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Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves

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Abstract. Plants from clonal cuttings of *Salix* sp. were subjected to a drying cycle of 10 d in a controlled environment. Gas exchange and fluorescence emission were measured on attached leaves. The light-saturated photosynthetic CO_2 uptake became progressively inhibited with decreased leaf water potential both at high, and especially, at low intercellular CO_2 pressure. The maximal quantum yield of CO_2 uptake was more resistant. The inhibition of light-saturated CO_2 uptake at leaf water potentials around −10 bar, measured at a natural ambient CO_2 concentration, was equally attributable to stomatal and non-stomatal factors, but the further inhibition below this water-stress level was caused solely by non-stomatal factors. The kinetics of fluorescence emission was changed at severe water stress; the slow secondary oscillations of the induction curve were attenuated, and this probably indicates perturbations in the carbon reduction cycle. The influence of light level during the drought period was also studied. Provided the leaves were properly light-acclimated, drought at high and low light levels produced essentially the same effects on photosynthesis. However, low-light-acclimated leaves became more susceptible to photoinhibitory treatment under severe water stress, as compared with well-watered conditions.

Key words: Chlorophyll fluorescence – Photoinhibition – Photosynthesis (drought effect) – *Salix* – Water stress.

Introduction

The rate of photosynthesis declines as leaf water stress increases. This is a consequence of both stomatal closure causing increased constraint on CO_2 diffusion, and decreased chloroplast activity (for a recent review see Bradford and Hsiao 1982). It is still a matter of controversy as to which chloroplast processes are most affected. There are studies reporting prominent water-stress effects on the thylakoid reactions (Fry 1972; Keck and Boyer 1974), but there are also those of a conflicting view that the dark reactions of the stroma are more affected (Kaiser et al. 1981; von Caemmerer and Farquhar 1984). The lack of consensus might partly be attributed to the use of different experimental designs; a wide range of both plant material and rate, degree and duration of water stress is being investigated. Also, it is possible that besides water status other environmental factors are allowed to contribute to the photosynthetic depression. For instance, if water stress is accompanied by an increased susceptibility to photoinhibition, as suggested by Björkman and Powles (1984), the concomitant light level should be taken into account.

The objectives of the present work were i) to quantify the contribution of stomatal and non-stomatal factors in the photosynthetic inhibition throughout a drying cycle of several days; ii) to elucidate the underlying causes of the non-stomatal effect; iii) to investigate whether water status and light level can interact to induce photoinhibition; iv) to evaluate the use of chlorophyll fluorescence as a probe for leaf water stress. This study uses a willow species with the potential of becoming a biofuel producer, but before it can be fully exploited its ability to withstand water stress and other unfavourable conditions must be investigated. Besides this, the use of a non-domesticated plant such as this may be advantageous when studying stress because domesticated plants may have evolved divergent stress responses.

Material and methods

Plant material. Cuttings of *Salix* sp. (clone No. 075 in the Swedish Energy Forestry Project, Swedish University of Agricultural Sciences, Uppsala, Sweden) were water-saturated after storage at 0°C by keeping the lower ends submerged in aerated water for about 24 h at room-temperature. They were

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Abbreviations and symbols: P_i = intercellular partial pressure of CO_2; PPFD = photosynthetic photon flux density; RuBP = ribulose 1,5-bisphosphate; ψ_L = leaf water potential

then planted in fertilized peat (Hasselfors Garden AB, Hasselfors, Sweden), first in small pots and after establishment in 5-dm^3 pots. During the growth period the plants were trimmed to carry two shoots without side-shoots and watered daily; two to three times a week a commercial, complete nutrient solution (Blomrika, Weibulls, Landskrona, Sweden) was used instead of pure water. The climate-chamber conditions were as follows: 17 h photoperiod, 23/15° C light/dark temperature, 70% relative humidity, and a photosynthetic photon flux density (PPFD) of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the tops of the plants was provided by metal-halogen lamps (HQI-TS 400 W Osram, Berlin, FRG). Before the drought treatment was started under the climate-chamber conditions (after approx. 14 d of growth), one shoot of the plant was cut off, leaving one shoot of a length of approx. 0.5 m. Two fully expanded leaves were selected for experimental purposes (the seventh and eighth leaf from the upper leaf that had attained a length of approx. 0.1 m), and nutrient solution was added to give a plant plus soil weight of approx. 2500 g. For the drought treatments, the weight was allowed to decrease by 150 g per day during the first 8 d; transpiration losses exceeding this figure were replaced by the alternate addition of water and nutrient solution. During the following 2 d the weight decrease was slower and nothing was added. The relationship between leaf water potential and duration of treatment is indicated in Fig. 2A. For the controls, the plant plus soil weight of 2500 g was maintained by daily additions of water or, one to two times a week, with nutrient solution.

The susceptibility of photosynthesis to photoinhibition under the drought conditions described above was studied in two different experiments. For this purpose a supplementary light from a projector (Liesegang Fanti Automat, Düsseldorf, FRG; with a 24 V/150 W Thorn lamp) was focused on the leaf samples. In one experiment, the leaves were exposed to a single 6-h period of a PPFD of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, followed by a 30-min period of a PPFD of 100–200 $\mu mol \cdot^{-2} \cdot s^{-1}$ before the start of gas-exchange measurements. The leaf temperature measured by copper-constantan thermocouples rose from 22.9 to 24.5° C upon the high PPFD exposure irrespectively of water status. In the second experiment, the supplementary PPFD exposed the leaves in a 13-h photoperiod (preceded and followed by 2 h of the background illumination) starting during their unfolding and gradually increasing over 2 d up to a PPFD of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This level was maintained, for 4–5 d of optimal watering, and during the subsequent period of restricted watering.

Measurements of gas exchange and photosynthetic photon flux density. Uptake of CO_2 and transpiration in single, attached leaves were measured with an open gas-exchange system identical to that described by Hällgren et al. (1982) except for the CO_2 analyser (Series 225 Mk3; Analytical Development Co., Hoddeson, Herts., UK) and the assimilation chamber (the leaf was held in position by the lid frames instead of screens). Calibration of the CO_2 analyser was made according to Thorpe (1978) at a range of known CO_2 concentrations obtained by the mixing pumps from CO_2-depleted air and CO_2-enriched cylinder air (analyzed for CO_2 at the Department of Inorganic Chemistry, University of Umeå). Rate of CO_2 uptake, conductances to CO_2 and H_2O, and intercellular partial pressure of CO_2 (P_i) were calculated according to von Caemmerer and Farquhar (1981; equations B5, 8, 14, 15, 16, and 18). The stomatal conductances were separated from the boundary-layer conductances but included cuticular conductances. The boundary-layer conductance to H_2O was estimated at 3.7 $mol \cdot m^{-2} \cdot s^{-1}$ by measuring the evaporation from a soaking-wet blotting paper with dimensions and position as for the average leaf. The saturation water-vapour pressure, assumed to be applicable to the intercellular spaces, was determined from leaf temperatures and the empirical formula of Riegel (1974). Uptake of CO_2 and conductances were expressed in terms of projected leaf area. Uptake of CO_2 at an external CO_2 pressure of 32.5 Pa, and contribution of stomatal and non-stomatal factors to its depression, were calculated as described in the legend of Fig. 1.

The measurements were conducted as follows. The plant was removed from the growth chamber 6–8 h after the onset of a photoperiod. The leaf midsection (55 mm length, 35–55 mm width) was enclosed in the gas-exchange chamber and illuminated with the projector specified above. The PPFD was measured outside the chamber next to the leaf and was varied by altering the length of the light path or attenuating the light with white nylon cloth. The rest of the plant was not exposed to the actinic light, and was occasionally lightly sprinkled with water to offset the decreased atmospheric humidity on the removal of the plant from the growth chamber. The leaf-air water-vapour pressure difference in the chamber was within 1.2–1.4 kPa. To measure the CO_2 response, a CO_2 pressure of 43.5 Pa was first supplied and the PPFD was gradually increased to above the point of saturation, usually to 1200 (stressed) or 1400 (control) $\mu mol \cdot m^{-2} \cdot s^{-1}$. A sequence of readings were then made by increasing the CO_2 pressure step-wise, and then decreasing it below that first supplied. No photoinhibition of photosynthesis was detectable after the completed measurement. The P_i was 60–100 Pa during measurement of the apparent quantum yield of photosynthesis.

The PPFD was measured with a quantum meter (Li-185A; Li-Cor Inc., Lincoln, Neb. USA), unless otherwise stated at the plane of the leaf.

Measurement of fluorescence. This was done on attached leaves using a fiber-optic-based system (Ögren and Öquist 1984) to excite and collect fluorescence as detailed in the legend of Fig. 6. About 5 min prior to excitation the leaf was gently fixed between two plastic-foam-coated Perspex bars (approx. 1 cm width) across its mid-section so that an attached Perspex rod (8 mm diameter), linking to the fiber-optic system, touched the upper leaf surface halfway between the midvein and the margin.

Measurements of leaf water potential (ψ_L) and relative water content. ψ_L was determined using a pressure chamber (Scholander et al. 1964), and using the leaf studied in the gas-exchange experiments, unless otherwise stated. (With only a few exceptions this ψ_L value differed less than 1 bar from a ψ_L value measured on an adjacent leaf immediately before the plant was transferred to the gas-exchange apparatus. This was done at a time of the day when the diurnal variation in ψ_L had reached a plateau minimum.) In the fluorescence experiments, ψ_L was determined on a leaf adjacent to that studied and excised prior to the dark pretreatment of the plant. Osmotic potential was estimated from the relation between the reciprocal of ψ_L and the relative water content on leaves that had experienced 10 d of restricted watering, and on leaves that had experienced 8–9 d of adequate watering. For this purpose four leaves per plant (the sixth to ninth leaves as defined in the plant material section) were excised under water, and placed with petioles submerged in a small container (the interior lined with wet blotting paper) that was sealed and kept at 4° C for about 16 h. A leaf was then rapidly weighed (at 100% relative water content) followed by repeated determinations of fresh weight and ψ_L; in the periods between, the leaf transpired freely outside the pressure chamber. Afterwards, the leaf dry weight was determined (after 24 h at 105° C). In all studies, the leaves were enclosed in plastic bags during transport and pressure-chamber measurements to minimize water losses. Also, the pressure chamber was pressurized, and depressurized, at a rate of about 10 $kPa \cdot s^{-1}$.

Results

Effect of water stress on the relationship between CO_2 uptake and intercellular CO_2 pressure (P_i). When water stress developed in attached willow leaves because of restricted watering, the light-saturated photosynthetic CO_2 uptake became progressively inhibited, both at high and low P_i. Representative photosynthetic CO_2 response curves of a control, a moderately and a severely stressed leaf are shown in Fig. 1. Figure 2 summarizes data of the CO_2-saturated maximal rate (A) and the initial slope (B) of CO_2-response curves, for control leaves (ψ_L within −5 to −6 bar) and leaves that had experienced up to 10 d of decreasing soil water content. Uptake of CO_2 was unaffected above a ψ_L in the interval −9 to −10 bar. Below this ψ_L, a marked decrease in both the maximal rate and the initial slope occurred over the following 3 d. The initial slope seems to be most sensitive since 50% inhibition of the initial slope and of the maximal rate was obtained at leaf water potentials of about −11 and −14 bar, respectively.

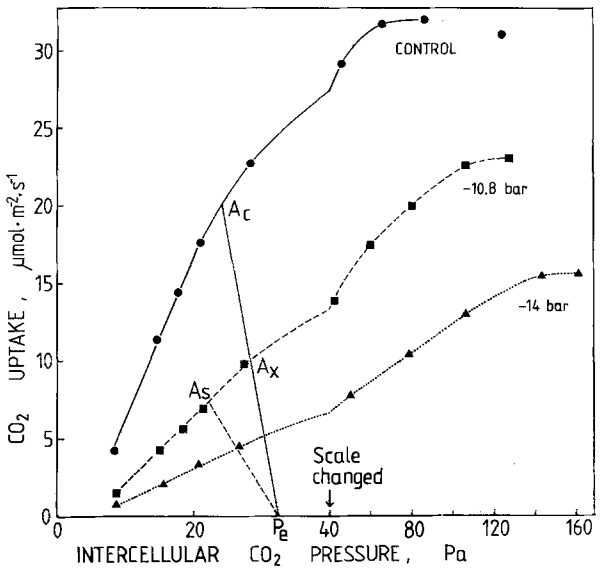

Fig. 1. Response of CO_2 uptake rate to intercellular CO_2 pressure (P_i) in willow leaves with water potentials of −5.5 (control), ●, −10.8, ■, and −14.0 bar, ▲. Measurements were made at saturating PPFDs and constant leaf temperature within 24.0–26.0° C. Supplementary characters and lines illustrate the calculations that yielded data of Figs. 3 and 4. The extent of stomatal and non-stomatal inhibition during a decline in CO_2 uptake at -10.8 bar was calculated as follows: A straight line, $A = g_{CO2} \cdot (P_e - P_i)$ P (the supply function), was drawn for the actual P (total gas pressure), for a P_e (external CO_2 pressure) of 32.5 Pa and for the value of g_{CO2} (conductance for CO_2 diffusion from turbulent air to intercellulars) measured at a P_e value within 29.0–32.0 Pa. The value of g_{CO2} was valid at P_e = 32.5 Pa as g_{CO2} was uninfluenced by such P_e variations in all leaves (data not shown). Accordingly, the estimated rate of CO_2 uptake at P_e = 32.5 Pa was A_c (control) and A_s (−10.8 bar). The control supply function was then applied to the stress situation to get A_x, i.e. the rate of CO_2 uptake that should have been observed with unchanged stomatal aperture. Thus, $A_c - A_x$ and $A_x - A_s$ represents the non-stomatal and stomatal inhibition, respectively. The control values of A and supply function are mean values of seven independent determinations

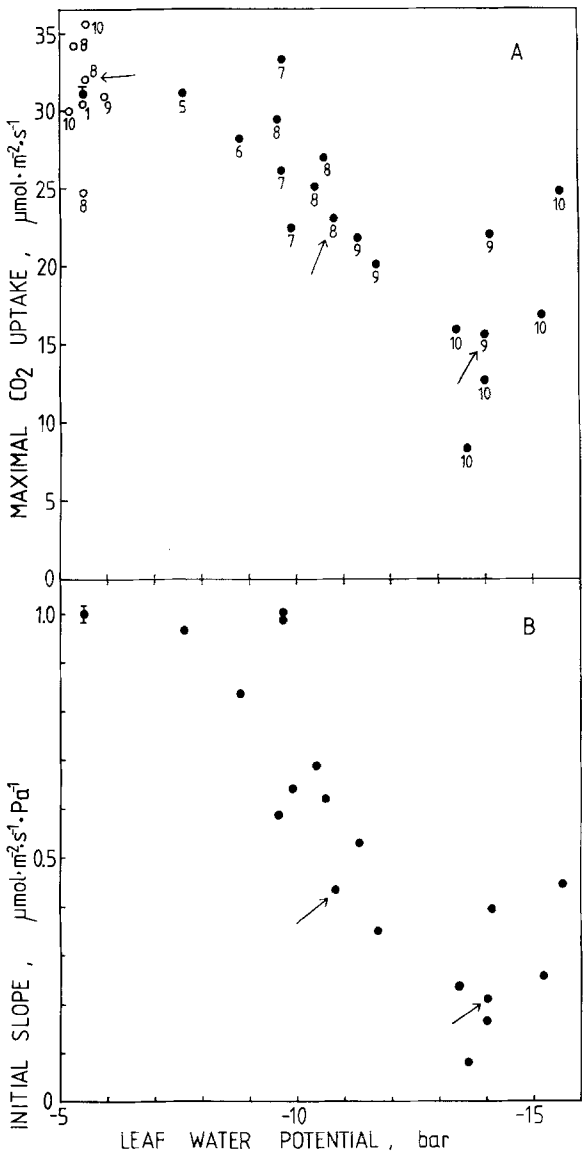

Fig. 2A, B. Effect of leaf water potential on the maximal rate of CO_2 uptake (**A**) and initial slope (**B**) of the plot of CO_2 uptake against intercellular CO_2 pressure in willow leaves. The experimental design was the same as in Fig. 1. Calculation of the initial slope was based on four to five, occasionally three, data points on the apparently linear, initial portion of the plot. Maximal rate of CO_2 uptake was usually a plateau value, but in a few cases of severely stressed leaves it was a near-plateau value. The control is given as the mean value with SE for seven experiments, and in **A** also as individual values (*open circles*). The figures in **A** represent number of days with restricted water supply, or, at *open circles*, corresponding time period for controls. The *arrows* indicate data points obtained from the curves of Fig. 1. Each plant gave one or two data points (different leaves); if two, at intervals of 1 or 2 d

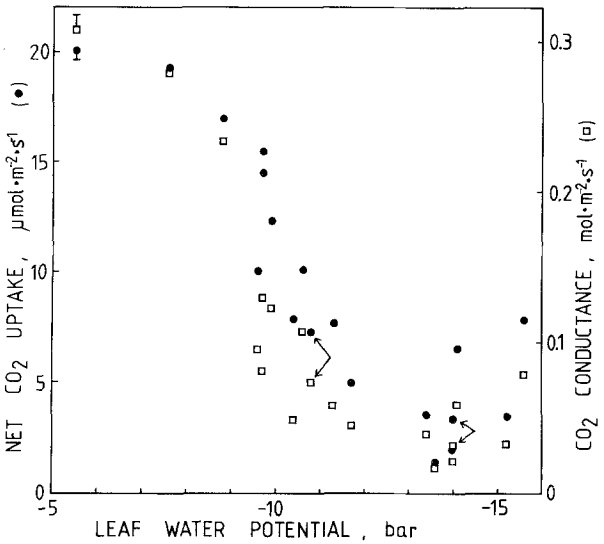

Fig. 3. Effect of leaf water potential on the rate of CO_2 uptake (●) and stomatal CO_2 conductance (□) in willow leaves at an external CO_2 pressure of 32.5 Pa. Calculation of CO_2 uptake is detailed in the legend of Fig. 1 and was based on the same photosynthetic CO_2 response curves as used in Fig. 2. *Arrows* indicate data points that are obtained from the curves of Fig. 1

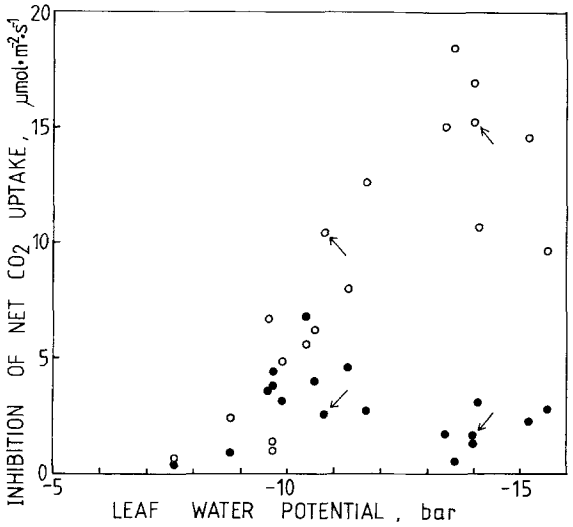

Fig. 4. Effect of leaf water potential on non-stomatal (○) and stomatal (●) inhibition of CO_2 uptake in willow leaves at an external CO_2 pressure of 32.5 Pa. The absolute rates of CO_2 uptake are shown in Fig. 3. Calculation were performed according to the legend of Fig. 1 with the same data as used in Fig. 2. *Arrows* indicate data points that are obtained from the curves of Fig. 1

Comparison of water-stress-induced stomatal and non-stomatal inhibition of CO_2 uptake. We also characterized the influence of water stress on the light-saturated CO_2 uptake at an ambient CO_2 pressure of 32.5 Pa which is close to that occuring in nature. Figure 3 shows that decreasing ψ_L below approx. –8 bar, sharply diminishes CO_2 uptake and stomatal conductance at this CO_2 concentration,

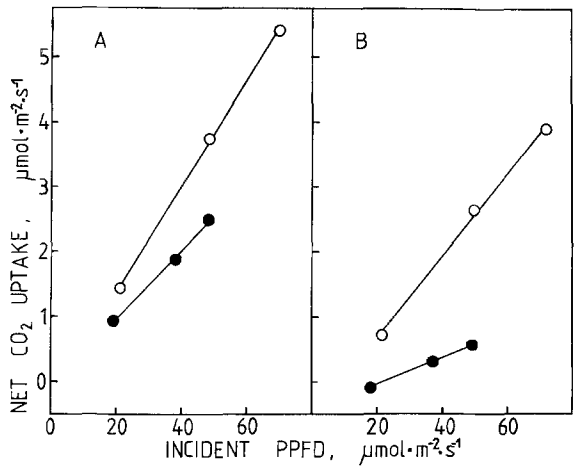

Fig. 5 A, B. Uptake of CO_2 at limiting incident PPFDs in willow leaves of water potentials of about –6 (○) and –18 bar (●). Before measurements the leaves had been exposed to: **A** the generally used PPFD of 100–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$; or **B** a 6-h period of a PPFD of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ that ended 30 min prior to measurements. Measurements were made at high CO_2 concentrations to eliminate photorespiration and at a leaf temperature of 22.0 ± 0.5° C

with the latter effect developing somewhat faster. The extent of stomatal and non-stomatal inhibition of CO_2 uptake appeared to be similar at moderate stress levels (around –10 bar; Fig. 4), but as the stress proceeded the non-stomatal inhibition increased and became by far predominant, whereas the stomatal inhibition tended to decrease.

Effect of water stress on the maximum quantum yield of CO_2 uptake. Apparent maximum quantum yields of photosynthesis were calculated from data of CO_2 uptake by willow leaves at three limiting PPFDs. The results are exemplified in Fig. 5 A for a control and a severely stressed leaf, and summarized in Table 1 (under A). As judged by measurements of leaf transmittance (with a measuring light beam of a perpendicular angle of incidence) and of leaf absorptance (with diffuse light in a light-integrating sphere), there were no significant differences in absorptance between the three classes of leaves in Table 1 (data not shown). Therefore, the changes in apparent quantum yield obtained can be ascribed to changes in the true quantum yield. The results of Table 1 indicate that a ψ_L below -12 bar is required if the quantum yield of CO_2 uptake is to be affected to any significant extent.

Photosynthetic responses of high-light- and low-light-acclimated leaves to water stress at a high PPFD. The question of whether stress caused by low ψ_L is influenced by the concomitant light regime was first considered in short-term experi-

Table 1. Apparent quantum yield of CO_2 uptake in willow leaves of three classes of Ψ_L, before (A) and after (B) a 6-h exposure to 1500 $\mu mol \cdot m^{-2} \cdot ^{-1}$. For further details see legend of Fig. 5

	$-1 \cdot \Psi_L$ (bar)		Apparent quantum yield[a]	Number of observations	Inhibition[b] (%)	Photoinhibition (%)
	Range	Mean				
A. Normal procedures						
Well-watered	5.3– 8.1	6.1	0.089 ± 0.002	6	0	–
Water-stressed	11.2–12.9	12.1	0.085 ± 0.002	3	4	–
Water-stressed	15.8–19.0	16.8	0.063 ± 0.003	5	29	–
B. Normal procedures + 1500 $\mu mol \cdot m^{-2} \cdot ^{-1}$ for 6 h						
Well-watered	6.2– 8.1	6.8	0.069 ± 0.003	6	22	22
Water-stressed	11.6–13.7	12.4	0.068 ± 0.003	3	23	20
Water-stressed	15.9–18.3	16.8	0.034 ± 0.004	5	61	45

[a] Mean ± SE
[b] Compared with "well-watered" in "normal procedures"

ments. Leaves of various ψ_L were produced by the normal routines (PPFD = 100–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and then exposed to a PPFD of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 6 h. Typical results for leaves of a high (–6 bar) and a very low (–18 bar) ψ_L are shown in Fig. 5B. Fig. 5A shows the corresponding results for normally exposed leaves. The changes in apparent quantum yield that occurred upon the high PPFD exposure, shown in Table 1 (under B), reflected changes in the true quantum yield, since the leaf absorptance was invariable. The high PPFD exposure caused a decrease in the quantum yield (photoinhibition), of the same magnitude in well-watered and mildly stressed leaves (about 20%), and of considerably greater extent in leaves of the lowest ψ_L (45%). It therefore appears that severe water stress is accompanied by increased susceptibility to photoinhibition.

High-light-adapted leaves which were water stressed in high light (PPFD = 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) showed a decline in the rate of CO_2 uptake (Table 2). This decrease was largest in saturating light but limiting CO_2, followed by saturating light and CO_2, and, limiting light. This pattern was also observed for leaves illuminated by a PPFD of 100–200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during growth and drought (Fig. 2, Table 1). Thus, provided the leaves were appropriately light acclimated, water stress affected photosynthetic properties in a manner that essentially was not modified by the long-term PPFD level between 100 and 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Effect of water stress on room-temperature chlorophyll fluorescence kinetics. Illumination of dark pre-treated leaves at room-temperature produces an induction of chlorophyll fluorescence emission which manifests the operation of key reactions in photosynthesis. Using the nomenclature of Papageorgiou (1975), the fluorescence rises from an initial level (O) to a peak (P) within a few seconds, reflecting the photoreduction of the primary stable electron acceptors Q in photosystem II (Duysens and Sweers 1963; Bradbury and Baker 1983). The fluorescence then decreases, largely as a consequence of electron-transport-mediated reoxidation of Q and build-up of transthylakoid $\triangle$pH (Krause et al. 1982; Quick and Horton 1984). Before the low steady-state level (T) is attained, a fluorescence oscillation with the minimum S and the maximum M occurs (Fig. 6; solid line representing a control willow leaf). It has been proposed that the S→M→T oscillation marks the onset of carbon assimilation (Walker 1981). This may result from the influence of regulatory reactions of carbon metabolism on electron transport via the levels of ATP and NADPH, accompanied by changes in the redox state of Q and the transthylakoid $\triangle$pH, and thereby also changes in the fluorescence intensity (Walker et al. 1983; Quick and Horton 1984; Cerovic et al. 1984).

Severe water stress caused altered kinetics of the fluorescence oscillation $S \rightarrow M \rightarrow T$, most apparently seen as an increased half-rise time for the S to M phase as shown by the examples of Fig. 6 and by Fig. 7. In the light of the theory previously referred to, this result appears to indicate that water stress most seriously affected carbon metabolism and possibly also photophosphorylation. Primary events in electron transport appeared to be less affected, as judged from an analysis of the early phases of fluorescence kinetics: the variation in the values of O and P levels, time for appearance of P, and initial rate of the P to S transient did not correlate with ψ_L (data not shown).

Table 2. Apparent quantum yield of CO_2 uptake, and maximal rate and initial slope of photosynthetic CO_2 response curves in willows leaves, grown and drought-stressed at a PPFD of 1000 $\mu mol \cdot m^{-2} \cdot ^{-1}$. The 7th and 8th leaf, as defined in Material and methods, was specifically illuminated on duplicate plants (plant 1 and 2) that otherwise followed the normal procedures. Maximal rate and initial slope was determined as in Fig. 2

Leaf position	Duration of restricted watering (d)	Ψ_L[a] (bar)	Apparent quantum yield	Inhibition[b] (%)	Maximal CO_2 uptake ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Inhibition[b] (%)	Initial slope ($\mu mol \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$)	Inhibition[b] (%)
Plant 1								
7	2	–	0.072	–	63	–	1.7	–
8	5	–	0.079	0	61	–	1.5	0
8	8	– 9.7	0.073	8	63	0	1.2	18
8	9	–10.0	0.063	20	45	30	0.6	60
8	10	–12.3	0.053	33	31	51	0.4	75
Plant 2								
7	2	–	0.062	–	57	–	1.8	–
8	2	–	0.060	–	52	–	1.6	–
7	6	–	0.067	0	64	0	2.1	0
8	6	–	–	–	64	0	1.7	0
7	8	–11.2	0.060	10	54	15	1.4	35
8	8	–11.2	0.061	–	53	17	1.1	35

[a] A leaf adjacent to the 7th and 8th leaf was measured before transfer of the plant to the gas-exchange apparatus
[b] Compared with the highest value observed for the given leaf

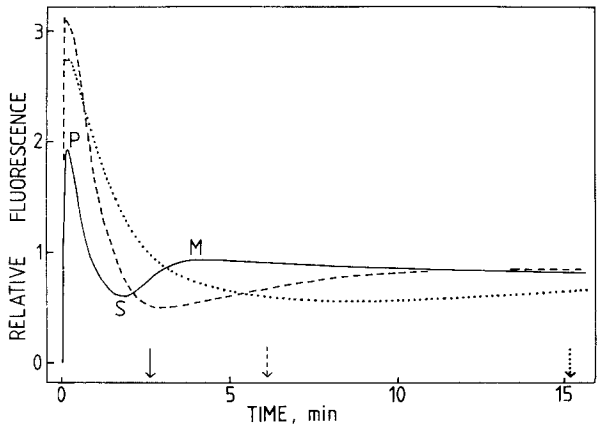

Fig. 6. Induction of chlorophyll fluorescence emission at 685 nm (6.4 nm halfwidth) in attached willow leaves at 20°C and at ψ_L of –4.9 (*solid line*), –12.8 (*broken line*) and -18.1 bar (*dotted line*). Plants were dark-pretreated for 45 min prior to broad-band excitation (390–560 nm; PPFD = 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Cardinal-points of the curve obtained at –4.9 bar are indicated. The *arrows* indicate points of half-rise from a minimum S to a last maximum M

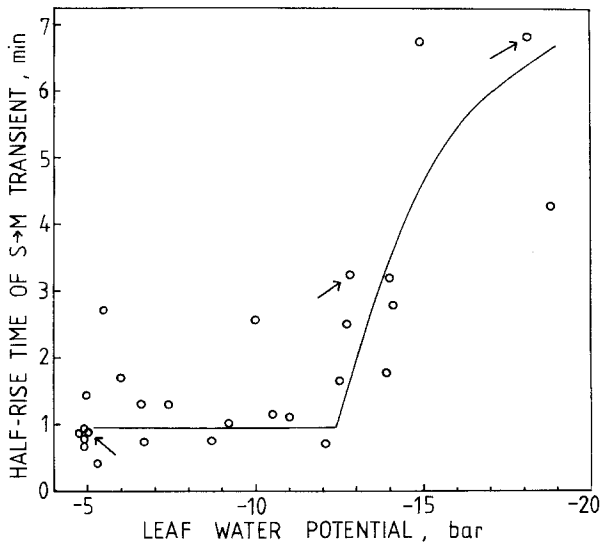

Fig. 7. Half-rise time of the S to M fluorescence transient in willow leaves as a function of leaf water potential. The experimental conditions are described in Fig. 6. The *arrows* indicate data points obtained from the curves in Fig. 6

Relationship between relative water content and water potential. Because of the considerable variation among the leaf samples (Fig. 8), it was not possible to determine numerically the osmotic potentials. However, the lower position of the curve for leaves that had undergone the 10-d period of limited water supply, clearly shows that these had adjusted the osmotic potential a number of bars, which resulted in a corresponding downward shift in the turgor loss point, as compared with the control. Figure 8 also reveals that the stressed leaves suffered rather marginal dehydration. For example, at –11 bar (associated with about 50% inhibition of the initial slope of the photosynthetic CO_2-response curve) and at –15 bar, the relative water content was decreased to about 0.92 and 0.87, respectively, from the control value of 0.97 (at –5.5 bar; data not shown).

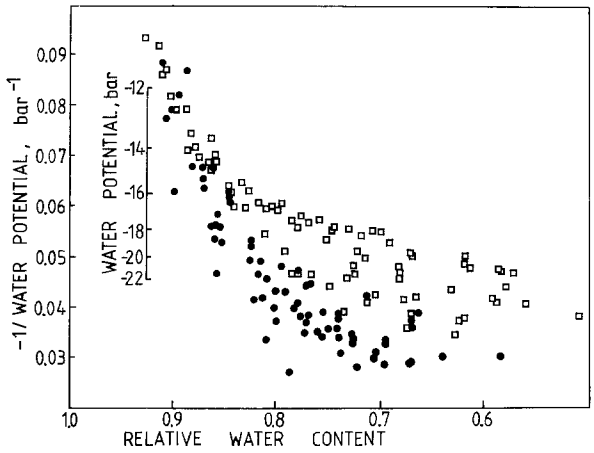

Fig. 8. The relationship between inverse water potential and relative water content in willow leaves that had experienced well-watered conditions (□) or 10 d of decreasing soil water availability (●). The data were gathered from four plants of each class (four leaves per plant)

Discussion

The drought-induced decline in CO_2 uptake, measured at saturating PPFD and natural ambient CO_2 concentration, was at an early stage equally attributable to stomatal and non-stomatal factors, but the further decline as drought continued was solely due to non-stomatal factors (Fig. 4). This conforms with earlier observations that non-stomatal factors are responsible for the photosynthetic depression to a considerable extent during water stress that is slowly applied through soil drought (Bradford and Hsiao 1982). The increased non-stomatal constraint on CO_2 uptake, at conditions of saturating PPFD and natural ambient CO_2 concentration, was mainly a consequence of decreased initial slope of the plot of CO_2 uptake versus P_i. This effect indicates that the activity of ribulose-1,5-bisphosphate (RuBP) carboxylase was decreased (Farquhar and Sharkey 1982). There are previous results derived from biochemical (Jones 1973; Johnson et al. 1974; O'Toole et al. 1976) and gas-exchange studies (Jones 1973; Jones and Fanjul 1983; Ehleringer and Cook 1984) that this enzymatic activity is decreased upon water stress induced by soil drought, but there are also results showing small or no changes (Huffaker et al. 1970; Beadle and Jarvis 1977; von Caemmerer and Farquhar 1984). The reason for this discrepancy is not clear but may involve incommensurable experimental designs, and errors inherent to the in-vitro assay of RuBP carboxylase. For instance, rehydration during the preparation procedure may result in recovery of the enzymatic activity (Plaut 1971). Besides drought stress, osmotic dehydration can cause a decrease in the initial slope of the photosynthetic CO_2-response curve of leaf slices (Kaiser 1984).

Next to the initial slope, the maximal rate of the same photosynthetic CO_2 response curves was most affected. According to the model (Farquhar and Sharkey 1982) the latter effect implicates a suppressed capacity for regeneration of the CO_2 acceptor (RuBP). From theoretical considerations, this may reflect effects on the electron-transport chain, on the associated phosphorylation process, or on the carbon-reduction cycle. The major effect is likely to be sought for in the carbon-reduction cycle, since the thylakoid reactions are less likely candidates as judged by the finding that the quantum yield of CO_2 uptake was rather stable. With important exceptions, for instance for sunflower (Keck and Boyer 1974), cotton (Fry 1972) and *Nerium oleander* (Björkman and Powles 1984), inhibition of photosynthetic electron transport is not prominent under mild and moderate water stress (Beadle and Jarvis 1977; Kaiser et al. 1981; Sharkey and Badger 1982; von Caemmerer and Farquhar 1984). In this context it is interesting to note that severe water stress caused an attenuation of the secondary oscillation of the chlorophyll fluorescence kinetics, which also may be explained in terms of changes in the carbon-reduction cycle (Walker 1981). This conformity with the gas-exchange measurements may open the possibility of using fluorescence kinetics as a rapid, non-intrusive method for monitoring photosynthetic responses to water stress. In contrast to the situation in willow plants, drought stress in plants of *Nerium oleander* (Govindjee et al. 1981; Björkman and Powles 1984) and maize (Havaux and Lannoye 1983) led to a decrease in the ratio of the maximum (P) to the minimum (O) fluorescence, indicating a decreased efficiency of photochemistry. However, the experimental conditions employed in those works differed from ours since high PPFDs were administered during the treatments and, in the maize leaves, the dehydration was greater. Photoinhibition could very well have been a contributing factor in their experiments.

At present, the link between water status and photosynthetic properties in leaves is poorly understood. It appears unlikely that the decrease in cell volumes in the stressed willow leaves of at most approx. 10% ($\psi_L = -18$ bar) would result in solute effects on enzymes as demonstrated with chloroplasts (Kaiser and Heber 1981) and leaf slices (Kaiser et al. 1983) under osmotic stress. Neither is the small relative decrease in the activity of water, i.e. ψ_L, a plausible triggering factor. However, one

of its components, the turgor pressure, underwent substantial relative changes. Cell growth and stomatal aperture are sensitive to changes in turgor pressure but the intrinsic photosynthetic processes are not known to be directly influenced. However, from a theoretical point of view, there are possibilities that the turgor-controlled processes in turn could affect the photosynthetic machinery, for example through feed-back inhibition upon decreased demand for carbohydrates for growth (Edwards and Walker 1983), or through an increased level of the hormone abscisic acid upon stomatal closure. There are reports of effects of abscisic acid on intrinsic photosynthesis (Tillberg et al. 1981; Cornic and Miginiac 1983) and on RuBP-carboxylase activity (Sankhla and Huber 1974).

In our drought regime, in conformity with the majority of studies, fertilizer was restricted in addition to water. It has been reported that nitrogen deficiency accelerates the photosynthetic decline during drought (Radin and Ackerson 1981). Also, in *Encelia farinosa* plants subjected to drought, decreased photosynthesis correlated well with decreased leaf nitrogen content (Ehleringer and Cook 1984). In our study, however, the change in the initial slope of the photosynthetic CO_2-response curve appears to be too rapid to be explained by a decreased availability of nutrients with a following net degradation of RuBP carboxylase; the slope was more than halved over 2 d. Moreover, no visible chlorosis occurred indicating that senescence was not markedly accelerated.

This study indicates that there are specific effects of water stress that are independent of photoinhibitory effects, since the relative sensitivity of certain component processes of photosynthesis to drought was the same at a low and a high long-term PPFD. However, no data are available for high PPFD at leaf water potentials below –12.3 bar, so the possibility of co-operative effects of illumination at severely depressed water potentials cannot be rejected. Our results are similar to those of Downton (1983) who used the loss of variable fluorescence as an indicator of photoinhibition; photoinhibition did not occur in slowly water-stressed grapevine leaves until they wilted. However, Björkman and Powles (1984) found that *Nerium oleander* plants water-stressed under full daylight showed effects similar to those obtained in photoinhibitory treatments. The reason for this apparent discrepancy is unknown. However, when the willow leaves were exposed to a PPFD considerably above the one they were adapted to, an inhibition of the quantum yield of CO_2 uptake also resulted. This situation is well known during photoinhibition (Björkman 1981). As photoinhibition was markedly greater at very low than at high ψ_L, we suggest that although photoinhibition is not a determining factor in water stress, it may occur under conditions of drought that are combined with widely fluctuating PPFDs. Its occurrence may also be triggered by other conditions that inhibit photosynthesis, for instance depletions of CO_2 and O_2 (Powles et al. 1984), and suboptimal temperatures above 0° C (Ögren and Öquist 1984; Ögren et al. 1984) or below (Öquist 1983). The primary cause is a decreased rate of photosynthesis and, possibly, of other processes involved in the orderly deactivation of excited chlorophylls. In general, the tendency towards excess excitation energy during stress results in an increased susceptibility to photoinhibition.

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References

Beadle, C.L., Jarvis, P.G. (1977) The effects of shoot water status on some photosynthetic partial processes in Sitka spruce. Physiol. Plant. **41**, 7–13

Björkman, O. (1981) Responses to different quantum flux densities. In: Encyclopedia of plant physiology, N.S., vol. 12A: Physiological plant ecology I: Responses to the physical environment, pp. 57–107, Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H., eds. Springer, Berlin Heidelberg New York

Björkman, O., Powles, S.B. (1984) Inhibition of photosynthetic reactions under water stress: interaction with light level. Planta **161**, 490–504

Bradbury, M., Baker, N.R. (1983) Analysis of the induction of chlorophyll fluorescence in leaves and isolated thylakoids: contributions of photochemical and non-photochemical quenching. Proc. R. Soc. London B **220**, 251–264

Bradford, K.J., Hsiao, T.C. (1982) Physiological responses to moderate water stress. In: Encyclopedia of plant physiology, N.S. vol. 12B: Physiological plant ecology II: Water relations and carbon assimilation, pp. 263–324, Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H., eds. Springer, Berlin Heidelberg New York

Cerović, Z.G., Sivak, M.N., Walker, D.A. (1984) Slow secondary fluorescence kinetics associated with the onset of photosynthetic carbon assimilation in intact isolated chloroplasts. Proc. R. Soc. London B **220**, 327–338

Cornic, G., Miginiac, E. (1983) Nonstomatal inhibition of net CO_2 uptake by (±) abscisic acid in *Pharbitis nil.* Plant Physiol. **73**, 529–533

Downton, W.J.S. (1983) Osmotic adjustment during water stress protects the photosynthetic apparatus against photoinhibition. Plant Sci. Lett. **30**, 137–143

Duysens, L.N.M., Sweers, H.E. (1963) Mechanism of two photochemical reactions in algae as studied by means of

fluorescence. In: Studies on microalgae and photosynthetic bacteria, pp. 353–372, Japan Soc. Plant Physiol. ed. Univ. of Tokyo Press, Tokyo

Edwards, G., Walker, D.A. (1983) C3, C4: Mechanisms, and cellular and environmental regulation of photosynthesis. Blackwell Scientific Publications, Oxford

Ehleringer, J.R., Cook, C.S. (1984) Photosynthesis in *Encelia farinosa* Gray in response to decreasing leaf water potenial. Plant Physiol. **75**, 688–693

Farquhar, G.D., Sharkey, T.D. (1982) Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. **33**, 317–345

Fry, K.E. (1972) Inhibition of ferricyanide reduction in chloroplasts prepared from water-stressed cotton leaves. Crop Sci. **12**, 698–701

Govindjee, Downton, W.J.S., Fork, D.C., Armond, P.A. (1981) Chlorophyll a fluorescence transient as an indicator of water potential of leaves. Plant Sci. Lett. **20**, 191–194

Hällgren, J.-E., Sundbom, E., Strand, M. (1982) Photosynthetic responses to low temperature in *Betula pubescens* and *Betula tortuosa*. Physiol. Plant. **54**, 275–282

Havaux, M., Lannoye, R. (1983) Chlorophyll fluorescence induction: A sensitive indicator of water stress in maize plants. Irrig. Sci. **4**, 147–151

Huffaker, R.C., Radin, T., Kleinkopf, G.E., Cox, E.L. (1970) Effects of mild water stress on enzymes of nitrate assimilation and of the carboxylative phase of photosynthesis in barley. Crop. Sci. **10**, 471–474

Johnson, R.R., Frey, N.M., Moss, D.N. (1974) Effect of water stress on photosynthesis and transpiration of flag leaves and spikes of barley and wheat. Crop. Sci. **14**, 728–731

Jones, H.G. (1973) Moderate-term water stresses and associated changes in some photosynthetic parameters in cotton. New Phytol. **72**, 1095–1105

Jones, H.G., Fanjul, L. (1983) Effects of water stress on CO_2 exchange in apple. In: Proceedings on effects of stress on photosynthesis, pp. 75–84, Marcelle, R., Clijsters, H., Van Poucke, M., eds. Martinus Nijhoff/Dr W. Junk Publishers, The Hague

Kaiser, W.M. (1984) Response of photosynthesis and dark-CO_2-fixation to light, CO_2 and temperature in leaf slices under osmotic stress. J. Exp. Bot. **35**, 1145–1155

Kaiser, W.M., Heber, U. (1981) Photosynthesis under osmotic stress. Effect of high solute concentrations on the permeability properties of the chloroplast envelope and on the activity of stroma enzymes. Planta **153**, 423–429

Kaiser, W.M., Kaiser, G., Prachuab, P.K., Wildman, S.G., Heber, U. (1981) Photosynthesis under osmotic stress. Inhibition of photosynthesis of intact chloroplasts, protoplasts, and leaf slices at high osmotic potentials. Planta **153**, 416–422

Kaiser, W.M., Schwitulla, M., Wirth, E. (1983) Reactions in chloroplasts, cytoplasm and mitochondria of leaf slices under osmotic stress. Planta **158**, 302–308

Keck, R.W., Boyer, J.S. (1974) Chloroplast response to low leaf water potentials III. Differing inhibition of electron transport and photophosphorylation. Plant Physiol. **53**, 474–479

Krause, G.H., Vernotte, C., Briantais, J.-M. (1982) Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae. Resolution into two components. Biochim. Biophys. Acta **679**, 116–124

Ögren, E., Öquist, G. (1984) Photoinhibition of photosynthesis in *Lemna gibba* as induced by the interaction between light and temperature. II. Photosynthetic electron transport. Physiol. Plant. **62**, 187–192

Ögren, E., Öquist, G., Hällgren, J.-E. (1984) Photoinhibition of photosynthesis in *Lemna gibba* as induced by the interaction between light and temperature. I. Photosynthesis in vivo. Physiol. Plant. **62**, 181–186

Öquist, G. (1983) Effects of low temperature on photosynthesis. Plant Cell Environ. **6**, 281–300

O'Toole, J.C., Crookston, R.K., Treharne, K.J., Ozbun, J.L. (1976) Mesophyll resistance and carboxylase activity. A comparison under water stress conditions. Plant Physiol. **57**, 465–468

Papageorgiou, G. (1975) Chlorophyll fluorescence: An intrinsic probe of photosynthesis. In: Bioenergetics of photosynthesis, pp. 319–371. Govindjee ed., Academic Press, New York London

Plaut, Z. (1971) Inhibition of photosynthetic carbon dioxide fixation in isolated spinach chloroplasts exposed to reduced osmotic potentials. Plant Physiol. **48**, 591–595

Powles, S.B., Cornic, G., Louason, G. (1984) Photoinhibition of in vivo photosynthesis induced by strong light in the absence of CO_2: an appraisal of the hypothesis that photorespiration protects against photoinhibition. Physiol. Vég. **22**, 437–446

Quick, W.P., Horton, P. (1984) Studies on the induction of chlorophyll fluorescence in barley protoplasts. II. Resolution of fluorescence quenching by redox state and the transthylakoid pH gradient. Proc. R. Soc. London B **220**, 371–382

Radin, J.W., Ackerson, R.C. (1981) Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis, and abscisic acid accumulation during drought. Plant Physiol. **67**, 115–119

Riegel, C.A. (1974) Comments on "A simple but accurate formula for the saturation vapor pressure over liquid water". J. Appl. Meteor. **13**, 606–607

Sankhla, N., Huber, W. (1974) Effect of abscisic acid on the activities of photosynthetic enzymes and $^{14}CO_2$ fixation products in leaves of *Pennisetum typhoides* seedlings. Physiol. Plant. **30**, 291–294

Scholander, P.F., Hammel, H.T., Hemmingsen, E.A., Bradstreet, E.D. (1964) Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. Natl. Acad. Sci. USA **52**, 119–125

Sharkey, T.D., Badger, M.R. (1982) Effects of water stress on photosynthetic electron transport, photophosphorylation, and metabolite levels of *Xanthium strumarium* mesophyll cells. Planta **156**, 199–206

Thorpe, M.R. (1978) Correction of infra-red gas analyser readings for changes in reference tube CO_2 concentration. Plant Cell Environ. **1**, 59–60

Tillberg, E., Dons, C., Haugstad, M., Nilsen, S. (1981) Effect of abscisic acid on CO_2 exchange in *Lemna gibba*. Physiol. Plant. **52**, 401–406

von Caemmerer, S., Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153**, 376–387

von Caemmerer, S., Farquhar, G.D. (1984) Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced $p(CO_2)$ on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. Planta **160**, 320–329

Walker, D.A. (1981) Secondary fluorescence kinetics of spinach leaves in relation to the onset of photosynthetic carbon assimilation. Planta **153**, 273–278

Walker, D.A., Horton, P., Sivak, M.N., Quick, W.P. (1983) Antiparallel relationship between O_2 evolution and slow fluorescence induction kinetics. Photobiochem. Photobiophys. **5**, 35–39

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