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EVALUATION OF PLANTS BY CHLOROPHYLL FLUORESCENCE AND OTHER SPECTROSCOPIC METHODS

R. Koepp[1], *H.J. Hellebrand*[2]

[1]Humboldt-Universität zu Berlin, Fachbereich Physik, Invalidenstr. 110, D-10115 Berlin, Germany
[2]Institut für Agrartechnik Bornim, Max-Eyth-Allee 1, D-14469 Potsdam, Germany

A b s t r a c t. Spectroscopic methods may be used for compositional analysis of plant materials or for the evaluation of properties of plants such as internal structure, metabolic activity or degree of damage. The state of plants very often is indicated by changes in the functioning of the photosynthetic apparatus (PSA) and, therefore, can be studied by measurements of the chlorophyll fluorescence yield (CFY).

A wide field of application of CFY and other spectroscopic methods is the determination of stress response of different plant species and cultivars. These methods were applied to monitor the plant reactions on environmental stress factors like: air pollution (SO_2, O_3, NO_x, NH_3 and other nitrogen compounds), - heavy metals (Pb, Cd,...) and other soil and water pollution, frost and unfavourable low temperature, high temperature and drought, radiation of high quantum energy (UV, X, and gamma rays), strong visible light and, of course, the combination of these effects, since the changes in the environmental conditions due to global problems (pollution, 'greenhouse effect', 'ozone hole') influence the state of plants.

K e y w o r d s: chlorophyll fluorescence, spectroscopic methods, environmental stress factors

INTRODUCTION

From the sowing up to the harvest the farmer is interested in the development of cultivated plants. The state of plant is defined by the developmental stage and the influence of different environmental factors. Especially the effects of environmental factors like frost, heat, drought, strong light, air pollution and diseases are of interest. At the end of the vegetation period the estimation of the degree of ripeness is important for the determination of the time of harvesting.

In the pre-industrial time the farmer gets most of the necessary information going over the crop field and looking at the crops. He seldom needed his other senses. In the same manner the housewife on the fruit marked tests the quality of fruits and legumes like ripeness, damage or rottenness mainly optically. Almost all information they got was optical one; smelling, tasting, feeling, and hearing is much less important.

In recent times, where the environment is more and more influenced and changed by mankind, the measurement of natural and anthropogenetic factors and its effects on plants play an increasing role and objective methods for the determination of the state of plants are necessary. In many research fields optical methods of investigation are highly important. The models of the atomic structure and the structure of solids based mainly on information got by optical methods. In the modern biosciences optical and other spectroscopic investigations are valuable tools. For the development of the model of the photosynthetic apparatus (PSA), especially the characterization of different pigments, optical and electron spin resonance (ESR) spectroscopy was used [47].

It was shown by Björkman and Demmig [4] for 44 plant species that the CFY is correlated with the quantum yield for oxygen evolution and is - with small deviations - the same for all vascular plants tested. The relation between fluorescence parameters and photosynthetic ozone evolution was investigated in many other papers [e.g., 17,46]. In this paper mainly results of experimental investigations of stress effects on plants with CFY and ESR methods were given. Other optical and spectroscopic methods like absorption, scattering and reflection of light, fluorescence of other (than chlorophyll) pigments and other characteristic plant compounds, acoustooptical spectroscopy and ultraweak luminescence were mentioned in connection with their application in plant evaluation. Fields of further applications were proposed.

DETECTION OF AIR POLLUTION EFFECTS ON GREEN PLANTS BY CFY AND ESR MEASUREMENTS

The most applied physical methods for the detection of damage in green plants are based upon measurements of the chlorophyll fluorescence induction curve. Complicated techniques are necessary for short time measurements of the increase or decrease of chlorophyll fluorescence after switching on and off of the exciting light, respectively [e.g., 8,43,50,52,56]. Chlorophyll fluorescence methods were often used parallel to gas exchange measurements [e.g., 6, 14, 20, 33,39,46] to detect inhibition of photosynthesis. Schmidt *et al.* [52] investigated the effects of air pollutants like SO_2 and NO_2 by a comparison of chlorophyll fluorescence (PS II) and P700 absorbance changes (PS I).

Sulphur dioxide and ozone

An example for a device for chlorophyll fluorescence measurements is sketched in Fig. 1 (for further details see 35). It is possible to measure simultaneously the prompt and the millisecond-delayed fluorescence. After exposition to air containing *ca.* 0.3 ppm sulphur dioxide (8 h/d during daytime) the ratio of the maximum fluorescence to the fluorescence after 3 min, $Rfd=F_m/F_{3\,min}$, decreases from 2.5 to 2 and from 4 to 3 for the prompt and delayed fluorescence, respectively [25]. Sudden increase of the ozone concentration in the ambient air in the concentration range 0.1 to 0.5 ppm causes rapid decrease of the *Rfd* value.

The effect of sulphur dioxide on the photosynthesis could be followed with ESR-measurements using the experimental arrangement sketched in Fig. 2. The characteristic

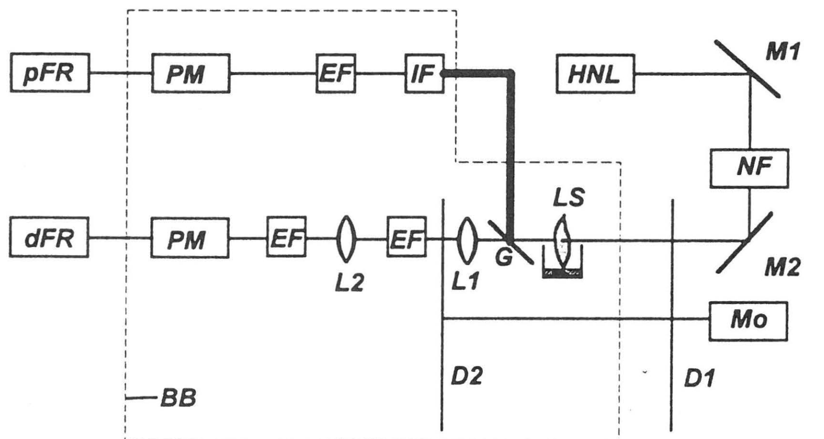

Fig. 1. Apparatus for simultaneous registration of prompt and the ms delayed fluorescence in two independent channels, pFR and dFR, respectively. The light source is a He-Ne laser with the wavelength 632.8 nm. The leaf sample is positioned between the two rotating discs D of the chopper. The half permeable mirror G reflects partly the (prompt) fluorescence light into a fibre optics. The delayed fluorescence passed the second disc 3-7 ms after the excitation. All measurements were done at room temperature.

6-line spectrum of Mn^{2+} increased by a factor of up to 10 after sulphur dioxide treatment and up to 8 by ozone treatment [29]. A treatment with both gases applying only half the concentration of each gas increases the Mn^{2+} signal only by a factor of 6, indicating that both effects do not superpose additively, but weaker. Ozone seems to weaken the effect of sulphur dioxide. This is in contradiction to Smith [57], who observed that the effects caused by sulphur dioxide in most cases increased by additional ozone,

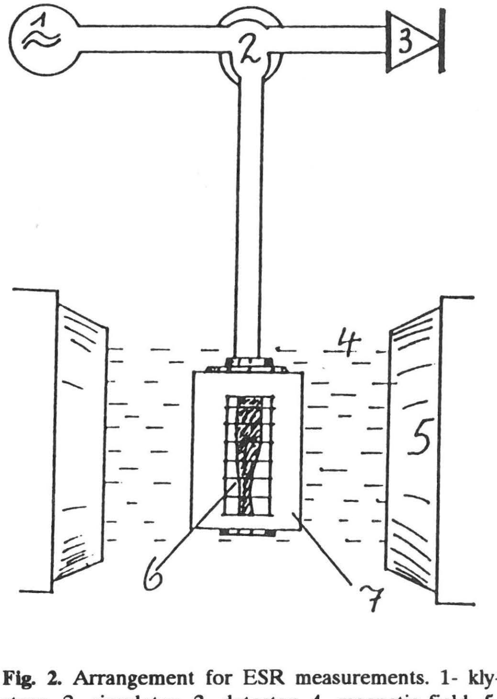

Fig. 2. Arrangement for ESR measurements. 1- klystron, 2- circulator, 3- detector, 4- magnetic field, 5- magnetic pole toe, 6- leaf in the quartz tube, 7- resonator with irradiation window. For measurements of the ESR signals of Mn^{2+} or of the PSA a Varian E3 (X band 9.5 GHz) spectrometer was used. Leaf or needle sections of about 2 cm were attached to a flat quartz glass rod or into a quartz glass tube and positioned in the resonator of the spectrometer. Normally measurements were done at a microwave power of 20 mW at room temperature. Illumination: 20-100 mW/cm^2, white light.

but in few cases decreased.

From the observation that the signal is much higher in the top region of the needles than in the basic region it may be concluded that the top region is more damaged. This corresponds well to the fact that needles start discolouring and dying at the top.

Sulphur dioxide and other air pollution effects could be followed, too, measuring the ESR signals of the photosynthetic apparatus PSA (at g values of 2.0026, 2.0046 and 2.014) in the dark and under illumination [29,30,36,59,60].

Brecht and Schulz [9] compared 8 different methods of optical spectroscopy for early detection of the damage of conifers by sulphur dioxide. They found that the *in vivo* measurements of chlorophyll fluorescence gave as good results as the methods where homogenization and biochemical separation of special components are necessary.

Koepp [24] compared the effect of sulphur dioxide on *Festuca arundinacea* Schreb. of different degree of ploidity on the chlorophyll fluorescence and the CO_2 assimilation (compare Fig. 3). The inhibition of both, the *Rfd* and the carbon dioxide uptake, was of the same degree. After six weeks of sulphur dioxide treatment the heat sensitivity [28] and the frost sensitivity [37] increased.

Nitrogen compounds

After prolonged immission of nitrogen compounds into the forest a tree dieback is observed. Measurements of the chlorophyll fluorescence yield using a PAM fluorometer (Walz, Effeltrich, Germany) developed and described by Schreiber *et al.* [54,55] give only weak lowering in the region of high nitrogen input, compared with control sites [27,34].

Under high light intensities (e.g., normal sunlight in the summer time) the variable chlorophyll fluorescence decreased much stronger for pine and beech trees from areas with high ammonium immission than from control areas [31].

Koepp *et al.* [27] investigated the frost resistivity of the needles of *Pinus sylvestris* L. from areas of high ammonium deposition by CFY measurements and found increased frost tolerance of the last year needles and decreased frost tolerance of current year needles.

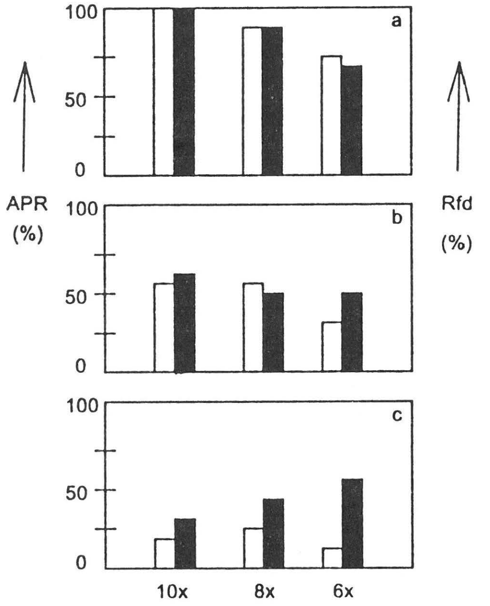

Fig. 3. Net photosynthetic rate (left columns) and Rfd values of chlorophyll fluorescence of Festuca leaves of different ploidity (indicated at the bottom): a - control, b - 0.5 h sulphite treatment, c - 1 h sulphite treatment (feeding of the detached leaves with a 10 mM Na_2SO_4 solution).

Ionizing and ultraviolet radiation, photoinhibition

Irradiation of seeds with gamma or X-rays causes changes in the development of plants. High radiation doses cause inhibition of growth and development, but low doses were often reported to have stimulating effects. The mechanisms of these effects are not well known. Kehrberg [22,23] investigated the fluorescence spectrum of gamma irradiated cucumber seeds using argon laser excitation and optical multichannel analyser (OMA II). They found in the envelope of the irradiated seeds (0.25 Gy Co^{60} gamma radia- tion) a 6 % enhanced blue fluorescence (400-480 nm), whereas in the inner part of the seeds the fluorescence in this range was 40 % lower. The same fluorescence decrease was observed after irradiation with laser light of the wavelength 337.1 nm. The results were interpreted as indication of oxidation of pyridin nucleotides by ionizing radiation.

Bornman and Vogelmann [7] measured the light absorption and scattering in leaves of *Brassica campestris* L., *Brassica carinata* L. and *Medicago sativa* L. grown under enhanced UV-B radiation with fibre optics. They found enhanced absorption and higher light scattering. CFY measurements give a decline for the plants grown under enhanced UV irradiation.

Cen and Bornman [12] found by chlorophyll fluorescence and gas exchange measurements that the effect of UV-B radiation depends on the background irradiance with visible light. At low irradiances with visible light there is a UV inhibition of photosynthesis, indicated by decline of variable fluorescence.

Hansen [20] measured the adaptation of elder spruce trees in a mountain forest to natural high light conditions by chlorophyll induction measurements. Bad adaptation indicates forest damage. Oberhuber and Bauer [44] found by CFY measurements that the reduction in potential photosynthetic yield in winter may be up to three times greater in ivy leaves subjected to increasing light levels.

Heavy metals

Treatment of *Festuca* leaves with lead via the transpiration way increases the ESR signals of Mn^{2+} and of the photosynthetic apparatus. The *Rfd* value of chlorophyll fluorescence decreases. After 2 days of treatment the leaves are yellow-brown but the turgor of the leaves is still normal after 8 days [36]. Lühmann and Kreeb [41] investigated combination effects of different toxic materials and proposed a mathematical model for the evaluation. Dubé and Bornman [15] observed in spruce plants exposed to UV-B radiation and cadmium the kinetics of chlorophyll fluorescence changed: the second maximum decreased. This was not observed by UV-B or Cd treatment alone. In the chlorophyll fluorescence spectrum the maximum at the longer wavelength (735 nm) increased by UV-B or Cd treatment but decreases by combination of both.

Heat, frost, chilling and drought stresses

Clement and van Hasselt [13] compared three different methods for the determination of frost hardiness in winter wheat and concluded that the frost hardiness can be determined by CFY measurements. Koepp *et al.* investigated heat sensitivity [28,35] and frost sensitivity [27,37] in different plant species by CFY measurements. A new method to investigate heat treatment effects was proposed by Koepp [26]. Olszewski and Herzog [45] made a frost resistance screening for many different cultivars of faba beans. Koepp [38] found that the inhibition of the electron transport rate or the fluorescence quenching are usable indicators of frost damage in faba beans.

Bauer *et al.* [3] investigated photosynthesis after freezing stress with gas exchange and chlorophyll fluorescence methods in plants with various degrees of freezing tolerance after different pretreatments and under different light conditions.

The temperature dependence of chlorophyll fluorescence and photosynthesis of thermophilic plants like cucumber and tomato was investigated by Janssen [21]. In the temperature region between 30 and 0 °C breakpoints were found in the fluorescence-temperature curve, dependent from the species and the cultivation conditions and characterizing the chilling sensitivity [21]. The ratio of laser excited fluorescence at 440 nm to that at 690 nm, F440/F690, increases significantly when the drought stress increases and this ratio increases again after watering the plants [19].

Water quality, plant diseases

Gerhardt and Putzger [18] developed an on-line biomonitor for the water quality monitoring. The principle based on the delayed fluorescence of algae. Panagopoulos [48] investigated the development of the *Cercospora* leaf spot disease in sugar beet plants using ultraweak luminescence to monitor the damage.

OBSERVATION OF THE DEVELOPMENT OF PLANTS AND PLANT CANOPIES BY OPTICAL METHODS

Pigment formation during greening

Stahl [58] measured the absorption and fluorescence spectra of etiolated wheat leaves during greening. The whole process from the beginning of greening (0.5 s after onset of illumination) up to the complete green leaf (300 min) was analysed by taking the whole absorption and fluorescence spectra in desired time distances. From the spectra the time-dependent quantities of the different pigments and their role in the PSA could be determined. Two phases of pigment formation in greening etiolated leaves were distinguished: in the first phase (*ca.* 2 h) mainly occur qualitative transformations between pigments and in the second phase the quantity of the main pigment, the chlorophyll, is accumulated.

Macyi and Stahl [42] compared absorption and remission spectra of the same spot of a maize leaf and they found that both types of spectra contain the information on physiological processes in plants. Elling and Knoppik [16] measured the reflection of green (healthy), yellow (damaged) and red-brown (severe damaged) spruce twigs. The main differences were observed in the spectral region between 530 and 730 nm.

Airborne measuring systems, evaluation of plant canopies

The reflectance and fluorescence spectra of green leaves, were measured by many authors under different conditions, mainly in the laboratory, but under outdoor conditions, too [e.g., 1,2,5,11,40,49,61]. Buschmann and Nagel [10] developed a new type of spectrometer (VIRAF=Visible Infrared Reflectance Absorption Fluorescence) for measurements in the spectral region 400-800 nm. For the leaf samples investigated the fluorescence induction could be measured without changing the position. The VIRAF spectrometer allows the determination of the physiological state of the leaves which need not to be detached from the plants.

Ammon and Schneider [2] measured the reflectance of a barley field in the spectral region from 400-2400 nm with a detector system positioned 10 m above the canopy. The spectral characteristics of vegetation are:

- -chlorophyll bands and green peak in the visible region,
- -increase of reflectance in the red/near infrared (700-1200 nm),
- -the water absorption spectra in the near infrared (1200-2000 nm).

The reflectance spectra for different field parts (different in soil quality) were compared with data taken at the ground (population density, stage of growth, height of growth, leaf area index, straw and grain yield). The variation between sites of different soil quality can be seen during the whole vegetation period. Lüdeker *et al.* [40] used laser induced fluorescence for the indication of vegetation stress.

Günther *et al.* [19], reported the possibilities of remote sensing of the vegetation status by laser induced fluorescence. The light source was a Nd:YAG laser with tripled emission wavelength 355 nm. The pulse length was, e.g., 6 ns, the energy 35 mJ. For the fluorescence detection a multichannel analyser (1024 channels) was used, but it was concluded that it is sufficient to monitor only the red chlorophyll fluorescence and the blue fluorescence from other leaf pigments in four channels.

CONCLUSIONS

1. Spectroscopic methods may be used for compositional analysis of plant materials or for the evaluation of properties of plants such as internal structure, metabolic activity or degree of damage.

2. The state of plants very often is indicated by changes in the functioning of the photosynthetic apparatus (PSA) and, therefore, can be studied by measurements of the chlorophyll fluoerscene yield (CFY).

3. A wide field of application of CFY and other spectroscopic methods is the determination of stress response of different plant species and cultivars.

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