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Laser-Induced Fluorescence Monitoring of Vegetation in Tuscany

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ABSTRACT

Field experiments using laser-induced fluorescence were performed on different vegetation species in Tuscany, Italy. Remote sensing fluorescence lidar experiments were performed at Pian di Novello studying mainly *Fagus Silvatica*, but also some other species occurring in the beech forest. One of the fluorescence lidar operated on the third harmonic of a Nd:YAG laser (355 nm) while the other one was based on a excimer-pumped dye laser operating et 480 nm. Both systems utilized optical multichannel detection. The lidar measurements were supplemented by fluorescence point monitoring using a fluorosensor based on a nitrogen laser and an optical multichannel analyzer, and using a portable He-Ne-based two channel instrument. High-quality remote spectra could be obtained from both lidar systems in distances ranging from 40-100m. In addition, point monitoring on *Picea Abies* was carried out at the CNR Camporgiano research station on experimental plants subject to controlled stress conditions. A discussion of the optimum choice of excitation wavelength is performed.

INTRODUCTION

During recent years it has become obvious that forests are endangered because of the influence of atmospheric pollution. There is a great need for methods of early detection and mapping of threatened areas. Satellites or airborne multi-spectral imagery provides excellent possibilities of area mapping with regard to major species. certain possibilities of forest damage assessment have also been demonstrated, but the techniques seem to have limited applicability. Laser-induced fluorescence (LIF) provides a further spectral dimension for vegetation assessment (Lichtenthaler and Rinderle 1988) and research efforts have been directed to using LIF for improved vegetation characterisation (See e.g., Cecchi and Pantani 1991, Hoge 1988, Moya *et al.* 1991, Pantani and Cecchi 1990, Svanberg 1990, Zimmermann and Gunther 1991). The concept of pont - and remote sensing monitoring of vegetation LIF is illustred in Fig. 1. Examples of remotely recorded fluorescence spectra of different species are shown in Fig. 2.

In the present paper we report on the result of field test employing point-monitoring and lidar LIF on vegetation in Tuscany (Italy). The field experiments were performed at the end of September 1990. An important aspect of the work is lidar remote measurements on beech (*Fagus Silvatica*) in Pian di Novello using two mobile systems. A photograph of the two measurement systems at the test area is shown in Fig. 3. In addition to the measurements on beech, point monitoring of *Picea Abies* at the CNR test site at Campogiano was carried out.

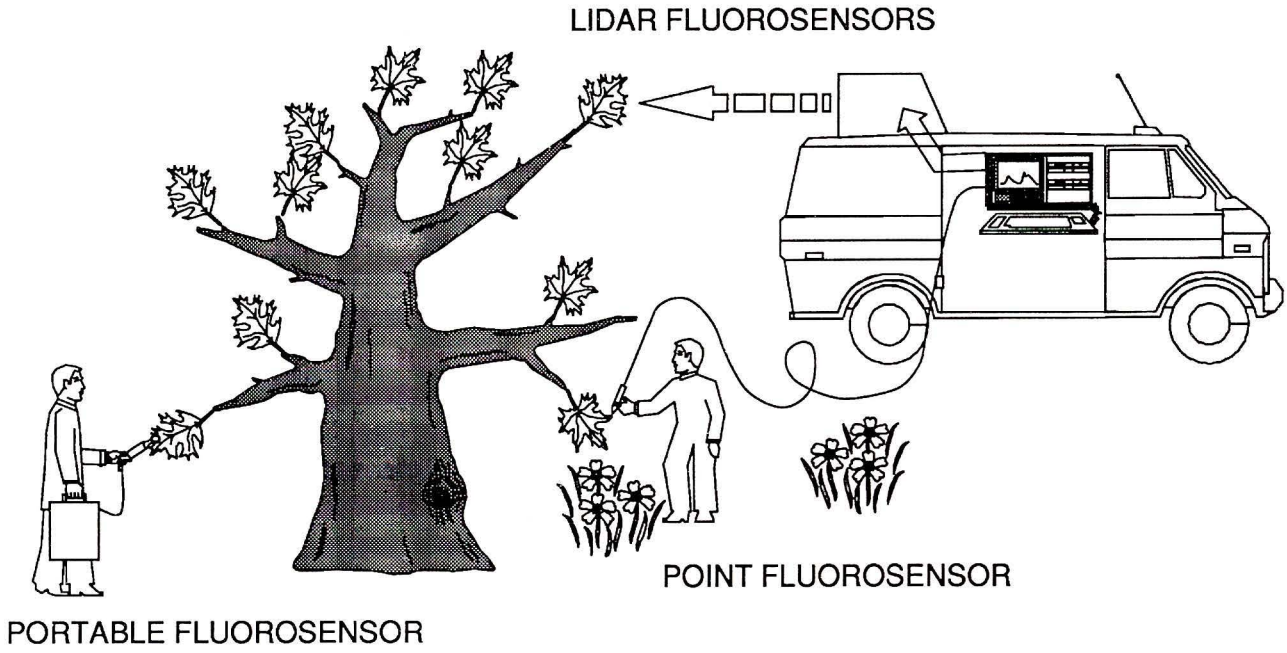


Fig. 1 - Artist's conception of point and remote LIF measurements on vegetation.

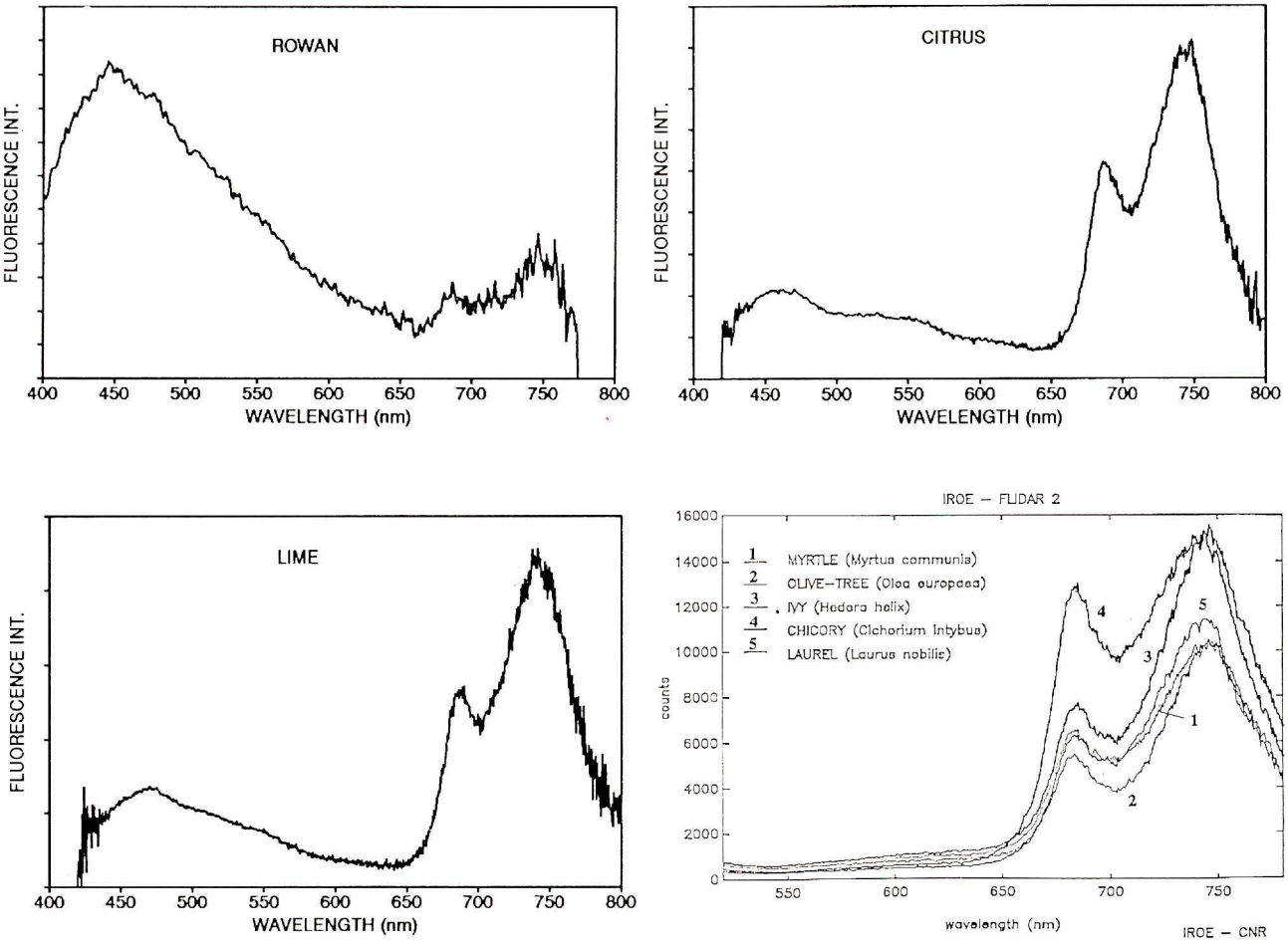


Fig. 2 - Examples of fluorescence spectra of vegetation recorded with a) the Swedish mobile system (excitation wavelength 355 nm) and b) the Italian mobile system (excitation wavelength 480 nm).

Fig. 3 - Photograph of two mobile fluorescence lidar systems at measurements site at Pian di Novello.

1. FLUORESCENCE EQUIPMENT

In the present measurements four different fluorosensor of capturing vegetation fluorescence spectra were employed. For point measurements a multi-channel and a two-channel instrument were used. Remote measurements were carried out with two independent mobile fluorescence lidar systems.

1.1 Point monitors

1.1.1 Multichannel fluorosensor

A multi-channel fluorosensor was used for point monitoring of the whole fluorescence spectrum of leaves and needles. The equipment has been described by ANDERSSON-ENGLES *et al.* (1991) and a schematic diagram is shown in Fig. 4. As an excitation source a Laser Science VLS337 pulsed nitrogen laser (λ = 337 nm) was used at a repetition rate of 10 Hz. Either this laser was used directly or as a pump source for a small dye laser (Laser Science Model VSL-DCM-3), which in the present measurements was used to generate light at 405 nm or 470 nm. The laser light was focused into a 600 m quartz fiber, that in the present experiment had a length of 5 m, but which could be extended to tens of meters. The fibre was put in direct contact with the plants to be studied and induced fluorescence was collected with the same fibre and conducted back to the measurement equipment. The fluorescence light passes through a 45° dichroic mirror, that is highly reflecting for the laser light and is used for injecting it into the fibre. A Schott high-pass coloured glass-filter was used to fully reject elastically back-scattered laser light while passing the Stokes-shifted fluorescence light. The fluorescence light was focused at the entrance slit of a Jarrel-Ash 0.275 m polychromator with a 400 m entrance slit.

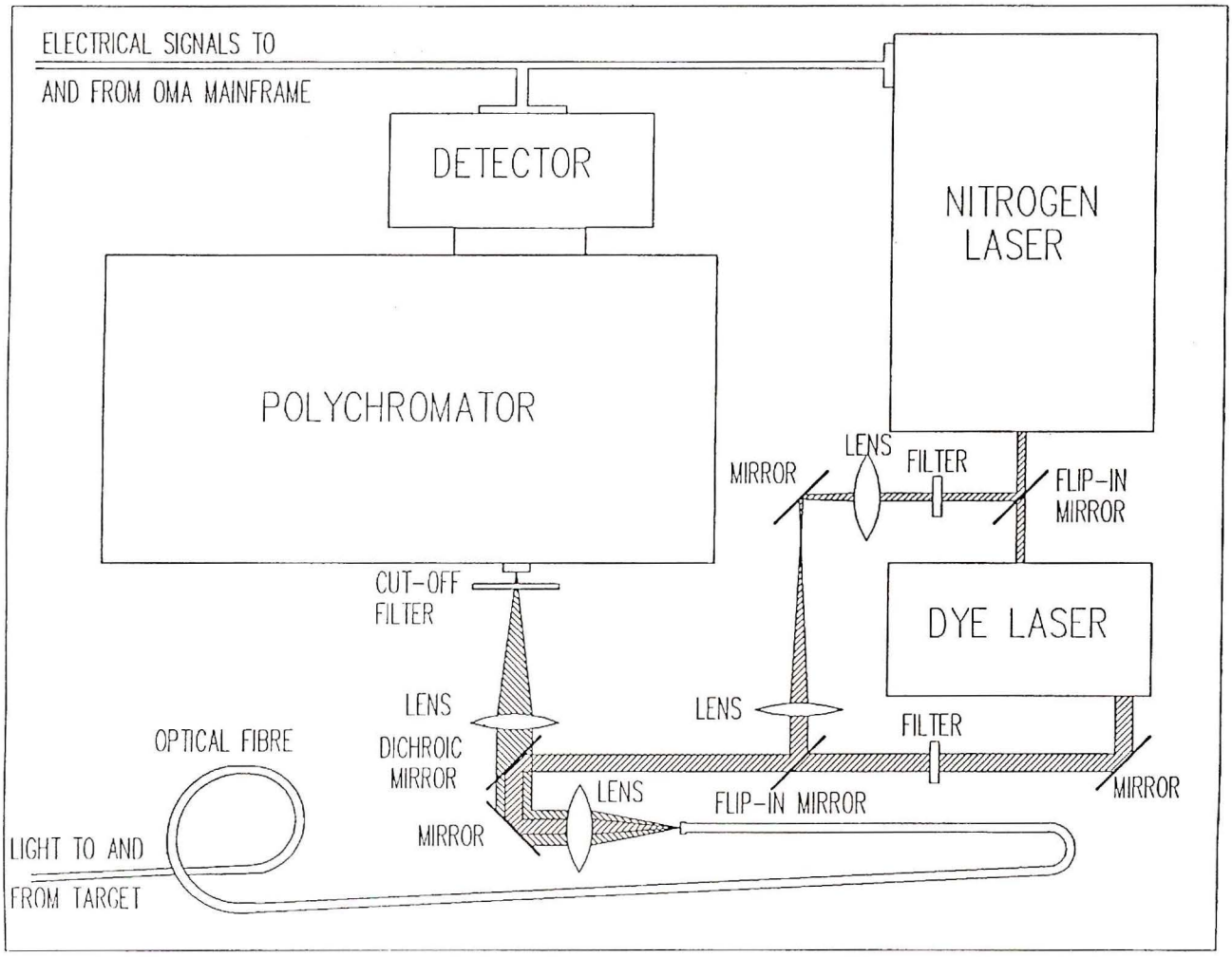


Fig. 4 - Schematic diagram of the Lund point monitoring multichannel fluorosensor.

In the focal plane of the spectrometer an image-intensified diode-array detector (EG&G Model 1421) was placed covering the wavelength range 300-800 nm. The captured fluorescence spectrum was transferred to an EG&G OMA mainframe. The spectra were stored on floppy disk for later evaluation usiong a specially developed software package.

In the field measurements the fluorosensor was transported in a van and electricity was provided from a small, portable motor generator.

1.1.2 Portable two-channel fluorosensor

Point measurements were also performed with the prototype of the portable, battery operated, two channel fluorometer, developed at IEQ and named LEAF (Laser Excited Automatic Fluorometer). LEAF uses a He-Ne laser (0.5 mW λ = 633 nm) as a fluorescence inducing source and a single optical fiber both to carry the excitation light to the leaf and to collect fluorescence. The returning beam is separated from the laser beam by a set of dichroic mirrors and interference filetrs (Fig. 5). The detectors are two miniature-size, head-om photomultipliers (Hamamatsu), with S20 photocathode for a good sensitivity in the red spectral region. The instrument contains all the detection electronics, including the analog amplifiers, sample-and-hold circuits and 12 bits analogue-to-digital converters. A microprocessor board acquires data and stores them in the internal RAM, for later dump via a RS 232 serial interface. The internal battery is sufficient for 4 hours of continuous use, but an auto power off software turns off the instrument when not in use. During field operation this battery capacity was found sufficient for a fully day of operation.

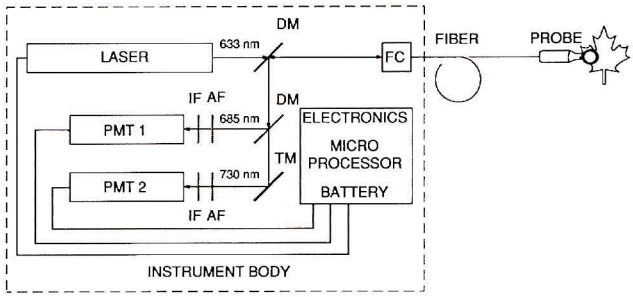


Fig. 5 - Schematic diagram of CNR-IEQ portable two-channel fluorosensor. FC=Fiber Coupler; DM=Dichroic Mirror; TM=Totally reflecting Mirror; IF=Interference Filter; AF=Absorption Filter.

LEAF can be measure chlorophyll fluorescence at 685 and 730 nm, with a band-pass pf $T\lambda$ = 5 nm. In spite of the different excitation wavelength it can perform the measurements of the F685/F370 ratio as the high resolution system. It s low weight (5.6 Kg, battery included) and its small waterproof aluminium case /37x29x11 cm^3) make it very useful as a "ground truth" instrument for the remote sensing systems and its simplicity is an advantage.

1.2 Mobile fluorescence lidar systems

1.2.1 Nd:YAG-based system

The Swedish mobile fluorescence lidar system employed in the present measurements is based on the remote sensing system described by ENDER at al. (1987). This system has been mainly beenused for atmospheric pollution monitoring as reviewed by SVANBERG (1991). For remote fluorescence monitoring the point multi-channel fluorosensor described above was connected to the output of the receiving optical telescope as also described by EDNER *et al.* (1991).

The system adopted to vegetation fluorescence measurements is schematically shown in Fig. 6. The lay-out of the optical and electroning system is given in Fig. 2 of the just mentioned reference (this volume). A short description of the system will be given here.

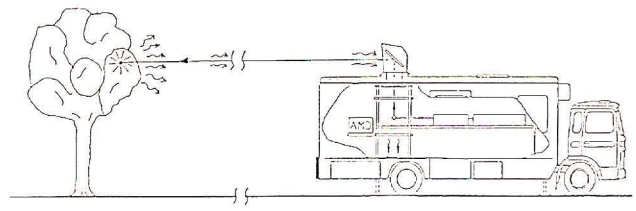


Fig. 6 - Lund mobile fluorescence lidar system.

The system is housed in a covered truck and eklectric power is supplied by a 20 KVA diesel motor generator, towed by the truck. As a laser transmitter a powerful pulsed Nd:YAG laser (Continuum Model YG 682) with a primary IR pulse energy of 1.2 J is used at a repetition rate of 20 Hz. For inducing fluorescence it was operated at its third harmonic (λ = 355 nm), where output pulse energies up to 250 mJ were available. The system is equipped with a Continuum Model TDL 60 dye laser, which is capable of producing pulses of about 20 mJ energy at 480 nm, where chlorophyll-a absorbs strongly. for practical reasons only the Nd:YAG third harmonic was used in the present experiments. The UV laser are transmitted into the atmosphere via a large first surface aluminum mirror on the top of the lidar van. With this cpmputer-controlled mirror the beam is directed onto the object under study. Fluorescence light induced in the target is reflected by the same mirror down into a vertical Newtonian telescope of the diameter 40 cm. In the image plane of the telescope a 600 m diameter quartz fibre is placed to collect fluorescence light and guide it to the 400 m wide entrance slit of the optical multichannel analyzer. The fibre is placed in the center of a small hole in a metal mirror, that directs the rest of the light collected by the telescope to a TV camera, used for aiming the system. On the TV screen the hole appears as black circle indicating the aiming point of the laser beam. The recording of the whole fluorescence spectrum from about 370 nm to 800 nm follows as described above in connection with the multi-channel point monitor. In order for the system to sensitively detect vegetation fluorescence in the presence of full sun light the multichannel plate of the image intensifier is gated to a time window of about 500 ns centered at correct time delay corresponding to the target distance.

1.2.2 Excimer-based system

The Italian nobile fluorescence lidar system used in the present experiments is basically the system described by CECCHI *et al.* (1991). It employes a XeCl pumped dye laser, operating at 480 nm as transmitter. The excimer laser can operate at 10 Hz with an output energy of 80 mJ. The dye laser conversion efficiency is about 10 percent. Mounted together with and aligned with a Newtonian telescope of diameter 25 cm, the integrated fluorosensor arrangement can be directed toward the target. Collected fluorescence light is focused into a fibre bundle and brought to the entrance slit of an intensified optical multichannel analyzer system. Spectra are stored on a hard disk, already preprocessed, for further analysis. During the field test the equipment was installed in a temporary vehicle awaiting the completion of the specially arranged

permanent vehicle. A schematic diagram of this fluorescence lidar system is shown in Figure 7.

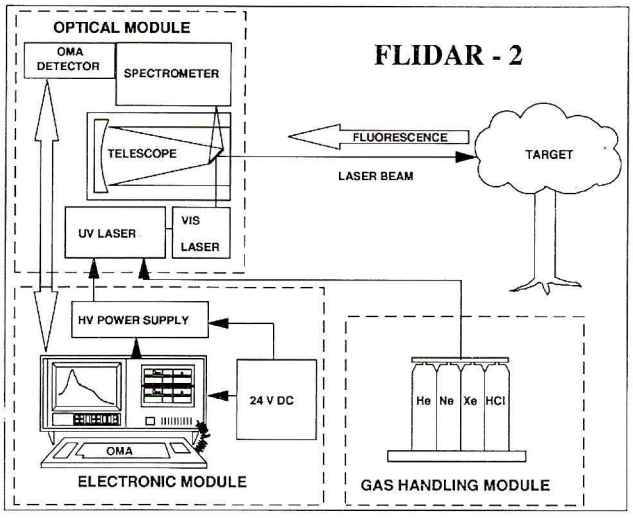


Fig. 7 - Schematic diagram of CNR-IROE mobile fluorescence lidar system.

2. REMOTE AND POINT MONITORING MEASUREMENTS IN PIAN DI NOVELLO

In Pian di Novello, close to Abetone Pass in northern Tuscany there are wide-spread forests of beech (*Fagus Silvatica*). Our measurements were performed at the beginning of the senescence period when fully green as well as yellowish leaves were available. The field experiments were started by point monitoring of beech leaves using the two point-monitoring devices described above.

As a starting point we consider the response of green leaves to excitation light of different wavelengths. Spectra were recorded using the multi-channel instrument and examples are given in Fig. 8. Using the N_2-laser-pumped dye laser, spectra were taken for 405 and 470 nm, where chlorophyll has a strong absorption and the radiation has a good penetration of the leaf. Correspondingly strong chlorophyll spectra featuring the characteristic peaks at about 690 and 735 nnl. The ratio I(690 nm)/I(735 nm) is of special interest, since it is an indicator of the proper functioning of the photosynthetic apparatus (LICHTENTHALER and RINDERLE 1988). Generally speaking a low ratio is obtained for a healthy plant while high ratios may indicate stress conditions. However, the ratio is also influenced by the amount of chlorophyll present and thus changes between the upper and lower side of a leaf. It is also strongly dependent on the ambient light level and other factors and thus no straight-forward relation between the ratio and the health status exists, but additional information is needed.

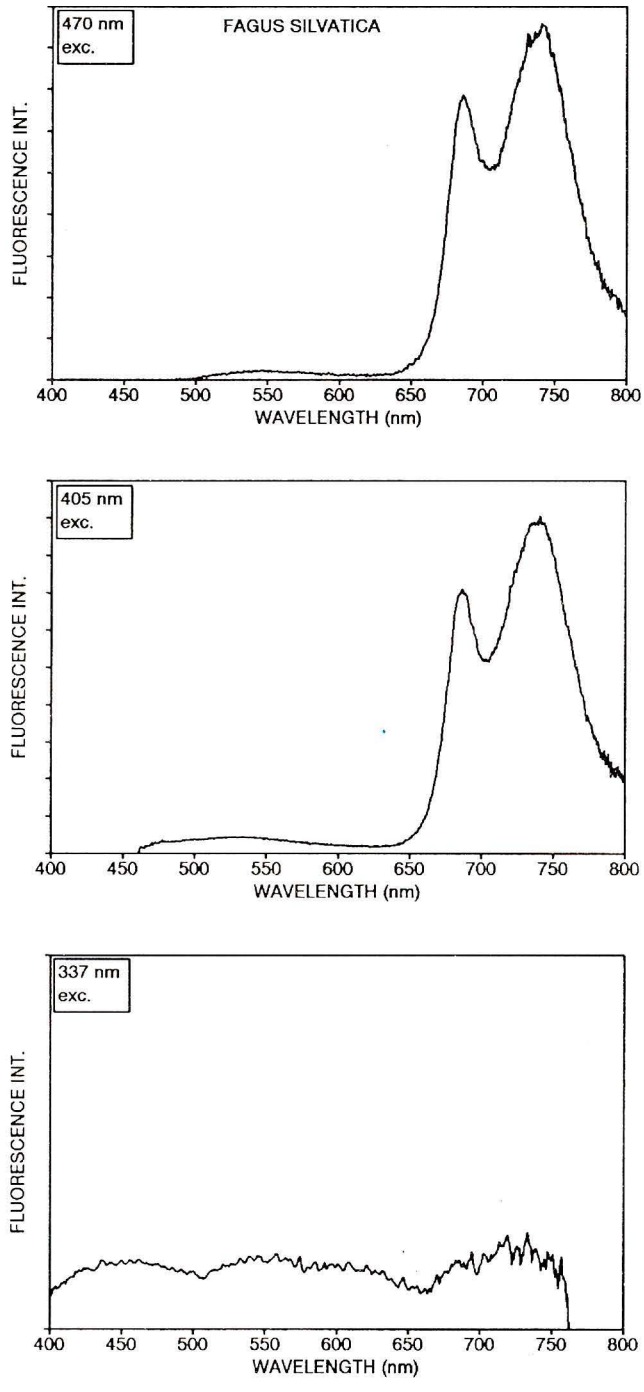


Fig. 8 - Point monitoring fluorescence spectra of beech leaves (Figus selvatica) measured at different excitation wavelengths using the Lund multichannel system.

It can be noticed from Fig. 8 that the broad-band fluorescence in the green spectral region gains in importance for the shorter excitation wavelength. For 337 nm excitation no chlorophyll fluorescence is induced, which is a common observation for most species (See e.g. CELANDER *et al.* 1978, CHAPPELLE 1990, SVANBERG 1990). The explanation is partly a spectral mismatch with the chlorophyll absorption but also reduced penetration into the leaf because of superficial "wax". For beech the wax is only weakly fluorescent as seen in Fig. 8. (Actually, 10 times as many laser shots were used for recording the 337 nm curve than for the other two). Normally the wax fluorescence is very strong, as e.g. seen in Fig. 14.

The change in fluorescence ratio between the upper and lower sides of beech leaves is illustrated in Fig. 9 showing recordings with the two-channel point monitoring system.

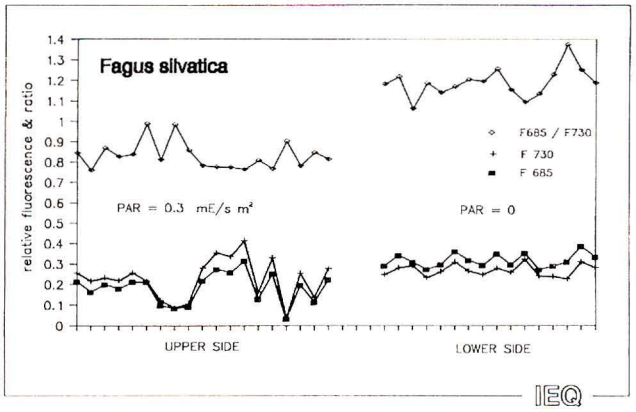


Fig. 9 - Point monitoring of upper and lower sides of beech leaves using the CNR-IEQ two- channel fluorosensor. Individual channel intensities and the ratio of the two channel readings are plotted.

tic ratio increase for the senescent leaves. In both spectra a signal possibly due to carotenoids is shown in the spectral region at 520 nm. Included in Fig. 10 are also spectra for the dead, brown leaves covering the ground of the beech forest and for the grey bark of the living beech trunks. It can be seen that the beech bark fluorescence is very strong and whitish and also features a chlorophyll signal. For airborne measurements this might be a problem and needs some considerations.

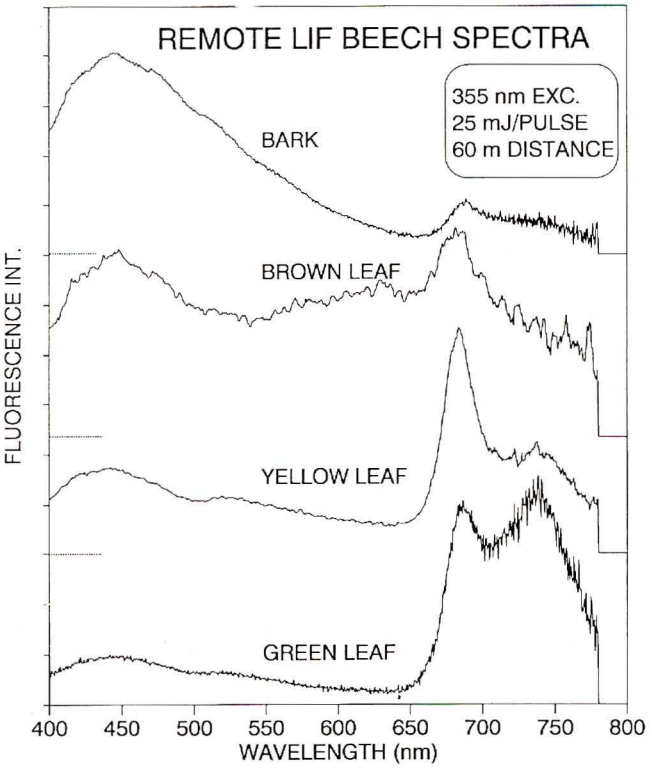


Fig. 10 - Examples of remote fluorescence recordings of beech trees at Pian di Novello using the Lund mobile fluorescence lidar system.

In the remote sensing experiments measurements were performed on leaves of young beech trees growing at the edge of an open area in the forest. The fluorescence lidar systems were placed as shown in the photograph in Fig. 3 shooting across the open area to hit targets in 40-60 m distance.

Examples of remote spectra recorded with the Nd:YAG-based system (λ_{exc} = 355 nm) are shown in Fig. 10 using a measuring distance of 60 m and a pulse energy of 25 mJ. Each spectrum was averaged for 500 laser pulses. The divergence of the exciting laser beam was chosen with a beam expanding telescope to a spot of selectable size, in our case with about 10 cm. diameter. Thus, 2-3 leaves were covered by the laser spot. Spectra are shown for green and yellow shadow leaves, featuring the characteris-

With the same system also passive remote monitoring of the leaf reflectance was performed with the laser switched off. The spectrally resolved reflected intensity from the leaves was first recorded and stored and then the sky light spectral distribution under the same circumstances was recorded by directing the telescope viewing to a first surface aluminized 30x40 cm^2 mirror angled to receive the vertical impinging light. By dividing the leaf spectrum with sky spectrum an approximate recording of the leaf reflectance is obtained. The results for green and yellow leaves are shown in Fig.11, showing the expected increased intensity towards the red spectral region. A prominent feature is the strong leaf reflectance in the near IR region, where the chlorophyll does not absorb any longer. In assessing forest decline the combination of using active (laser) and passive remote sensing may prove to be important.

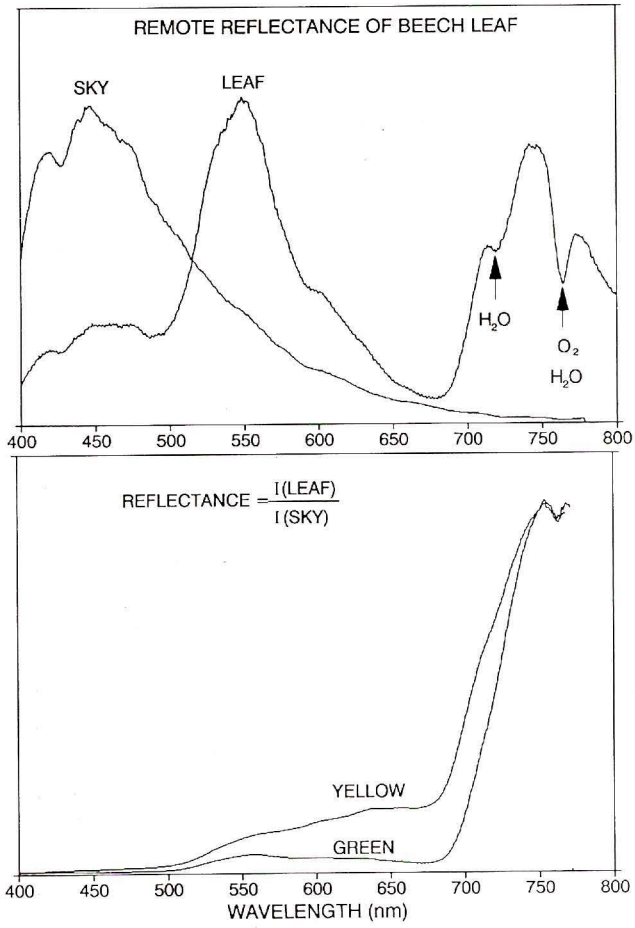


Fig. 11 - Remote recording of green leaf reflected intensity and quasi-simultaneous sky intensity distribution for a beech tree at Pian di Novello. In the power part of the figure the resulting reflectance curve is shown for the green leaves and also for yellow leaves. The recordings were taken with the Lund fluorescence lidar system operating in its passive (non-laser) mode.

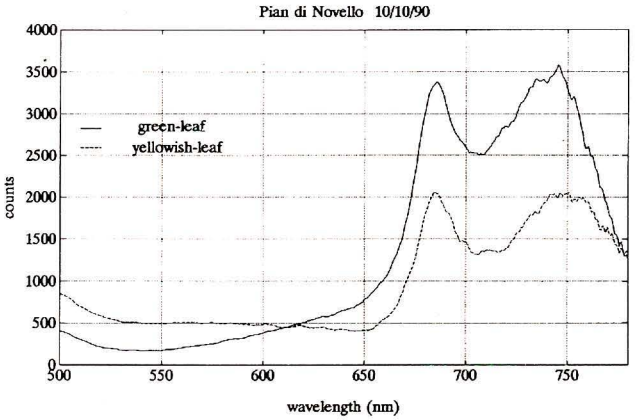
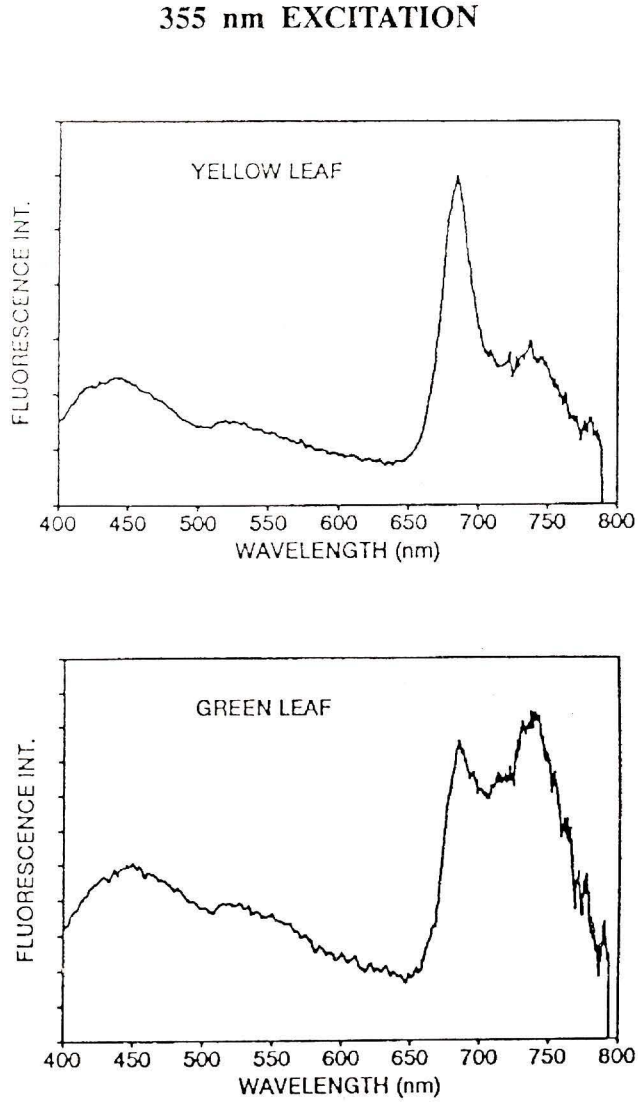


Fig. 12 - Examples of green and yellow beech leaf spectra recorded remotely with the CNR- IROE fluorescence lidar system.

Examples of remotely recorded spectra obtained with excimer-based fluorescence lidar system are given in Fig. 12 for 40 m measuring distance.

Simultaneous measurements on the same leaves using both fluorescence lidar systems were performed. Examples of such recordings are given in Fig. 13 for green and yellow shadow leaves. A quite detailed and very satisfying agreement between the chlorophyll signals recorded with the two lidar systems is obtained.

Night-time remote recordings on green shadow leaves were taken for the case of dark adaption following light exposure of the leaves from a flash-light. Signals obtained with the two fluorescence lidar systems investigating the same leaves are shown in Fig. 14.



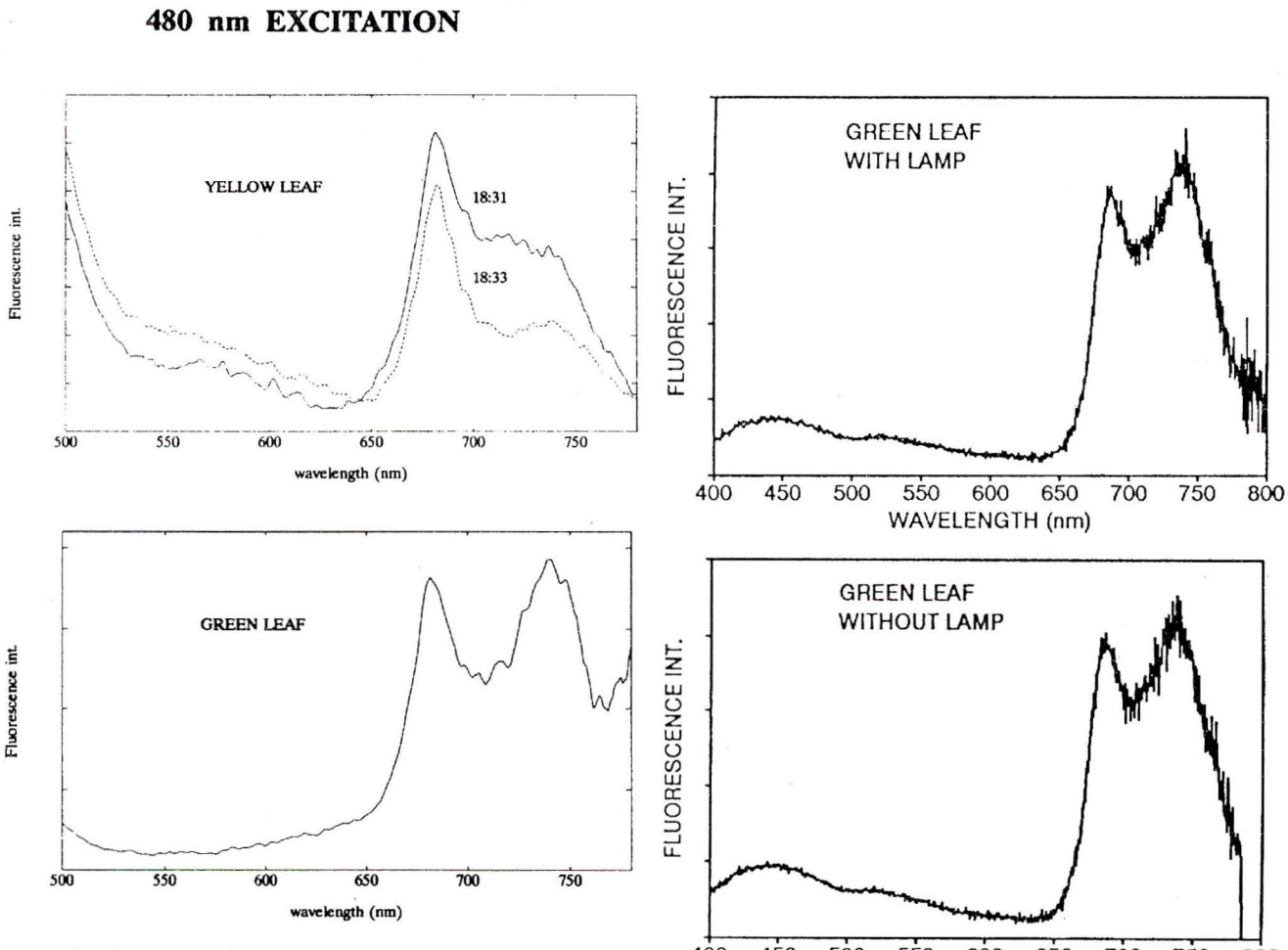


Fig. 13 - Comparison between simultaneous remote recordings of the same green and yellow leaves obtained with the two mobile fluorescence lidar systems.

The difference obtained with the two lidar systems might be due to the two different excitation wavelengths, and/or an actinic effect due to the 20 Hz 355 nm excitation.

3. POINT MONITORING MEASUREMENTS ON *PICEAS ABIES* AT CAMPORGIANO

In addition to the remote fluorescence lidar measurements on beech trees in Pian di Novello point monitoring on spruce plants (*Picea Abies*) was performed at the CNR test site at Camporgiano (Garfagnana). The studied plants had a height of about 80 cm, and consisted of normal reference specimen and plants that had been subject to detergent (ABS) exposure affecting the wax surface and/or had been subject to water (dehydration) stress. In particular the water stress was terminated six months before experiment, while the ABS stress was on going (once a week).

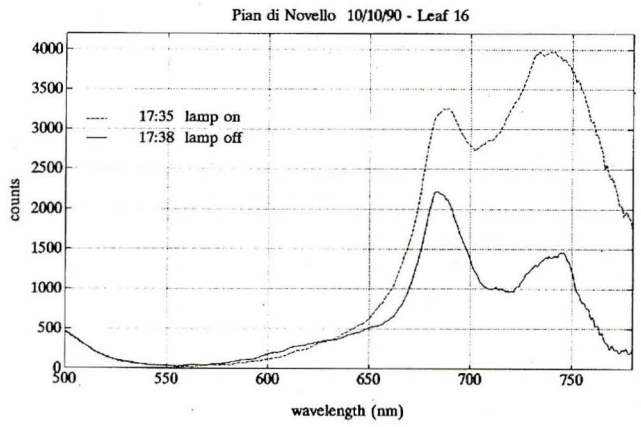


Fig. 14 - Remote night-time fluorescence recordings of green beech leaves. Spectra were recorded in darkness and during light exposure with a flashlight. a) Recordings with the Lund system (lexc-355 nm). b) Recordings with the CNR-IROE system (lexc=480 nm).

Spectra for different excitation wavelengths are shown in Fig. 15 as recorded with the multi- spectral fluorosensor. We have already commented on the strong wax fluorescence and the absence of chlorophyll fluorescence when using 337 nm excitation.

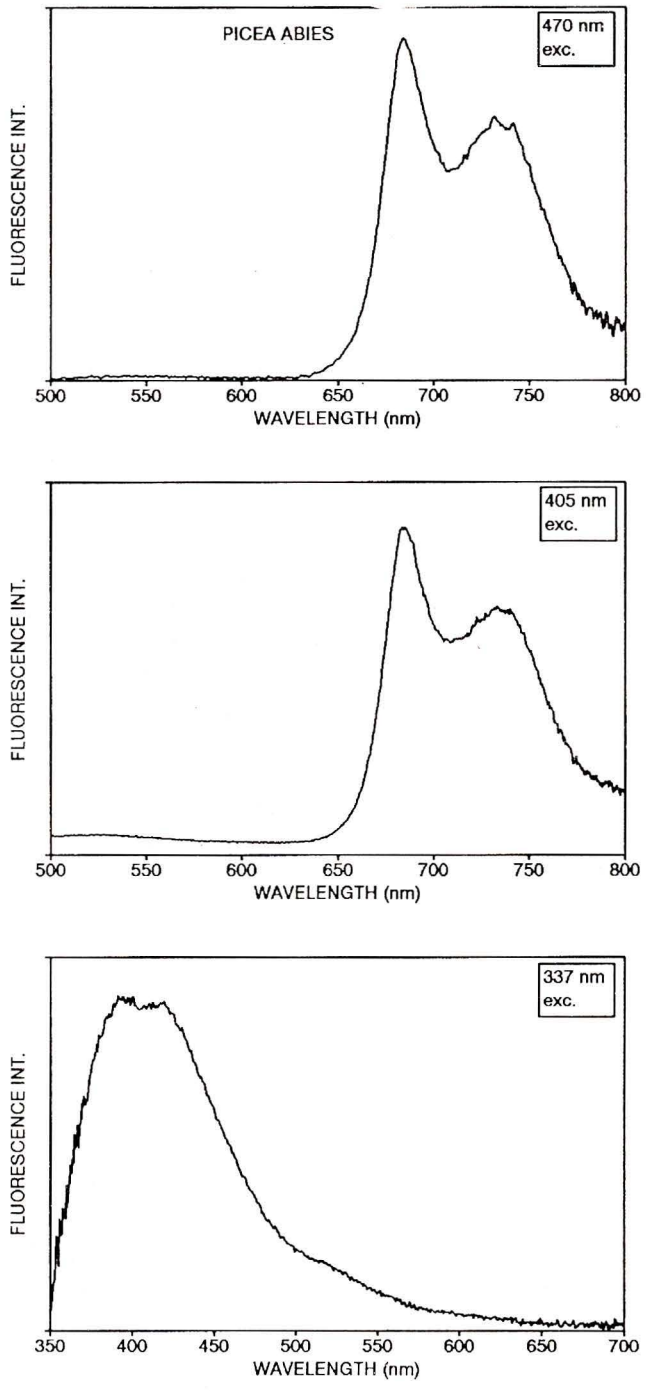


Fig. 15 - Point monitoring of fluorescence spectra of Picea Abies obtained with the Lund multichannel fluorosensor operating at three different wavelengths.

In Fig. 16 data on the ratio of the two chlorophyll fluorescence peaks intensities I(690 nm)/I(735 nm) are given for a healthy plant and an even visually damaged plant subject to both detergent and water stress. Results obtained with the two point monitors are presented in separate diagrams. In these measurements the multi-spectral instrument was operated with 470 nm excitation. The fluorescence data can here clearly distinguish the two plants from each other as could the naked eye.

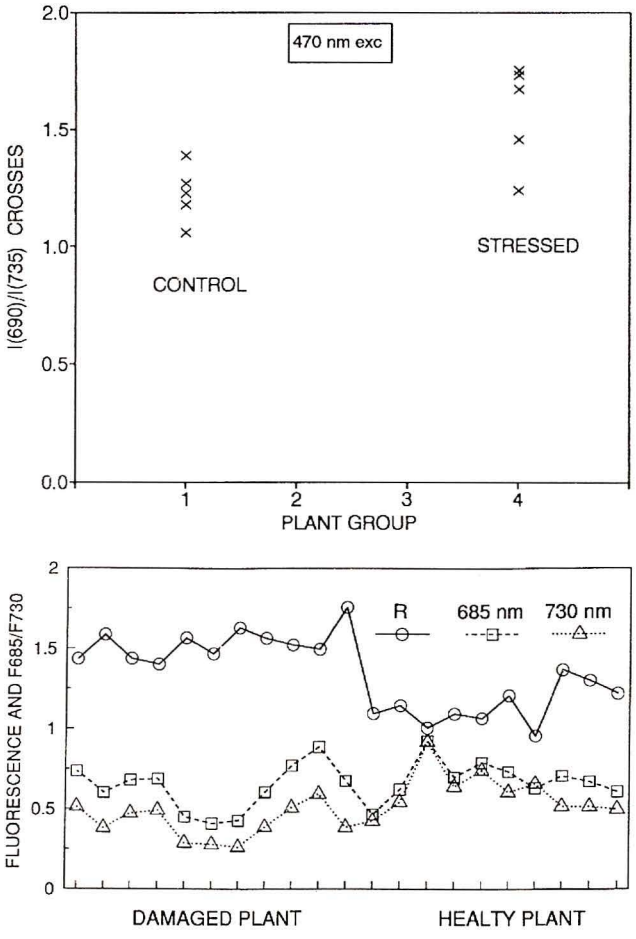


Fig. 16 - Comparison between fluorescence data for a normal and even visually strongly stressed spruce plant. a) Data obtained with the Lund multichannel point-monitoring fluorosensor (lexc=4700 nm). b) Data obtained with the CNR-IEQ two-channel point-monitoring fluorosensor (lexc=633 nm).

Measurements were performed on four classes of plants as shown in Fig. 17 for an irradiation corresponding to partly cloudy skies (PAR values were also measured). The excitation wavelength was chosen to 405 nm and one spectrum recorded for each of 5 plants in each group. The different plants could not be visually distinguished from each other. The chlorophyll peak ratio I(690 nm)/I(735 nm) as well as the blue to red fluorescence

ratio I(490 nm)/I(735 nm) are given. As can be seen the statistical spread is quite large. Two more series of data of the same kind were recorded for other light intensities, and again a large statistical scatter was evident. Using 337 nm excitation no chlorophyll is excited. However, the bluish wax fluorescence does not provide any marked demarcation either. The conclusion from these data is that, based on fluorescence ratio measurements alone, only strongly stressed plants can be distinguished from healthy ones. This is in accordance with earlier findings by ANDERSSON- ENGELS *et al.* (1988), who investigated young Swedish spruce and pine plants subject to ozone stress.

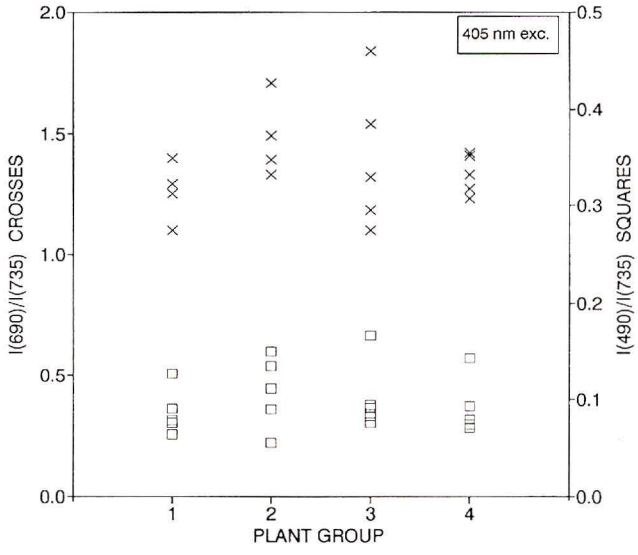


Fig. 17 - Fluorescence data on 4 classes of Picea Abies, subject to different environmental conditions. Class 1: normal reference plants, Class 2: water stress, Class 3: detergent (ABS) stress, Class 4: water and detergent stress. Excitation wavelength was 405 nm.

4. DISCUSSION

Valuable experiences in remote fluorescence monitoring was obtajned in the field experiments described in the present paper. High-quality remote spectra could be recorded with both mobile systems. Measurements ranges of 40-100 m were used. Excitation in the blue wavelength region yields optimum chlorophyll fluorescence while a shorter wavelength also provides information on the wax layer covering the leaf. The 355 nm output from a frequency tripled Nd:YAG laser is readily available at high power levels and is eyesafe in practical measurements, but cannot excite the chlorophyll fluorescence in sun leaves of beech trees. An optimum wavelength in view of eye-safety consideration might be just below 400 nm.

The 633 nm excitation is also useful for comparisons with many existing data (LICHTETHALER and RINDERLE 1988) on F685/F730 ratio changes due to different chlorophyll concentration and/or stress states. The red wavelength is also a suitable probe of chlorophyll fluorescence since it directly excites the first triplet states of chlorophyll molecule, without any transfer from other pigments. However this wavelength is not very suitable for lidar operation for the above-mentioned reasons of eye-safety and for the poor availability of high peak power red lasers.

Point measurements on spruce subject to dehydration stress and/or detergent exposure did not provide any statistically significant demarcation between stressed and normal plants. Only when changes were visible to the eye a fluorescence demarcation was also observed. For the remote beech monitoring clear fluorescence differences were also observed when the visible color of the leaves changed from green to yellow. However, the material collected for beech was not well controlled enough to make any conclusive observation on the possibilities to detect stress on beech using fluorescence.

LIF measurements directly interfere with the photosynthetic process and thus might have the potential to reveal stress conditions to vegetation before these are detectable by conventional remote-sensing techniques based on external characteristics of the plant such as spectral reflectance. The present study shows that early detection of stress is quite difficult. Probably it is necessary to use different pieces of information collected in fluorescence measurements (at different excitation wavelengths, and possibly also time-resolved) and in reflectance measurements to assess early damaged to forests. In view of the paramount importance of such an assessment the effort is motivated even if no easy way directly presents itself.

ACKNOWLEDGEMENTS

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