidar instruments design and operation), and, to a minor extent, to task I and II, dealing with correlation of spectral and time resolved measurements of chlorophyll emission with plant photosynthetic activity, respectively.

Within task IV, activities at ENEA have been devoted to the development and to the test of a lidar fluorosensor equipped with both spectral and temporal analysis capabilities, which is suitable for remote sensing of chlorophyll emission by a tree at a distance of few hundred meters. Within the other tasks, joint campaigns of measurements with other participating groups have been planned.

In october 6th to 18th, 1991, the ENEA group took part to the first LASFLEUR joint field campaign together with groups collaborating to tasks I, II and IV, as listed in Table 1. Goals of the joint campaign, held in Viterbo in a controlled University farm, were both to cheek the field-operation of different local and remote sensing instruments and to ascertain their sensitivity to plants' health. A specific objective of our group was to assess the capabilities of the ENEA lidar fluorosensor to the active remote surveillance of vegetation, by demonstrating the feasibility and the utility of both spectral and time resolved data taken by the same instrument, which is described in sect. II.

TABLE 1

List of groups with specific activities in tasks I, II and IV in the EUREKA/LASFLEUR Project participating to the campaign at Viterbo University farm

	TASK I Spectral Ratio Plant Physiology Reflectance	TASK II Life Time Plant Physiology Reflectance	TASK IV Development and Test of Far Field Ground Based Instruments
Operating Agent	Univ. Viterbo	CNRS (France)	DLR
Partecipants	Univ. Karlsruhe Univ. Munich Univ. Viterbo CNR IROE CNR IEQ ENEA	ENEA	DLR ENEA

Actually, we were able to measure with our system both the trees' active fluorescence spectrum (taking into account passive background) and the fluorescence decay time constant. Moreover, a preliminary investigation of the fluorescence efficiency vs transmitted wavelength has been attempted during this campaign. Experimental results are summarized in sect. III and discussed in sect. IV, where correlations with plant photosynthetic activity are attempted. Conclusions are reported in sect. V, where a list of major improvements for a set-up dedicated to remote sensing of vegetation is also suggested on the basis of the experience we gained during the field activity.

II. EXPERIMENTAL

Before starting the campaign, the existing lidar fluorosensor has been upgraded to become more suitable to remote sensing of vegetation. With respect to the former set-up [1], main changes have concerned the laser transmitter and the detection configuration. In order to make possible field operation, the instrumentation has been enclosed in proper avionic boxes and powered by a dedicated Diesel generator.

The existing UV laser transmitter has been implemented by adding a unit for shifting laser radiation to the blue-green wavelength range, where chlorophyll absorption has a maximum. The new transmitter is now constitued by a low divergence excimer laser, coupled to a Raman shifter providing different emission wavelengths. The Raman cell has been filled with CH_4, and Stokes frequencies from first to fourth have been generated, with output energies up to few mJ. The final outcoming beam has been characterized, and methods for wavelengths selection have been tested. However, difficulties encountered during field operation of this new part of the set-up, indicated the needs of further improvements before ending up with a system in a movable configuration.

Additional work has also been devoted to the receiver electronics. Efforts have been concentrated on the study of optical and electronic triggering modes allowing for independent acquisitions both of the spectral and the time features of the return signals.

The experimental set-up used for measurements is shown in Fig. 1. The XeCl excimer laser emits at 308 nm, with typical pulse energy and width as reported in Table 2 together with the characteristics of the complete send-receive sub-system. The laser beam impinges upon the investigated leaves of the selected tree, and the induced fluorescence is collected by a Newtonian telescope collinear with the transmitter.

A quartz fiber optic (10 m long, 1.0 mm dia.), coupled with the receiver telescope, allows a fast switch of the collected radiation between an Optical Multichannel Analyzer system (OMA III by EG&G), and a Streak Camera (SC by Hamamatsu) equipped with a new slow trigger unit. Table 3 shows the characteristics of the optical acquisition sub-system.

Synchronization of detectors with the transmitted laser pulse signal has been accomplished by sending a small fraction of the 308 nm radiation onto a fast photodiode, and properly setting electronic delays. Sketches of the electronic com-

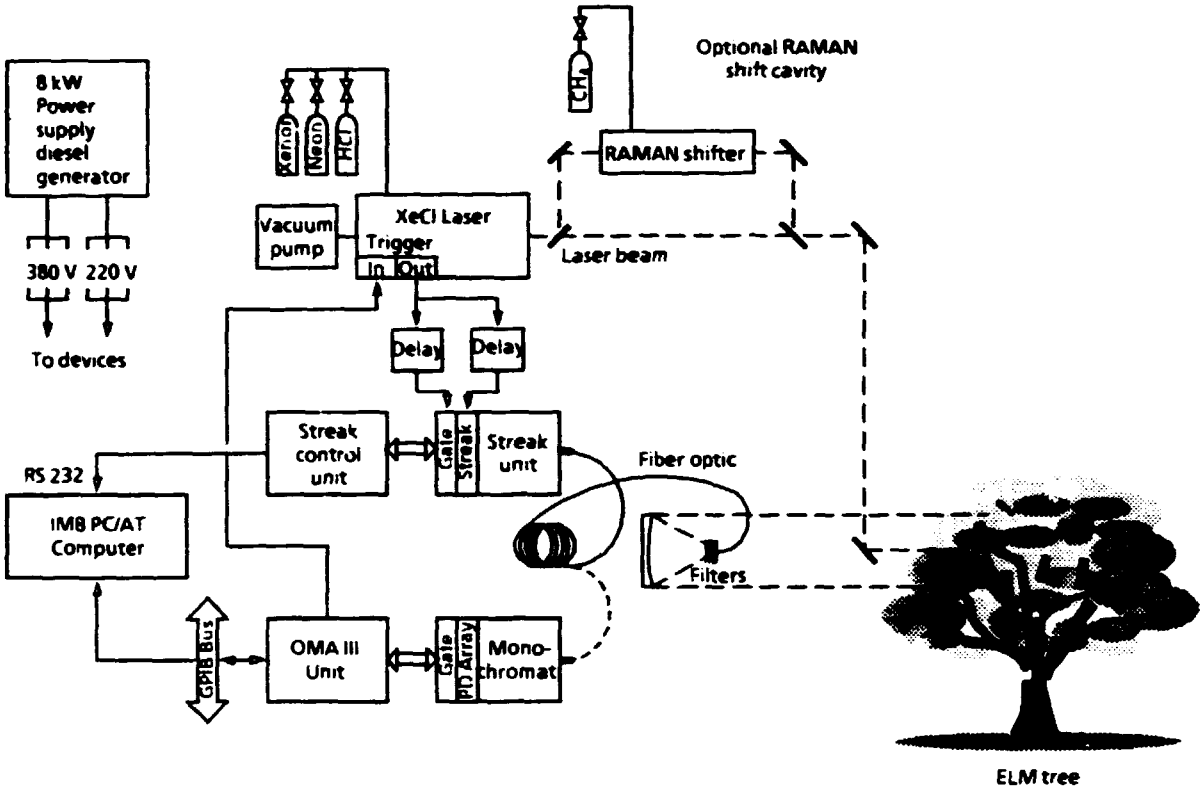

Fig. 1 Experimental set-up used in field experiments on vegetation

TABLE 2
Main characteristics of the send/receiver sub-system

U.V. laser **Lambda Physics EMG 1003i**	**XeCl excimer laser** **Positive branch unstable cavity** **Emission wavelength** **Pulse energy** **Pulse width** **P.R.R.** **divergence $\theta_{1/2}$** **ver. 0.2 mrad**		**308 nm** **typically 150 mJ** **20 ns (3 long. modes)** **max. 200 Hz** **hor. 0.4 mrad**
Vis. laser **Lambda Physics RS75**	**Raman shifter in CH_4 (5-30 atm)** **wl 308 nm** **wl 338 nm** **wl 374 nm** **wl 421 nm** **divergence $\theta_{1/2}$ 7.0 mrad**	**pe 40 mJ** **pe 5 mJ** **pe 1.5 mJ** **pe 0.3 mJ**	**pw 20 ns** **pw 16 ns** **pw 16 ns** **pw 16 ns**
Receiver	**Newtonian telescope d=40 cm** **Aluminum f# 2.8**		

TABLE 3
Main characteristics of the optical acquisition sub-system

O.M.A.III: EG&G	Intensified photodiode array detector 1421 Gate unit 1304 HR-320 monochromator Spectral channels Grating Spectral resolution	 f = 0.32 m 1024 147 grooves/mm 0.5 nm (0.1 mm slit) 5 nm (1.0 mm slit)
Streak Camera: Hamamatsu	M2548 slow control unit C2830 temporal disperser Temporal channels 512 Temporal ranges	 from 10 ns to 1 ms
Dichroic Filter	T = 90% (@λ > 350 nm)	
Interference Filters	λ = 450 nm (60 nm bandwidth) λ = 600 nm (80 nm bandwidth) λ = 650 nm (60 nm bandwidth) λ = 750 nm (60 nm bandwidth)	
Computer control	IBM PC/AT CPU 80286 8MHz	

ponents and trigger systems used for both detectors are shown in Fig. 2.

II. - RESULTS

Spectral and time resolved measurements were performed almost contemporarily, during the same days. In the first case, signals were acquired by the OMA detector within a 100 ns time gate; in the second case, the wavelength bandwidth entering the SC analyzer was selected by interference filters (see Table 3). All the measurements reported in the following, except a few data in sect. III.3, have been obtained by directly transmitting the XeCl UV radiation not followed by the Raman shifter.

As previously observed [3,1], the vegetation fluorescence induced by near UV radiation is strongly peaked on the blue spectral region, where different pigments present in the wood and on the leaf surface are emitting. It has also been already proposed to use this blue fluorescence emission to trace different families of plants

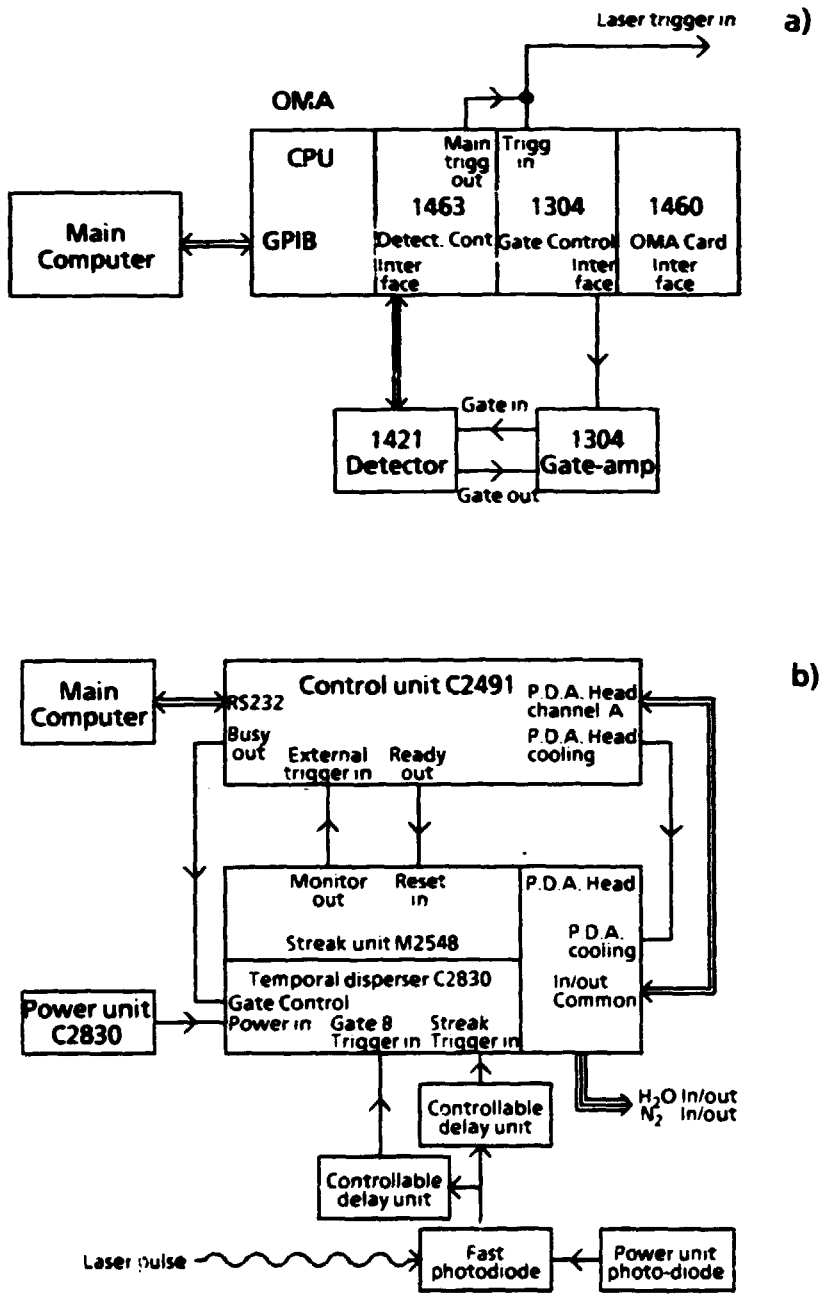

Fig. 2 Block diagrams with triggering schemes for OMA (a) and SC (b) detectors

present in a forest during a laser remote sensing experiment [3]. In our case, this intense blue emission has been used for alignment purposes. Although chlorophyll absorption is strongly peaked in the visible spectrum, it has been already

demonstrated [1] that the typical chlorophyll emission bands in the red (680 nm-760 nm) can be detected after excitation by an intense laser at 308 nm.

III.1 SPECTRALLY RESOLVED MEASUREMENTS

Before starting remote sensing data collection, the set-up reassembled at the chosen test site has undergone repeated experimental checks. In all the measurements reported here, the OMA spectra have been properly corrected by taking into account the detector spectral sensitivity curves given by the manufacturer throughout the whole visible range [4].

Fluorescence by leaves detached from different trees has been detected after pressing them in methanol for a few minutes, in order to extract in solution the different organic pigments. As an example, we report in Fig. 3 the emission obtained from grass leaves in methanol throughout the whole visible spectrum where the two

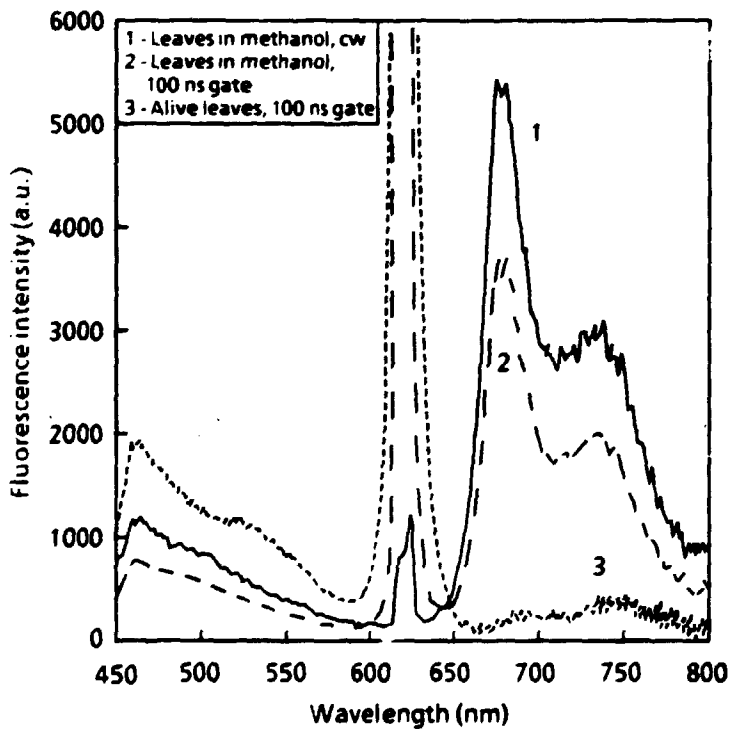

Fig. 3 Fluorescence spectrum from grass leaves (Dycondra Repens) in methanol, and alive as measured in a laboratory experiment

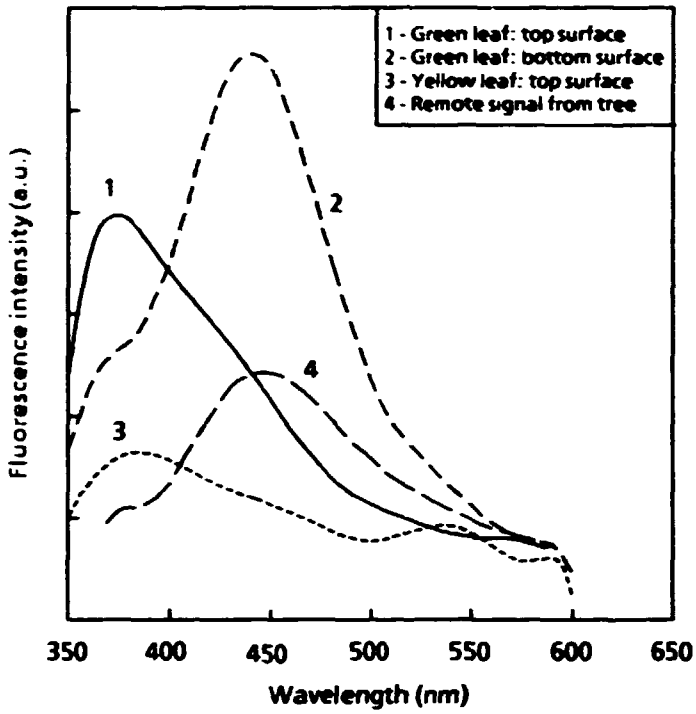

Fig. 4 Fluorescence signals from single elm leaves, sampled with the fiber optic as compared with the remote signal from elm tree

main chlorophyll bands peaked at 690 nm and 735 nm, respectively, are resolved. The strong peak appearing around 620 nm is merely instrumental, being due to the back-scattered light at the excitation wavelength, diffracted at the second order, entering the OMA monochromator in the absence of the dichroic low pass filter.

Single different leaves have been examined as well, by collecting the fluorescence signal on a fiber optic coupled to the monochromator. As shown in Fig. 4 for elm leaves, the blue spectral emission resulted to be sensitive to leaf orientation. Remote sensing data appear to be clearly averaged over all possible orientations of leaves in the tree.

First remote sensing signals have been measured on different available trees, in order to optimize the target choice. Some results in the blue region are reported in Fig. 5, which supported the choice of holm-oak and elm trees to be used as targets during this campaign of measurements.

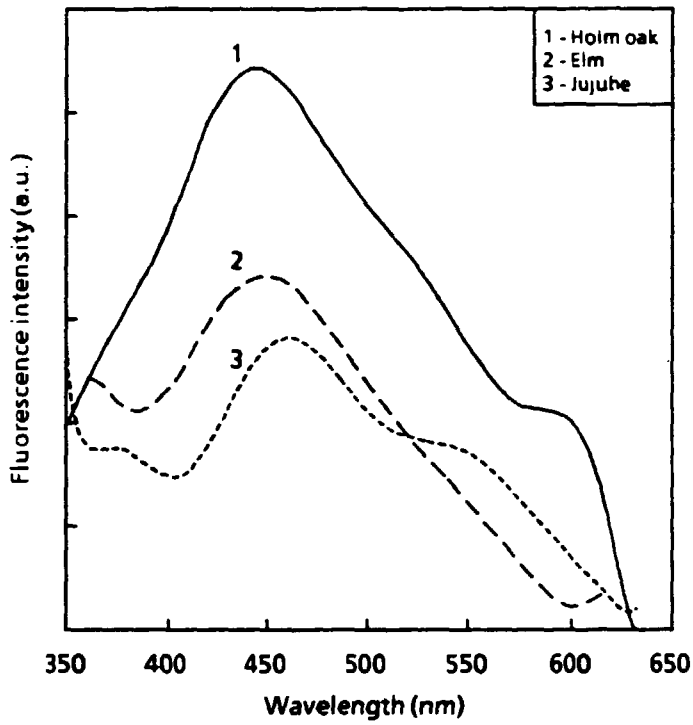

Fig. 5 Fluorescence signals from different Mediterranean trees at the Viterbo controlled site for field experiments

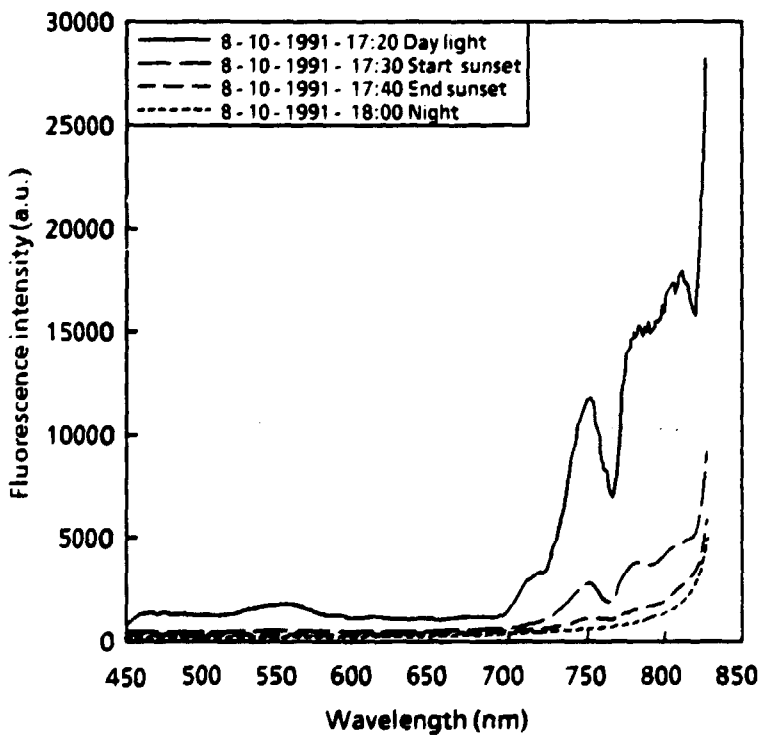

Fig. 6 Background emission from holm-oak tree measured without gating the OMA (exposure time 16 ms), results on the elm tree were quite similar

In field measurements, the role of background visible emission from the vegetation exposed to solar irradiation has been investigated. In particular, as shown in Fig. 6, we have verified that background emission was quite flat at wavelengths shorter than 750 nm, and did not interfere with the laser induced fluorescence signal measured with a narrow (100 ns) gate width. In the present measurements, the small feature peaked at 750 nm resulted to be always negligible, except for data taken on Oct. 17th, 1991, which was a strongly sunny day. In the latter case, a corresponding set of background emission spectra has been measured with the same gate width and subtracted to the laser induced fluorescence spectra before data analysis.

The laser induced fluorescence spectra taken from the elm tree, in conjunction with sun radiance measurements performed by different instruments available at the test site, form a self-consistent data set describing the daily cycle of fluorescence spectra. Typical signals from elm tree are reported in Fig. 7. Measurements have

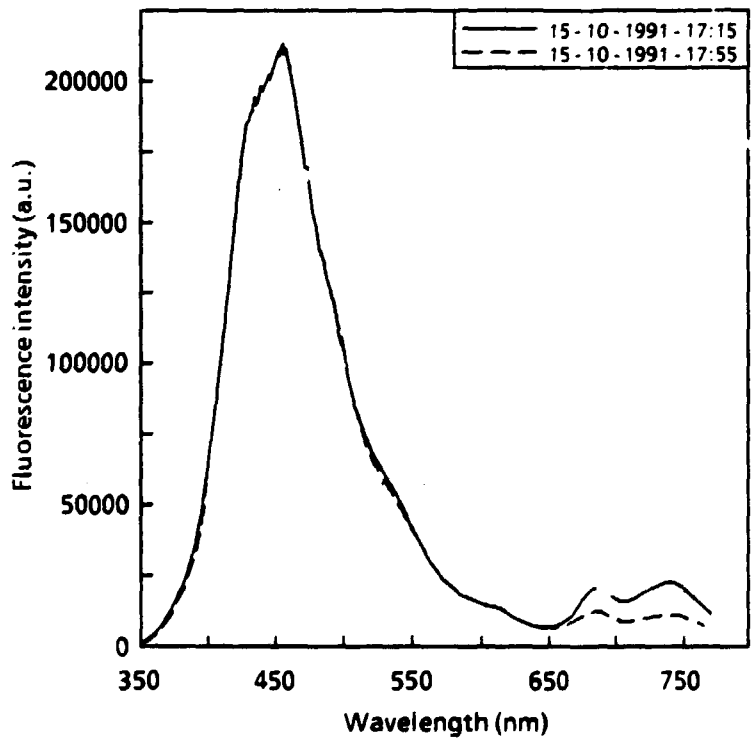

Fig. 7 Laser induced fluorescence spectra from elm tree (sunset was at 17:30)

also been done on leaves in a state of *water stress*, simulated by cutting a branch from the target tree: results obtained on healthy and stressed branches are compared in Fig. 8. Quite strong differences appear among spectra taken under different weather conditions, spanning almost all intermediate situations from rainy to sunny. Data show a large variability of the ratio F690/F735 with changing visible illumination, and the sensitivity of this feature to water stress is confirmed.

A temptative analysis has been done, trying to correlate the fluorescence peak intensity measured at various wavelenghts with some other physical quantities. The correlation with biological parameters is not yet possible due to the lack of local measurements performed on our tree in coincidence with the remote signal acquisition. Actually, the only biological parameter available for our elm target is its water potential, which is typical of a healthy tree.

As far as the fluorescence red-red peak ratio (F735/F690) and blue-red ratio (F450/F690) are concerned, Figs. 9a and 9b show their time evolution, together with

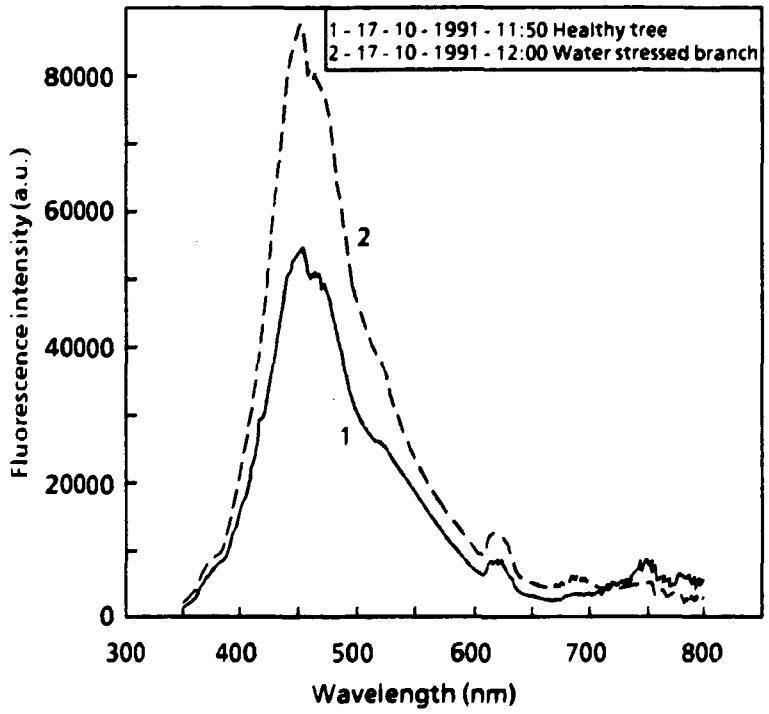

Fig. 8 Laser induced fluorescence from elm tree measured on a healthy and a water stressed branch (the latter cut from the plant one hour before the measurement)

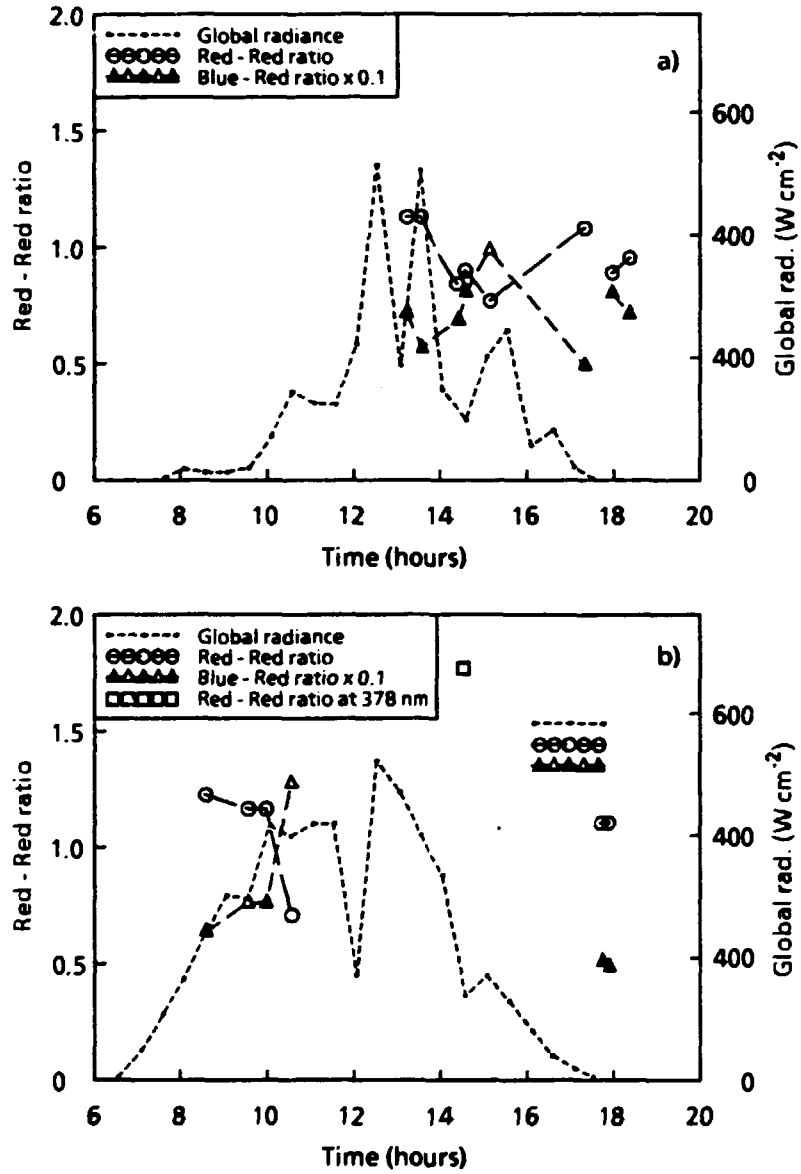

Fig. 9 Diurnal measurements of red-red and blu-red ratio fluorescence peaks compared with solar global irradiance. a) October 15th daily measurements. b) October 16th daily measurements

the global solar radiance, as measured in two different days. A part from data taken after sunset (t> 17:30) a direct correlation with global solar radiance is evident for the blue-red ratio. On the other hand, present data seem to suggest a sort of anticorrelation between global radiance and the red-red ratio, including the datum measured by exciting a single leaf at 378 nm. This is also indicated in Fig. 10, where the red-red ratio has been plotted vs the red-blue one with the data taken during

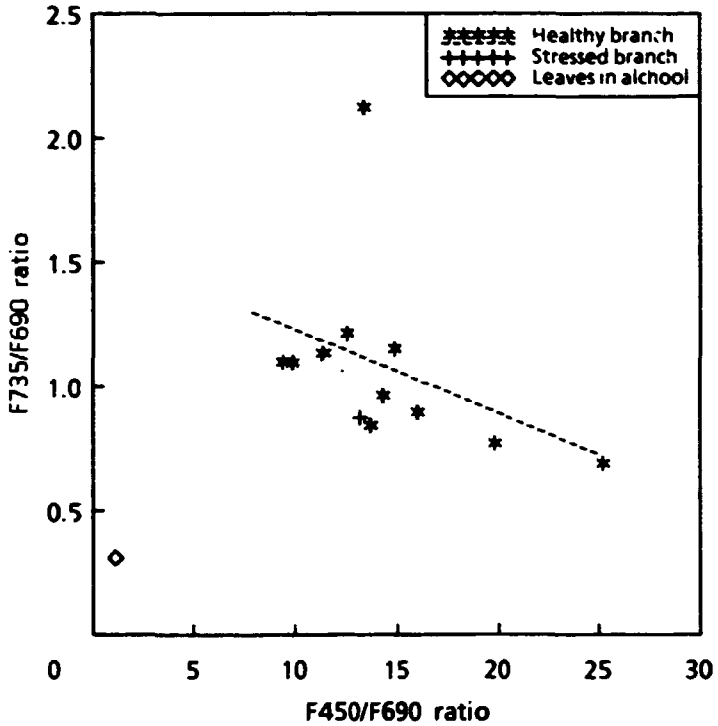

Fig.10 Correlation diagram between red-red and blue-red fluorescence peaks measured on Oct. 15, 16 and 17

three different days of measurements. Notice that the only datum clearly out of correlation refers to Oct. 17th, 1991, which was a strongly sunny day for which radiance data are not available. Possible relationship of this results to plant physiology will be shortly considered in the discussion.

III.2 TIME RESOLVED MEASUREMENTS

Due to pulse to pulse time profile fluctuations and to electronic jitter of the excimer laser, measurements of the fluorescence decay time constants resulted to be quite difficult, so that we were not able to take a complete diurnal set of data. Some results obtained on Oct. 16th, 1991, are reported in the following. Time resolved measurements both of blue and red fluorescence, were performed at wavelengths selected by suited filters (see Table 3). The analyzed spectral regions are shown in Fig. 11 for the blue and red case, respectively.

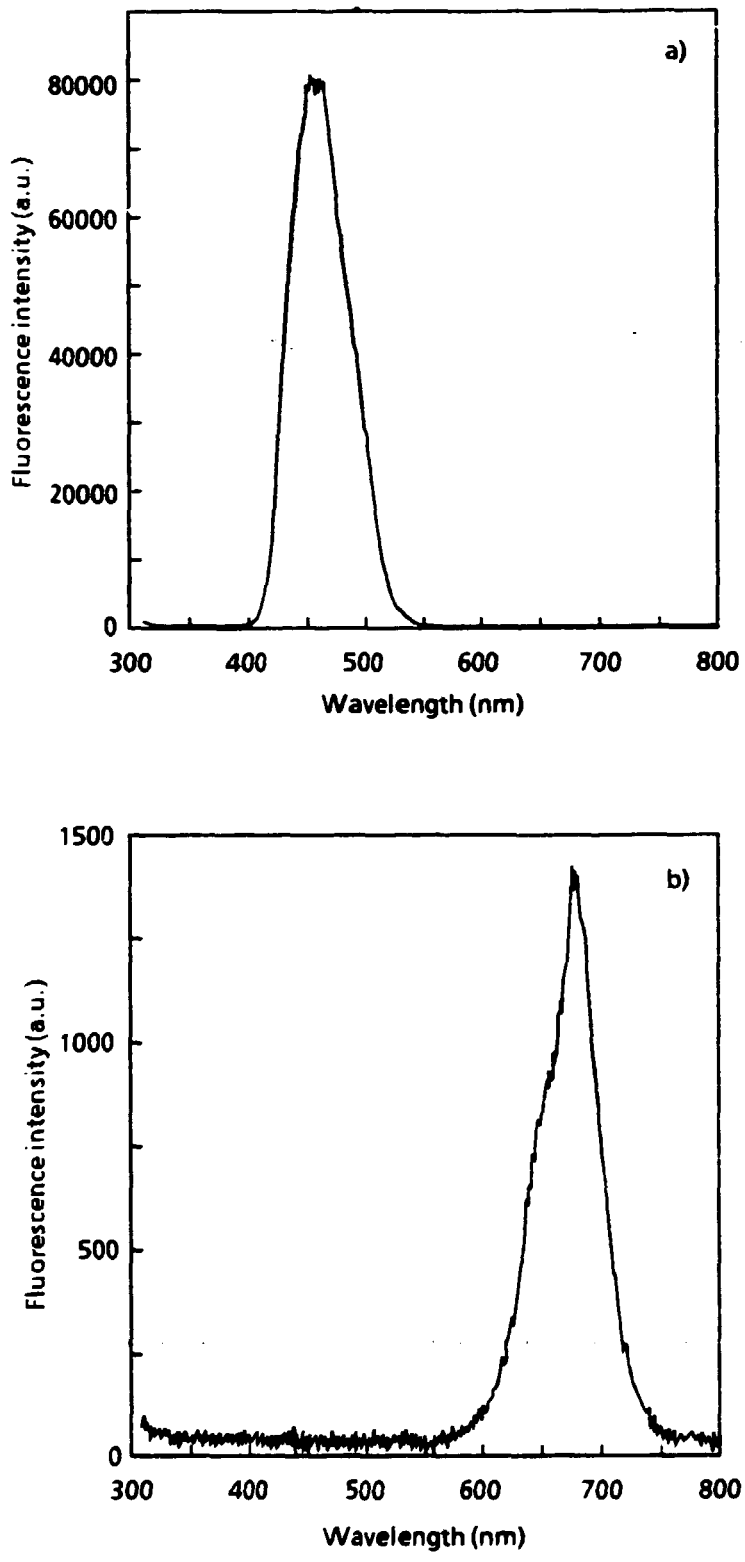

Fig. 11 Regions in blue (a) and red (b) spectral range selected in time resolved measurements reported in Fig. 12

From a consistent set of time resolved curves, reported in Fig. 12, an unexpected trend has been observed. Data show that blue fluorescence (Fig. 12a) begins to be emitted just at the rising edge of the laser pulse with a time profile which roughly reflects the exciting pulse [1]. Conversely, the red emission (Fig. 12b) appears with a typical 5 ns delay after the beginning of the excitation and is narrower than the exciting laser pulse width. Hypotheses on the relationship of this observation to plant physiology will be considered in the discussion.

Reliable time decay constants could not be obtained from present data, due to the relatively long excitation time (20 ns) as compared to the expected chlorophyll decay constants [6] and also to possible secondary laser induced processes. Actually, present data could not be analyzed by the fast Fourier transform technique applied by us in the case of oil fluorescence measurements which were characterized by decay constants in the range 1 to 20 ns [2].

III.3 EXCITATION AT DIFFERENT WAVELENGTHS

A preliminary discussion upon the field operation of our lidar fluorosensor with the Raman shifter is reported in this section. Since chlorophyll absorption exhibits a maximum for exciting wavelengths in the range 380 nm to 430 nm, the 308 nm wavelength we used was not optimized for chlorophyll fluorescence. Furthermore, the results obtained and presented in Sect. III.1 and III.2, can be related only to the outermost leaf surface signature, since the 308 nm laser light is strongly absorbed by inner leaf pigments.

In order to improve the absorption and possibly the penetration into the leaf, thus increasing the chlorophyll absolute and relative fluorescence efficiency, a Raman shifter cell filled with CH_4 at 25 atm has been added to the laser, obtaining the first, second and third Stokes lines at 334 nm, 378 nm and 421 nm, respectively. With this configuration, adopted for the first time in the present field experiment, remote sensed data could not be obtained because of a poor spatial beam quality of

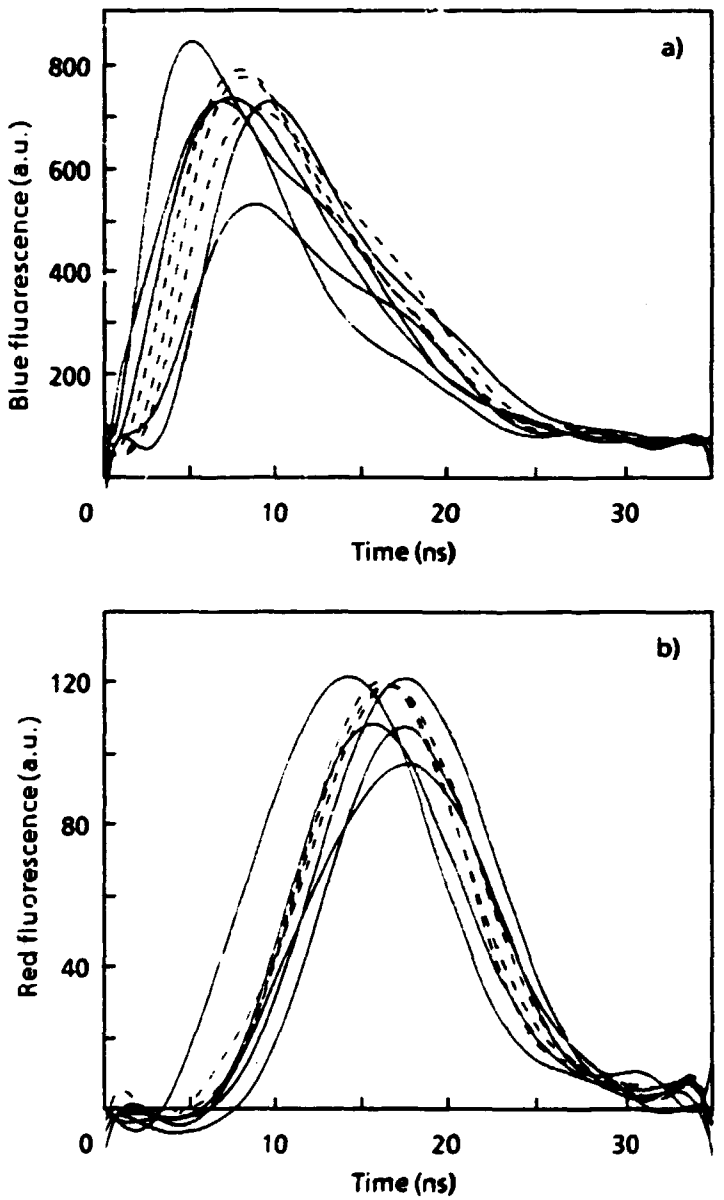

Fig.12 Single shot time resolved chlorophyll fluorescence signals from elm tree in the blue (a) and red (b) spectral regions

the Raman shifted radiation and of the difficulties in building a recollimating device directly on the field. However, some near field measurements have been performed on elm leaves, sending the Raman shifter output on a single leaf and collecting the fluorescence with a lens and a fiber optic placed behind suitable filters.

The measured fluorescence spectra (a typical one is shown in Fig. 13), exhibit a very strong background in the blue region. Actually, the poor dispersion power of the

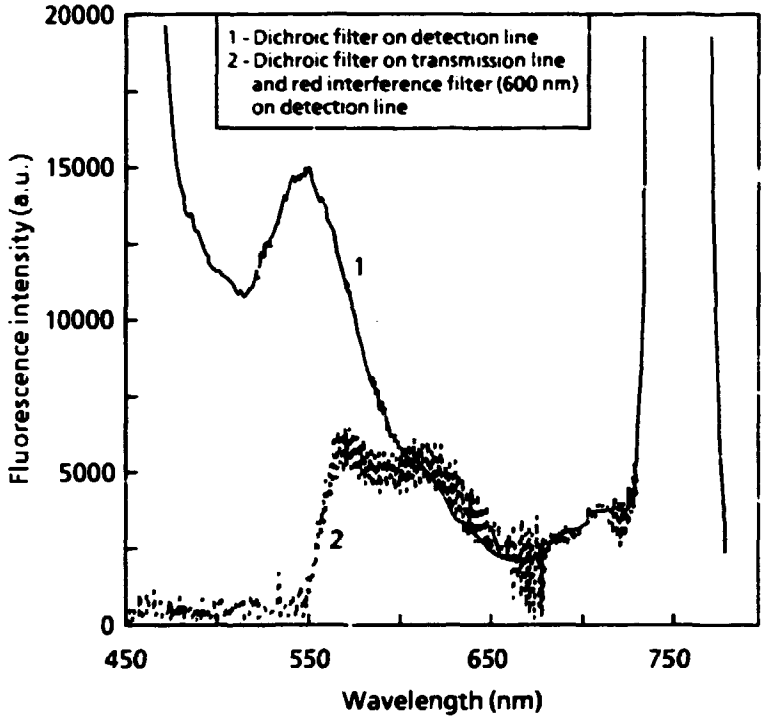

Fig. 13 Fluorescence spectrum of a single elm leaf measured at 378 nm excitation wavelength

prism placed at the Raman cell output caused unwanted contamination by the backscattered radiation generated at different transmitter wavelengths. The red region was partially covered by second order diffraction peaks of the exiciting wavevelengths and the available interference filters, peaked in the red region to cut off the source wavelengths, were not completely able to solve the problem. In spite of these difficulties and of the low intensity of the exciting pulse at each Raman shifted wavelength, the red chlorophyll emission could be detected in some cases, and for data shown in Fig. 13 also the red-red ratio was obtained. The latter value, measured on Oct. 16th, 1991, at 378 nm, is in quite reasonable agreement with the set of data measured at 308 nm.

System improvements including a better separation of Raman shifted wavelengths, beam collimation at the cell output and the use of proper dichroic filters will be needed in order to enable a reliable field operation of this system in a

movable configuration. These improvements, as discussed in the following, have been already planned.

IV. - DISCUSSION

Before discussing results, we must stress that all but data of Oct. 17th, have been collected under cloudy weather conditions with a large component of diffuse light.

In spectral resolved measurements, the following items deserve a deeper discussion in their connection with plant physiology:

correlation of the blue-red fluorescence emission ratio with global radiance;

anticorrelation of red-red ratio with global radiance.

The above results may be due to the fact that global radiance is a measure of available light over a broad spectral range from UV to near IR, and the UV component prevailed under present conditions of diffuse light. The blue fluorescence emission with a large UV absorption followed the global radiance, whereas the chlorophyll red-red ratio was sensitive mostly to the unmeasured blue-visible component of the available light. Under this hypothesis, quite different results migth be obtained in sunny days under conditions of clean sky.

Chlorophyll emission, giving rise to variations of red-red ratio, was measured also after sunset, in the absence of solar light.

This was unexpected and it might indicate the presence of a laser induced photosynthesis process. In this case we note, however, that the total fluorescence signal was lower than during the day, as expected. A shorter laser pulse should avoid the problem of possible laser induced photosynthesis and simplify the physiological interpretation.

Leaf penetration of the UV excitation wavelength.

Excitation in the UV (308 nm) takes place in a layer close to the leaf surface, and the question whether a deeper penetration is desired or not for plant physiology

interpretation, is up to now controversial (for istance, chlorophyll antennae are located on the leaf surface). However, a few data taken with excitation at 378 nm, corresponding to a deeper light penetration, do not indicate a different spectral signature, as far as the red-red emission ratio is concerned.

In time resolved measurements, two issues seem to be of major interest:

Which biological parameter, if any, is related to the time decay constant of the fluorescence or to the ratio of the decay constants taken at different wavelengths?

This is still an open question, but, from an instrumental point of view, a laser source with shorter pulses is certainly required to measure these decay constants.

What is the meaning of the observed delay between the start of the red and blue fluorescence?

This delay might be related to the presence of coating layers on the upper face of leaves and supply different information on the overall plant health connected, for instance, with the occurrence of acid rains or damage due to chemicals.

According to our opinion, a deeper analysis of data acquired during the campaign has to be done from a plant physiology point of view, in order to answer more properly to the above risen questions.

V. CONCLUSIONS AND FUTURE PLANS

In conclusion, the ENEA fluorosensor has proven to be able to measure vegetation fluorescence spectra although the apparatus should be revised in order to obtain a more easily transportable version. Apparatus revision, data analysis, and understanding of the vegetation signatures in collaboration with biologist groups, will be the topics of the work in the near future at ENEA as far as the LASFLEUR Project is concerned.

The following improvements of the lidar fluorosensor are planned, in order to make it more suitable to remote sensing of vegetation health:

a) rearrangement of the receiver, to provide it with optimum optical matching;

b) splitting of received signals for contemporary spectral and time resolved measurements;

c) installation of a new set of filters to select narrower spectral regions for the time resolved signals;

d) optical matching of the Raman cell;

e) installation of new dichroic filters to cut off excitation wavelengths from 310 to 430 nm.

f) shortening of the laser pulse width by at least one order of magnitude with commercially available electro-optical devices [7].

ACKNOWLEDGMENTS

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