The Nature of the Different Laser-Induced Fluorescence Signatures of Plants

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ABSTRACT

Spectroscopic methods, in particular reflectance spectra and reflectance ratios, have been used for a long time in ecophysiology and remote sensing to characterize the physiological state of plants and terrestrial vegetation. Present scientific research concentrates on establishing the complete laser-induced fluorescence-emission spectra of plants (LIF-signatures) from 400 to 800 nm with the aim to eventually develop active LIDAR fluorosensor systems for application in remote sensing. This research is reviewed in this contribution. The LIF signatures of green plants, as induced by a UV-laser (excitation 337 nm), consists of two to three components: a) the blue fluorescence (BF) with an emission maximum near 450 nm, b) a second, mostly smaller maximum (or shoulder) in the 530 nm region (= green fluorescence GF) and c) the well-known red chlorophyll fluorescence (RF) with maxima in the 690 and 735 nm region.

In contrast to chlorophyll fluorescence, the nature and shape of the UV-laser induced blue-green fluorescence is not yet well understood. It appears to be a mixed signal composed of the fluorescence of mainly phenolic substances (candidates are: coumarins and cinnamic acids for BF emission and quercetin and other flavonols for GF emission near 530 nm). Short-term and long-term stress not only increases the chlorophyll fluorescence ratio F690/F735, but changes the intensity of the ratio of BF to RF. Whether laser-induced blue and red fluorescence signatures are suitable for remote sensing of the physiological status of plants is matter of investigation in several laboratories. Attempts are made to screen the vitality of terrestrial vegetation via the ratio F690/F735 using an active LIDAR System (EUREKA project LASFLEUR), which in future will also include the blue fluorescence as well as reflectance measurements. Other approaches may be the application of a Fraunhofer line discriminator FLD, which senses the stress-increased chlorophyll fluorescence in a Fraunhofer line.

INTRODUCTION

Leaves, plants and terrestrial vegetation as a whole can be judged and investigated by means of spectroscopic methods. Several optical methods can be applied to characterize the leaves and plants by particular spectral signatures. These are absorption spectra, reflectance spectra, transmission spectra, fluorescence-emission spectra as well as photoacoustic spectra. Some of these spectroscopic methods can only be applied at a near distance between target and sensor of several mm or cm, i.e. in a fairly close contact of leaves with the particular detector system, as is the case with absorption, photoacoustic and transmission spectroscopy. The two other spectral signatures, reflectance and fluorescence-emission spectra, can, however, not only be sensed from a short distance, but also when the sensor is far away from the leaf or plant target. Hence, reflectance and fluorescence of plants can be applied in the remote sensing of plants and terrestrial vegetation.

In plant leaves, solar radiation in the visible region is absorbed by the photosynthetic pigments (chlorophylls and carotenoids) of the leaf mesophyll. The absorption characteristics of leaves can be determined via absorption spectra. A part of the incident light is reflected at the epidermis layer, which is free of photosynthetic pigments, and also at the upper part of the mesophyll cells, where the photosynthetic pigments determine the reflectance characteristics of leaves. A small proportion of the light passes through the leaf and permits determination of transmission spectra. In many cases of normal green plant leaves, absorption and transmission spectra cannot be taken, since the leaves are too thick and/or the pigment concentrations (chlorophylls and carotenoids) per leaf area unit are too high. In contrast, reflectance spectra can be registered also from thicker, dark green leaves.

The reflectance spectra are much influenced by the chlorophyll and carotenoid content of leaves, as can be
seen in Fig. 1a. The higher the pigment content per leaf area unit, the lower the leaf reflectance in the visible range (Lichtenhuler and Buschmann, 1987). In the red and blue regions the reflectance spectrum of leaves is determined by the absorption bands of chlorophylls in the red and blue spectral regions and those of carotenoids (blue spectral region). In the green region there exists a gap in the light absorption by chlorophylls, which is seen in a higher reflectance near 550 nm. Since the photosynthetic chlorophylls do not absorb in the near infra-red, the reflectance rises considerably at the red edge (> 690 nm) and is then solely determined by the arrangement of cells, cell size as well as size and distribution of aerel interspaces and cavities within the leaf mesophyll. In the second derivative of the reflectance spectrum one can see a “blue shift” of the inflection point of the red edge to shorter wavelengths in cases of a lower chlorophyll content, e.g. in needles of a damaged spruce (Fig. 1b). With decreasing chlorophyll content the reflectance in the chlorophyll absorption band near 680 nm increases and the chlorophyll content of a leaf can be estimated using the ratio of the reflectance, e.g. that near 750 nm to that near 680 nm (R750/R680). Other ratios (e.g. R740/R650) can also be applied with success. The values of such ratios decrease with decreasing chlorophyll content.

Terrestrial and airborne reflectance measurements have been applied to detect stress in plants and to classify forest damage with great success (Schmuck et al., 1987; Huss, 1984; Rock et al., 1986a and b). In damaged spruces which showed the typical forest- decline symptoms (needle loss, yellowing of needles etc.) the reflectance in the near infra-red (780 - 900 nm) was considerably decreased in the years 1983 - 1985 as compared to healthy spruces. This allowed a good classification of spruce stands, e.g. in the Northern Black Forest, into different damage classes by airborne reflectance measurements (Schmuck et al., 1987). The decrease of the reflectance in the near infra-red of damaged spruces was, however, lost due to partial regeneration of damaged spruces after 1986, which resulted in almost normal morphology and pigment content of the newly formed needle years. In addition, the blue shift of the red inflection point is no longer so predominant as shown in Fig. 1 for the 1983 spruce needles.

This excludes at present the possibility of performing a damage classification of forests by reflectance measurements alone. This situation inspired the search for additional optical methods to complement the passive airborne reflectance measurements. The laser-induced red chlorophyll fluorescence, which is a very suitable stress indicator of plants (Lichtenhuler, 1988 and 1990; Lichtenhuler and Rinderle, 1988), appears to be one possibility. Therefore the possible development of active airborne fluorescence LIDAR sensors for future remote sensing of the state of health of plants is matter of present research approaches in several laboratories. The extension of such measurements to the simultaneous sensing of the only more recently detected blue fluorescence of plants may provide more detailed fluorescence information on the physiological state of plants.

**Fig. 1 - Reflectance spectra (a) and second derivative (b) of two-year-old spruce needles (needle year 1983) of Picea abies Karst. Green needles of a healthy tree ( ) and yellowish-green needles of a damaged spruce (.....) with forest- decline symptoms (damage class: 3 - 4, 70 % needle loss). The blue shift of the inflection point at the red edge (a) is also seen in the second derivative (b) as points of intersection (arrows).**

### 1. THE LASER-INDUCED RED CHLOROPHYLL FLUORESCENCE (LIRF)

The red *in vivo* chlorophyll fluorescence (RF) of intact leaves was first observed by Miller, 1874. 60 years ago Kautsky described the chlorophyll fluorescence in more details (Kautsky and Hirsch, 1931) and showed that it is inversely correlated to the photosynthetic activity of plants (Kautsky and Franck, 1943; Kautsky et al., 1960). This was later confirmed by many authors using more sophisticated equipments for measuring CO₂ assimilation and chlorophyll fluorescence (Franck et al., 1969; Papageorgiou, 1975; Krause and Weis, 1984; Lichtenhuler et al., 1986; Walker, 1988). Kautsky, whose 100th
anniversary we commemorate 1991 (he was born on April 13th, 1891 in Vienna), gave decisive impulses to photosynthesis research, which influences our research even today.

Under optimum conditions for photosynthesis the largest part of the light energy absorbed by the photosynthetic pigments (chlorophyll, carotenoids) is used for photochemical quantum conversion to drive plant photosynthesis i.e. the fixation and conversion of inorganic CO₂ into sugars and biomass. Smaller proportions of the absorbed light energy E are re-emitted either as heat (ca. 15 - 20 %) or as red chlorophyll fluorescence (1 - 3%). The exact amounts of energy distribution are difficult to estimate and can vary considerably according to stage of development and stress conditions. This general relationship is given by the equation:

\[ E_{\text{absorbed}} = E_{\text{photochemistry}} + E_{\text{heat}} + E_{\text{fluorescence}} \]

When the process of photosynthetic quantum conversion is reduced due to environmental stress or general senescence, the de-excitation of absorbed light energy via heat and fluorescence emission increases. In contrast to heat emission, which is only detectable by special techniques such as photoacoustic spectroscopy (Buschmann et al., 1984; Nagel et al., 1987), the chlorophyll fluorescence is easy to measure (Krause and Weis 1984; Lichtenhalter et al., 1986). A higher emission of red chlorophyll fluorescence RF occurs not only under stress conditions, but also when predarkened leaves are illuminated. RF rises very fast via the ground fluorescence to a maximum level at within 100 to 500 ms and then - with the onset of photosynthetic quantum conversion - declines to a steady-state value. This transient in the chlorophyll-fluorescence emission (variable fluorescence) is known as Kautsky effect and as chlorophyll fluorescence induction kinetics. An example of the induction kinetics measured at two different wavelengths is shown in Fig. 2.

Variable chlorophyll fluorescence (vF or vRF) is only detectable in photosynthetically active plant tissue, but not when the photosynthetic light reactions are blocked e.g. by the herbicide dioxon (Lichtenhalter and Rinderle, 1988). In fact, the larger the decrease of RF from fm to the steady-state level fs and the lower the steady-state level fs, the higher the photosynthetic activity of the illuminated leaf part. This can be quantified by the chlorophyll fluorescence decrease ratio Rfd, introduced by Lichtenhalter, which is a vitality index of leaves and plants (Lichtenhalter and Rinderle, 1988):

\[ Rfd = \frac{fd}{fs} = \frac{(fm-fs)}{fs} \]

High Rfd-values of 3 to 5 indicate high photosynthetic capacity, whereas values below 1.5 point to low photosynthetic rates and damage to the photosynthetic apparatus.

![Excitation: continuous He/Ne-laser (632.8 nm)](image)

**Excitation: continuous He/Ne-laser (632.8 nm)**

Aurea leaf of tobacco (Su/su)

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**Fig. 2 - Chlorophyll-fluorescence-induction kinetics (Kautsky effect) of an aurea leaf of tobacco measured with the OMA III LIDAR fluorosensor (cw He/Ne laser: 632.8 nm, 10 mW). The fast fluorescence rise to fm is not resolved in this case. The ratio of the fluorescence decrease fd to the steady-state fluorescence fs (Rfd = fd/fs) is a good measure of the photosynthetic capacity of the illuminated leaf (s. Lichtenhalter and Rinderle, 1988).**

### 1.1 The chlorophyll fluorescence emission spectra

The red chlorophyll-fluorescence-emission spectra are characterized by two maxima in the 690 and 735 nm regions (French, 1960; Lichtenhalter et al., 1986; Lichtenhalter and Rinderle, 1988) as shown in Fig. 3. Due to the fact that the shorter fluorescence-emission band near 690 nm overlaps with the in vivo absorption bands of chlorophylls, the relative intensity of the fluorescence maximum near 690 nm decreases with increasing chlorophyll content of the leaf, as is shown in Fig. 4. Consequently the chlorophyll-fluorescence ratio F690/F735 decreases with increasing chlorophyll content. This had independently been observed in an investigation of the chlorophyll content of nitrogen-deficient plants (Kochubey et al., 1986), during forest decline research.
(Lichtenthaler et al., 1986) and during autumnal chlorophyll breakdown (Lichtenthaler, 1987b).

In fact the ratio F690/F735 is inversely correlated to the chlorophyll content (curvilinear relationship) and can be used as a non-destructive determination method of the chlorophyll content (Hak et al., 1990; Lichtenthaler et al., 1990a). It can also be applied to detect short-term and long-term stress effects on plants. Under short-term and low stress conditions photosynthesis declines, without yet affecting the chlorophyll content, and the ratio F690/F735 rises by about 20 to 30%. Under long-term stress conditions photosynthesis may in part recover (due to adaptation and regeneration mechanisms), whereas the chlorophyll content declines and is maintained at a much lower level. This then causes a 1.5 to 4-fold increase in the fluorescence ratio F690/F735 (Rinderle and Lichtenthaler, 1988). Therefore the chlorophyll-fluorescence ratio F690/F735 appears to be a very suitable stress indicator (Lichtenthaler and Rinderle, 1988; Rinderle and Lichtenthaler, 1988), which can also be applied in stress detection of terrestrial vegetation via remote sensing (Lichtenthaler, 1990).

A European research initiative with several laboratories from different countries has been established (EUREKA projekt No. 380 LASFLEUR) to investigate the possibilities for remote sensing of plants on the basis of fluorescence ratio F690/F735 (registration of laser-induced chlorophyll-fluorescence spectra) and to develop an active fluorescence LIDAR (FLIDAR) system based on the ratio F690/F735. Some of the first results and approaches of this joint European research will be described on the occasion of the EARSeL workshop 1991 in Florence (LIDAR Remote Sensing of Land and Sea) in this contribution and also in the following papers of other research groups.

**Fig. 4 - Change in the shape of the chlorophyll-fluorescence-emission spectra with increasing chlorophyll content of beech leaves.** The shorter wavelength maximum near 690 nm is suppressed with increasing chlorophyll content due to a preferential reabsorption of the 690 nm fluorescence. 1. light green, 2. green and 3. dark green leaf. Excitation with blue light (470 30 nm) (from Lichtenthaler, 1988).

**1.2 Sensing of chlorophyll fluorescence and F690/F735 with a LIDAR system**

With respect to the induction and registration of complete chlorophyll-fluorescence-emission spectra we established a LIDAR fluorosensor equipped with an optical multi-channel analyzer (OMA III), which can sense either the red fluorescence spectra from 650 to 800 nm (induced by red or blue lasers) or alternatively the complete fluorescence-emission spectra of plants from 400 to 800 nm. In the latter case a pulsed UV-laser, 337 nm, is applied as excitation source. The principal components of this LIDAR system with the nitrogen laser are shown in Fig. 5. The OMA III LIDAR fluorosensor can be modified so that the UV-laser pumps a dye-laser which emits blue light (around 440 nm; using coumarins as blue light-emitting substances). A further modification is the application of a red cw He/Ne-laser (632.8 nm; 10 mW) as excitation source in place of the UV-laser or dye laser. Chlorophyll fluorescence can be excited by the blue dye laser or the red He/Ne-laser, the shape of the fluorescence spectra is,
Setup for measuring blue and red fluorescence spectra

Excitation: pulsed UV-laser (nitrogen laser)

Fig. 5 - OMA III LIDAR fluorosensor system applied to measure the complete blue fluorescence and the red chlorophyll-fluorescence-emission spectra of green plants as induced by a UV-laser. In the standard position the fluorosensor permits to sense the fluorescence signatures in a near distance (5 to 30 cm) to the target (plant), but can be rearranged also for larger distances (up to 6 m). Excitation is performed by a pulsed nitrogen laser (Laser Photonics UV-12; 337 nm; 10ns pulse length; 2.5mJ per pulse). The emitted blue and red fluorescence is sensed via a KV 380 cut-off filter by a polychromator diode array system and transferred to the optical multichannel analyzer (OMA III, EG & G). Standard pulse frequency 10 Hz, integration time 100 ms. In the case of excitation by blue light (pulsed dye laser 440 nm) or red light (cw He/Ne laser, 632.8 nm) a RG 665 cut-off filter was applied to exclude excitation light. The pulse amplifier permits gating, i.e. the measurement of the fluorescence signal of samples in the presence of white or sun light. The delay device compensates the time delay between the laser trigger signal and the laser light pulse (0.6 ls).

however, different. Blue light is readily absorbed by chlorophylls and carotenoids of the upper outer leaf half, whereas red light (632.8 nm) penetrates much deeper into the leaf layers. Consequently the red light-induced chlorophyll fluorescence near 690 nm undergoes a higher reabsorption process than the blue light-induced fluorescence, which is emitted more from the outer leaf regions. Hence the chlorophyll-fluorescence ratio F690/F735 of green leaves exhibits higher values at blue light excitation (F690/F735: 0.8 to 1.1) than at red light excitation (values of ca. 0.4 to 0.6) (Rinderle and Lichtenthaler, 1988; Theisen 1988). Even though the absolute values for the ratio F690/F735 are different at blue and red light excitation, the differences in F690/F735 due to short-term or long-term stress are evident in both cases.

1.3 Measurements with the OMA III LIDAR fluorosensor

The dependence of the fluorescence ratio F690/F735 on the chlorophyll content of leaves, as determined during the course of the autumnal chlorophyll breakdown in 1990, is shown in Fig. 6. The increase in F690/F735 with decreasing chlorophyll content was demonstrated by measurements with the diode array OMA III LIDAR fluorosensor system (pulsed dye laser: 440 nm) as well as using our portable two-wavelength-band chlorophyll fluorometer LITWAF (excitation: red light by a cw He/Ne laser, 632.8 nm). Although the absolute values for F690/F735 are different due to the differences in excitation light (blue and red), the same curvilinear correlation (according to a
power function $y = c x^d$ shows up with good values of $r^2 = 0.90$ in both cases.

In standard position of the diode array OMA III fluoroscope the plants are kept in a short distance of ca. 15 to 30 cm from the laser and the detector system. These short distance measurements allow the screening of many different leaf samples and stress conditions. By rearrangement of the instrumental components the fluorescence sensing distance of plant to polychromator can be enlarged up to 6 m or more. This makes it possible to test whether the differences in F690/F735 values as found in near distance measurements, between controls and stressed plants or leaves of high and low chlorophyll content, can also be sensed when the distance of the plant to the detector is larger. This knowledge is a basic requirement for the future remote sensing of terrestrial vegetation via an active LIDAR fluorosensor.

In the comparison of two tobacco varieties, a normal green variety su/su and a yellowish-green mutant Su/su, the differences in chlorophyll content could be detected by measuring complete chlorophyll fluorescence spectra and determination of the ratio F690/F735 at a near distance of 20 cm (Fig. 7). At high chlorophyll content (55.6 µg a+b cm$^{-2}$ leaf area) the ratio F690/F735 is much lower (0.6 green plant) than at low chlorophyll content (15.4 µg a+b cm$^{-2}$; F690/F735 of 1.7 in aurea plant) as shown in Table 1.

![Fagus sylvatica: autumal chlorophyll breakdown](image)

**Fig. 6** Dependence of the chlorophyll-fluorescence ratio F690/F735 on the chlorophyll content in beech leaves (Fagus sylvatica L.) during the autumnal chlorophyll breakdown. The fluorescence spectra and the ratio F690/F735 were determined using a) the OMA III LIDAR system with a pulsed dye laser (440 nm) as excitation light () and b) the two wavelength chlorophyll fluorometer LITWAF with a cw He/Ne laser (632.8 nm) as excitation source (). The curvilinear relationship, as demonstrated by good values of $r^2 = 0.9$ in both cases, is independent of the wavelength of the excitation light.

### Table 1. Intensity of chlorophyll fluorescence at 690 and 735 nm (5 nm), ratio F690/F735 as well as content of chlorophylls and carotenoids and pigment ratios (a/b and a+b/x+c) in leaves of a green and an aurea variety of tobacco (Nicotiana tabacum L.) in near (0.2m) and far distance measurements (2 and 6m). Excitation was performed with a He/Ne laser (see Fig. 7). Mean of 6 measurements with separate leaves from 2 intact tobacco plants. The differences in the fluorescence and pigment ratios and in the pigment content between green and aurea leaves are highly significant (P 0.005). The relative fluorescence intensity given is that of broad 10 nm bands at 690 and 735 nm (5 nm) measured in predarkened plants.

<table>
<thead>
<tr>
<th></th>
<th>F690</th>
<th>F735</th>
<th>F690/F735</th>
<th>a+b*</th>
<th>x+c*</th>
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<tr>
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<tr>
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<td>2 m</td>
<td>2207</td>
<td>3488</td>
<td>0.63</td>
<td>(1.3)</td>
<td>(0.3)</td>
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<tr>
<td>6 m</td>
<td>961</td>
<td>1102</td>
<td>0.87</td>
<td></td>
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<td><strong>aurea leaf</strong></td>
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* The content of pigments is given in lg cm$^{-2}$ leaf area, a/b = the ratio of chlorophyll a to chlorophyll b; a+b/x+c = the ratio of chlorophylls to carotenoids. The values in parentheses indicate the standard deviations.
fluorescence spectra and the ratios F690/F735 between the green and the aurea mutant was, however, maintained even at 6 m (Fig. 7 and Table 1). When adjusted for the same intensity of the excitation light, the ratios F690/F735 were the same for all three distances i.e. 0.61 ± 0.04 and 1.66 ± 0.07 for the predarkened green and aurea leaves, respectively. This observation demonstrates that the remote sensing of plants and of differences in chlorophyll content via registration of laser-induced chlorophyll fluorescence is quite possible. In a further experiment we could also remotely sense the differences in the F690/F735 between healthy and a damaged spruce which showed typical forest decline symptoms (e.g. lower needle and chlorophyll content), again the ratio F690/F735 was the same when measured in near (20 cm) and far distance (6 m).

1.4 Fluorescence LIDAR (FLIDAR) systems of other research and LASFLEUR groups

With a very preliminary airborne system the laser-induced chlorophyll fluorescence could be remotely sensed as two bands in the 690 and 735 nm region (Zimmermann and Günther, 1986). A different approach to remote sensing of complete chlorophyll-fluorescence spectra with a FLIDAR system from a ground platform is reported by Rosema et al., 1988. In both cases blue light (pulsed dye lasers) was applied as excitation source. Further details on the development and the characteristics of the FLIDAR system are found in Castagnoli et al., 1988, Cecchi et al., 1989. The newest development of this LASFLEUR research group is FLIDAR 3, which allows the registration of passive reflectance data and laser-induced chlorophyll-fluorescence spectra (Cecchi and Pantani, 1991). A further approach to the development of a fluorescence LIDAR system for measurements of chlorophyll fluorescence within the LASFLEUR research programme is given by Günther et al., 1991.

The general aim of the EUREKA research programme LASFLEUR is to assay the laser excited in vivo chlorophyll fluorescence as a possibility for a further remote sensing of the physiological state of terrestrial vegetation in combination with the more classical passive reflectance measurements. Although the chlorophyll-fluorescence ratio F690/F735 appears to be a very suitable parameter for remote sensing and description of the state of health of plants, parallel research is also devoted to investigating, whether the life-time and intensity of the chlorophyll fluorescence after a single laser shot (Hodges and Moya, 1986; Holzwarth, 1988) provides possibilities to detecting and eventually remotely sensing stress effects in plants. An additional fluorescence parameter which
will be included in the future LASFLEUR research is the blue fluorescence of plants. The latter, and its relative intensity compared with that of chlorophyll fluorescence, may open new possibilities for stress detection in plants, which seems to contain complementary information to the hitherto investigated chlorophyll fluorescence.

2. THE BLUE FLUORESCENCE EMISSION SPECTRA OF PLANTS

The blue fluorescence-emission spectra of green plants, first described by Chapelle et al., 1984, can be induced by UV-light of the wavelength region of ca. 260 to 390 nm (Theisen, 1988) using either a commercial spectrofluorometer or a pulsed UV-laser (nitrogen laser) with e.g. 337 nm as excitation light. The blue fluorescence BF is characterized by a broad maximum in the blue region near 450 nm and a second maximum/shoulder near 530 nm (which we have termed green fluorescence GF), as is shown for a beech leaf in Fig. 8. In addition to the blue-green fluorescence, UV-light also induces the red chlorophyll fluorescence RF with the two maxima at 690 and 735 nm.

The blue fluorescence BF of plants as induced by UV-B radiation was apparently already known in the last century, at least for some plant parts (see Förster, 1951) and re-detected only more recently by Chapelle et al. 1984. That the blue fluorescence is a genuine property and characteristic of green plants has been confirmed in the last 3 years by different research groups (Chapelle et al., 1990; Goulas et al., 1990; Lichtenthaler et al., 1990b; 1991a and b; Theisen, 1988). The origin and the physiological significance of the blue and green fluorescence of green plants is, however, not yet clarified (Lichtenthaler and Stober, 1990; Lichtenthaler et al., 1990b) and needs much further investigation.

2.1 The blue emitting plants substances

Candidates of the emitted blue and green fluorescence are diverse phenolic substances which are known to occur in plant tissue and which can differ in concentration and composition from plant species to plant species and which may primarily be associated with the vacuoles of the epidermal layers and partially also the cell walls. In a detailed study we could show that blue fluorescence with a maximum near 440 or 450 nm is emitted by the coumarins aesculetin and scopoletin, diverse cinnamic acids such as caffeic acid, sinapic acid, and ferulic acid, the depside chlorogenic acid and the tan compound (+)-catechin (Lang et al., 1991). Green fluorescence GF with a maximum near 530 nm is emitted e.g. by the ubiquitous quercetin and possibly other flavonoids and the alkaloid berberine, which shows up, however, only in few plants (Lang et al., 1991). Although riboflavin, NADPH and β-carotene are also discussed as candidates of the blue fluorescence emission of leaves (Chapelle et al., 1990), riboflavin and NADPH do not seem to contribute much to the blue fluorescence of leaves and purified β-carotene does not exhibit any blue fluorescence (Lichtenthaler and Stober, 1990; Lang et al., 1991).

The blue-green fluorescence emission is not only seen in green plant leaves but also during the autumnal chlorophyll breakdown. It seems to consist of at least two components, of which the blue fluorescence declines with autumnal leaf senescence, whereas the green fluorescence near 530 nm remains (Lang and Lichtenthaler, 1991). Removal of the epidermis from a tobacco leaf gave different blue-green fluorescence signals for the epidermis and the remaining leaf mesophyll (Lang et al., 1991). From all the data available hitherto, it appears that, in contrast to the red chlorophyll fluorescence, the blue-green fluorescence of plants is a mixed signal composed of the fluorescence emission of various blue and green fluorescing primarily secondary plant products, possibly including also some primary plant substances (riboflavin ?, NADPH?, phyllohydroquinone K1 ?).
Table 2. Fluorescence ratios of blue to green (BF/GF), blue to red (BF/RF) and chlorophyll fluorescence ratio F690/F735 in leaves of different plants (beech: Fagus sylvatica L., soybean: Glycine max, spruce: Picea abies). The content of total chlorophylls (a+b) and carotenoids (x+c) is given in lg cm⁻² leaf/needle area and also the ratio of green to yellow pigments (a+b)/(x+c). The fluorescence ratios are based on the fluorescence emitted in the bands of 10 nm width at 450, 530, 690 and 735 nm (5 nm). The data are based on 3 (beech, spruce) and 6 measurements (soybean) per plant and condition.

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<td>48</td>
<td>9</td>
<td>5.3</td>
</tr>
<tr>
<td>lower leaf side</td>
<td>1.8</td>
<td>0.7</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picea abies**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991 needles</td>
<td>1.3</td>
<td>0.9</td>
<td>1.6</td>
<td>19</td>
<td>5</td>
<td>3.8</td>
</tr>
<tr>
<td>1990 needles</td>
<td>1.7</td>
<td>19.4</td>
<td>0.9</td>
<td>64</td>
<td>12</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* The pigment content and ratios a+b/x+c are given for whole leaves, a differentiation between upper and lower leaf halves (beech, soybean) was not made.
** The differences in fluorescence ratios between upper and lower leaf sides (beech, soybean) and first-year and second-year needles are significant (P 0.01) except for the BF/GF ratio in beech.

2.2 Blue and green emission spectra of leaves

Blue and green fluorescence emission is not only found in the upper leaf sides but also on the lower sides of leaves (Fig. 8). The shape and intensity of the complete UV-light induced fluorescence emission spectra in the blue, green and red regions are quite different for the upper and lower leaf sides, not only in beech but also in soybean (Fig. 9). The higher fluorescence emission in all spectral regions of the lower leaf sides, which in C₃-plants contain less cells and lower amounts of chlorophylls and carotenoids, indicates that the emitted blue-green as well as the red chlorophyll fluorescence are partially reabsorbed by the photosynthetic pigments. That the blue-green and the red chlorophyll fluorescence of the lower leaf side do not, with respect to the upper leaf side, increase to the same degree (Figs. 8 and 9), is an indication that, besides reabsorption processes, differences in the content in blue-green fluorescing substances may also exist between upper and lower leaf half. The differences in the relative expression of the blue (BF), green (GF) and red fluorescence (RF) emission between two plant species and between the upper and lower leaf sides (Figs. 1 and 2) can be quantified by forming the ratios of blue to green fluorescence (BF/GF) and blue to red fluorescence (BF/RF), whereby the ratios F450/F530 and F450/F690 are formed (Table 2).

Whereas BF of green plants is mostly higher than GF, the ratio BF to RF appears to be very variable between different plant species and also between upper and lower leaf sides (Lang et al., 1991). In soybean the BF of the upper leaf side is higher than RF, but this is opposite in the lower leaf side. In beech leaves the fluorescence ratio BF/RF is about 1 in the upper leaf side, but 1.7 in the lower leaf side. That the fluorescence ratios BF/GF and BF/RF might be used for plant species identification seems to be an exciting possibility, but needs much further investigation, before a final decision can be taken.

![Fig. 9 - Complete fluorescence-emission spectra of the upper and lower leaf side of a soybean leaf (Glycine max L.) as obtained with the OMA III LIDAR fluorosensor system (excitation: 337 nm, pulsed nitrogen laser). Concerning fluorescence ratios BF/GF and BF/RF see Table 2.](image-url)
2.3 Blue and red fluorescence emission spectra of spruce

In the youngest needles of this year 1991 (new sprouts induced in spruces kept in the greenhouse) BF and GF can be seen which are lower than the chlorophyll fluorescence (Fig. 10), as also seen in the ratio BF/RF in Table 2. The chlorophyll fluorescence ratio F690/F735 is high (value of 1.6) since these young needles are not yet fully greened, as is also indicated by a lower value of the ratio green to yellow pigments a/b/x/c of 3.8. In the dark green, one-year-old spruce needles of 1990, in turn, the intensity of the laser UV-laser induced BF and GF emission is increased as compared to the young needles of 1991, whereas the chlorophyll fluorescence unexpectedly decreased very much and is hardly detectable (Fig. 10). Consequently the ratio BF/GF exhibits very high values in older spruce needles (Tab. 2). When blue light (dye laser) or red light (He/Ne-laser) are applied, the older spruce needles, however, exhibit a red chlorophyll fluorescence 4 to 6 times higher than at UV-laser (337 nm) excitation. This indicates that UV-B radiation light of 337 nm is almost fully absorbed in the epidermis layer, presumably by particular plant phenolics which exhibit broad absorption bands in this UV-B region. Very little UV-B radiation seems to reach the chlorophylls in the subepidermal mesophyll layers of the one year old needles which then results only in a weak chlorophyll fluorescence. In contrast, blue and red excitation light are not absorbed in the chlorophyll-free epidermis; these visible light qualities pass the epidermis, reach the chlorophylls in the mesophyll cells unhindered and there induce the typical chlorophyll-fluorescence spectrum. Hitherto spruce needles are the only example where little of the exciting UV-laser light penetrates through the epidermis. Other conifer needles will be tested. In leaves of mono- and dicotyledonous plants this effect has not yet been observed.

2.4 Blue fluorescence as a stress indicator?

Of the variation of the BF and GF emission during development and under environmental stress very little is known. It appears that changes in the BF intensity and in the ratio BF/GF as well as blue to red fluorescence BF/GF might be a very powerful complementary indicator of stress conditions in plants, in addition to the hitherto applied chlorophyll-fluorescence ratio F690/F735. This topic, however, needs a careful detailed study with different plants and stress conditions to eventually establish it as a complementary stress indicator. In a preliminary investigation we could show that emission of BF and GF increased a) in Douglas fir (Pseudotsuga menziesii) with increasing age of needles, b) in Platanus leaves during autumnal senescence and c) pine needles (Pinus sylvestris) during water stress (Lichtenthaler et al., 1991 b). When the increase of BF and of the ratio BF/RF under stress conditions can be further confirmed, the measuring of blue fluorescence and of the ratio BF/RF can and will be integrated in the LIDAR fluorosensors for remote sensing of terrestrial vegetation. Simultaneous registration of the blue and red plant fluorescence (ratios BF/RF and F690/F735), together with sensing of the reflectance between two laser shots, will open interesting and very promising possibilities for remote sensing of the state of health of terrestrial vegetation.

3. THE FRAUNHOFER LINE DISCRIMINATOR FLD

3.1 The Fraunhofer lines

The incoming solar radiation (continuous sun spectrum) contains a large number of dark lines, which were detected in 1814 by Fraunhofer and are caused by absorption of particular wavelength bands by various gases on the sun’s surface. The major Fraunhofer lines in the visible region range from 397 to 761 nm (Table 1). These dark lines are very narrow and are much reduced in intensity as compared with the intensity of the continuum. The 486.1 nm band is the H-β absorption line, the 589.0 band the Na-D2 line and the 656.3 nm band the H-α absorption line etc.
The major Fraunhofer lines in the visible region and near infra-red are:

<table>
<thead>
<tr>
<th>colour region</th>
<th>band</th>
<th>position (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>violet</td>
<td>H</td>
<td>396.8</td>
</tr>
<tr>
<td>blue</td>
<td>G</td>
<td>430.8</td>
</tr>
<tr>
<td>blue</td>
<td>I</td>
<td>486.1</td>
</tr>
<tr>
<td>green</td>
<td>E</td>
<td>527.0</td>
</tr>
<tr>
<td>yellow</td>
<td>D</td>
<td>589.0</td>
</tr>
<tr>
<td>red</td>
<td>C</td>
<td>656.3</td>
</tr>
<tr>
<td>red</td>
<td>B</td>
<td>686.7</td>
</tr>
<tr>
<td>infra-red</td>
<td>A</td>
<td>760.8</td>
</tr>
</tbody>
</table>

3.2 Fraunhofer lines and fluorescence

Solar light reflected from plant material will also show the Fraunhofer lines. In many targets, however, which are exposed to the sun, the Fraunhofer lines are partially filled by radiation. This increased radiation at the central wavelength of Fraunhofer lines can be attributed to fluorescence emission of the sun-exposed target. Fluorescence derives from originally absorbed light quanta which are re-emitted as fluorescence light, which exhibits a longer wavelength than the absorbed exciting light. Hence the infilling of particular Fraunhofer lines contains information on the fluorescence characteristics of a target. Some minerals will have a stronger fluorescence than others. This as well as the particular position of the emitted fluorescence will show up in the Fraunhofer lines and can in principle be used to identify minerals, soil types etc. This methods can also be developed and applied to the remote sensing of soils and soil composition (Plascyk, 1975; Placyk and Gabriel, 1975).

Green plants exposed to solar radiation also reflect sunlight. The amount being reflected largely depends upon the surface of plant leaves (rough, smooth, type and structure of waxes) as well as on the absorption characteristics of the leaves. Green leaves contain in their mesophyll the photosynthetic pigments. The green chlorophylls strongly absorb in the blue-green and red spectral region and the yellow carotenoids solely in the blue-green region (Lichtenthaler, 1987a). Light absorbed by the photosynthetic pigments is transferred to chlorophyll a and used either for photosynthesis or re-emitted as heat or as red chlorophyll fluorescence with fluorescence maxima near 690 and 735 nm. The red chlorophyll fluorescence which starts near 640 nm should not only be seen in the red Fraunhofer lines at 656.3 and 687 nm but possibly also in the line 761 nm. Plants also possess additional pigments e.g. the faintly yellow flavonoids and other colour-less-phenol-type compounds which absorb in the blue and/or UV-B spectral region. Some of these compounds, which seem to be primarily located in the epidermis of leaves, exhibit a blue and/or green fluorescence (Lichtenthaler et al., 1991a and b) and cause the blue-green fluorescence-emission spectrum of plant leaves which possesses maxima near 450 and 530 nm (Lang et al., 1991). This blue-green fluorescence, as induced by solar radiation, which is different from plant species to plant species, should show up in the Fraunhofer lines between 397 and 527 nm. It is certainly of interest to check whether the solar-radiation-induced blue-green fluorescence can remotely be sensed via the Fraunhofer lines. In the case of the red chlorophyll fluorescence there was a successful approach using a particular set-up termed the Fraunhofer line discriminator or Fraunhofer luminescence detector.

3.3 Application of the Fraunhofer line discriminator

The device called the Fraunhofer line discriminator FLD was built by the U.S. Geological Survey in order to identify ore deposits, industrial wastes, contamination of water systems by fluorescent materials (Plascyk and Gabriel, 1975). The sun is used as excitation source. Variations in solar radiation are eliminated by taking a ratio of the radiance in the line center to that of the continuum next to the Fraunhofer line. Three lines are selectable with the FLD 486.1, 589.0 and 656.3 nm. The 656.3 nm line has successfully been applied to measure chlorophyll fluorescence. In non-irrigated lemon trees, which showed water-stress symptoms, the chlorophyll fluorescence in the 656.3 line was increased as compared with the irrigated trees (Mc Farlane et al., 1980). This increase in chlorophyll fluorescence was expected, since the photosynthetic quantum conversion declines under water-stress conditions, whereas the fluorescence emission of the absorbed light energy increases (Lichtenthaler and Rinderle, 1988).

The Fraunhofer line-depth principle was also applied more recently to measure the Kautsky effect i.e. the chlorophyll-fluorescence-induction kinetics as induced in predarkened leaves by exposure to sun light (Carter et al., 1990). The principles of the detection are measurements in a broad and narrow band at 656.28 ± 0.50 nm (=Fraunhofer line + adjacent continuum) and in the line centre at 656.28 ± 0.35 nm. Two measurements each at the broad and narrow band are made of solar irradiance and radiance from the fluorescing object (plant) and a fluorescence factor F is calculated from these measurements. If the
target were non-fluorescent, the target reflectivity would be about equal in both measurements. When fluorescence occurs in the Fraunhofer waveband, the factor F increases.

CONCLUSION

A detailed investigation and understanding of the various kinds of the laser-induced fluorescence signatures in the blue, green and red spectral region will open new ways for a better judgement of the state of health of plants via active remote sensing using fluorescence LIDAR systems. The fluorescence ratios blue to green (BF/GF), blue to red (BF/RF) and the chlorophyll-fluorescence ratio F690/F735 will be essential elements in the stress detection of terrestrial vegetation by active florosensors. The future remote sensing of the various fluorescence signatures and ratios is seen as a complementary information to the hitherto applied reflectance spectroscopy. From the present state of knowledge it appears feasible that the sensors to be developed should possess the ability to passively and actively sense reflectance and fluorescence, respectively.

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