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# Photoinhibition of photosynthesis in willow leaves under field conditions

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**Abstract.** Chlorophyll fluorescence of leaves of a willow (*Salix* sp.) stand grown in the field in northern Sweden was measured on several occasions during the growing season of 1987. For leaves that received mostly full daylight, the $F_V/F_P$ ratio declined roughly 15% in the afternoon on cloudless days in July ($F_P$ is the fluorescence at the peak of the induction curve obtained at the prevailing air temperature after 45 min of dark adaptation, and $F_V$ is variable fluorescence, $F_V = F_P - F_0$, where $F_0$ is minimal fluorescence). There was no decrease in the $F_V/F_P$ ratio on cloudy days, while the effect was intermediate on changeable days. In view of this light dependence, together with the fact that the decline in the $F_V/F_P$ ratio was paralleled with an equal decline in the corresponding fluorescence ratio $F_V/F_M$ at 77K, and a similar decline in the maximum quantum yield of $O_2$ evolution, it is suggested that the decline in the $F_V/F_P$ ratio represents a damage in photosystem II attributable to photoinhibition. Recovery of the $F_V/F_P$ ratio in dim light following a decline on a cloudless day took 7–16 h to go to completion; the $F_V/F_P$ ratio was fully restored the following morning. When all active leaves of a peripheral shoot were compared, the $F_V/F_P$ ratio in the afternoon of a day of bright light varied greatly from leaf to leaf, though the majority of leaves showed a decline. This variation was matched by a pronounced variation in intercepted photon flux density. When leaves developed in the shade were exposed to full sunlight by trimming of the stand an increased sensitivity to photoinhibition was observed as compared to peripheral leaves. The present study indicates that peripheral willow shoots experienced in the order of 10–20% photoinhibition during an appreciable part of their life. This occurred even though the environmental conditions were within the optimal range of photosynthesis and growth.

**Key words:** Chlorophyll fluorescence – Photoinhibition – Photosynthesis – Quantum yield – *Salix*.

*Abbreviations and symbols:* $F_0$ = minimum fluorescence; $F_P$ = fluorescence at the peak of the induction curve obtained at normal ambient temperatures; $F_V$ = variable fluorescence; $F_M$ = maximum fluorescence obtained at 77K; PPFD = photosynthetic photon flux density

## Introduction

It has been recognized for a long time that inhibition of photosynthesis can be produced by exposure to high light intensities (Powles 1984). This phenomenon called photoinhibition is manifested at the level of the leaf as a decline in the quantum yield and, in most cases, in the maximal capacity of photosynthesis. Recently, much effort has been aimed at elucidating the molecular mechanisms of photoinhibition. Though it seems clear that the primary damage occurs within the electron-transfer processes of photosystem II, much controversy still exists as to the precise site. Another matter of discussion is whether photoinhibition is of any importance for the productivity of plants, or just an artefact brought about by laboratory conditions not normally encountered by plants. There are only a few studies that have considered photoinhibition in plants growing under natural conditions. Nevertheless, from the studies available it seems clear that under conditions unfavorable for growth and photosynthesis, plants become more susceptible to photoinhibition. When exposed to light intensities corresponding to bright sunlight, plants growing in the field have been shown to suffer photoinhibition under stresses of drought (Björkman and Powles 1984), chilling temperatures (Farage and

Long 1987), freezing temperatures (Öquist and Ögren 1985; Strand and Lundmark 1987), high temperatures (Ludlow and Björkman 1984; Adams et al. 1987), and in the glasshouse, under stress of limiting nitrogen supply (Osmond 1983). Also, a preceding period of shade long enough to induce shade adaptation predisposes plants to photoinhibition (Powles and Critchley 1980). However, it has yet to be demonstrated whether photoinhibition can occur in natural situations that, besides photoinhibition, are not associated with any stress.

In evaluating photoinhibition, chlorophyll fluorescence has proved to be a useful indicator. The decline in quantum yield of photosynthesis upon photoinhibition is paralleled by a corresponding decrease in the variable component of chlorophyll fluorescence (Ögren and Öquist 1984a; Ögren et al. 1984; Demmig and Björkman 1987). This correlation and the simplicity and rapidity of the technique has opened the possibility of its use in the extensive search for photoinhibition in nature. Aiming at this goal, a portable battery-powered instrument for chlorophyll fluorescence measurements was designed and built at the department (Öquist and Wass 1988). This instrument resolves the rapid kinetics of chlorophyll fluorescence induction equally well as the traditionally used but more cumbersome techniques that require liquid-nitrogen temperatures or an oscilloscope. As each measurement only takes a few seconds, and can be performed on attached leaves, the instrument enabled a large number of leaves to be assayed in the field. In the present study this technique for fluorescence measurement was applied to address the question of whether photoinhibition occurs under non-stress conditions. Evidence is presented indicating the frequent occurrence of photoinhibition throughout the growing season, even at light intensities less than full sunlight, for a plant growing in a field amply supplied with water and nutrients, and at temperatures that are within the optimal range for photosynthesis.

## Material and methods

*Plant material.* Under investigation was a stand of a single clone of *Salix* sp. (originating in Finland; denoted No. 075 in the Swedish Energy Forestry Project, Swedish University of Agricultural Sciences, Uppsala, Sweden), relatively dense, about 5 years old, 2–4 m in height, covering a ground area of about 15 $m^2$, and growing in well-fertilized soil in the yard outside the department in Umeå (63°50′N, 20°20′E, altitude 30 m). As a consequence of shading from buildings, reception of direct solar radiation by the stand was restricted to the hours between 9 am and 4 pm (Swedish normal time). The study was carried out in July 1987, with the exception of a single day in September. In July, mean daily and absolute maximum/minimum air temperatures at leaf height were 19.4/9.1° C and 27.3/4.6° C, respectively; 3 d were cloudless between the hours 7 am and 4 pm, 12 d were totally cloudy and the rest (16) were changeable. On cloudless days the maximum photosynthetic photon flux density (PPFD) at solar noon was 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on a horizontal plane, and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on a plane at right angle to the solar beam. With the exception of the study of whole shoots of Fig. 7, the sample leaves were oriented towards the sector SE–SW; one leaf per shoot was selected among the third- to fifth-uppermost fully expanded ones. The shoots were either peripheral and not subjected to shading from outside, or interior and shaded by neighbouring shoots for most of the time.

*Measurement of chlorophyll fluorescence at the prevailing air temperature.* This was done using a fibre-optic-based, portable instrument designed and built at the department (Öquist and Wass 1988). It is provided with a magnetic shutter that allows resolution of the initial, minimum fluorescence level, $F_0$, that is emitted when the leaf is illuminated with the built-in light source. The fluorescence-induction curve from $F_0$ to the peak, $F_P$, and beyond is monitored by a photodiode and stored in a microprocessor unit that also calculates the ratio of $F_V/F_P$, where $F_V = F_P - F_0$. The actinic illumination is set to be strong enough to produce maximum fluorescence ($F_M$) at the *P*-peak. The whole procedure is completed within a few seconds. The particular instrumental settings used in the present study are detailed in the legend of Fig. 1. Prior to measurement the leaf was dark pre-treated for 45 min using a lightweight clamp cuvette. This was done on attached leaves if not otherwise stated. Without removing the cuvette, the fibre end (7 mm diameter) was applied onto the leaf surface through a gate while bright light was prevented from entering the gate by the aid of a black cloth. By this means the leaf remained dark adapted until excitation. The instrument, under the name of Plant Stress Meter, is now manufactured by Polartech, Umeå, Sweden.

*Measurement of chlorophyll fluorescence at 77K.* This was done using a fibre-optic-based system described elsewhere (Ögren and Öquist 1984a). Fluorescence was sensitized by 433-nm excitation (half-band width 10 nm) of 1.0–1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and was detected at 689 nm (half-band width 6.4 nm); the photosystem-II band peak appeared at 689 nm in emission-spectra measurements at 77K of intact willow leaves (half-band width of monochromator 3.2 nm). The fluorescence induction from the initial level, $F_0$, to the maximum steady-state level, $F_M$, was slow enough (4–5 min) to be resolved on a chart recorder.

*Measurement of quantum yield.* Exchange of $O_2$ was measured at $CO_2$ saturation with a leaf-disc electrode (Model LD2; Hansatech Ltd., King's Lynn, Norfolk, UK) connected to a readout control box (Model CB1; Hansatech), and a chart recorder. The chamber was maintained at 25° C by attaching it to a waterbath. White light provided from the Hansatech light source (Model LS2), was passed through a Calflex-C heat-reflecting filter (Balzers AG., Fürstentum, Liechtenstein), a heat-absorbing filter (KG4; Schott AG, Mainz, FRG) and, to obtain the desired attenuation, Schott neutral-density filters. For each filter combination used, the PPFD at the position of the leaf was determined with a quantum sensor (Model Li-185 A; Li-Cor Inc., Lincoln, Neb., USA) as the mean value of measurements at 17 evenly spaced spots. The calibration of the quantum sensor was checked after completion of this study with an authorized standard lamp (Techtum Instruments, Umeå, Sweden) and was found to be correct within 1%. The measurements

of quantum yields of $O_2$ evolution were conducted as follows: within 2 min after detaching a leaf outdoors, or terminating a high-light exposure in the laboratory, a 10.0-$cm^2$ disc was cut, transferred to the chamber and immediately illuminated by a PPFD of 96 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The chamber was flushed with a stream of 5% $CO_2$ in air from a gas cylinder, that before entering the chamber was adjusted to a flow rate of about 20 $cm^3 \cdot min^{-1}$ using a thermal-mass-flow controller (Model 5810/5835; Brooks instrument Co, Veenendaal, Netherlands), and water-saturated at 25° C by bubbling through a water column held at this temperature. This flow rate was high enough to maintain 5% $CO_2$ in the chamber and low enough not to cause any detectable loss of the leaf water. The leaf disc was then allowed to equilibrate for 60 min prior to taking the first reading (at 96 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Pilot studies with control willow leaves had proved that it takes 40–50 min to reach a steady-state rate of $O_2$ evolution at this light level. The PPFD was then decreased in steps (70, 43 and 26 $\mu mol \cdot m^{-2} \cdot s^{-1}$). At each step the inlet and outlet valves were first held open for about 5 min to maintain the high-$CO_2$ atmosphere; the valves were then alternatively open or closed until a constant rise in $O_2$ concentration was obtained with close valves. Throughout these procedures the $CO_2$ content of the chamber atmosphere did not drop below 4.9% (assuming the exchange ratio $O_2$:$CO_2$ to be 1:1). When the response to the lowest PPFD had been recorded, the chamber was darkened and the rate of respiration was recorded. It was ascertained that this rate was not dependent on the PPFD of the previous light period: the steady-state rate of $O_2$ evolution at the PPFD of 96 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was once again established and the measurement of dark respiration was repeated. For all leaves the two dark measurements gave the same result. Immediately after removal of the leaf disc, the electrode was calibrated using streams of air, assumed to contain 21% of $O_2$, and of pure $N_2$. Rates of $O_2$ exchange were calculated from the ideal gas law. The volume of the empty chamber with all accessories present (metal and foam discs, fibre matting) was 5.68 $cm^3$. It was determied by recording the change in signal when 1.0 $cm^3$ of air was injected from a gas-tight Hamilton syringe into the air-filled chamber (Delieu and Walker 1981). From this value was subtracted the volume of the leaf ($cm^3$) assumed to be equal to its weight (g) and the dead volume of the syringe holder of the valve (0.12 $cm^3$). After finishing $O_2$-exchange measurements, the leaf was kept in the dark between wet filter papers for 45 min prior to fluorecence measurements. Following fluorescence measurements a 3-$cm^2$ disc was punched from the centre of the original 10-$cm^2$ disc. Using this smaller disc the leaf-absorptance was determined in an Ulbricht integrating sphere (15 cm diameter) coated on the inside with magnesium oxide. Light from a projector lamp was focused onto the sample held at the centre of the sphere by a holder. The Li-Cor quantum sensor, level with the inner wall, was shielded from the light beam and from light directly reflected from the sample. The PPFD was read with the leaf sample inside ($I$) and outside ($I_0$) the beam, and absorptance calculated as being equal to $1-(I/I_0)$. The spectral distribution of the projector lamp was, within the range of 400–650 nm, identical with the lamp used in the $O_2$ measurements and, over the range of 650–700 nm, 20% lower than the lamp. This was determined with a quanta spectrometer (Model QSM-2500; Techtum Instruments). The $O_2$-exchange data were plotted against absorbed PPFD and a straight line was fitted for four to five data points by the method of least squares. In all cases a correlation coefficient of $R^2 > 0.999$ was obtained.

*Measurements of integrated PPFD.* The PPFD data in the field were obtained using a Li-Cor quantum sensor connected to a millivolt integrator (Model MVI; Delta-T Devices, Cambridge, UK).

*Photoinhibition under artificial light.* The data represented by open symbols in Fig. 4, and the broken line in Fig. 1, were obtained using leaves that had been removed from the study stand and exposed to a PPFD of 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided from a metal-halogen lamp (HQI-TS 400 W; Osram, Berlin, West Germany) at 16° C (measured with 0.05-mm-diameter chromel-alumel thermocouples). The leaf upper surface was illuminated behind a 7-cm water filter, while the petiole was immersed in water and the opposite leaf surface covered with a soaking-wet filter paper.

## Results

In Fig. 1. the induction of chlorophyll fluorescence emission at room temperature following 45 min of dark pre-treatment is compared for a control and a photoinhibited willow leaf. The rise in fluorescence from the initial, minimum level ($F_0$) to the peak ($F_P$; nomenclature from Papageorgiou 1975) reflects the gradual accumulation of photosystem II centres with a photoreduced electron-acceptor $Q_A$ (Duysens and Sweers 1963; Bradbury and Baker 1983). Evidently, upon photoinhibition the variable component ($F_V = F_P - F_0$) is decreased. Provided $F_0$ is correctly resolved, all $Q_A$ acceptors are reduced at $F_P$, and no significant trans-thylakoid proton gradient is yet attained at $F_P$, the $F_V/F_P$ ratio would reflect the maximal efficiency of primary photochemistry of photosystem II (Lavorel and Etienne 1977). To check if these requirements were met, comparison was made with fluorescence induction at 77K. At this low temperature the elec-

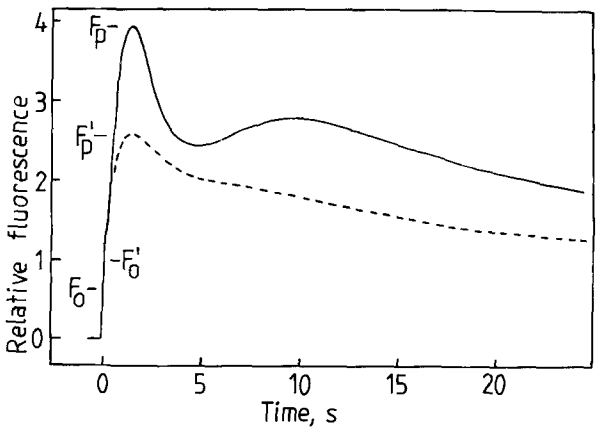


**Fig. 1.** Induction of chlorophyll fluorescence emission at 690 nm (half-band width 12 nm) in a detached willow leaf at about 20° C, before (*solid line*) and after (*broken line*) exposure to a PPFD of 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants were pre-treated with 45 min of darkness prior to broad-band excitation (330–660 nm, peaking at 500 nm; PPFD = 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Throughout the rest of this study measurements were terminated after 5 s

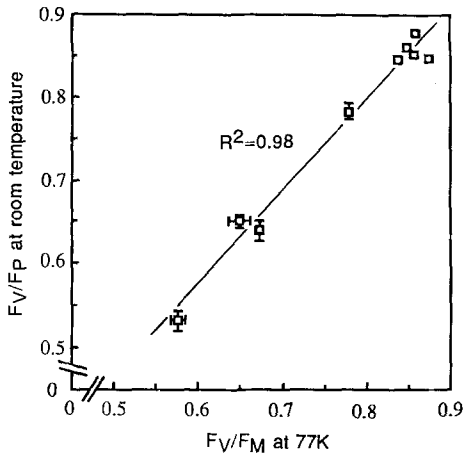


**Fig. 2.** Relationship between the $F_V/F_P$ ratio at room temperature and the $F_V/F_M$ ratio at 77K in willow leaves that at their natural positions had experienced various photoinhibitory PPFDs, as described in **Fig. 5**. Room-temperature measurements (detailed in **Fig. 1**) were completed within 5 min and immediately followed by the 77K measurements (detailed in *Material and methods*). The mean values of 3 (77K) or 13 (room temperature) replicate measurements of one leaf are given. Only SEs > 0.006 are represented. The equation of the regression line is $y = 1.12x - 0.10$

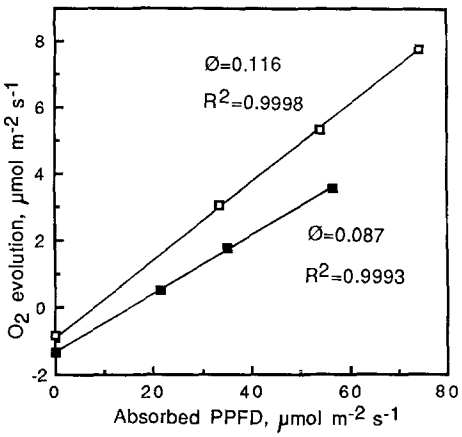


**Fig. 3.** Oxygen evolution at limiting absorbed PPFDs in shade-acclimated willow leaves, for a sample that has experienced full sunlight for 5 h (■), and for a control sample (□). Prior to measurements the leaf samples were kept in the electrode chamber for 60 min at an incident PPFD of 96 μmol·$m^{-2}$·$s^{-1}$. Values of quantum yields (slopes) are given

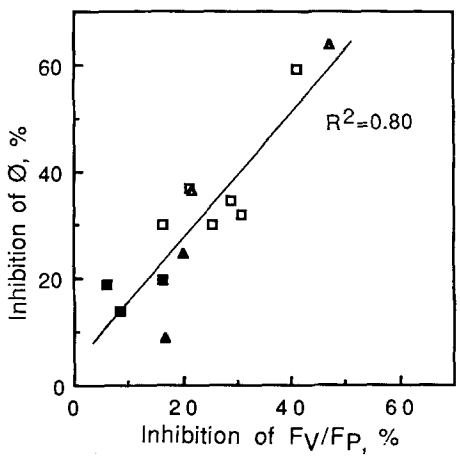


**Fig. 4.** Relationship between inhibition of the quantum yield of $O_2$ evolution and inhibition of the $F_V/F_P$ ratio in willow leaves that have been exposed to sunlight for 5.5 h (*closed symbols*), and to a PPFD of 2000 μmol·$m^{-2}$·$s^{-1}$ from a lamp for various periods of time at 16° C (*open symbols*). Leaves were developed on shade (*triangles*) or peripheral (*squares*) shoots. Following treatment, each leaf sample was equilibrated in the electrode chamber as described in the legend of **Fig. 3**, followed by $O_2$ measurements for about 20 min, dark pre-treatment for 45 min, and finally, 10–13 replicate measurements of fluorescence. The control values of the quantum yield of $O_2$ evolution and of the $F_V/F_P$ ratio were 0.115 (SE = 0.001) and 0.865 (SE = 0.009), respectively (mean values of five leaves, developed and kept in the shade)

tron-transfer reactions are limited to only those occurring at the reaction centres and the ratio of $F_V/F_M$ at 685–695 nm, where $F_M$ is the maximum steady-state level and $F_V = F_M - F_0$, is known to represent the maximal efficiency of primary photochemistry of photosystem II (Butler 1978). A close correspondence between the two methods was found using leaves that had been exposed to a range of natural, photoinhibitory light regimes at their undisturbed positions within a stand (Fig. 2). Therefore, it seems well justified to use the $F_V/F_P$ ratio at the prevailing air temperature as a measure of the damage of the photochemistry of photosystem II that occurs when willow leaves are exposed to bright light in the field.

To assess to what extent photoinhibition of photosystem-II photochemistry is expressed at the leaf level, the rate of $O_2$ evolution of leaf discs was measured at limiting absorbed PPFD. The results are exemplified in Fig. 3 for a control shade leaf and a shade leaf that had been exposed to full sunlight for 5 h. As the rates of $O_2$ evolution are linearly related to absorbed PPFD, the slopes of the lines represent maximal quantum yields of $O_2$ evolution. For a number of photoinhibition states, measurement of the quantum yield of $O_2$ evolution was followed by measurement of fluorescence induction on the same leaf disc. Figure 4 shows that for both experimentally (open symbols) and naturally (closed symbols) photoinhibited leaves, damage to the photosystem-II photochemistry, as probed by fluorescence measurements, and damage to the quantum yield of $O_2$ evolution correlated fairly well. Furthermore, the relationship is roughly 1:1, indicating that the magnitude of

inhibition of the ratio of $F_V/F_P$ can be taken as an estimate of the magnitude of inhibition of the quantum yield of $O_2$ evolution.

Above it is stated that in field-grown willow plants, parallel effects on the photosystem-II photochemistry and on the quantum yield of $O_2$ evolution were observed that could be assigned to photoinhibition. A full account of data and argument underlying this interpretation follows. Diurnal changes in fluorescence characteristics of attached leaves of a willow stand grown outdoors were followed in the summer of 1987. Even though sample leaves were selected on criteria that made them liable to receive direct sun light (situated near the top of peripheral shoots; oriented towards the sector SE to SW), shading from neighbour leaves still frequently occurred as the leaves were undisturbed and free to move in the wind. The results, gathered from several days, are summarized in Fig. 5, where the $F_V/F_P$ ratios at 9:00 and at 14:30 h (Swedish normal time) are expressed as a function of the average value of external PPFD of the time interval in between. On clear days in July with mean values of PPFD around 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the $F_V/F_P$ ratio dropped roughly 15% (triangles). There was no decrease in the $F_V/F_P$ ratio on cloudy days (PPFD $< 500$ $\mu mol \cdot m^{-2} \cdot s^{-1}$) and an intermediate decrease was observed on changeable days (PPFD $= 600$–$1000$ $\mu mol \cdot m^{-2} \cdot s^{-1}$). This light dependence indicates that photoinhibition is the underlying mechanism. Other possibilities could be excluded on the grounds that none of the environmental factors, temperature, humidity, wind speed and direction, were correlated with the $F_V/F_P$ ratio (data from local climate station; not shown). Nor could water stress be responsible, since the leaf water potential remained high even on clear days ($-4.0$ to $-8.5$ bar measured with Scholander bomb; data not shown).

The data discussed so far refer to leaves with positions on peripheral shoots and therefore can be considered to be acclimated to bright light. It is a general feature of photoinhibition that leaves acclimated to shade are more prone to become photoinhibited. With this knowledge in mind an experiment was conducted where the stand was trimmed such that shade leaves became fully exposed to sunlight. The result obtained was an even more pronounced decrease in the $F_V/F_P$ ratio (Fig. 5, squares), which finding once again gives support in favour of photoinhibition.

On all occasions of photoinhibition presented in Fig. 5, fluorescence characteristics were fully restored the following morning. This reversibility of photoinhibition was examined in more detail

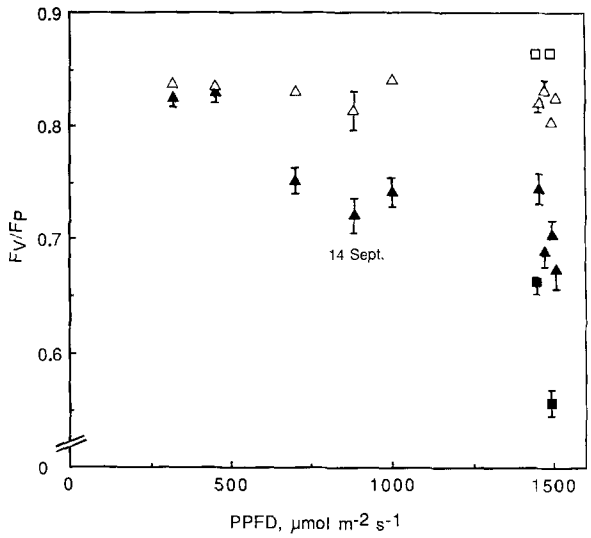


**Fig. 5.** The $F_V/F_P$ ratio of attached willow leaves at 9:00 (*open*) and at 14:30 h (*closed symbols*) versus the average, external PPFD of the intervening time period. Leaves were naturally exposed, i.e. on peripheral shoots (*triangles*), or developed in the shade but exposed to direct sunlight by trimming of the stand (*squares*). With one exception, 14 Sept. (indicated), measurements were performed over the period 3–29 July 1987. Mean values and SEs $> 0.007$ are given for 25 replicate leaves (*triangle*) and 10 replicate measurements on a single leaf (*square*)

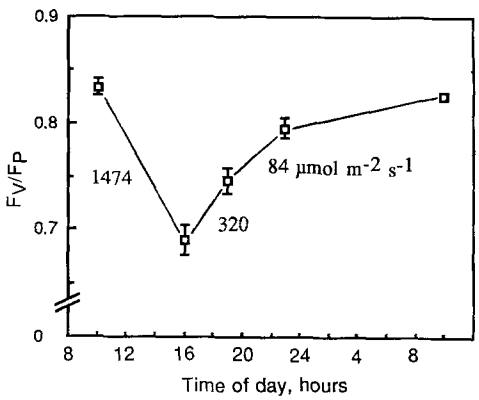


**Fig. 6.** The time course of recovery of the $F_V/F_P$ ratio from photoinhibition in naturally exposed leaves on peripheral shoots on a clear day (16 July). Experimental conditions were the same as in **Fig. 5**. The mean values of the external PPFD of the time periods in between measurements are indicated. Mean values of SEs $> 0.006$ of 25 replicate leaves are given

in a study presented in Fig. 6. Following 5.5 h of full sunlight, the leaves recovered from most of the photoinhibitory damage on the same day. During the recovery phase the leaves experienced diffuse light only.

The late measurement of $F_V/F_P$ on 14 September fits into the general pattern (Fig. 5). Thus the response of photoinhibition to light does not

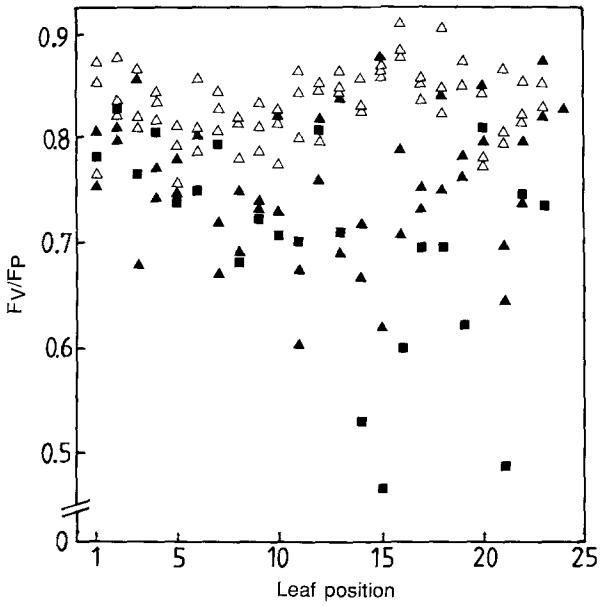


**Fig. 7.** The $F_V/F_P$ ratio at 9:00 h (*open symbols*) and at 15:30 h (*closed symbols*) on three successive days (29–31 July) as a function of the position of the leaf, from the uppermost fully expanded (*position 1*), down to the lowest non-chlorotic one (*position 24*), within a single peripheral shoot. The average value of external PPFD of the intervening time period was about 1000 and 1300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for, respectively, the first two days (*triangles*) and the third day (*squares*)

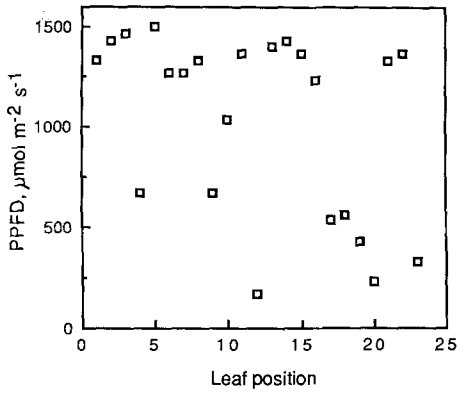


**Fig. 8.** The PPFD as a function of leaf position on the third day (at 12:00) of the experiment of **Fig. 7**. Average values for 1-min periods of integration are given. The sensor was held on a horizontal plane

seem to vary over the growing season. However, less photoinhibition would be expected as a result of a general decrease in maximal PPFD towards the end of the season.

In addition to examining the temporal variation in photoinhibition, an examination of the spatial variation was made by measuring the fluorescence characteristics of all fully expanded leaves of a single, peripheral shoot. In contrast to the situation above, the leaves were oriented in all directions. This increased the leaf-to-leaf variation in intercepted PPFD in comparison to the temporal study. On three successive days of bright light, the two first with average PPFDs about 30% below that of the third due to clouds, the ratio of $F_V/F_P$ was decreased in the afternoon for the majority of the leaves, even for those on the lowest parts on the shoot (Fig. 7). However, the degree of photoinhibition varied greatly from leaf to leaf. This can presumably be ascribed to variation in intercepted PPFD among the leaves. Indeed, the PPFD at the various leaf positions showed great variability (Fig. 8). It is especially important to note that high PPFD could occur even on the lowest leaves of the shoot (Fig. 8), which matches the occurrence of photoinhibition among the lowest leaves (Fig. 7). Also the data illustrate once again the light dependence of photoinhibition, since the most pronounced photoinhibition occurred on the third day which had the highest PPFD (Fig. 7, squares).

## Discussion

Willow leaves with exposed, undisturbed positions in a field-grown stand frequently exhibited a depression in the $F_V/F_P$ ratio in the afternoon. The mechanism responsible is suggested to be photoinhibition of the well-known type with primary damage residing within photosystem II. This idea is supported by several lines of evidence that are discussed in the following.

(i) The prevailing PPFD appears to be the determining factor for the decline in $F_V/F_P$ ratio. The decline was largest (up to about 20%) on cloudless days, smaller on changeable days, and altogether absent on cloudy days Fig. 5). This observation is important, since one of the most marked features of photoinhibition is its light dependence (Powles 1984). Other environmental factors that potentially can undergo diurnal changes, such as water relations and temperature, could not be related to the depression in the $F_V/F_P$ ratio (data not shown). Also, when comparing leaves of different positions within the same shoot, there was a marked variability in the afternoon values of the $F_V/F_P$ ratio on clear days (Fig. 7), and an even more marked variability in PPFD measured at the leaf level (Fig. 8). It is unlikely that other factors, environmental or physiological, could match the high degree of variability in PPFD, making them less likely candidates.

(ii) Leaves developed in the shade appeared to be more sensitive to bright light than leaves devel-

oped at exposed places (Fig. 5), which finding is consistent with the differential sensitivity to photoinhibition of shade and sun leaves (Powles an Critchley 1980).

(iii) The recovery in dim light of the $F_V/F_P$ ratio following a decline on a clear day took 7–16 h to go to completion (Fig. 6). This rate of recovery agrees well with other reports on photoinhibition in higher plants, for instance in Ögren et al. (1984) where complete recovery of photosynthesis by *Lemna* plants from 60% photoinhibition took about 10 h. Processes other than photoinhibition known to affect the fluorescence characteristics generally show kinetics of relaxation that are about an order of magnitude faster, except for those associated with excessively high (above 35° C) or freezing temperatures (below −4° C) that are irrelevant in this context. The best defined of these operate in the time scales of seconds or minutes, and are related to the trans-thylakoid proton gradient (Krause et al. 1983), the redox state of the primary photosystem-II electron acceptors (Robinson and Croft 1983), or the distribution of excitation energy within the photochemical apparatus (Hodges and Barber 1983). Probably, the willow leaves became fully relaxed with respect to these processes during the 45-min period of darkness prior to the fluorescence measurements. Recently, evidence has appeared for an additional kind of fluorescence quenching, showing time periods of recovery approaching but not reaching that of photoinhibition after effecting decreases in both $F_0$ and $F_M$ at 77K (Björkman and Powles 1984; Demmig and Björkman 1987). This is in contrast to the situation with the willow leaves where $F_0$ invariably underwent an increase in parallel with the decrease in $F_V/F_M$ ratio (data not shown).

(iv) Evidence is presented that the decline in $F_V/F_P$ ratio at room temperature was paralleled by an equal decline in the corresponding ratio at 77K (Fig. 2), and also a similar decline in the quantum yield of $O_2$ evolution (Fig. 4). In fact, the inhibition of the quantum yield somewhat exceeded that of the $F_V/F_P$ ratio, though this might be attributable to the fact that measurement of the latter was extra delayed (10 min at dim light plus 45 min in darkness), during which time period, additional recovery might have occurred. These results are in agreement with the current literature, for instance (Ögren and Öquist 1984a, b; Ögren et al. 1984; Demmig and Björkman 1987) where it is shown that the primary photodamage, residing within the primary photochemistry of photosystem II as probed by the $F_V/F_M$ ratio at 77K, is fully expressed by the quantum yield of photosynthesis.

Even if there appears to be little doubt that photoinhibition of photosynthesis occurred in the willow leaves, the question as to the importance of this still remains. From the knowledge that it is restricted to clear and changeable days, one can, from climate data, extrapolate the fluorescence data for July (Fig. 5) to the whole season (essentially the period 1 June to 30 August). Provided this extrapolation is correct and the $F_V/F_P$ ratio and the quantum yield are equally affected (as indicated by Fig. 4), it can be estimated that on roughly one-third of the days of the growing season of 1987, the willow leaves of exposed, natural positions became photoinhibited in the order of 10–20%. These figures are essentially applicable to whole, peripheral shoots as well (Fig. 7). For the peripheral shoots, therefore, it seems reasonable to guess that photoinhibition resulted in a depression of the potential carbon gain or 1987 that was in the order of a few percent.

There are a number of reports demonstrating diurnal changes in the photosynthetic rate by leaves growing in the field. Different possible mechanisms have been suggested. Under conditions of high light and temperatures above 30° C, so-called midday depression of $CO_2$ exchange has been observed in many plants. This is shown to be associated with inhibition of the photosynthetic capacity, independent of stomatal effects, which is reversed in the afternoon on the same day (Tenhunen et al. 1984). This is different from the present study where the temperature did not reach above the optimum, and the kinetics of recovery was slower. Other workers have, in field-grown eucalyptus, observed a decrease in photosynthetic capactiy on clear days that is hypothesized to manifest the operation of endogenous factors triggered by high doses of light (Küppers et al. 1986). Though this effect shows some similarities with that of the present study (it is not particularly dependent on temperature and it is reversible on the hours scale), it is principally different since the quantum yield of $CO_2$ uptake is reported to remain constant. Distinct from this, sun-exposed leaves of *Nerium oleander* are reported to exhibit as diurnal variation in the fluorescence ratio of $F_V/F_M$ at 77K, but no effect on the photosynthetic functioning could be demonstrated unless water stress was applied (Björkman and Powles 1984). Under well-watered conditions this effect was reversed within about 1 h, i.e. much faster than for the willow leaves. In view of the current literature, therefore, the present paper describes a novel situation of photoinhibition in the sense that partial photoinbibition is indicated to occur frequently in plants

growing under the moderate conditions of light and temperature that prevail during the summers of high latitudes.

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