

## Seasonal variations in photosynthetic activity of spruces as determined by chlorophyll fluorescence

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### Introduction

In photosynthetically active, green plant tissue (leaves, needles), the largest part of the light energy absorbed by the pigments (chlorophylls, carotenoids) is used for photosynthesis (photosynthetic quantum conversion). A minor part is re-emitted as chlorophyll fluorescence, whose spectrum exhibits maxima near 690 and 735 nm (Lichtenthaler *et al.*, 1986; Lichtenthaler and Rinderle, 1988a). The light-induced *in vivo* chlorophyll fluorescence of a pre-darkened green leaf or needle sample shows a transient which is known as fluorescence induction kinetics and variable fluorescence (Kautsky effect). Upon illumination, one observes a fast fluorescence rise (ca 400 ms) to a maximum ( $f_{max}$ ) followed by a slow fluorescence decrease ( $f_d$ ) to the steady state fluorescence ( $f_s$ ). The fluorescence decrease ratio ( $Rfd = f_d/f_s$ ;  $Rfd$ -values at 690 nm), which indicates the potential photosynthetic activity of a leaf area, has successfully been established as a very suitable vitality index and stress indicator in plants (Lichtenthaler and Rinderle, 1988a, b; Lichtenthaler *et al.*, 1986; Strasser *et al.*, 1987). The

height of the  $Rfd$ -values (measured in the 690 and 730 nm regions) reflects the potential photosynthetic activity of leaves as is demonstrated by parallel measurements of the net  $CO_2$ -assimilation rate  $P_N$ . The  $Rfd$ -values are an indicator of the intactness of the internal photosynthetic apparatus. Though they usually parallel the net  $CO_2$ -assimilation rates, they are a different parameter and independent of stomatal opening.

With an additional apparatus, the PAM fluorometer (Schreiber *et al.*, 1986), one can determine the photochemical  $Q$ - and the non-photochemical  $E$ -quenching and the rate of  $Q_A$ -reoxidation in the photosynthetic electron transport chain. Measurement of the chlorophyll fluorescence emission spectra enables the determination of a further stress indicator: the ratio  $F_{690}/F_{735}$  of the 2 fluorescence maxima. The height of  $F_{690}/F_{735}$  mainly reflects the chlorophyll content of the needles and, to a lower degree, its photosynthetic activity (Rinderle and Lichtenthaler, 1988). The registration of the different chlorophyll fluorescence parameters (Lichtenthaler, 1987; Lichtenthaler *et al.*, 1986; Lichtenthaler and Rinderle, 1988) permits a fast screening of seasonal and short-term variations

in photosynthetic activity and in chlorophyll content of trees as well as damage to the photosynthetic apparatus. This is documented here for spruce trees of the Northern Black Forest by measurement of the different fluorescence signatures of needles during a 1 yr period from 1987 to 1988.

## Materials and Methods

The fluorescence signatures of different needle years, of mainly healthy (Althof, damage class 0/1) and of damaged spruce trees (Mauzenberg, damage class 3/4) were measured using 3 different fluorescence methods. 1) The red laser-induced chlorophyll fluorescence kinetics (determination of *Rfd*-values as a vitality index of needles) measured near 690 and near 730 nm in a portable field fluorometer (Lichtenthaler and Rinderle, 1988b). 2) The chlorophyll fluorescence emission spectra at room temperature induced by blue light ( $470 \pm 30$  nm) recorded with a Shimadzu MPS 5000 spectrometer under steady-state conditions of the chlorophyll fluorescence (5 min after onset of illumination). 3)

The differentiation between photochemical *Q*-quenching and non-photochemical *E*-quenching using the new PAM fluorometer of A. Walz, Effeltrich (Schreiber *et al.*, 1986). In this new fluorometer, the excitation light to measure chlorophyll fluorescence is separately applied to the actinic light, which drives the photosynthetic reactions. Ground fluorescence *F* is excited repetitively by 1  $\mu$ s pulses of low intensity.

The photosynthetic prenyl pigments (chlorophylls and carotenoids) were extracted with 100% acetone and the pigments quantitatively determined using the newly established extinction coefficients of Lichtenthaler (1987). The  $\text{CO}_2$ -assimilation rates were determined at room temperature and light saturation ( $2000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) using the  $\text{CO}_2/\text{H}_2\text{O}$ -porometer system of Walz (see Nagel *et al.*, 1987).

## Results

### *Rfd*-values and net $\text{CO}_2$ -assimilation

The height of the fluorescence decrease ratio (*Rfd*-values at 690 or 730 nm)

**Table 1.** Values of the fluorescence decrease ratio (*Rfd*-values at 690 and 730 nm as a vitality index) and net  $\text{CO}_2$ -assimilation  $P_N$  in green needles of healthy (Althof) and of light green needles of damaged spruces (Mauzenberg).

Damage class	<i>P. abies</i> needle yr	<i>Rfd</i> -values		$P_N^a$ (per $\text{m}^2$ )	$P_N^b$ (per a+b)	Transpiration <sup>c</sup>	g $\text{H}_2\text{O}^c$
		690 nm	730 nm				
0/1 (Althof)							
	1984	4.7	3.2	5.4	0.7	0.6	50
	1985	5.2	3.4	6.7	0.8	1.4	54
	1986	5.4	3.6	7.0	1.0	1.1	45
	1987	5.4	3.6	7.2	1.6	1.0	48
3/4 (Mauzenberg)							
	1984	4.4	3.0	2.6	0.8	0.82	35
	1985	4.0	2.9	2.4	1.0	0.45	24
	1986	4.4	3.2	4.0	1.3	0.73	30
	1987	5.9	3.9	5.5	1.9	0.95	38

Measurements in August 1987 with light-exposed N3- and N4-needles. Mean of 3–6 determinations at each stand. Maximum deviation 12% or less.

<sup>a</sup> $P_N$  in  $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

<sup>b</sup> $P_N$  in  $\text{mg CO}_2\cdot\text{mg a+b}^{-1}\cdot\text{h}^{-1}$

<sup>c</sup> Transpiration and stomatal conductivity in  $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

reflects the photosynthetic activity  $P_N$ , as shown for several needle years of the healthy and damaged spruces (Table I). This is valid for normal physiological conditions during summertime, when the stomata are open and can be regulated. The  $Rfd$ -values in the needles of damaged trees were also very high and only slightly lower than those of healthy spruces. The high  $Rfd$ -values thus indicated that the chlorophyll in the needles of damaged trees, though lower in content, was photosynthetically active. Under water stress conditions and in wintertime when the stomata are closed, the  $Rfd$ -values (e.g., values of 2.5–4) indicated that the internal photosynthetic apparatus was functional, though the net  $\text{CO}_2$ -assimilation rates were very low or even zero. Photosynthetic quantum conversion then depended upon the  $\text{CO}_2$  set free by respiration.

Needles from fully green healthy spruces possessed a higher chlorophyll content per needle area unit than the corresponding needle years of damaged spruces, which were light green and often showed yellowish–green parts at the upper needle part. Net photosynthesis  $P_N$  per needle area unit was therefore always higher for green control needles than for needles of damaged trees (Table I).

#### *Seasonal variations*

The chlorophyll content of summer 1987 decreased in the spruce needles in the winter months of 1988 up to 25% in the older needles and to a somewhat lower degree in the youngest needle year 1987. With the start of the new vegetation period, the chlorophyll content increased again. This increase was particularly strong in the 1987 needles, which in the 1st yr still had a very low chlorophyll content. In the case of the damaged

spruce, the 1987 needles showed, however, a much lower increase in the new vegetation period than the older needle years and those of the healthy, fully green spruce.

The photosynthetic activity of the spruce needles ( $P_N$  measured with a  $\text{CO}_2/\text{H}_2\text{O}$  porometer) decreased in October and November from original values of 4–8  $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  to very low values in December (frost period; values of 0–2  $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) with some recovery in a rather warm January. In March 1988, the  $P_N$ -values increased again to reach maximum values at the end of April (6–8  $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), just before the new shoots were formed. Thereafter, the  $P_N$  showed lower values again. These characteristics were found at the Althof and the Mauzenberg sites. The low  $P_N$  values in winter appear to be mainly due to closed stomata, but in part also to damage of the photosynthetic apparatus as seen from the lower  $Rfd$ -values.

In contrast, the  $Rfd$ -values as a vitality index and as an index of the intactness of the photosynthetic apparatus, showed a different behavior. There was a clear decrease of the values in December, with considerable increase in January and again a decrease in March 1988. Thereafter, higher values between 4 and 5 were reached (Fig. 1). These characteristics were found in the 1986 and 1987 needles at the Althof and Mauzenberg sites. The very high  $Rfd$ -values of 6–7 were only reached in the very young current year needles. The decrease of the  $Rfd$ -values in December and March indicated damage of the photosynthetic apparatus, the increase in January (during a warm period) demonstrated the fast regeneration rate of the photosynthetic apparatus.

With the new PAM-fluorometer, one can determine the fluorescence kinetics with saturating light-pulses. The resulting fluo-

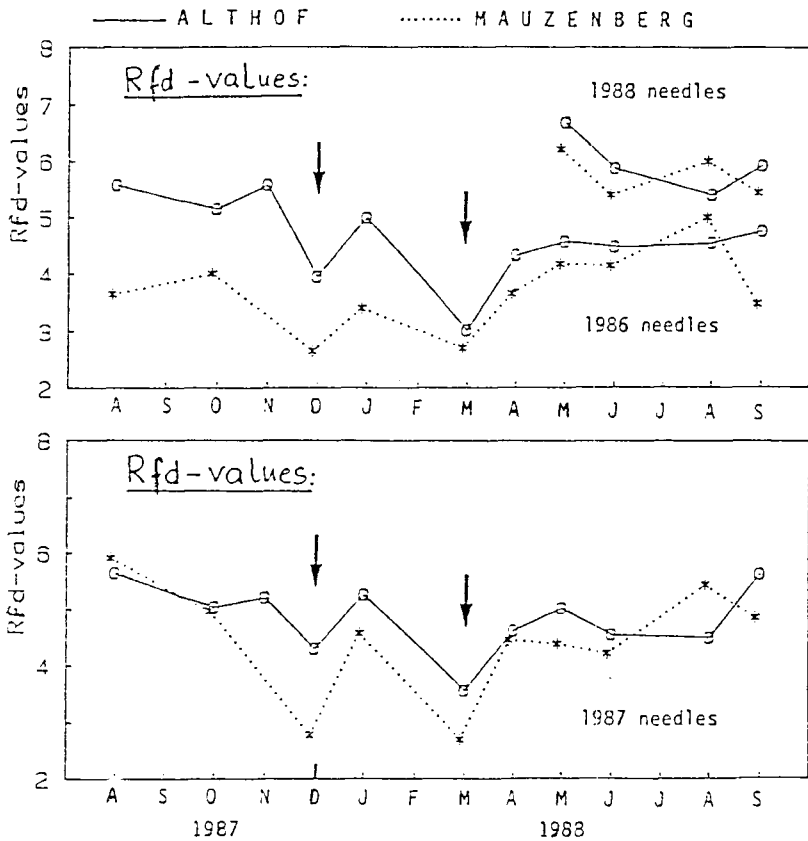


Fig. 1. Development of the internal photosynthetic activity (fluorescence decrease ratio: *Rfd*-values) of different needle years of spruces during 1 yr starting in August 1987. Althof (healthy trees) and Mauzenberg (damaged trees). The arrows point to a strong decrease in *Rfd*-values in January and March 1988. Each point is the mean of 8 determinations from 2 trees (standard deviation  $\leq 15\%$ ).

rescence spikes (distance  $g-h$  in Fig. 2), which indicate the reoxidation capacity of the primary photosynthetic quencher  $Q_A$ , were higher for the normal green needles (Althof) than those of the Mauzenberg site. The height of the spikes decreased in the cold winter months together with the *Rfd*-values.

From the kinetics of the PAM-fluorometer, one can calculate the coefficients for photochemical ( $qQ$ ) and non-photochemi-

cal quenching ( $qE$ ) (see Schreiber *et al.*, 1986; Lichtenthaler and Rinderle, 1988). The  $qQ$ -values were more or less the same in summer and winter (values of 0.83–0.96 at the Althof and Mauzenberg sites). In contrast, the  $qE$ -values (energy quenching), which contain information, *e.g.*, of the light-mediated formation of a proton gradient across the membrane, were higher in winter (values of 0.55–0.68) than at the time of highest pho-

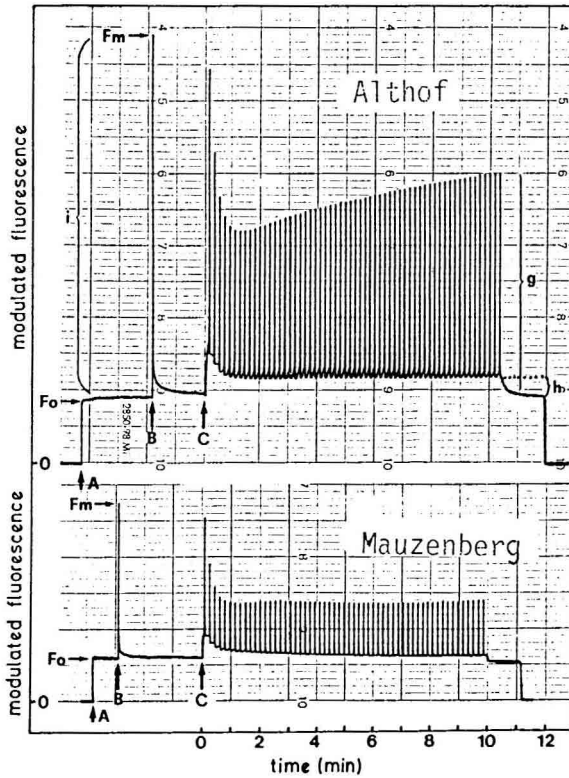


Fig. 2. Light-induced modulated chlorophyll fluorescence induction curve of spruce needles with saturating light pulses obtained using a PAM-fluorometer (Schreiber *et al.*, 1986). The 2 yr old green needles of the healthy tree (Althof) exhibit much higher fluorescence spikes (higher  $Q_A$ -reoxidation capacity) than the yellowish-green needles of the damaged spruce (Mauzenberg).

tosynthetic activity, *e.g.*, in springtime (values of 0.35–0.45).

The ratio of the chlorophyll fluorescence intensity at the 2 maxima near 690 and 735 nm ( $F_{690}/F_{735}$ ) was about 0.98–1.08 in normal green needles and *ca* 1.2–1.6 in the light green or yellowish-green needles of the damaged spruces. The differences, mainly due to the differing chlorophyll content of the needles, were larger in summer than in winter. The values for the ratio  $F_{690}/F_{735}$  tended to increase by

about 20% in the winter months, which paralleled a lower chlorophyll content and photosynthetic activity.

## Conclusion

The photosynthetic activity of spruce needles undergoes seasonal variations with a maximum in springtime (April), before and at the time of the formation of

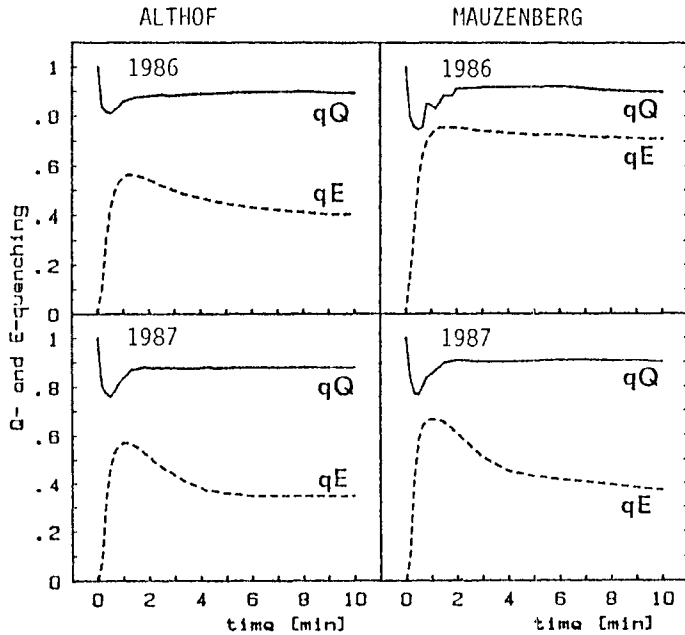


Fig. 3. Time course of the quenching coefficients  $qQ$  (photochemical quenching) and  $qE$  (energy quenching) during chlorophyll fluorescence induction kinetics in spruce needles. The values are calculated from the original tracings obtained from a PAM-fluorometer in August 1987, according to Schreiber *et al.* (1986) and Lichtenthaler and Rinderle (1988). Althof: green needles (healthy). Mauzenberg: yellowish-green needles (damaged tree).

the new year's needles. The current year needles reach their maximum in May and June. The chlorophyll fluorescence signatures of the needles of spruces ( $Ffd$ -values as well as the values for  $qE$  and the ratio  $F690/F735$ ) are very suitable to describe the seasonal variation in photosynthetic activity. These fluorescence signatures reflect the intactness of the internal photosynthetic apparatus even at closed stomata and are much better parameters to describe the internal photosynthetic activity than measurements of net  $CO_2$ -assimilation alone.

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