Spectral and Temporal Analysis of Laser Induced Fluorescence Signatures of Heat Treated Tobacco Leaves

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ABSTRACT

This article describes spectral - and temporal resolved fluorscence signatures of tobacco plants under different temperatures. Laboratory measurements, performed with the JRC time resolved laser fluorosensor equipped with a streak camera system, in the temperature range from 20°C to 45°C will be presented. After UV excitation the fluorescence is characterized by a broad emission band in the blue-green region of the spectrum (400 nm - 60 nm) and the well known chlorophyll fluorescence in the region of 650 nm to 800 nm. An attempt has been made to analyze the temporal behaviour of each emission peak as well as to compare different fluorescence ratios in view of a possible application for a remote screening of the state of vegetation.

INTRODUCTION

Because of the sensitive, rapid and non-destructive nature of the two different chlorophyll fluorescence techniques (fluorescence lifetime determinations and fluorescence emission ratio) a possible application in the remote detection of vegetation stress is under discussion. Up to now these methods have been used in laboratory in basic research to understand the complex organisation and functioning of the photosynthetic apparatus as well as in applied research for vegetation stress detection (Lichtenthaler and Riderle, 1988). Within the frame of the EUREKA - project LASFLEUR (EU 380) the Institute for Remote Sensing Applications of the Joint Research Center is investigating the possible use of the Fluorescence lifetime as a parameter for remote detection of plant stress. Laboratory studies on green algae and isolated pigment-protein complexes in the picosecond time range have been used to describe the energy transfer processes and the functional organisation of the photosynthetic apparatus (Moya et al, 1980).
About the first lifetime studies on the in-vivo plant system in combination with stress effects was reported in 1986. Green needles with intact photosynthesis were characterized by lifetime components of 0.1 ns and 0.5 ns. After exposition of these needles to ozone the second lifetime component increased and a third component of 2.5 ns appeared (Schneckenburger and Frenz, 1986). Fluorescence lifetime measurements on spinach leaves with and without the PS II herbicide DCMU showed that a blocking of the overall fluorescence decay (Schmuck et al., 1990). Therefore the authors proposed to use the average lifetime as a simple indicator of vegetation stress.
Beside the known fluorescence emission of the chlorophylls a fluorescence in the blue and green region appears after UV - excitation (Duysens and Amesz, 1957 - Olson and Amesz, 1960). Recent work has demonstrated that the ratio of the red to blue-green fluorescence can be applied to distinguish different plant types and certain stresses (Chapelle and Williams, 1987). The nature and origin of this emission is at present not well understood. Time - resolved measurements showed that this signal is composed of at least three elementary components of unknown origin (Goulas et al, 1990).

In this paper we describe our first results about the influence of temperature on the fluorescence lifetime and on different fluorescence emission ratios. For a better understanding of the temperature effects on the photosynthetic system fluorescence induction kinetics (KAUTSKY effect) have been recorded in parallel.

1. MATERIAL AND METHODS

Tobacco plants were grown for 4 weeks in a phytochamber (photoperiod 14th; day temperature 24°C, rel. humidity 50%, 300 umol quanta m-2 s-1; night temperature 17°C, rel humidity 70%). Freshly cut leaf discs were placed at room temperature into vapour-saturated chambers which were slowly adjusted (for 20 minutes) to the different temperatures by a water circulating system. The tempera-

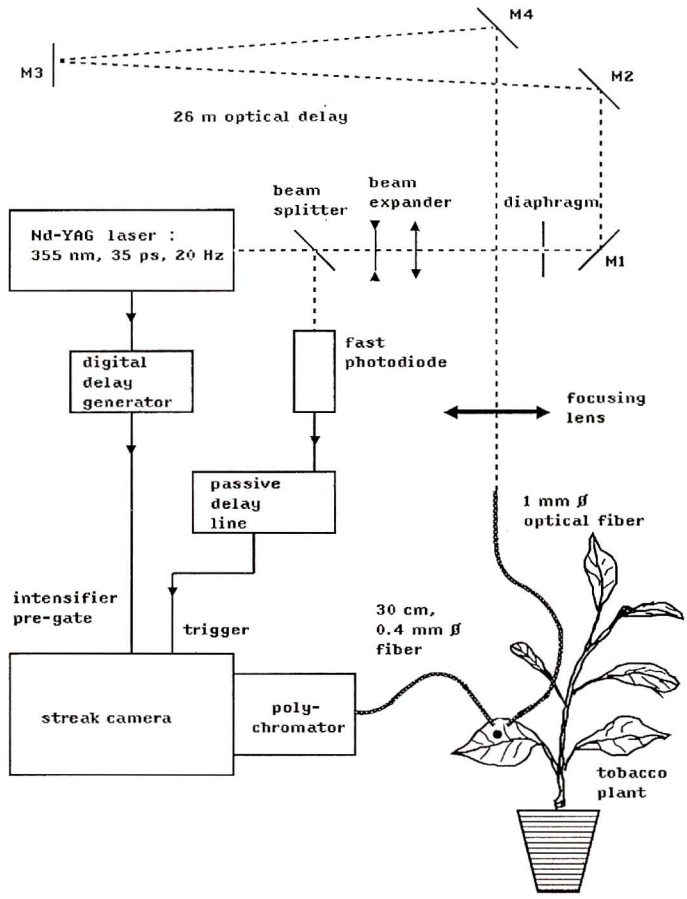

Fig. 1 - Experimental set-up lifetime measurements using pslaser excitation and a streak camera detection system.

ture of the leaves were controlled via a copper-constant thermocopule which was attached to the upper leaf surface.

Figure 1 shows the set-up used for fluorescence lifetime measurements. The excitation source was a model-locked Nd-YAG laser delivering pulses ao about 35 ps duration at 355 nm. The delay necessary for the streak camera operation was realized by an optical path line (26m) built with high energy mirrors. The beam was attenuated with a diaphragm and focussed on the upper leaf surface by an optical fiber. The fluorescence light was collected via a swcond optical fiber an d brought to the entrance of the polychromator coupled with the streak camera. The streak camera was a Hadland Photonics Imacon 500 equipped with an image intensifier and a CCD camera. The trigger signal for the streak camera was generated by a fast vacuum photodiode (Hamamatsu R1328U-01)receiving a portion of the laser beam deviated at the laser output. A passive delay line was used to adjust the delay between the streak camera intensifier was obtained from a pulse, synchronized with the flash discharge, which was appropriately delayed by means of a digital delay generator.
The 200 ps/mm scale of the streak camera (time window 3.2 ns) was used. The FWHM of the instrumental resolution comprimising the laser pulse duration, the triggering jitter and the effect of the fiber diameter was estimated to be 230 ps. The laser spot on the leaf was about 2 mm in diameter; the excitation energy was estimated to be in the order of 10E14 photons cm^{-2}, in order to avoid artifacts due to annhilation processes (Schmuck et al. 1991). For statistical reasons the images are the results of 2400 laser shots.
All the measurements were performed by exciting the upper leaf surface and by collecting the fluorescence from the same side. Before starting the measurements the leaves were adjusted to a steady state fluorescence level.

Fluorescence induction kinetics were recorded with the PAM Fluorometer (Schreiber, 1986). For the determination of the ground fluorescence Fo a weak measuring light of about 10 µmol photons $m^{-2} \cdot s^{-1}$ was used. The actinic light was 150 umol photons $m^{-2} \cdot s^{-1}$: saturation pulses of 5000 µmol photons $m^{-2} \cdot s^{-1}$ (pulsewith 700 ms) were applied every 10s. The fluorescence quenching analysis was performed according to van Kooten and Snel, 1990. Before starting the measurements the samples were always dark adapted for 20 minutes.

2. RESULTS AND DISCUSSIONS

Figure 2 shows the spectro-temporal image of the laser-induced fluorescence of a tobacco leaf at 20°C and 40°C, following excitation at 355 nm. The emission spectra is characterized by a broad emission in the blue-green region (emission maximum at around 480 nm and 530 nm) and the well known chlorophyll fluorescence with a maximum around 690 nm. The second chlorophyll fluorescence peak in the region of 730-740 nm is depressed beacuse of the spectral response of our detection system at this wavelength. An increase of the temperature of the leaves from 20°C to 40°C has no effect on these maxima of this emission spectra. Table I shows the fluorescence ratios F690/F480 and F690/F530 as a function of the leaf temperature. It can be seen that these ratios remain quite constant during the heat treatments of the samples.

	F690/480	F690/F530
20°C	3.30	3.3
30°C	3.30	3.3
32°C	3.30	3.3
34°C	3.30	3.3
36°C	3.28	3.3
40°C	3.28	3.3

Table I - Fluorescence ratios F690/F840 and F690/F530 of tobacco leaves as a function of heat pretreatment.

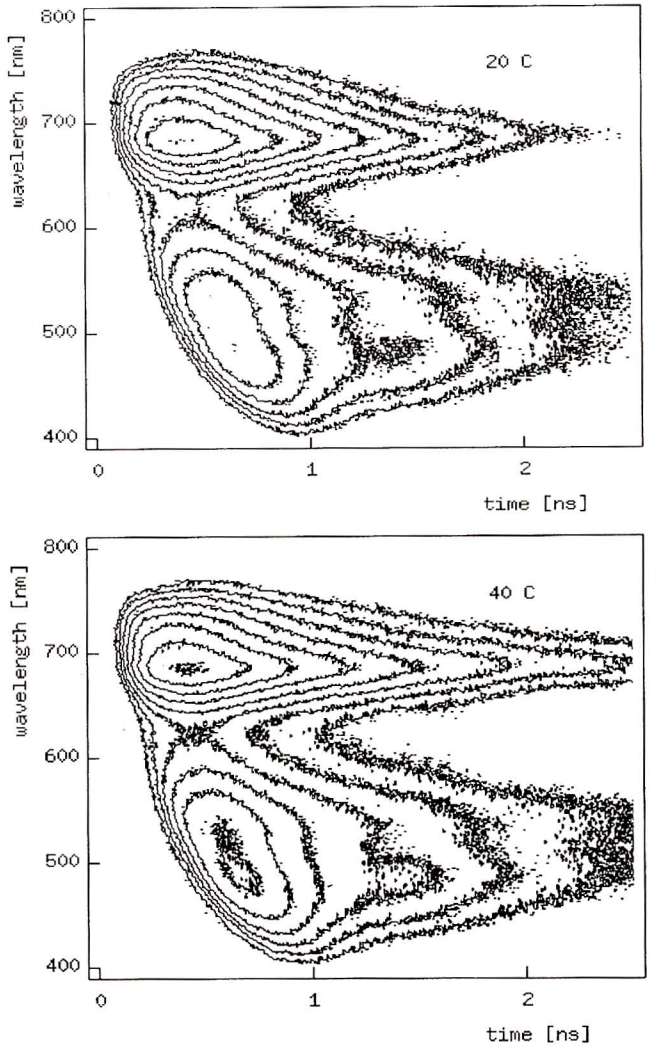

Fig. 2 - Spectro-temporal image of the laser-induced fluorescence of a tobacco (29°C and 40°C) as a function of the emission wavelenght and the decay time.

Figure 3 shows the results of the analysis of the decay measurements (at 20°C, 30°C, 40°C and 45°C) in a 20 nm spectral band centered at 690 nm. The overall decay at a stationary fluorescence level was analysed by a least square fitting programme using the Marquardt algorithm. In the temperature range from 20°C to 36°C the fluorescence decay remains constant. Starting from a leaf temperature of 40°C the decay in the fluorescence maximum is lengthened (Fig.3). Table II shows a comparison of the mean lifetimes during the heat treatment of the samples. It became obvious that a steady state conditions of photosynthesis the fluorescence decay can be characyerized by the simple parameter of the mean lifetime (Schmuck et al. 1990). In the temperature range from 20°C to 36°C the overall decay is characterized by a mean lifetime of 385 ps. Starting from leaf temepratures of 40°C the mean lifetime increases to 510 ps. At 45°C mean lifetime values of about 600 ps are reached. The results are quite similar if the heat treatment was performed on the same leaf if samples from different leaves have been used for the studies.

	20°C	30°C	32°C	40°C	45°C
τ [ps]	374	378	390	507	607

Table II - Comparison of the mean lifetime of the laser-induced chlorophyll fluorescence decay at 690 nm of tobacco leaves after heat-pretreatment tons m-2 s-1).

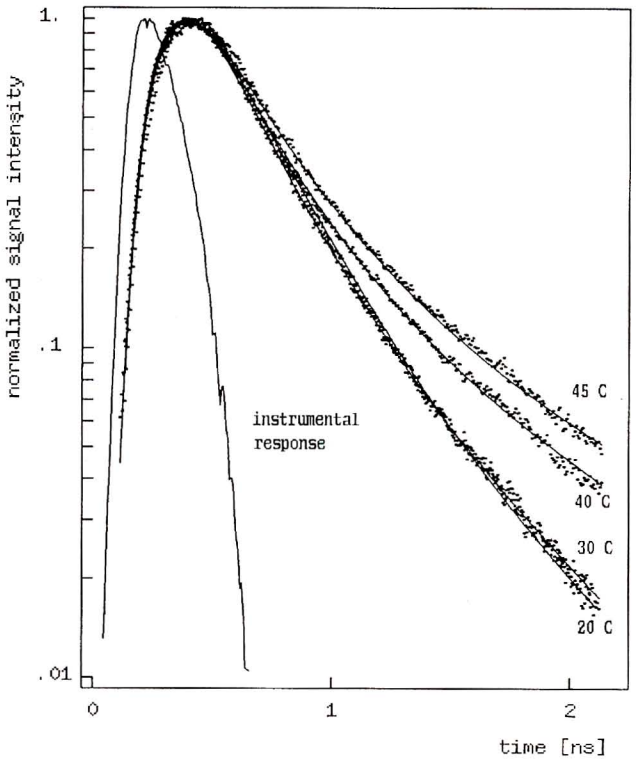

Fig. 3 - Chlorophyll fluorescence decay (measuring wavelength: 690 nm 10 nm) of a tobacco leaf measured with a streak camera at 20°C, 30°C, 40°C and 45°C.

Concerning the lifetimes of fluorescence emission at 480 nm and 530 nm, no changement was observed due to the increase of the temperature from 20 to 40°C. At 480 nm the mean lifetimes remain constant ($\tau = 1{,}122$ ns), whereas the decay at 530 nm is characterized by mean lifetime values of 623 ps.

To characterize the samples used for time decay analysis fluorescence induction kinetics and quenching analysis have been performed to get information about the heat-induced effects on the stability and organization of the thylakoid membrane and the embedded pigment-protein complexes.

Figure 4 shows the behaviour of the dark fluorescence yield Fo as a function of the temperature of the sample. The Fo-level represents emission from excited chlorophyll molecules in the pigment antenna, when all the PS II reaction centers are photochemically active. By incresing the temperature from 10°C to 36°C the fluorescence increases slowly . Starting from 40°C the Fo level increases significantly, which indicates that this temperature can be regarded as the heat tolerance limit of the studied tobacco plants. The usefullness of such T-Fo curves for determining heat tolerance of plants adapted to different climates has been described by Bilger et al., 1984. The higher Fo-levels at higher temperatures reflect changes in yhe deexcitation pathways inside the antenna. An explanation for the increase of the Fo-level could be a disconnection of the light collecting antenna from the photosystem II reaction center. For a better localisation of the primary site of the heat induced stress, saturation pulseinduction curves and quuenching analysis have been recorded on the samesampes which were used for the chlorophyll lifetime determinations.

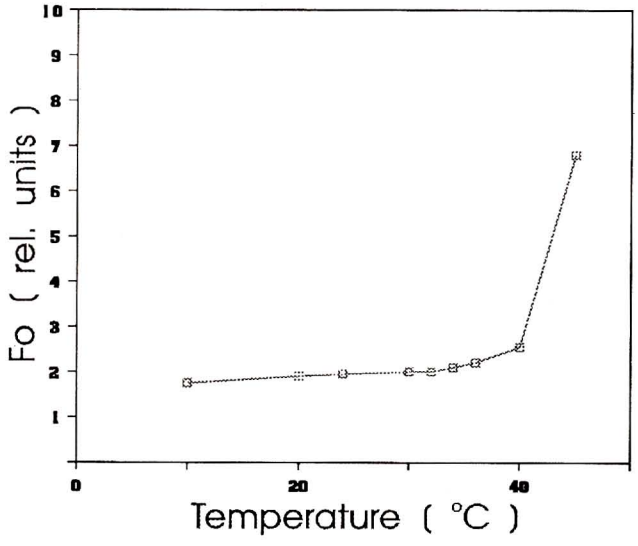

Fig. 4 - T-Fo curve of dark adapted tobacco leaves (measuring light intensity 10 mmol photons m-2 s-1).

Figure 5 shows the effect of the heat pretreatment on the non-photochemical quenching coefficient qNP. With increasing temperature qNP is increasing which indicates an increasing limitation of the Calvin cycle activity (Bilger et al. 1984). Future measurements will be necessary to distinguish between these two possibile explanations for damages of the photosynthetic system due to the heat stress.

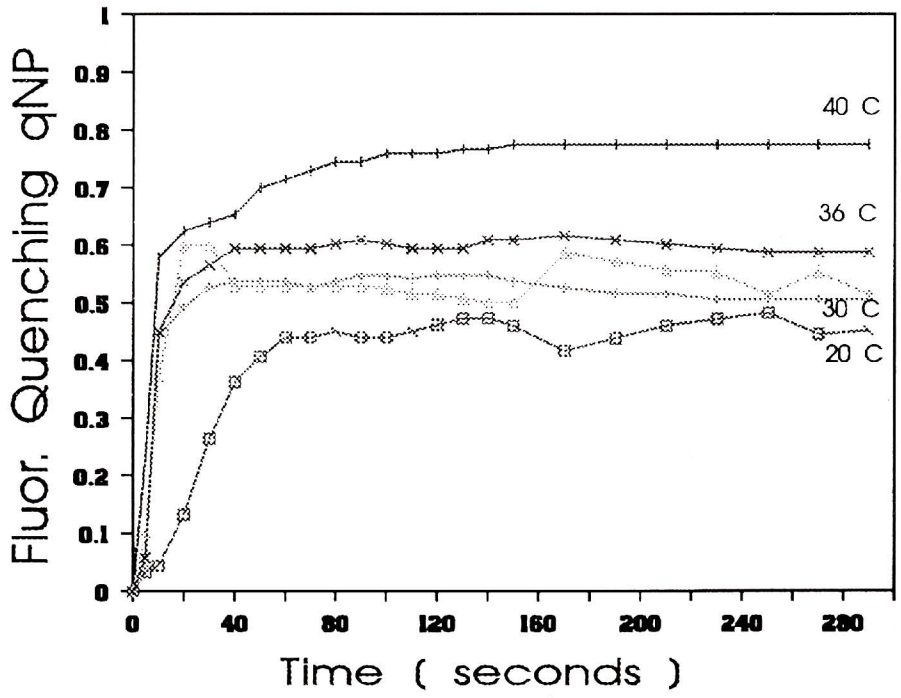

Fig. 5 - Effect of heat pretreatment on the nonphotochemical quench coefficient qNP. Fluorescence induction kinetics and quenching analysis were performed with leaves, adapted to 20°C, 30°C, 32°C, 36°C and 40°C.

CONCLUSIONS

The first study about the effect of temperature stress on the spectral and temporal behaviour of the laser-induced fluorescence has shown that only the chlorophyll fluorescence lifetime parameter can be used as an indicator of heat damage. Increasing temperature does not modify the fluorescence ratios F690/F480 and F690/F530 of tobacco plants under the above mentioned conditions. This work has shown that the lifetime detection canreveal a lengthening of the chlorophyll fluorescence decay due to temperature stress.

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