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Time-Resolved Chlorophyll Fluorescence of Spruce Needles Under Various Light Conditions

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Different branches of a declined spruce were exposed to full sunlight, reduced sunlight (using a fine wire mesh), and natural shadow. Subnanosecond decay kinetics and time-gated fluorescence spectra of individual needles were measured and compared with their chlorophyll concentration. Sunlight-exposed needles showed lower chlorophyll concentrations and higher intensities I_3 of a long-lived fluorescent component (decay time about 3 ns) than shadow needles. This seems to be due to a reduced energy transfer from the chlorophyll antenna molecules to the reaction centres of Photosystem II. After light reduction to 15–20% during one summer season the chlorophyll concentration increased, whereas I_3 decreased, thus proving some recovery of the photosynthetic apparatus.

KEY WORDS: Forest decline; chlorophyll fluorescence; light stress; time-resolved spectroscopy.

INTRODUCTION

Spruces growing at Freudenstadt-Schöllkopf (Black Forest) are submitted to various stress factors, including Mg deficiency (<0.3 mg Mg/g needle dry matter), O_3 pollution (>100 μg/m³ monthly means, May through August), and high global irradiance at 840 m a.s.l. At this site, apparently healthy spruces are growing among trees showing typical symptoms of yellowing and needle loss. In an early state of yellowing a simultaneous decrease in chlorophyll (a+b) and other pigments, as well as a reduction of the photosynthetic capacity, was measured [1]. Using picosecond decay kinetics, the energy transfer from the chlorophyll antenna molecules to the photosynthetic reaction centers was investigated. A long-lived fluorescent component, I_3 (decay time, 2.5–4.0 ns, compared with 100–600 ps for intact photosystems [2,4], indicated that the energy transfer was somehow obstructed. So far, the relative intensity I_3 varied between 1–2% in winter and 4% in summer for healthy spruces but increased in summer up to 8–20% for declined trees. A pronounced stress situation during the summer period might be due to high levels of incident light, drought and increased ozone concentrations. Therefore, in a first step, part of a declined spruce was exposed to reduced light intensity by using a wire mesh, whereas other parts remained exposed to full sunlight.

In addition to measurements of fluorescence decay kinetics using a picosecond laser diode [3], a novel setup for time-gated (nanosecond) microspectrofluorometry was employed in the current investigations [5].

MATERIALS AND METHODS

Small twigs of a damaged spruce [*Picea abies* (L.) Karst.; damage class 2, about 25% needle loss and 40% yellowing] were harvested at intervals of 4–8 weeks during the summer season 1993, and needles of the years 1991 and 1992 (8th–10th whorl) were measured. The twigs had been growing either in full sunlight, in re-

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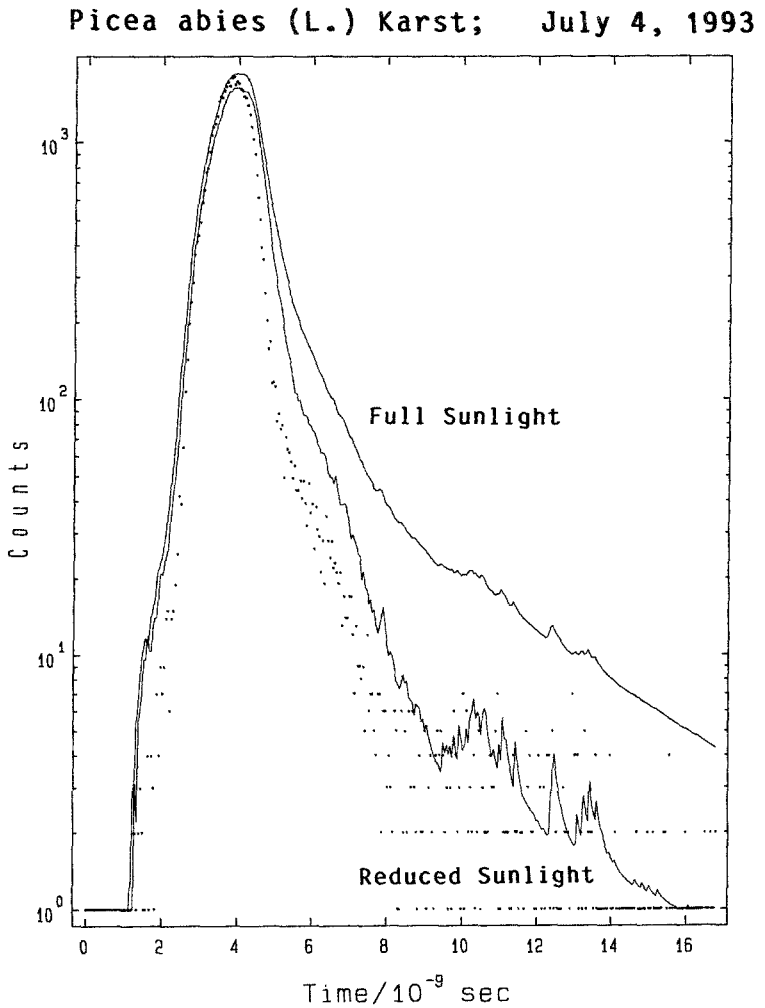

Fig. 1. Decay curves of chlorophyll fluorescence from dark-adapted spruce needles exposed to full and reduced sunlight (July 1993; age class, 1992; excitation wavelength, 668 nm; emission measured at 690–800 nm). The dotted curve corresponds to the response of the apparatus.

duced sunlight (two whorls with different degrees of initial yellowing, which were covered by a wire mesh with 15–20% light transmission since May 1993), or in natural shadow (northeastern side of the tree). In addition, light and shadow needles from a healthy reference spruce were investigated.

Fluorescence decay kinetics of 8–10 densely packed needles were measured using a (first-generation) picosecond laser diode (Hamamatsu Photonics, PLP-01, 668 nm, 90 ps, 100 kHz, average power of 0.7 μW reduced by a factor of 20), two long-pass filters for 690 nm, and time-correlated photon counting equipment (Hamamatsu R 928 photomultiplier, Tennelec NIM Electronics, IBH-199M software by Edinburgh Instruments for reconvolution and 3-exponential curve fitting). When using this miniaturized apparatus [3], the half-width of the response curve was 1.2 ns, thus giving a time resolution of about 100 ps of the fluorescence decay curves after reconvolution analysis. Fluorescence spectra of individual needles were detected within short intervals of 5 ns at various delay times after the exciting pulses of a dye laser (428 nm, 2 ns, 10 Hz, pulse energy <10 μJ) pumped by the third harmonic of a Nd:YAG laser. A self-constructed polychromator and an optical multichannel analyzer with a time-gated image intensifier (Hamamatsu, IMD C4562) were therefore adapted to the fluorescence microscope [5]. All fluorescence spectra were integrated over 5 s, corresponding to 50 exciting laser pulses.

In addition, the chlorophyll concentrations of individual needles were determined by absorption spectroscopy according to Lichtenthaler and Wellburn [6].

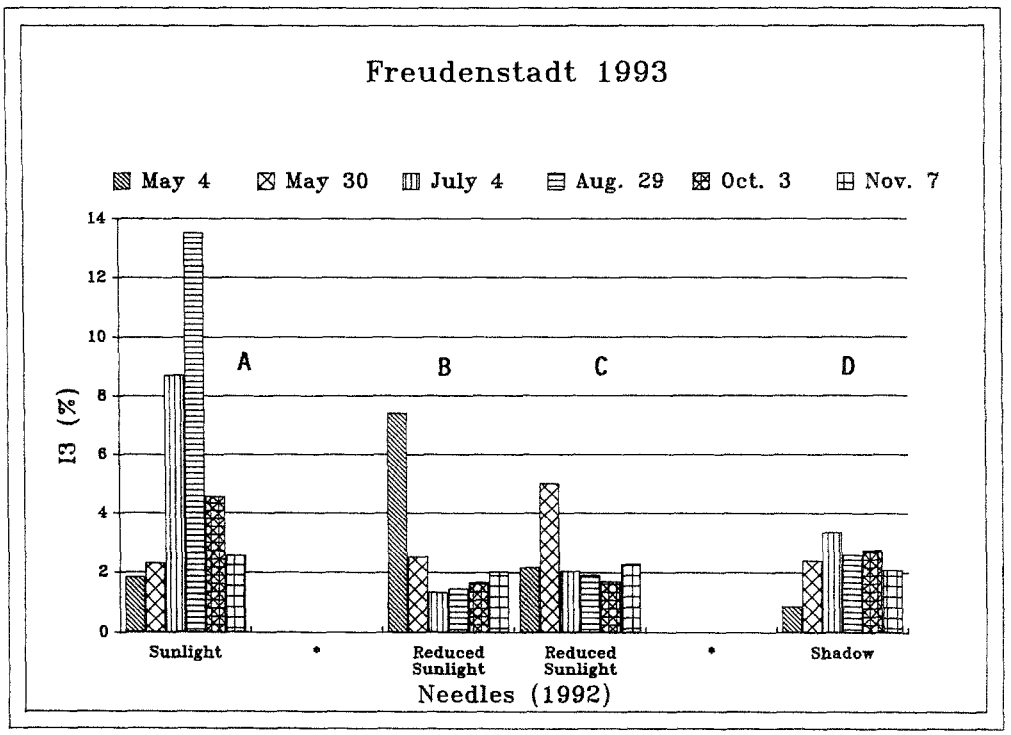

Fig. 2. Relative intensity I_3 of the long-lived component of chlorophyll fluorescence obtained at different harvesting dates of the needles after exposition to full sunlight, artificial shading and natural shadow (age class, 1992; reproducibility of individual values within ± 10%).

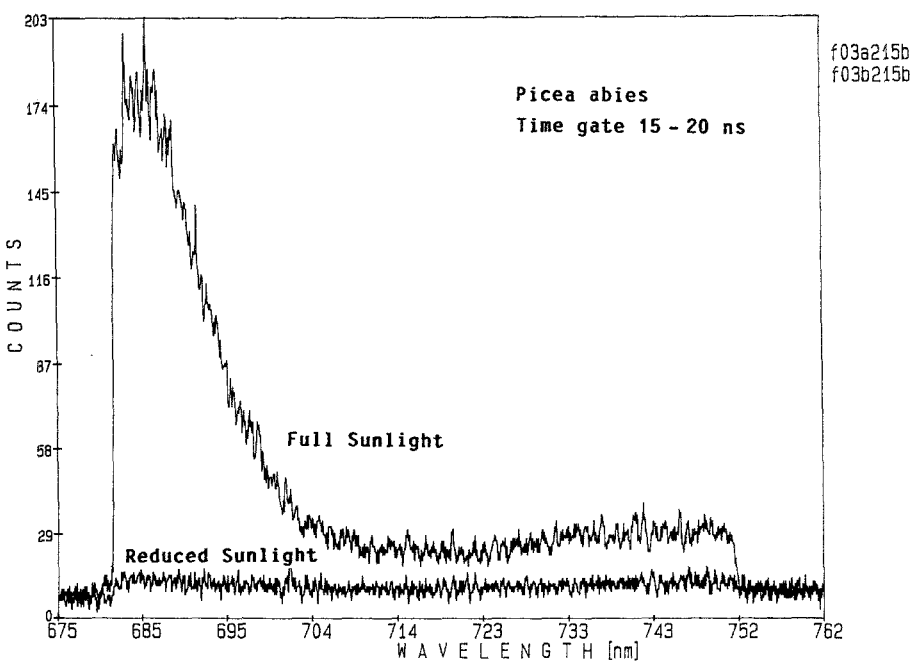

Fig. 3. Time-gated spectrum of chlorophyll fluorescence of spruce needles at 15–20 ns after the exciting laser pulse (428 nm, 2 ns.).

RESULTS AND DISCUSSION

Figure 1 shows the fluorescence decay curves obtained in July 1993 from spruce needles (age class 1992) exposed to full (upper curve) and reduced sunlight (lower curve), respectively. The curves could be fitted by three exponentially decaying components with lifetimes in the ranges of 100–200 ps, 400–600 ps, and 2.5–4.0 ns. Within the given limits the lifetimes varied with the season and also between individual samples. Obviously, the relative intensity (I_3) of the long-lived component was more pronounced for the fully light-exposed needles. The results of a more detailed analysis over 6 months are given in Fig. 2. Needles growing in full sunlight (A) or natural shadow (D) showed the annual time courses of I_3, which are typical for damaged and intact spruces, respectively [3] (a similar time course as for the shadow needles was obtained for the needles of the healthy reference spruce). In contrast to this, after artificial shading, the I_3 values decreased or remained on a constant low level. This is demonstrated by the measurements of needles from two branches under reduced sunlight with higher (B) or lower (C) levels of initial yellowing. The time course observed for I_3 was somehow reciprocal to the course of chlorophyll concentration. This concentration was comparatively low at full sunlight (1000–1400 μg/g fresh matter), but higher and strongly fluctuating (1300–2000 μg/g) in natural shadow and continuously increasing after artificial shading (from 1300–1400 μg/g on May 4 to 1800–1900 μg/g on Oct. 3 and Nov. 7). Visually, the needles exposed to full sunlight could be distinguished from the needles growing in natural or artificial shadow by a higher degree of yellowing.

Needles of the age class 1991 showed a behavior similar to that of the needles of 1992 (as reported above) at full or reduced sunlight, respectively. However, in natural shadow, the annual time course was similar to that of the sunlight-exposed needles. This means that the same kind of damage occurred with a delay of 1 year.

More detailed information about the location of the light-induced damage may be obtained from the time-gated fluorescence spectra. Whereas the spectra measured at 0–5 ns (during or directly after the laser pulse) reflected mainly the chlorophyll concentration, the spectra detected 10–15 or 15–20 ns (Fig. 3) after the laser pulse selectively exhibited the long-lived fluorescent component. According to the literature (see, e.g. Ref. 7) the pronounced peak at 685 nm can be attributed to photosystem II. Therefore, the energy transfer from the antenna molecules to the reaction center of photosystem II appears to be obstructed.

CONCLUSION

Due to high environmental light doses, the energy transfer from the antenna molecules to the reaction centers of photosystem II appears to be partly obstructed. A certain recovery was observed after reduction of the incident light. The impact of additional stress factors, such as high ozone levels and nutrient deficiencies, remains to be investigated.

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