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# Temperature and light dependent modifications of chlorophyll fluorescence kinetics in spruce needles during winter

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**Abstract**. Prompt chlorophyll a fluorescence kinetics at room temperature were measured from intact spruce needles. The fluorescence signal was recorded after varying light pretreatments. During the winter, induction curves showed characteristic changes in both the initial peak of fluorescence FV/FP (FP-FO/FP) and the steady state level $F_{dr}$ (FP-FT/FP). Winter stress induced decreases in both values which showed close correlation to the light and temperature pre-history of the plants. In February changes in fluorescence induction indicative of a restoration of photosynthesis were detected and these corresponded to a rise of temperature above zero in combination with low light levels. In March increasing light intensity combined with chilling temperatures induced again decreases of both values of chlorophyll fluorescence induction suggesting the occurrence of photoinhibition.

## Introduction

A large part of the Northern Hemisphere – and even regions of the Southern – consist of coniferous forests. These forests usually occur in climatic zones with pronounced seasonal changes in temperature. During winter, temperatures below zero combined with physiological drought and occasionally in interaction with high light intensities may be particularly damaging to the plants (Björkmann 1986).

Most coniferous trees retain their needles for at least two years. The photosynthetic apparatus has therefore to survive the harsh winter conditions and retain the capacity to photosynthesis when warmer conditions return.

The photosynthetic apparatus reflects both structural alterations and physiological dysfunction during winter (Senser and Beck 1979, Martin et al. 1979). It is well documented that winter stress induces changes in the function of the photosynthetic apparatus and, as a consequence, modifica-

tions of the chlorophyll fluorescence induction curve depend at least partly on the intensity of incident light in combination with freezing temperatures (Öquist 1983).

However, there has been no detailed study of changes in the function of the photosynthetic apparatus in relation to short-term fluctuations in temperature and light over the winter period. The aim of this work is to detect how the characteristics of the fluorescence induction curve respond to these climatic fluctuations. This information will be an important prerequisite for analysing the effects of air pollutants when superimposed on natural changes in photosynthetic activity.

## Material and methods

*Material*

Samples of branches were collected weekly from trees in the experimental garden of the University of Vienna (N 48° 13′35″ E 16° 22′30″, altitude 164 m) and from potted plants of same age and origin cultivated in the cold division of the "Phytotron" at the same location. The impact of air pollution expressed in maximal day mean values obtained from the City-Service, Vienna, in this district are: 0.15 mg $SO_2 \cdot m^{-3}$; 20 ppb $NO_x$, 60 ppb $O_3$. It was assumed that the average concentration of air pollutants in the experimental garden was comparable to the concentrations in the "Phytotron". After sampling, the branches were recut under water and put upright into water and stored in a climatic cabinet under constant conditions (RUBARTH, Rumed 1002, FRG). During day time the temperature was 15 °C, during night time 5 °C. During the photoperiod the branches were exposed to "dim"-light with an intensity of $10\,\mu mol \cdot m^{-2} \cdot s^{-1}$ and an intensified emission in the red and blue absorption band of chlorophyll (GROW LUX 20 W, Silvania, FRG). Relative humidity was 70%.

Conditions in the "Phytotron" are presented in Fig. 4. Temperature ranges were controlled by a specifically developed computer program. In case of weak day light intensities, plants were provided with additional light with an intensity of $300\,\mu mol \cdot m^{-2} \cdot s^{-1}$ between 11 a.m and 3 p.m (1000 W SONT and 2000 W HPIT, Philips, FRG).

*Set up and methods*

The fluorescence induction curves were measured with a microscopic spectrophotometer (MPV I, Leitz, FRG) connected to a red-sensitive photomultiplier tube (RCA 1P21) (Fig. 1). For measurement, an individual needle was placed onto a micro slide. The objective (32 ×/0.40) allows a high

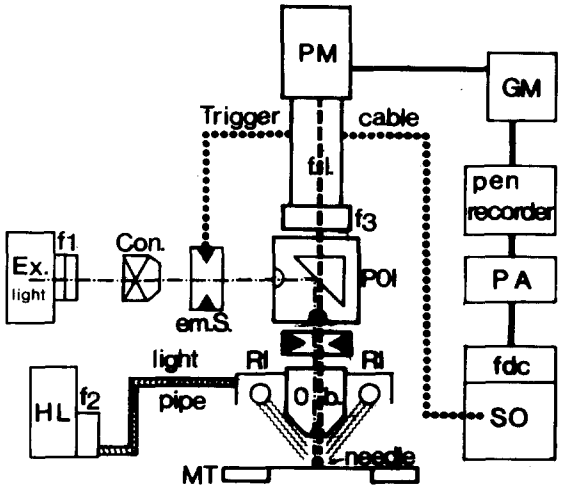


*Fig. 1.* Schematic diagram of the set up. Abbreviations: Ex. light = excitation light (435 nm), fl = filters (BG 38, BG 12), Con. = condenser, em. S. = electromagnetic shutter, HL = halogen lamp (250 W), f2 = filter (699 nm), RI = ring illumination, Ob. = objective, MT = microscopic table, POI = ploem opak illuminator, f3 = filter 695 nm, fl. = fluorescence light beam, PM = photomultiplier, GM = mirror galvanometer, PA = preamplifier, fdc = floppy disk computer, SO = storage oscilloscope.

working distance and exact focusing of the penetrating measuring beam into the assimilatory parenchyma of the needle. Around the objective a halogen ring illumination was mounted (LEITZ, 250 W, FRG). Light is transferred from the halogen lamp via a fibre optic light pipe. This construction renders possible preillumination of the plant material with bright white light and with red light (Interference filter DAL 699 nm, Schott, FRG).

Blue excitation light (435 nm) is provided by a stabilised DC mercury high pressure lamp (HANAU S.T. 75, FRG). After passing a heat absorbing filter (BG 38, Schott, FRG) and a coloured glass filter (BG 12, Schott, FRG) the light is focused by a condenser. The light is passed through a ploem opak illuminator (Eichler and Walter 1967). The diameter of the light beam incident upon the needle is 0.125 mm with a photon flux density of $627\,\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

An electromagnetic shutter (PRONTOR, FRG) is opened to allow entry of excitation light and this electronically triggers the signal output to a storage oscilloscope (VUKO digitalscope, VKS 220-16, 8 kByte memory, FRG). Duration of shutter opening is 1.5 ms, allowing measurement of the fast kinetics of fluorescence induction.

Transmission of blue light to the photomultiplier is eliminated by the use of an additional blocking filter (695 nm). The photomultiplier is supplied

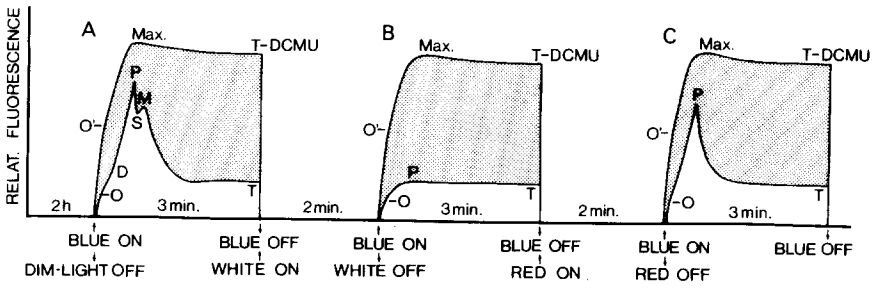


*Fig. 2.* The kinetics of fluorescence emission after excitation with 435 nm blue light ($627\,\mu mol \cdot m^{-2} \cdot s^{-1}$ from A) a dim-light adapted needle, B) after pre-treatment with white light ($900\,\mu mol \cdot m^{-2} \cdot s^{-1}$), C) after pre-treatment with PS I-activating red light ($3.0\,\mu mol \cdot m^{-2} \cdot s^{-1}$). FO′ = apparent increased FO-level after DCMU-treatment. Further explanations are given in the text.

with a stabilised high voltage unit (KNOTT, NSHM BN 600 S/N 430, FRG). The signal is fed to a mirror galvanometer (NORMA, 251 22, Austria) and then transferred to a pre-amplifier. The amplified signal is captured digitally on the storage oscilloscope. A floppy disk computer (VUKO FDC-2, FRG) stores the screen contents onto diskettes for further investigation of fast kinetics (FO-FP). Slow kinetics are recorded with a potentiometric pen recorder (BBC Goerz Servogor 310, Austria) over a period of three minutes for registration of the steady state-level T. (Fig. 1).

*Light pre-treatments and measurements*

After two hours of "dim"-light pre-treatment in the controlled environment, needles were transferred to the measuring stage and a first fluorescence induction curve was recorded. After the first measurement the needle while remaining mounted in the same position was illuminated with white light ($900\,\mu mol \cdot m^{-2} \cdot s^{-1}$). After two minutes of illumination a second fluorescence induction curve was recorded. Finally, the needle was illuminated with red light (699 nm, $3.0\,\mu mol \cdot m^{-2} \cdot s^{-1}$) for two minutes and a final induction curve recorded.

Adaptation to "dim" light generates weak electron transport activity. The induction curve measured after this pre-treatment was found to exhibit a more distinct and uniform P-S-M-T transition compared to that after simple pre-darkening (Fig. 2A). However, pre-treatment with bright white light provokes a strongly modified fluorescence induction curve (Fig. 2B). The short period of exposition to high light induces in any case a complete loss of fast kinetics and favours radiationless deactivation (Fork et al. 1986) (data not shown). The loss of induction kinetics is reversible by illumination with red light. Red light of 700 nm provides reoxidation of the Plastochinon-

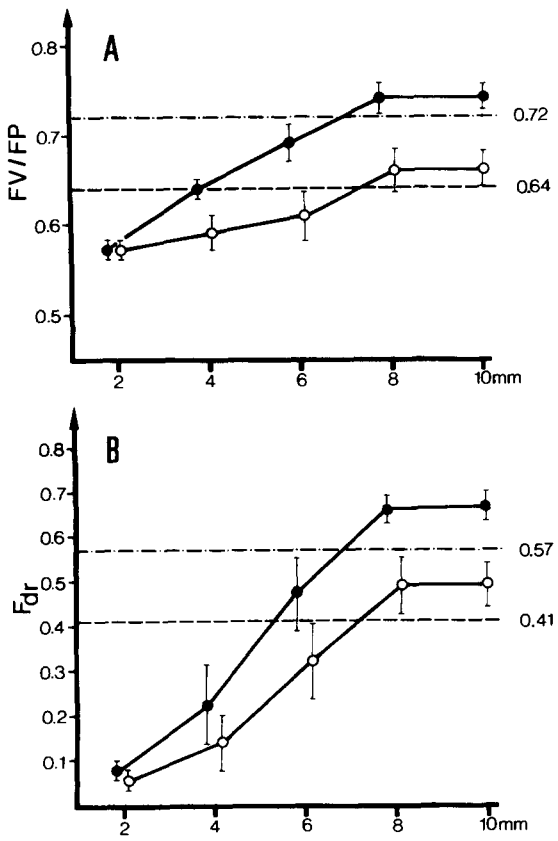


*Fig. 3.* Effect of uptake of 1 mmol DCMU intact needles: Distance from the needle base. A) Mean values of fast kinetics FV/FP. B) Mean values of slow kinetics $F_{dr}$. ● = after dim-light, ○ = after red light. Both calculations of the confidence limits with a probability level of 10%. Distance from the needle base is given in mm on the abscissa. -·-·-·- = limit of disturbance for determination of damage for measurement after dim-light, - - - - = limit of disturbance for measurement after red light pre-treatment.

Pool (Fig. 2C) by generating electron flow through the Photosystem I. After renewed excitation with blue light the induction curve exhibits a steeper decline from P to T by elimination of the P-S-M transition (terminology according to Lavorel and Etienne 1977).

The characteristic values of fast kinetics FV/FP (FP-FO/FP) were as well calculated as the values of slow kinetics $F_{dr}$ (FP-FT/FP). $F_{dr}$ provides information about the decrease of fluorescence from P to the steady-state level T reflecting the activity of Calvin Cycle and related processes (cp. Lichtenthaler et al. 1986, Baker and Horton 1987). On account of a more

distinctive variability of the maximum P-Peak the fluorescence decline was related to P.

Current year needles were measured each week and characteristic values of fast kinetics FV/FP and slow kinetics $F_{dr}$ of at least 8 measurements determined.

Climate data were gained by a computerised climate station (Honeywell Delta 100): temperature sensor: Balco 500 SL, Honeywell, FRG; light sensors: star pyranometer Schenk, Austria; quantum sensor LI-COR, USA; humidity sensor; Hygrotest 6000, Testotherm, FRG).

A limiting value for detection of an impairment of the photosynthetic apparatus was determined by treatment with the photosynthetic inhibitor DCMU (3-(3,4-Dichlorphenyl)1,1-dimethylurea). This "limit of disturbance" indicates an impairment of the photosynthetic activity regardless of the origin of the dysfunction. Therefore seasonal variations of photosynthetic activity are as well detectable as effects of artificial origin.

Leaf base was cut off the needles. The needles were standing upright in a styropor block and the cut ends were placed in a solution of DCMU (1 mmol/l) and stored in the climatic cabinet. After 24 hours of uptake into the plant material, measurements were made starting from base to top at regular intervals of 2 mm. FV/FP and $F_{dr}$ of at least 10 measurements were calculated.

## Results

The extent of damage caused by herbicidal effect decreases with distance from the base to the top of the needle because of reduced penetration of the herbicide solution (Fig. 3). Measurements 8 mm above the needle base show no or only a slight herbicidal effect and are therefore used as control level. The limits of disturbance for FV/FP and $F_{dr}$ are determined by the mean value of the measurements at 6 and 8 mm.

The pronounced decrease of the variable fluorescence is caused by an apparent increase of the O-level which is due to an extremely reduced half-rise time of the induction curve to the maximal value. On account of the shutter velocity after herbicidal treatment the registration of the O-level occurs after FO has reached the I-level and is therefore designated FO'.

Broader varying of $F_{dr}$ values is caused by appearance of a secondary M-Peak. The $M_2$-Peak occurs within a period of 3 minutes after starting of excitation. Nevertheless the T-value is rigidly determined after 3 minutes. Thus, the T-value may be read off during the phase of the secondary M-Peak (cf. also Fig. 5).

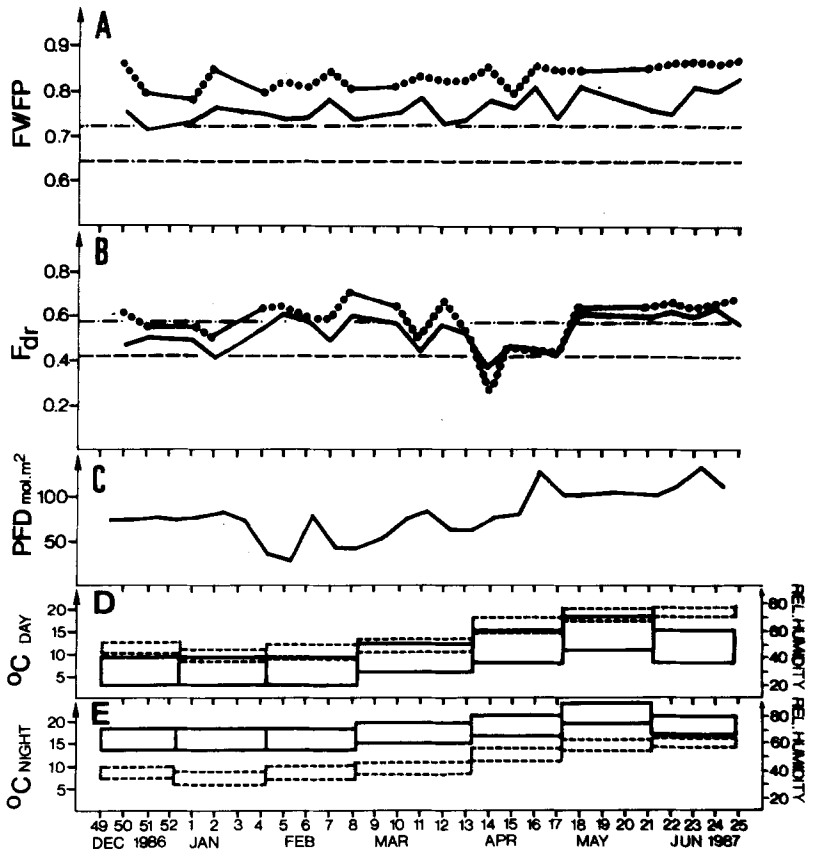


*Fig. 4.* Chlorophyll fluorescence of spruce trees cultivated in the "Phytotron". Abscissa: numbers designate week of the year. A) mean values of FV/FP. B) mean values of $F_{d_r}$. Both calculations were made from 8 measurements. ●●● = after dim-light, —— = after red light illumination. —— = missing data. Limits of disturbance: -·-·-·- = after dim-light, - - - - = after red light. Climatic data in the "Phytotron": C) Photon flux density (400–700 nm): sum of irradiation in $mol \cdot m^{-2}$ and week. D) - - - - = range of day temperature in °C, —— = range of relative humidity in % (day). E) - - - - = range of night temperature in °C, —— = range of relative humidity in % (night).

A comparison between spruce trees exposed to natural climatic conditions and spruce trees exposed to cold house conditions in the "Phytotron" is shown in Figs. 4 and 6. The plants of the green house exhibit only slight variations of the fast kinetics of fluorescence induction during the whole observation period. This is due to the fact that the minimum temperature always exceeds + 5 °C.

Contrary to this a remarkable decrease of $F_{dr}$ was detectable in April. This fall of the values is closely linked with the physiological activity of the plant

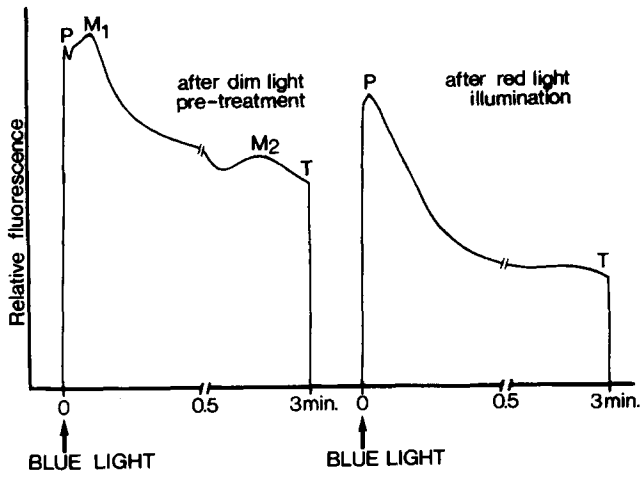


*Fig. 5.* Slow kinetics of chlorophyll fluorescence emission during the period of flushing. $M_1$ = M-Peak, $M_2$ = delayed M-Peak.

induced by the new flushing. During the flushing period no differences between the fluorescence characteristics of older and of young needles were detectable, either. After "dim"-light exposure the S-M transition showed a marked biphasic feature and in addition a well pronounced secondary M-Peak. The occurrence of the $M_2$-Peak causes a relatively low $F_{dr}$ value. After red light-exposure the decline P to T is more pronounced thus causing higher $F_{dr}$ (Fig. 5).

Unlike the cold house conditions in the "Phytotron" the climatic conditions in the natural stand are submitted to variations in both temperature and light conditions (Fig. 6c, d). Owing to sub-zero temperatures the kinetics of fluorescence induction are affected by winter stress. It can be seen that both the level of fast kinetics characteristics and the level of slow kinetics characteristics show a remarkable decline in the course of January. Decline of FV/FP and $F_{dr}$ is caused by a substantial decrease of the maximal value P. To a minor extent FO and the steady state-level T are declining during winter.

In February a relaxation of the fluorescence response with a tendency to reach normal values was observable. This change of the characteristics of induction kinetics corresponded to a change of climatic conditions. During this period temperature increased above zero in combination with relatively low light levels. It must be noted that calculation of the confidence limit with a probability level of 10% shows a significant difference of values of FV/FP after dim-light exposure between the data of week 5 and the following data of week 6, 7 and 8. After red-light illumination significant difference of

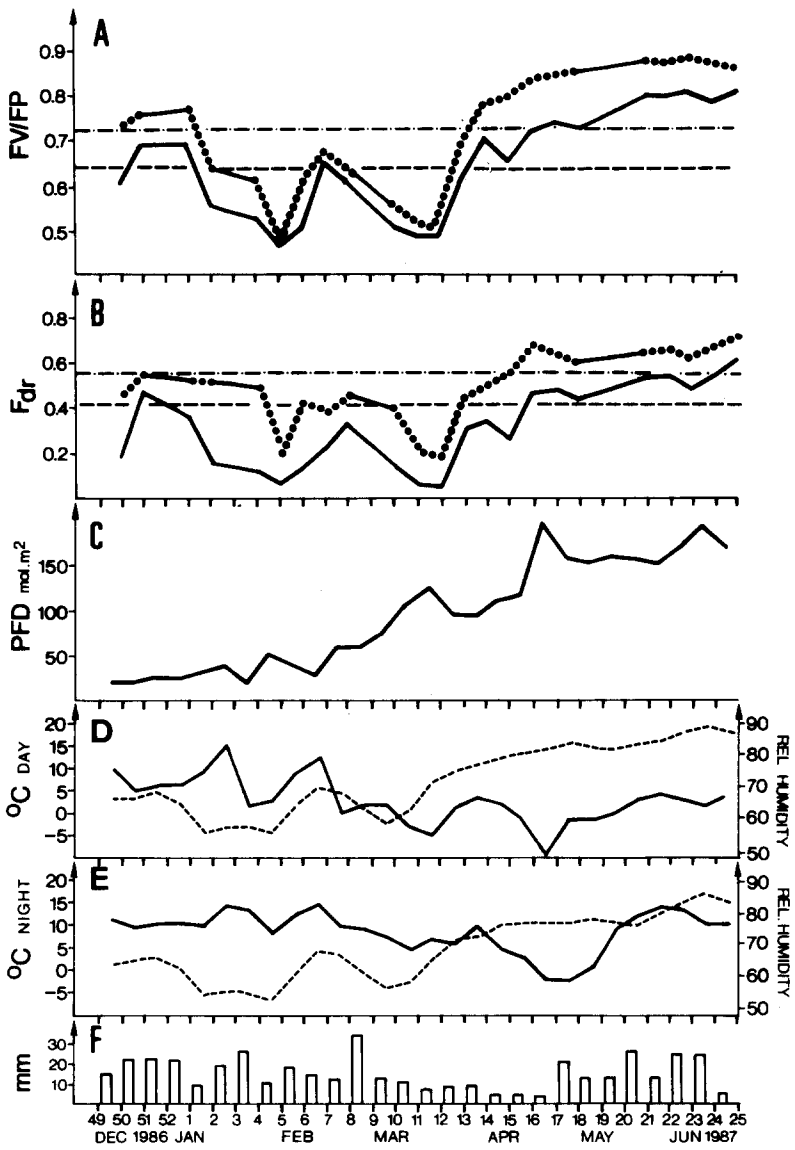


*Fig. 6.* Chlorophyll fluorescence of trees cultivated outside in the experimental garden. Abscissa: numbers designate week of the year. A) Mean values of FV/FP. B) Mean values of $F_{dr}$. Both calculations were made from 8 measurements. ●●● = after dim-light, —— after red light, —— = missing data. Limits of disturbance: -·-·-·- = after dim-light, ---- = after red light. Climatic data: C) Photon flux density (400–700 nm): sum of irradiation in mol·m$^{-2}$ and week. D) ---- = day temperature: mean values of the week calculated from day mean values in °C. —— = relative humidity (day): mean values of the week calculated from day mean values in %. E) ---- = night temperature: mean values of the week calculated from night mean values in °C. —— = relative humidity (night): mean values of the week calculated from night mean values in %. F) week sums of precipitation in mm $H_2O$.

FV/FP is calculated only between the data of week 5 and those of week 7 and 8. Values of slow kinetics following both light treatments exhibit a significant difference only between the data of week 5 and 8.

At the beginning of March once more a loss of photosynthetic activity and decline of FV/FP and $F_{dr}$ was observable. In contrast to the preceding periods day light intensity increased enormously. Low values of both FV/FP and $F_{dr}$ seem to be attributable to a photoinhibition effect, enforced by low, but not freezing temperatures. From our experiments it is not detectable whether this decline of fluorescence results from an increased radiationless deactivation of the photosystem II reaction centre, as suggested by (Bradbury and Baker 1986).

This period is followed by a rapid reactivation at the beginning of April. Statistically significant differences for FV/FP and $F_{dr}$ can be calculated from the data of week 12 and 13. In April, during flushing, the needles of the trees of the natural stand exhibited a less pronounced decline of $F_{dr}$ due to a protracted course of flushing.

## Discussion

It is well documented that plants exposed to chilling temperatures are much more sensitive to high light intensity causing photoinhibition phenomena (Bongi and Long 1987) and even photooxidative damage (Wise and Naylor 1987a, b).

It is of particular interest that the extent of winter stress was diminished during one period in February: levels of low light intensities provoked restoration of the "unstressed" pattern of chlorophyll fluorescence when day and night temperatures exceeded 0 °C.

In March (and even April) increasing day length and the different position of the sun favours higher irradiation levels. Low temperatures, e.g. frost at night, in interaction with high light levels enhance the development of a severe photoinhibitory damage (Strand and Lundmark 1987). It seems possible that a snow cover could act as a protection against damage caused by high light intensities (Martin et al. 1978).

Comparable changes of $CO_2$ assimilation over the winter period were described previously (Pisek 1973) in accordance with our results. The rate of $CO_2$ fixation as well as the pattern of fluorescence induction can be modified during leaf development, too (Ireland et al. 1985). It is well documented that the pattern of slow kinetics of fluorescence induction is correlated to the onset of carbon assimilation and the appearance of the quenching-mechanism $q_E$ (Walker 1981, Bilger and Schreiber 1986). As our results suggest,

the activity of the Calvin Cycle is low in both needle generations and/or is affected by deceleration of the induction phase of the photosynthetic carbon assimilation during flushing. Contrary to this the fast phase of fluorescence induction, mainly determined by photochemical quenching $q_Q$ (Duysens and Sweers 1963) is not affected in this period.

Herbicide action, however, causes both changes of the fast kinetics and changes of slow kinetics indicating that the primary site of herbicide action is near Photosystem II (Renger 1986), even if needles are not fully damaged (see Figs. 2 and 3). It can be concluded that a disturbance of the electron transport chain always induces changes of FV/FP and $F_{dr}$, whereas a weak disturbance of the Calvin Cycle activity induces only changes of $F_{dr}$. If the point of attack is situated in the Calvin Cycle causing strongly diminished activity, a feed back reaction on the capacity of the electron transport chain may be observed and as a result FV/FP will decrease.

As our results show, the pattern of chlorophyll fluorescence changes flexibly in accordance with the prevailing climatic conditions. On the other hand specific conditions of the physiological activity of the plant may induce modifications in the kinetics of chlorophyll fluorescence. If the effect of man-made stresses, e.g. the effect of chronic or temporary air pollution, are to be detected, all possible natural changes of physiological activity have to be taken into consideration first to eliminate those naturally caused modifications. For field-studies it is of particular importance to distinguish between stress phenomena caused by natural environmental conditions and stress phenomena of anthropogenic origin.

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