Investigation of the processes involved during the photoinhibition of *Zea mays* L. seedlings.

by
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My supervisors Dr A.M. Amory and Prof. N.W. Pammenter, for their kind assistance and advice; My colleague Lesley Ford for her friendship; My father Dr S.H. Ripley for his comments on the final draft and the CSIR for financial support.

PREFACE

The experimental work presented in this thesis is the original work of the author and has not been submitted in any other form to any other university. This work was carried out in the Department of Biological Sciences, Durban, from January 1988 to December 1989.

B.S. Ripley

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ABSTRACT

It has been proposed that the protective systems (photorespiration, the anti-oxidant system and non-radiative energy dissipation) alleviate or reduce photoinhibitory damage under high light conditions. To investigate the role of these mechanisms in C_4 photosynthetic species, nine day old *Zea mays* seedlings were photoinhibited (30 minutes of 2500 μmol m^{-2} s^{-1} PPFD) in the presence of various concentrations of O_2 or CO_2; or by photoinhibiting leaves in N_2 after they had been fed glycolate or phosphoglycerate via the transpiration stream. The extent of the photoinhibition and the subsequent recovery from the photoinhibitory treatments was monitored with both CO_2 gas exchange and chlorophyll fluorometry.

Photoinhibitory treatments resulted in both a decrease in the rate of CO_2 fixation and an interruption of PSII electron transport. CO_2 response curves were used to monitor the efficiency of the carboxylation processes and the level of carbon metabolism substrate cycling during recovery following photoinhibitory treatments. Both were decreased by the treatment and recovered once leaves were returned to normal conditions. Low concentrations of O_2 (2%) markedly reduced the extent of the photoinhibition. This protection could not be accounted for by photorespiration, which would be inoperative at such a low O_2 concentration. Leaves fed glycolate exhibited enhanced photoinhibtion. It is also unlikely that the anti-oxidant system (Mehler reaction and associated glutathione and ascorbate reactions) could utilize sufficient reductant at such low O_2 concentrations to produce the observed protection.

Leaves inhibited in the presence of O_2 had decreased maximum fluorescence yields (F_m) and little altered initial fluorescence yields (F_o), resulting in decreased PSII efficiency (F_v/F_m). Photoinhibition resulted in a small increase in the slow relaxing component (60 minute) of non-radiative energy dissipation. This component became more predominant as the O_2 concentration was increased. The rate constant for photochemistry was also decreased by the inhibitory treatment.

Leaves supplied with CO_2 at a concentration above 50 μmol mol^{-1} exhibited little photoinihibition suggesting that the protection was not due to a quantitative utilization of energy. PGA, fed via the transpiration stream, enhanced the photoinhibition, suggesting that more than just the Benson-Calvin cycle is required to protect

C_4 plants from photoinhibition. At CO_2 concentrations below this, the F_v/F_m ratio was decreased due to large increases in the F_o values. F_m was little altered. These changes are characteristic of a decrease in the rate constant for photochemistry. The rate constant for non-radiative energy dissipation was little altered by the photoinhibition.

The protection observed in the presence of either CO_2 or O_2 was not due to a quantitative utilization of energy and the different responses of F_o, F_m and the rate constants K_D and K_P, suggest that different mechanisms were operative in the presence or absence of oxygen.

(1) INTRODUCTION

1.1) Characteristics of Photoinhibition

Injury to the photosynthetic apparatus of green plants exposed to high light intensity is a well documented phenomenon and has been termed photoinhibition. Photoinhibition has recently been defined as the loss of photosynthetic activity without the loss of chlorophyll (photo-oxidation) and this phenomenon also includes the protective mechanisms that are related to reversible photoinhibition (Powles, 1984; Critchley, 1988; Krause, 1988). Photoinhibition, in both isolated chloroplasts and intact leaves, is characterized by: an exponential decrease in light saturated CO_2 assimilation (Osmond, 1981); decreased O_2 evolution (Baker and Horton, 1987); a decrease in the quantum yield of photosynthesis as indicated by decreased CO_2 assimilation at limiting light levels (Powles, 1984); and a reduction in photosystem II (PSII) electron transport (Powles and Osmond, 1979). Following the removal of photoinhibitory conditions there is considerable recovery manifested by the reversal of the above characteristics.

Photoinhibition has been recorded in a wide range of plant species, ranging from unicellular algae (Neale, 1987) to important crop species such as maize (C_4) and barley (C_3) (Huber and Edwards, 1975). The extent of the photoinhibition appears to be dependent upon the plant species, its prehistory and the severity of the inhibitory treatment. In general shade grown plants are more susceptible to photoinhibition than sun grown plants (Anderson and Osmond, 1987).

1.2) Photoinhibition under conditions of limited metabolism.

In accordance with the hypothesis of Jones and Kok (1966), Osmond (1981) proposed that photoinhibition occurs under any circumstances in which the rates of transfer of excitation energy from the light harvesting complexes (LHC) to the photosystem reaction centres exceeds the rate of transfer from reaction centres to the electron transport chain. This has been substantiated to some degree by the fact that under conditions of limiting CO_2, and hence limited utilization of reductant by the carboxylation processes, photoinhibition is enhanced (Powles and Osmond, 1979; Nilsen, Chaturvedi and Dons, 1984). This has been shown in the laboratory by the reduction of ambient CO_2 concentrations (Krause and Cornic, 1987) and in the

field during periods of stomatal closure (Ludlow, 1987). The inhibition is also enhanced if O_2 is removed during photoinhibition, suggesting that alternative energy dissipating processes operate in the presence of O_2. Low temperature (Oquist, Greer and Ogren, 1987) and water stress (Boyer, Armond and Sharp, 1987) have similar exacerbating effects, both of which are likely to directly or indirectly reduce the capacity of the biochemistry to utilize reductant.

1.3) Sites and proposed mechanisms of photoinhibition.

The mechanism of photoinhibition is as yet unknown but certainly involves disruption of the thylakoid light reactions. Jones and Kok (1966) showed that the action spectrum that induces photoinhibition was coincident with the chlorophyll absorption spectrum. This, together with changes in both fluorescence yield and fluorescence transients of photoinhibited leaves (Critchley and Smillie, 1981), suggested that the reduced quantum yield of photosynthesis was due to disruption of the photosynthetic membranes. Disruption of the primary reactions of photosynthesis would also account for the decreased light saturated CO_2 assimilation and oxygen evolution characteristic of photoinhibited leaves or chloroplasts.

The kinetics of chlorophyll fluorescence may be used as an indicator of photoinhibition. On illumination PSII fluorescence exhibits an instantaneous rise to a low level (F_o), followed by a relatively rapid rise to a maximum (F_m). The difference between F_o and F_m represents the variable component of fluorescence (F_v), arising from the energy generated by the charge recombination of the radical pair $P_{680}Q_A^-$. Fluorescence is low if the electron acceptor Q_A can be reduced and increases if no further Q_A reduction can occur. Thus, F_v reflects the extent of Q_A reduction in PSII (Krause and Weis, 1984).

F_v has been found to decrease on photoinhibition (Björkman, 1987). Powles and Osmond (1979) and Critchley and Smillie (1981) suggested that the decrease in F_v was due either to changes at the PSII reaction center or at the electron transport reactions on the oxidizing side of PSII. The change in F_v was not due to stomatal closure as similar results could be obtained with either isolated chloroplasts or algae (Powles and Osmond, 1979; Critchley and Smillie, 1981). The PSIIα centres appear also to be more susceptible to photoinhibiton than the PSIIβ centres, probably due

to their larger antennae sizes (Cleland, Melis and Neale, 1986). PSI is held to be far less susceptible to photoinhibition and although conflicting results exist, these have been attributed to the photoinhibitory treatments being administered to either intact leaves or isolated systems (Critchley, 1988). Little or no effect on PSI has been observed *in vivo*, and in isolated systems the inhibition of PSI is considered to be a secondary effect requiring O_2 (Krause, 1988). Photoinhibitory light supplied at wavelengths that excite only PSII, produces greater inhibition than do wavelengths that excite PSI (Chaturvedi and Nilsen, 1987).

The present understanding of the PSII reactions are summarized in Figure 1.1. Electron transport is initiated by charge separation between the pigment P_{680} in the first excited singlet state (P^*_{680}) and pheophytin (Pheo) and results in the transfer of an electron from the secondary donor (Z) to the primary and secondary acceptors Q_A and Q_B (Krause, 1988).

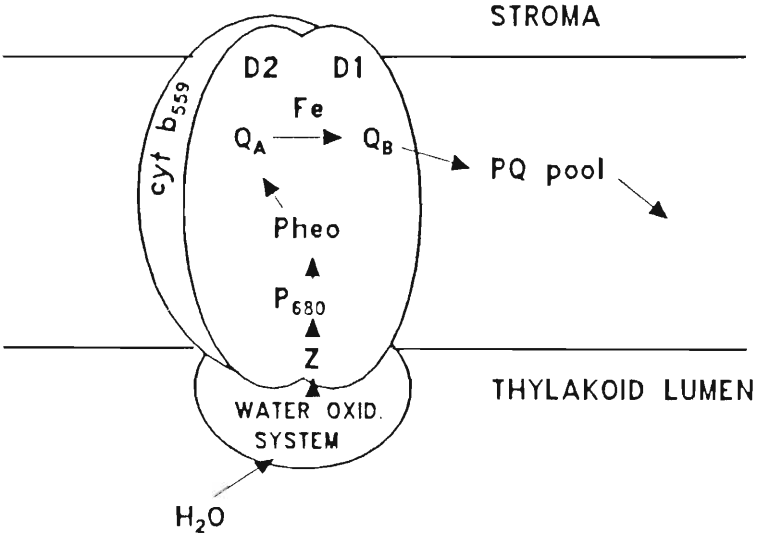


Figure 1.1. Schematic representation of electron transport reactions of PSII containing: the heterodimer D1 and D2 proteins and cytochrome b_{559}; Z, the secondary electron donor; P_{680}, the reaction center chlorophyll; the primary electron donor Pheo, pheophytin; and PQ, plastoquinone. Redrawn from Krause (1988).

Kyle and Ohad (1986) and Kyle (1987) proposed that the 32kDa Q_B binding protein was the primary site of photoinhibition based on the observation that PSII activity

was lost, following the inhibition of *Chlamydomonas reinhardii* cells, if diaminodurene was supplied as the terminal electron acceptor. Diaminodurene accepts electrons from Q_B. However, if silicomolbdate, which is proposed to accept el ectrons from Q_A, was supplied little or no PSII activity was lost. Further evidence for this proposed site was that photoinhibited cells lost their ability to bind atrazine, a herbicide that binds to the Q_B protein. The validity of using the electron acceptor silicomolybdate has been questioned by Graan (1986), who found that it could accept electrons from both Q_A and Q_B. Photoinhibition has also been shown to occur in both *Microcystis aeurginosa* (Tytler, Whitelam, Hipkins and Codd, 1984) and in isolated thylakoids (Cleland and Critchley, 1985; Ohad, Kyle and Hirschenberg, 1985) in the presence of both silicomolybdate and aminodurene. Inactivation of the Q_B binding protein has been proposed to occur due to the reaction of molecular O_2 with quinone anions, generating oxygen radicals at the Q_B binding sites. This is thought to occur continuously; constant protein synthesis replacing damaged Q_B protein. However, during photoinhibition the rate of damage exceeds protein synthesis (Ohad, Kyle and Arntzen, 1984). This is thought to alter chromophore orientation and hence quench chlorophyll fluorescence.

The water-oxidizing function of PSII can be inhibited with hydroxalimine and such treatment leads to enhanced photoinhibition, as indicated by the quenching of F_v. This quenching can be reversed with electron donors such as catechol or diphenyl carbazide (Critchley, 1988). This suggests that the water-oxidizing component is not the primary site of photoinhibition.

Photoinhibited thylakoids show impaired electron transport from P_{680} to Q_A; shown by both the quenching of F_v (Demmig, Cleland and Björkman, 1987) and by the decreased light induced absorbance at 320nm and at 540 - 500nm. Such absorbance changes may indicate the formation of Q_A^- semiquinone (Satoh and Fork, 1982; Cleland, Melis and Neale, 1986). Based on similar observations Ohad, Kyle and Arntzen (1984) proposed that the water-oxidizing component could reduce P_{680} while stabilizing Pheo, thereby forming a stable reaction centre which would be responsible for the quenching of variable fluorescence. Absorbance changes at 680nm have also been used to monitor changes in the P_{680} reaction centre during photoinhibition and have yielded conflicting results. Powles and Osmond (1979) and Demeter, Neale and Melis (1987) found a decrease in absorbance at 680nm suggesting reaction center inactivation while Allakhverdiev, Setlikova, Klimov and

Setlik (1987) found no such change and concluded that the primary site of inactivation was beyond Pheophytin.

1.4) Chlorophyll fluorescence of PSII during photoinhibition.

Photoinhibition is consistently related to a decrease in F_v of PSII and is frequently expressed as the decrease in the ratio F_v/F_m. Butler (1978) describes fluorescence emission as one of several competing first order reactions of deactivation of excited chlorophyll molecules. These reactions are fluorescence, non-radiative energy dissipation, the transfer of excitation energy from PSII to PSI, and photochemistry. Their rate constants are termed K_F, K_D, K_T and K_P, respectively. The fluorescence yield of PSII is given by:

$$\phi F = \frac{F}{I} = \underbrace{\frac{A\,K_F}{K_F+K_D+K_T+K_P}}_{\text{(open units)}} + \underbrace{\frac{(1-A)\,K_F}{K_F+K_D+K_T}}_{\text{(closed units)}}$$

where ϕF is the fluorescence yield, I the absorbed light and A the fraction of 'open' centres (Q_A in the oxidized state). When all the reaction centres are open, a minimal fluorescence yield F_o is obtained. Fluorescence is maximal (F_m) when all centres are 'closed' and the potential yield (F_v/F_m) of the photochemical reaction can be calculated as follows:

$$\frac{F_v}{F_m} = \frac{F_m - F_o}{F_m} = \frac{K_P}{K_F+K_D+K_T+K_P}$$

The ratio F_v/F_m has been found to be linearly related to the photon yield of photosynthesis (Demmig and Björkman, 1987) and is decreased by photoinhibition, indicating a loss of photochemical efficiency. The decrease in F_v/F_m may be due to either an increase in F_o, a decrease in F_v or a combination of both. Any damage to the system which decreases the rate constant for photochemistry (K_P) would increase the fluorescence emitted from of the 'open' traps (F_o) but would have no effect on the fluorescence emitted from the closed traps (F_m). An increase in the rate constant F_o non-radiative energy dissipation (K_D) would result in the quenching, although not to the same extent, of both F_o and F_m. As long as K_D remains relatively small in relation to K_P, an increase in K_D can result in the pronounced quenching of

F_m with only a small decline in F_o (Demmig and Björkman, 1987; Baker and Horton, 1987).

The quenching due to photoinhibition in plants under aerobic conditions, originates predominantly from the decrease in F_m and less, or not at all from an increase in F_o (Powles and Björkman, 1982; Baker and Horton, 1987). This implies an increase in non-photochemical quenching. Plants inhibited in anaerobic conditions exhibit substantial increases in F_o which have been attributed to damaged PSII reaction centres (Satoh and Fork, 1982; Krause, Koster and Wong, 1985). The increase in non-photochemical quenching has been attributed mainly to the increase in the rate constant F_o non-radiative energy dissipation (K_D), rather than to the rate of energy transfer from PSII to PSI (K_T). An increase in K_T would result in the quenching of F_v and Horton and Lee (1985) suggested that the phosphorylation of the LHCs during photoinhibition may diminish the excitation energy of PSII. Phosphorylation saturates at low light intensities (Horton and Hague, 1988) and decreases at high light intensities (Horton and Hague, 1988; Fernyhough, Foyer and Horton, 1984; Demmig, Winter, Kruger and Czygan, 1988; Horton and Hague, 1988). Demmig and Björkman (1987) were also able to find no increase in PSI fluorescence during photoinhibition, confirming that the increase in non-photochemical quenching was due to an increase in K_D and not K_T. The increase in the dissipation of energy as heat has been substantiated by Havaux (1989) who showed an increase in the photoacoustically monitored heat yield during photoinhibition.

There are two components to the fluorescence quenching resulting from an increase in the rate constant for non-radiative energy dissipation: high energy quenching (q_E) and photoinhibitory quenching (q_I). High energy quenching is related to the light induced increase of the trans-thylakoid pH gradient. Exactly how the pH gradient can influence non-radiative energy dissipation is not known and ultrastructural alterations of the thylakoid membrane are usually invoked (Krause and Weis, 1984). q_E has a limited protective effect as it saturates at high light intensities. Q_E has been shown to relax within seconds on darkening, which distinguishes it from a second quenching mechanism which relaxes with a half time of approximately 30 minutes (Demmig, Cleland and Björkman, 1987).

This slower relaxing form of quenching is termed photoinhibitory quenching (q_I) and sun adapted plants appear to have an increased capacity for this form of quen-

ching (Demmig and Björkman, 1987). The molecular mechanism of q_I is not known but a correlation between q_I and the formation of zeaxanthin at the expense of violaxanthin and β-carotene has been shown (Demmig, Winter, Kruger and Czygan, 1988). The accumulation of zeaxanthin has also been shown in chilled spinach (Yamamoto, Nakayama and Chichester, 1962) and in photoinhibited soybeans (Musser, Thomas, Wise, Peeler and Naylor, 1984).

It remains unclear whether the initial site of photoinhibition is the P_{680} reaction centre or the Q_B protein. However, since all components in the reaction sequence Z to Q_B are located in the PSII heterodimer entity it is conceivable that any structural changes may have various results. Photoinhibition may in fact result from more than one mechanism. Bradbury and Baker (1986) observed two phases of photoinhibition, only the faster of which could be related to the loss of functional Q_B protein. Demmig and Björkman (1987) and Greer (1988), from observed changes in F_o and F_v, suggested that the two responses to photoinhibition were non-radiative energy dissipation and damaged PSII reaction centres. Presumably, if the maximum capacity for non-radiative energy dissipation is exceeded then PSII damage occurs. This may account for the varied responses of F_o observed under different conditions and with different species (Krause, Koster and Wong, 1985; Demmig and Björkman, 1987). Another mechanism has been suggested for chilling-sensitive *Zea mays* mesophyll chloroplasts, where photoinhibition was related to the inhibition of electron transport from the LHCs to PSII (Hayden, Baker, Percival and Beckwith, 1986).

1.5) Consequences of photoinhibition

Irrespective of the primary sites or mechanisms of photoinhibition the net result is the cessation of electron transport. Hence no further NADPH or ATP synthesis can occur resulting in both the inactivation of many enzymes requiring reduction for activation, including NADP-malic dehydrogenase, pyruvate dikinase (Powles, 1984) and Ribulose 1,6-bisphosphate carboxylase/ oxygenase (Rubisco) (Farineau, Suzuki and Morot-Gaudry, 1988), and the reduction or halting of energy dependent processes. Net CO_2 assimilation is halted and plant growth is prevented.

The inactivation of Rubisco has also been shown by the decrease in the initial slopes

of CO_2 response curves following photoinhibitory treatment of C_3 plants (Krause, Koster and Wong, 1985; Anderson and Osmond, 1987). A similar decrease in the initial slope for C_4 plants (Powles, Chapman and Osmond, 1980) indicates an inactivation of the more complex carboxylation processes found in these species. The level of ribulose 1,6 bis-phosphate (RuBP) in C_3 plants (Powles and Osmomd, 1979) and the carboxylation substrates in C_4 plants are also reduced due to photoinhibition (Powles, Chapman and Osmond, 1980), presumably due to the reduced energy supply for substrate cycling.

1.6) Recovery from Photoinhibition.

The extent and rate of recovery from photoinhibition is dependent on the severity of the inhibitory treatment (Powles, Cornic and Louason, 1984; Greer and Laing, 1988; Lee and Vonshak, 1988). Recovery saturates between 60 and 300 minutes (Powles, Cornic and Louason, 1984; Greer, 1988; Greer, Berry and Björkman, 1986), but is not necessarily complete. This suggests that plants have a certain capacity for reversible inhibition, which if exceeded results in more permanent damage.

Recovery involves the reversal of non-photochemical quenching (Powles and Björkman, 1982) as well as the repair of inactivated photosystems (Greer and Laing, 1988). The reduction in non-radiative energy dissipation involves the relaxation of both high energy quenching (q_E) and photoinhibitory quenching (q_I). Q_E relaxes within 30 seconds of darkening (Demmig and Björkman, 1987) whereas, q_I has the longer relaxation time of 30 minutes (Demmig, Winter, Kruger and Czygan, 1987). The relaxation of q_I has yet to be correlated with the conversion of zeaxanthin to violaxanthin and β-carotene, although the conversion of zeaxanthin to violaxanthin and β-carotene is known to occur once photoinhibited plants are returned to the dark (Yamamoto, Nakayama and Chichester, 1962) or when inhibitory conditions are removed (Demmig, Winter, Kruger and Czygan, 1988). Recovery has also been shown to require the *de novo* chloroplast directed synthesis of protein (Greer, Berry and Björkman, 1986) and this protein was shown to be the Q_B binding protein (Ohad, Kyle and Arntzen, 1984). In order that damaged Q_B protein does not accumulate in the thylakoid membranes, it would need to be removed in parallel with the insertion of newly synthesized protein. Photoinhibition has been shown to involve the removal of prelabeled Q_B protein (Ohad, Kyle and Hirschenberg, 1985;

Kyle, 1987). The prelabled protein is not released as low molecular weight membrane bound polypeptides (the standard protease effect), suggesting that it is removed by a highly efficient and specific protease (Ohad, Kyle and Hirschenberg, 1985). The repair processes are temperature dependent (Greer and Laing, 1988) and require light. The light requirement suggests either a light regulated step in protein synthesis or an energy requirement (Ohad, Kyle and Arntzen, 1984).

1.7) Protective Mechanisms.

Several systems have been proposed to protect plants from photoinhibition by either utilizing reductant and maintaining carbon flow, or by preventing further reductant production (Krause, 1988). Plants maintained in normal air under light which is not much above light saturation level exhibit stable photosynthesis and appear to be optimally protected against photoinhibition. Under such conditions, the carboxylation processes are the major sinks for photochemically produced ATP and reductant. Oxidation of NADPH continuously restores the terminal electron acceptor to the electron transport chain. This permits the continuous steady deactivation of the reaction centres and protects against photoinhibition. The carboxylation processes' utilization of reductant can be restricted by reducing or removing ambient CO_2 which exacerbates photoinhibition (Powles and Thorne, 1981; Nilsen, Chaturvedi and Dons, 1984; Chaturvedi and Nilsen, 1987). Only a low concentration of CO_2 is required to prevent photoinhibition and concentrations at or close to the CO_2 compensation point of the order 30 to 60 μmom mol^{-1} prevent photoinhibition (Powles, Chapman and Osmond, 1980; Powles, Cornic and Louason, 1984).

Photorespiration, with the associated refixation of ammonia via glutamine, also utilizes reductant (Singh, Kumar, Abrol and Naik, 1985) and this could protect plants against photoinhibitory damage (Powles and Osmond, 1979; Powles, 1984; Cornic and Louason, 1984). Photorespiration also maintains carbon cycling as it releases CO_2 by the decarboxylation of glycine and this CO_2 may be refixed by the Benson-Calvin cycle. This hypothesis was based on the observation that O_2, in the absence of CO_2, could prevent photoinhibition (Powles and Osmond,1979; Osmond, 1981; Powles, 1984; Krause, Koster and Wong, 1985; Chaturvedi and Nielsen, 1987) and that this effect was more noticeable in C_3 plants than in C_4 plants (Brown and Morgon, 1980; Powles, Chapman and Osmond, 1980; Powles, Cornic and Louason,

1984). C_4 plants reputably have lowered capacities for photorespiration (Osmond and Harris, 1971).

Molecular oxygen may act as the terminal electron acceptor of PSI, generating oxygen free radicals (the Mehler reaction). The oxygen radicals may then be metabolized by the ascorbate- glutathione reactions (anti-oxidant system), superoxide dismutase, catalyse and the carotenoids (Asada and Takahashi, 1987). The ability of catalase to metabolize oxygen free radicals is questionable as it is peroxisomal, has been shown to be inactivated by high light (Feierabend and Engel, 1986), and shows no increased activity in plants exposed to hyperoxic conditions (Foster and Hess, 1980). The anti-oxidant system utilizes reductant (Radmer and Kok, 1976) and may offer protection against photoinhibition (Powles, Chapman and Osmond, 1980) by returning NADP to the electron transport reactions. The activity of glutathione reductase has also been shown to increase two or three fold if plants under light saturating conditions are transferred from normal conditions to an atmosphere containing 75% O_2 (Foster and Hess, 1980). The Mehler reaction also allows the formation of the transthylakoidal pH gradient which has been shown to be related to the dissipation of light energy as heat (Krause and Cornic, 1987). If the capacity of the reactions that metabolize oxygen free radicals is exceeded, oxidative damage may result. Postuka, Wroblewska and Mikulska (1976) showed that photoinhibition in the presence of 100% O_2 rather than 21% greatly enhanced the inhibition of CO_2 assimilation.

The increase in the rate constant for non-radiative energy dissipation (K_D), which serves to non-destructively dissipate energy as heat, has also been proposed to be a 'protective' or 'regulatory' mechanism (Demmig and Björkman, 1987; Horton and Hague, 1988; Krause, 1988). Both high energy quenching (q_E) and photoinhibitory quenching (q_I) reduce reductant production during photoinhibition and are reversible. Horton and Hague (1988) showed that fluorescence quenching was constant over a three hundred fold increase in light intensity and that at low light photochemical quenching predominated but that as light intensity was increased non-photochemical quenching became more important. High energy quenching (q_E) was found to saturate at the same light intensities as does photosynthesis and declined at higher light intensities, whereas photoinhibitory quenching (q_I) continued to increase. These findings emphasize the regulatory nature of non-photochemical quenching.

1.8) Photoinhibition in C_3 and C_4 plants.

The carbon metabolism in C_3 and C_4 plants is different and although this has no effect on the proposed mechanisms of photoinhibition, it may effect the plants protective abilities. C_4 plants under normal environmental conditions show a limited capacity for photorespiration (Osmond,1981; Volk and Jackson, 1972) which is restricted to the bundle sheath cells (Chollet and Ogren, 1972). As photoinhibition occurs in the mesophyll cells (Powles, Chapman and Osmond, 1980; Hayden, Baker, Percival and Beckwith, 1986) the protective function of photorespiration is doubtful. The bundle-sheath cells of C_4 species malic-NADP, such as maize, have little or no PSII activity and display little PSII variable fluorescence (Li and Nothnagel, 1989) and subsequently cannot produce NADPH. It is proposed that reductant is shunted from mesophyll cells to bundle-sheath cells by the transfer of malate which is decarboxylated to pyruvate and CO_2 with the release of reductant. The only way in which photorespiration of the bundle cells could utilize reductant during photoinhibitory conditions is if the malate shuttle transfers reductant from mesophyll to bundle-sheath cells. The production of malate in the mesophyll cells requires CO_2 and thus experiments performed in the absence of CO_2 or during stomatal closure would prevent this cycle.

The Mehler reaction is not restricted to one cell type and the mesophyll and bundle-sheath cells of maize and sorghum were equally effective in light dependent H_2O_2 scavenging. The activities of ascorbate peroxidase in these cell types was consistent with there capacity to scavenge H_2O_2 (Nakano and Edwards, 1987). Thus, the Mehler reaction and anti-oxidant system may offer some protection against photoinhibition but in view of the limited protective effect observed when O_2 is supplied to C_4 plants, Powles, Chapman and Osmond (1980) suggested that this was not an important protective mechanism in these plants. As photorespiration and the anti-oxidant system appear to have limited protective capacity, it may be that non-radiative energy dissipation may be the most important protective mechanism in C_4 plants.

The relative contributions of the various proposed protective mechanisms are little understood, particularly in C_4 plants. The aim of this study was to assess the roles of

non-radiative energy dissipation and the mechanisms influenced by O_2 and CO_2 in the photoinhibition of *Zea mays* seedlings. In an attempt to boost carbon metabolism and hence reductant utilization during photoinhibitory treatments, intermediates of the Benson-Calvin cycle or of photorespiration were fed via the transpiration stream. A comparison of these results with results from experiments where O_2 and CO_2 were supplied, allowed the roles of photorespiration and the Benson-Calvin cycle to be assessed. The extent of inhibition immediately following photoinhibition and the subsequent recovery from photoinhibition was monitored by chlorophyll fluorescence and gas exchange techniques. CO_2 response curves constructed during recovery, allowed both the recovery of the efficiency of the carboxylation processes and the level of substrate cycling to be determined.

(2) MATERIALS AND METHODS

2.1) Growth conditions.

Zea mays L. (Hickory King) seeds were germinated and grown in acid washed sand within a high light, constant temperature chamber (Figure 2.1.1). The chamber was constructed from six cm polystyrene foam sheet and consisted of two chambers: an upper glass topped chamber which was separated from the lower chamber by a light-safe, slatted floor. The lower chamber was constructed with double glazed windows and was well ventilated with cold air from an electric fan. For thermostatically controlled infra-red heating lamps were situated such that they irradiated the lower chamber via the windows and heated air to 26 $\pm$ 1°C. Plants were grown in the upper chamber.

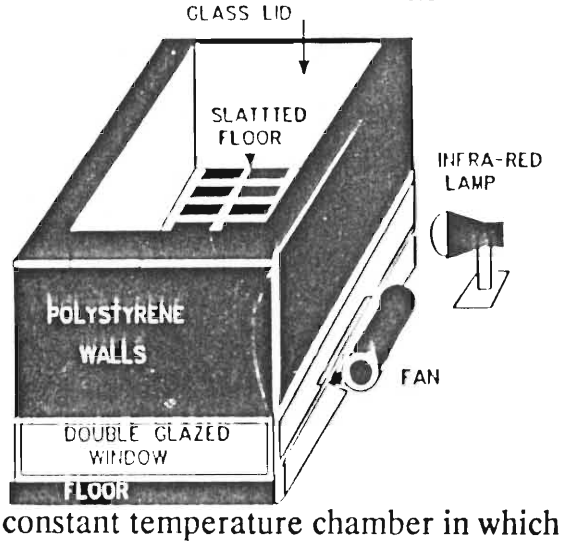


Figure 2.1.1 High light, constant temperature chamber in which maize seedlings were grown.

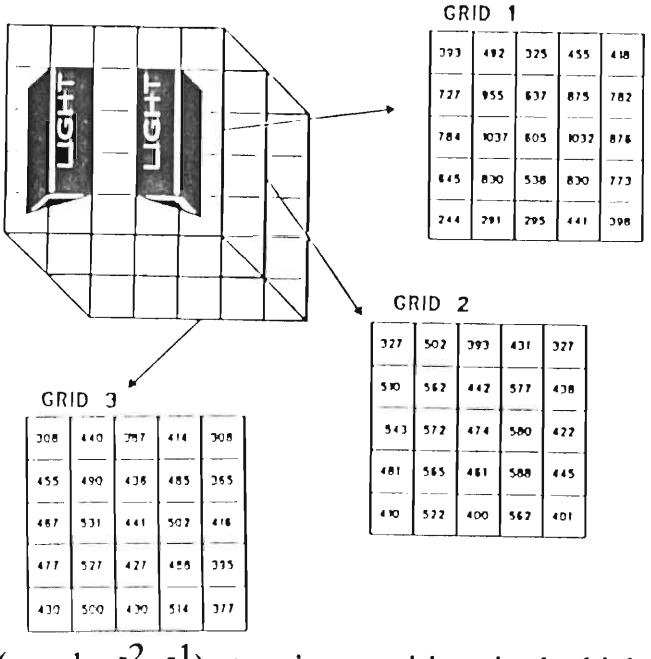


Figure 2.1.2. PPFD (μmol m^{-2} s^{-1}) at various positions in the high light chamber. Grids 1,2 and 3 are 30, 40 and 50cm from the light scources, respectively.

Photosynthetically active radiation was supplied by two Wotan Power Star HQI-T 400W/DH metal halide lamps (14 hours light, 10 hours dark). The photosynthetic photo flux density (PPFD) conditions in the growth chamber varied with both position and distance from the light source (Figure 2.1.2).

Plants were moved in a cyclic fashion such that at the end of the growth period each had been exposed to the same light conditions. Plants were watered daily and on days 4 and 8 were fed Long Ashton growth solution (Hewitt, 1952) which contained the fungicide Benlate (0.1% m/v). The second leaf of plants, nine to ten days after shoot emergence, were excised under water and used in all experiments. The cut end of the leaf was inserted in a pill vial of tap water.

2.2) Cuvette design.

Leaf blades (middle third) were enclosed in a glass, water jacketed, cuvette for gas exchange experiments (Figure 2.2.1); or in an ADC 12357 leaf cuvette (ADC, Hoddesdon, U.K.) for chlorophyll fluorescence measurements. The ADC cuvette was modified by replacing the upper window with a rubber stopper through which the fluorescence probe was inserted.

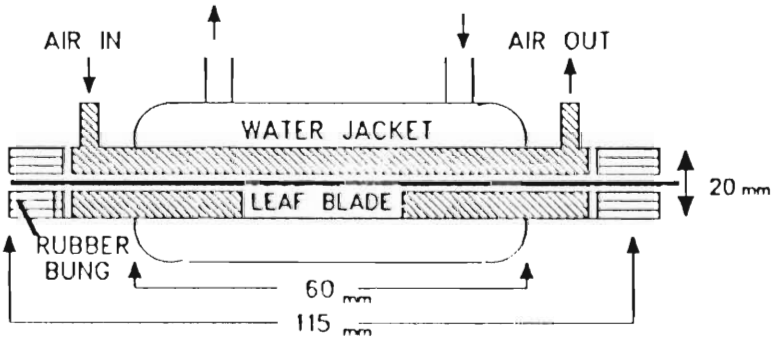


Figure 2.2.1. Glass water jacketed cuvette used for gas exchange experiments.

2.3) Photoinhibitory treatments.

The photoinhibitory treatment used a Wotan Power Star HQI-T 400W/DH metal halide lamp intensified through a large lens such that the PPFD at the leaf surface

was 2450 μmol m^{-2} s^{-1}. For the fluorescence measurements photoinhibitory PPFD was supplied by means of fiber optics connected to a Schott KL1500 light source and the intensity at the leaf surface was 2550 μmol m^{-2} s^{-1}. Photoinhibitory treatments lasted 30 minutes, except where indicated, and were performed in the presence of N_2 or various concentrations of O_2 or CO_2. Gases were supplied at a flow rate of approximately 2 l min^{-1}. Leaf temperature was maintained at 25.5 $\pm$ 0.5 °C during fluorescence and gas exchange measurements, but rose to a maximum of 29.5 °C during photoinhibitory treatments.

To investigate the effect of photoinhibition on the photosynthetic processes the extent and the subsequent recovery from photoinhibition was monitored. Measurements of CO_2 exchange were used to assess the effects on the carboxylation and reduction processes and chlorophyll fluorescence to study effects on the light reactions.

2.4) CO_2 exchange measurements.

Infra-red gas analyzers (ADC 255-MKIII, ADC, Hoddesdon, U.K.) were used to measure both CO_2 and water-vapour exchange. Measurements were made before and for 80 minutes following photoinhibitory treatments. Leaf temperature was maintained at 25 $\pm$ 0.5 °C and the leaf to air water-vapour gradient was maintained at 14 mmol mol^{-1}. PPFD before and following photoinhibition was 550 μmol m^{-2} s^{-1}. Flow rates were such that boundary layer resistance was negligible. Assimilation rates (A), transpiration rates (E), stomatal conductances (g_{CO2}) and internal CO_2 concentrations (c_i) were calculated according to Farquhar and Sharkey (1982). All assimilation rates were normalized by dividing post-inhibitory assimilation rates by the assimilation rates obtained for each leaf at ambient CO_2 (350 μmol mol-1) and O_2 concentrations, determined before the commencement of the experimental treatments.

2.5) Chlorophyll fluorescence measurements.

A pulse modulated fluorimeter (Waltz PAM 101, H. Waltz, Effeltrich, F.R.G.) was used to measure the initial fluorescence (F_o) and the maximal fluorescence (F_m), before and for 80 minutes following photoinhibition. The variable fluorescence

($F_v = F_m - F_o$) and the fluorescence ratio (F_v/F_m) were calculated (Krause and Weis, 1984). F_o was measured with a weak light from a pulsed light emitting diode. To ensure that Q_A was fully oxidized before the measurement of F_o the leaf was kept in total darkness for 5 minutes. F_m was determined by the application of a 700 ms pulse of saturating light from a Schott KL 1500 light source. During the recovery from photoinhibition leaves were illuminated with a PPFD of 380 μmol m^{-2} s^{-1}, except for the five minute periods of darkness. In all experiments the fiber optic probe was maintained at a constant distance from the leaf surface. The intensities of the weak and the saturating light, as well as the gain of the measuring system, were also maintained constant. Therefore differences in the fluorescence values between different samples reflect true differences in fluorescence yields. Fluorescence units are arbitrary.

To calculate the rate constants for PSII photochemistry (K_P) and for non-radiative energy dissipation (K_D) the formulations of Bjorkman (1987) and Greer (1988) were used. The rate constants were calculated using the following assumptions.
(1) K_F is constant at all times and set to equal 1.
(2) The correction coefficient for fluorescence measurement is constant at all times.
(3)$K_P = 70\ K_F$ at $t=0$.
Using these assumptions, the value of ϵ at $t=0$ is calculated. Then using this value of ϵ, K_P and K_D can be calculated for all $t > 0$ where:

$$K_D = (\epsilon - F_m)/F_m; \qquad K_P = [(\epsilon - F_o) - K_D \times F_o]/F_o$$

These calculations are based on the assumption that non-photoinhibited leaves have a F_v/F_m ratio of 0.864. In this study the non-photoinhibited leaves have F_v/F_m ratios in the order of 0.77. These F_v/F_m values appear to be real as a plot of fluorimeter output voltage against the reciprocal of saturating pulse intensity (results not shown) did not increase F_m values when extrapolated to infinite light intensity. Hence the calculations underestimate K_D and K_P but do allow for comparisons between treatments.

2.6) O_2 and CO_2 effect.

The O_2 and CO_2 concentrations supplied to leaves are proposed to affect the systems that offer protection against photoinhibition. This effect was investigated by

supplying O_2 or CO_2 during photoinhibitory treatments. Plants were photoinhibited in the presence of various concentrations of O_2 (0, 2, 21, 29, 50 and 100%) or CO_2 (0, 10, 19, 36 and 50 μmol mol^{-1}) and then recovered at ambient O_2 and CO_2 concentrations. Non-photoninhibited plants were used as controls and assimilation rates remained constant over the entire experimental period. The various gas concentrations were blended either in gas cylinders or with mass flow controllers (Hastings Hi Teck F100, Holland). The concentrations of CO_2 and O_2 were measured with an ADC MKIII infra-red gas analyzer and an oxygen analyser (Beckman OM-14, U.S.A.), respectively.

2.7) Intermediates of the Benson-Calvin cycle and photorespiration.

Phosphoglycerate (PGA) and glycolate are intermediates of the Benson-Calvin cycle and photorespiration, respectively, and were fed to the leaves in an attempt to boost the intermediates of these cycles. If these cycles protect against photoinhibition, boosted intermediate levels may enhance this protective effect. Prior to the photoinhibitory treatment the cut leaves, enclosed in the glass IRGA cuvette, were fed PGA or glycolate (10 mM) for 45 minutes. PPFD, leaf temperature and leaf to air water vapour gradient were as described for gaseous exchange measurements (see 2.4). Leaves were supplied with ambient air at a flow rate of 2 l min^{-1}. Leaves were photoinhibited as described (see 2.3) in the presence of N_2 and recovered at ambient CO_2 and O_2 conditions. Controls were non-photoinhibited leaves fed either PGA or glycolate.

To ensure that the intermediates were metabolized, C^{14} labeled glycolate (0.5 mCi in 10ml of 10 mM PGA) was fed to the leaves via the transpiration stream. After 45 minutes the leaf samples were homogenized in cold ethanol and the sugars, organic acids, amino acids and PGA separated chromatographically, according to the method of Amory (1982). The separated fractions were air dried and resuspended in 3 ml of distilled water. A 500 μl sample of each was added to 10ml of Beckman Readysolv scintillation fluid and the radioactivity measured with a Beckman LS7500 (Beckman, U.S.A.) scintillation counter. Results were corrected for quenching and counting efficiency and were expressed on a fresh weight basis.

2.8) CO_2 response curves.

CO_2 response curves constructed for C_4 plants allow both the efficiency of the carboxylation processes and the rate of RuBP regeneration to be investigated. Both of these processes are effected by photoinhibition and CO_2 response curves were used to monitor their recovery following photoinhibition. Plants were exposed to a photoinhibitory treatment (0% O_2 and CO_2) and then recovered (for 80 minutes) at a particular CO_2 concentration and gas exchange rates recorded. CO_2 assimilation rates were normalized to the initial rates (assimilation rates of uninhibited leaves measured at 350 μmol mol^{-1} CO_2) and the internal CO_2 concentrations were calculated. This was repeated for CO_2 concentrations of 20, 60, 120, 345, and 650 μmol mol^{-1}. Curves were then hand-fitted to each data set (normalized A and c_i) and values interpolated at 10, 20, 40 and 80 minutes. Normalized A and c_i values were then used to construct A versus c_i plots at 20, 40 and 80 minutes. Controls were non-photoinhibited leaves exposed to the different CO_2 concentrations. Each leaf was exposed to a range of CO_2 concentrations and allowed to reach steady state at each CO_2 concentration before gaseous exchange measurements were recorded. Normalized A and c_i were calculated and A verses c_i plots constructed. Assimilation rates were normalised to rates measured at 350 μmol mol^{-1}.

2.9) Statistics.

O_2, CO_2 and metabolite feeding experiments were replicated a minimum of three times. The number of replicates is indicated in figure legends. A Kolmogrov-Smirnov two sample test was used to check if treatments were significantly different from the 0% O_2 and CO_2 treatments.

The recovery of photoinhibited leaves at the various CO_2 concentrations (for the construction of CO_2 response curves) was replicated a minimum of three times, the exact number of replicates is indicated in the figure legends. Kolmogrov-Smirnov test was used to find whether curves constructed at 20, 40 and 80 minutes time intervals during the recovery were significantly different from control CO_2 response curves.

(3) RESULTS

3.1) General photosynthetic characteristics.

In order to quantify the general photosynthetic characteristics of nine day old maize *Zea mays* seedlings, CO_2 and PPFD response curves were constructed. The second leaves of such plants showed photosynthetic characteristics typical of C_4 plants. CO_2 dependent CO_2 assimilation saturated at an internal CO_2 concentration of approximately 110 μmol mol^{-1} and the steep initial slope of the curve indicated a high carboxylation efficiency (Figure 3.1.1). These characteristics are typical of C_4 species (Osmond and Björkman, 1972). The maximum CO_2 assimilation rates for the different replicates varied from 10 to 15 μmol m^{-2} s^{-1}; this variation was removed by normalizing the data (Figure 3.1.2). Although CO_2 assimilation rates are low for a C_4 photosynthetic species, they are not untypical for plants of this age (Crespo, Frean, Cresswell and Tews, 1979).

CO_2 assimilation saturated at a PPFD of approximately 550 μmol m^{-2} s^{-1} (Figure 3.1.3); variation in the rates of CO_2 assimilation could be removed by normalizing the data (Figure 3.1.4). As normalizing data successfully removed between leaf variation, data from all other gas exchange experiments was normalized as previously described (see 2.4).

The CO_2 assimilation of leaves (in ambient air) was inhibited when exposed to light intensities in excess of 1 500 μmol m^{-2} s^{-1} (data not shown). Based on this, a light intensity of approximately 2 500 μmol m^{-2} s^{-1} was chosen for photoinhibitory treatments. During non-inhibitory treatments leaves were exposed to a PPFD of 550 μmol m^{-2} s^{-1} which was sufficient to just saturate CO_2 assimilation.

To determine the optimal conditions required to photoinhibit tissue without causing irreversible inhibition of CO_2 assimilation leaves were exposed to a light intensity of 2 450 μmol m^{-2} s^{-1} in 100% N_2 for different time periods, and the photosynthetic rate determined for 80 minutes on the return of leaves to 21% O_2, 350 μmol mol^{-1} CO_2 and a light intensity of 550 μmol m^{-2} s^{-1}. Photoinhibitory treatments longer than 1.5 hours caused complete and irreversible inhibition of CO_2 assimilation (Figure 3.1.5); The 30 minute treatments resulted in decreased CO_2 assimilation which upon return to ambient conditions, recovered to within 20% of the pre-

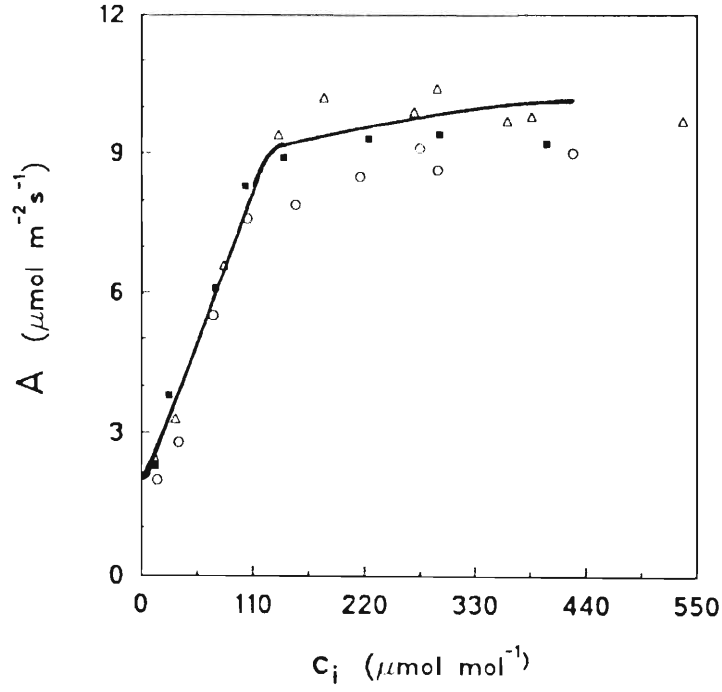


Figure 3.1.1 Response of CO_2 assimilation (A) of non-photoinhibited leaves to internal CO_2 concentration (c_i). (N=3)

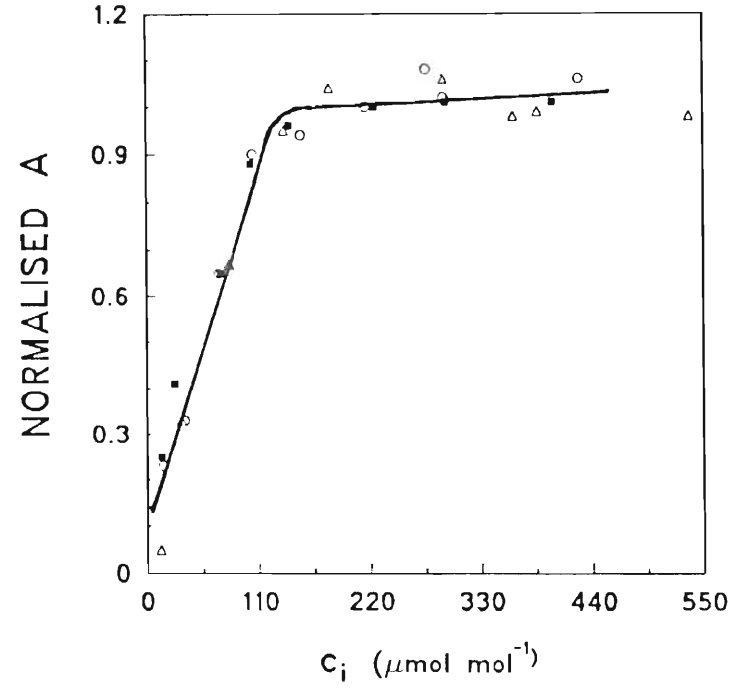


Figure 3.1.2 Response of normalised CO_2 assimilation (A) of non-photoinhibited leaves to internal CO_2 concentration (c_i). (N=3).

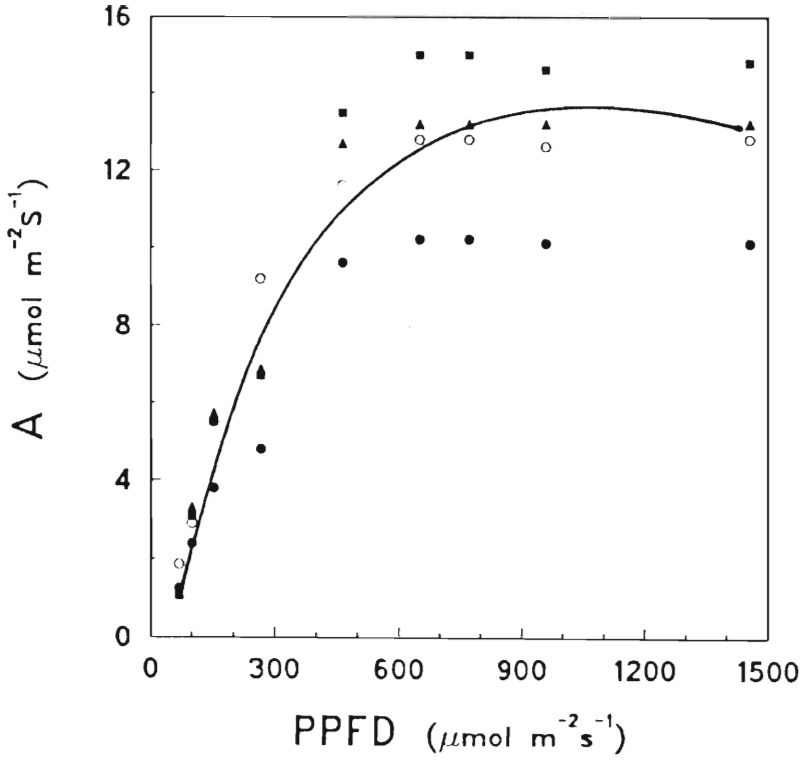


Figure 3.1.3 Response of CO_2 assimilation (A) to photosynthetically active photon flux density (PPFD). (N=3)

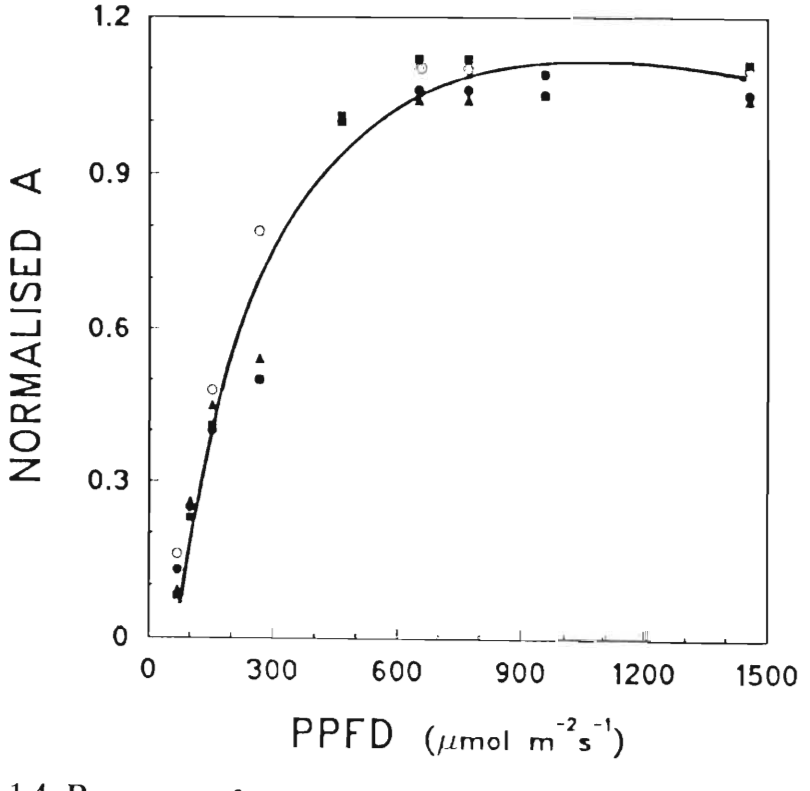


Figure 3.1.4 Response of normalised CO_2 assimilation (A) to photosynthetically active photon flux density. (N=3)

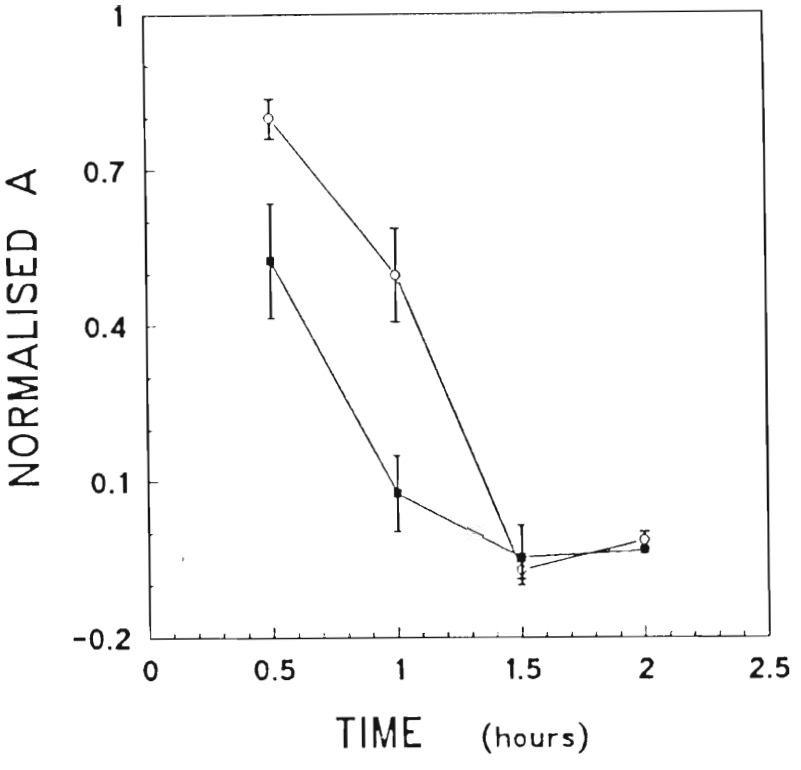


Figure 3.1.5 Effect of length of exposure to photoinhibitory treatments (0 % O_2, 0 % CO_2 and 2450 μmol m^{-2} s^{-1} PPFD) on normalised CO_2 assimilation (A) 10 (■) and 80 (o) minutes after leaves were returned to ambient conditions (21 % O_2, 350 μmol mol^{-1} and 550 μmol m^{-2} s^{-1} PPFD). (N=3)

photoinhibitory values over the following 80 minutes (Figure 3.1.5). For all subsequent experiments, unless stated otherwise, the photoinhibitory treatment is defined as a 30 minute treatment at a light intensity of 2 450 μmol m^{-2} s^{-1} in 100% N_2.

3.2) Effect of supplying O_2 in the absence of CO_2.

Photorespiration and the anti-oxidant system are influenced by the ambient oxygen concentration (Krause and Cornic, 1987). O_2 has also been associated with non-radiative energy dissipation, both due to its involvement with the trans-thylakoidal pH gradient (Krause, 1988) and its function in the proposed mechanisms of non-radiative energy dissipation such as the conversion of violxanthin to zeaxanthin (Goodwin and Mercer, 1983). Hence supplying leaves with various O_2 concentrations during photoinhibitory treatments allows the protective function of these systems to be assessed.

The oxygen concentration supplied during the photoinhibitory treatments effected both the extent to which CO_2 assimilation was inhibited and its subsequent recovery at ambient conditions (Figure 3.2.1). In the absence of O_2 the photoinhibitory treatment decreased the CO_2 assimilation rate by about 60%; within 80 minutes the rate had recovered to a maximum of 85% of the pre-inhibitory rate (Figure 3.2.1 A) Photoinhibition in the presence of 2% and 21% O_2 resulted in a decrease in assimilation rate which was less marked and the tissue recovered more rapidly to 90% of pre-inhibitory rate within 60 minutes (Figure 3.2.1 B and C). These rates are significantly different from those of leaves inhibited in the absence of O_2 (Table 3.2.1).

O_2 CONCENTRATION (%)	SIGNIFICANT DIFFFERENCE BETWEEN TREATMENT LEAVES AND LEAVES INHIBITED WITH 0% O_2 AND CO_2 (TREATMENT A)
2	P< 0.001
21	P= 0
29	P< 0.02
50	P< 0.001
100	P< 0.001

Table 3.2.1 Significant differences between leaves inhibited in the absence of O_2 and in the presence of various O_2 concentrations.

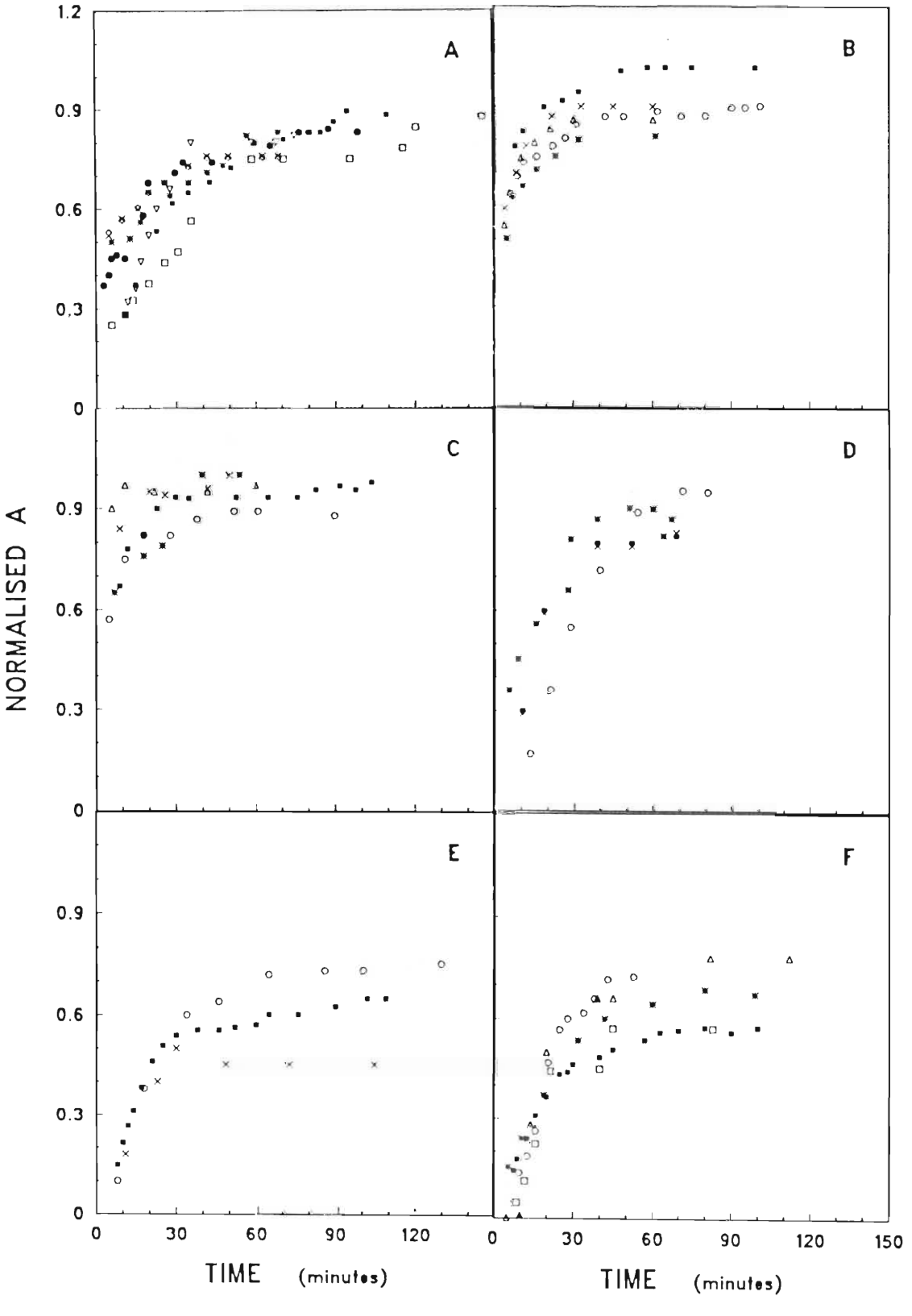


Figure 3.2.1 Recovery of normalised CO_2 assimilation following inhibitory treatments in the presence of O_2 at concentrations of 0% (A), 2% (B), 21% (C), 29% (D), 50% (E) and 100% (F). Different symbols represent replicates with individual leaves. (N=6, 5, 4, 3, 3 and 3 for A, B, C, D, E and F, respectively)

High concentrations of oxygen (50 and 100%) enhanced the photoinhibitory damage and assimilation rates were decreased by 90% ten minutes after the inhibition and recovered to 60% after 80 minutes (Figure 3.2.1 E and F). Assimilation rates of these leaves were significantly different from the leaves inhibited in the absence of O_2 (Table 3.2.1).

To summarise the effect of the various O_2 concentrations supplied during the photoinhibitory treatments, mean normalised assimilation rates 10 and 80 minutes after treatment were plotted against O_2 concentration (Figure 3.2.2). The protective nature of oxygen at concentrations between 2 and 21% is evident particularly after only 10 minutes of recovery. Leaves supplied with O_2 at concentrations of two to 29% recovered to within 90% of pre-inhibitory levels. O_2 at these concentrations appears to effect the extent of the photoinhibition rather than the subsequent recovery. O_2 supplied at concentrations of 29% or more enhanced the photoinhibition, and concentrations of 50 or 100% reduced the extent of the subsequent recovery.

Photorespiration and the anti-oxidant system may be operative at O_2 concentrations between 2 and 21%, utilizing reductant and maintaining carbon flow, and hence offering some protection against photoinhibition, but the lack of a difference in effect between plants supplied 2 or 21% suggests that another protective mechanism may be involved. O_2 concentrations above 21% may increase oxidative damage, due to oxygen radical production (Halliwell, 1987), and may account for the enhanced photoinhibition observed at 50 and 100% O_2.

Other workers (Brown and Morgan, 1980; Powles, Chapman and Osmond, 1980; Krause and Cornic, 1988) generally used inhibitory treatments of much longer duration than used in this study and found that supplying oxygen to C_4 plants had little effect on the extent of the short term recovery . To check that the observed effect of O_2 on the extent of photoinhibition and the subsequent recovery was not a consequence of the short (30 minute) photoinhibitory treatment, leaves maintained in 21% O_2 were exposed to inhibitory treatments for various lengths of time. Supplying O_2 at 21% offered protection against photoinhibition irrespective of the duration of the inhibitory treatment; the inhibition of leaves treated for one hour was, however, greater than leaves inhibited for 0.5 or 2 hours. Subsequent recovery was unaffected by the duration of the treatment (Figure 3.2.3).

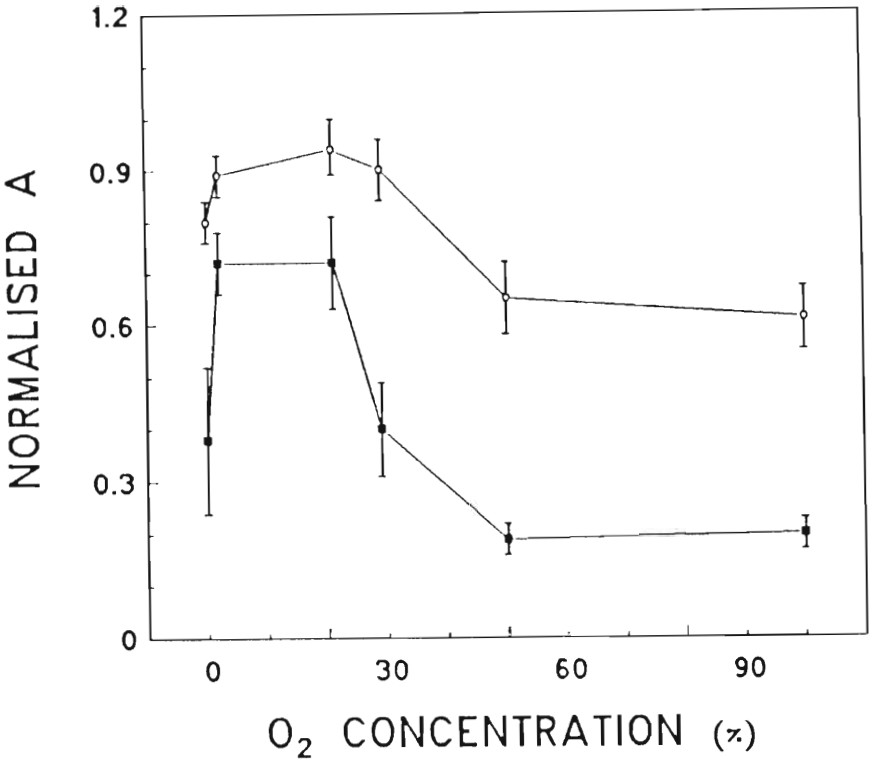


Figure 3.2.2 Summary graph of the effect of O_2 supplied during photoinhibitory treatments on the normalised assimilation rates 10 (■) and 80 (o) minutes after leaves were returned to ambient conditions.

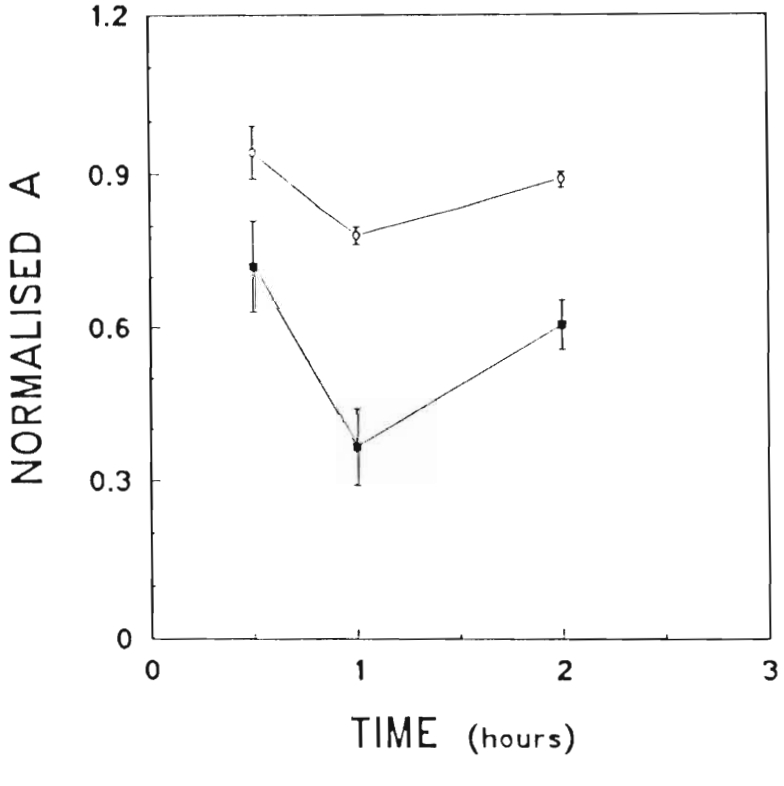


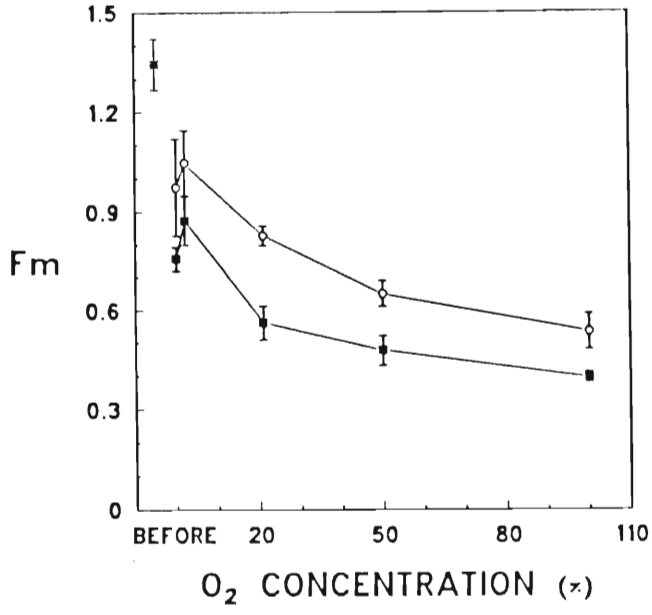
Figure 3.2.3 Effect of length of photoinhibitory treatment in the presence of 21 % O_2 o

The light reactions of photosynthesis are effected by photoinhibition (Powles, 1984) thus decreasing the production of reductant which would affect the rate of CO_2 assimilation. To investigate the effect of photoinhibition of the PSII light reactions, the initial fluorescence (F_o) and maximum fluorescence yield (F_m) of leaves supplied with various concentrations of O_2 during the photoinhibitory treatment were measured. Maximal fluorescence yield (F_m) was decreased by photoinhibitory treatments; five minutes after the return of leaves to ambient conditions, leaves inhibited in the presence of 21, 50 or 100% O_2 had F_m values lower than leaves inhibited in the absence or in 2% O_2 (Figure 3.2.4). After 80 minutes of recovery trends were similar, as F_m values recovered by approximately the same amounts at all concentrations. Recovery was incomplete at all concentrations and was maximal for leaves supplied with 2% O_2, (F_m recovered to 78% of the pre-inhibitory value).

Initial fluorescence yield (F_o) was little altered if O_2 was present during the photoinhibition but increased slightly over the 80 minute recovery period (Figure 3.2.5). If leaves were inhibited in the absence of O_2, F_o values increased such that after 5 minutes at ambient conditions values were 85% higher than pre-inhibitory values and recovered to preinhibitory values over the following 80 minutes. F_o results, although difficult to interpret may indicate that when oxygen was present no alterations to open reaction centres occurred, but that this was not so in the absence of oxygen. This is a phenomenon which has been noted in other species (Krause, Koster and Wong, 1985).

As F_o is fairly constant, the variable fluorescence ($F_v = F_m - F_o$), as effected by the oxygen concentration supplied during the photoinhibitory treatment showed similar trends to the F_m values (Figure 3.2.6). F_v values both five and 80 minutes after leaves were returned to ambient conditions decreased with increasing O_2 concentration. Owing to the large increase in F_o when leaves were photoinhibited in the absence of O_2, F_v was decreased but recovered over the following 80 minutes. F_v indicates the extent of Q_A reduction (Krause and Weis, 1984) and F_v is reduced during photoinhibition due to primary photochemistry inactivation (Krause, 1988). Presumably PSII inactivation is enhanced at higher O_2 concentrations.

The ratio F_v/F_m is a measure of the efficiency of the PSII photochemistry (Björkman, 1987). The F_v/F_m ratio was decreased by photoinhibitory treatments and showed some recovery on return to ambient conditions; both the extent of the reduction in efficiency and the subsequent recovery were dependent on the O_2 con-



gure 3.2.4 Effect of O_2 supplied during photoinhibitory treatments on the maximum fluorescence yield (Fm), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. Fm before photoihibitory treatment is shown by the symbol (*). (N=3)

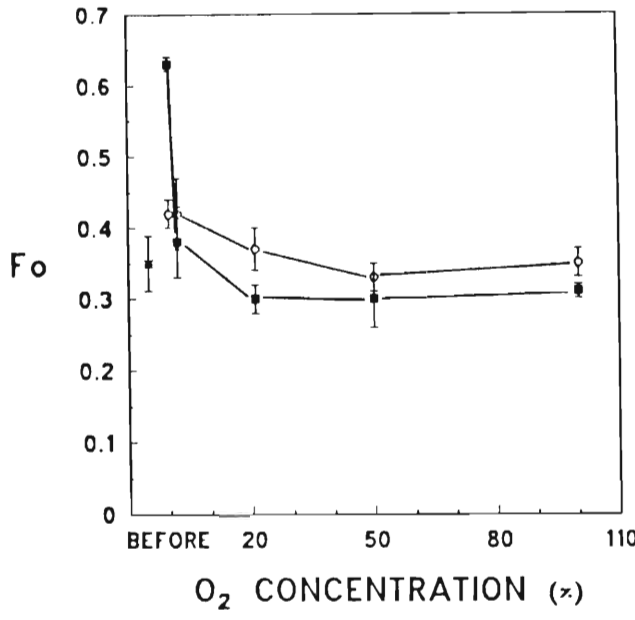
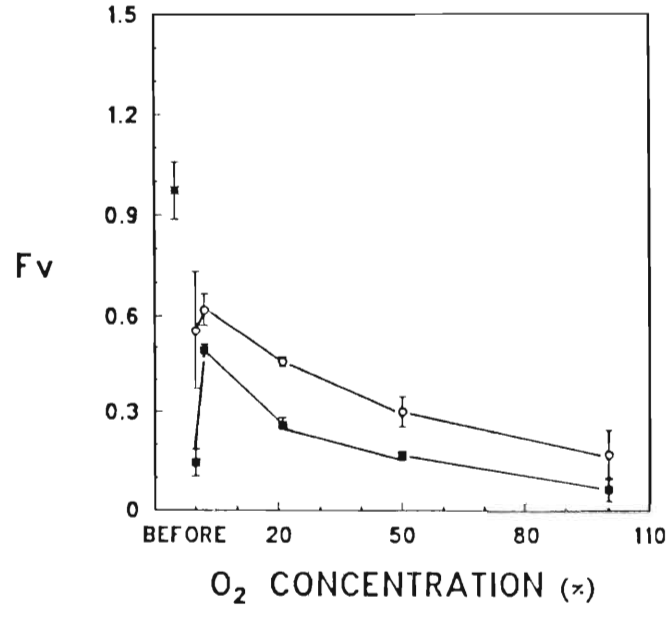


Figure 3.2.5 Effect of O_2 supplied during photoinhibitory treatments on the initial fluorescence yield (Fo), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. Fo before photoinhibitory treatment is shown by the symbol (*) (N=3)



gure 3.2.6 Effect of O_2 supplied during photoinhibitory treatments on the variable fluorescence (Fv), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. Fv before photoinhibitory treatment is shown by the symbol (*) (N=3)

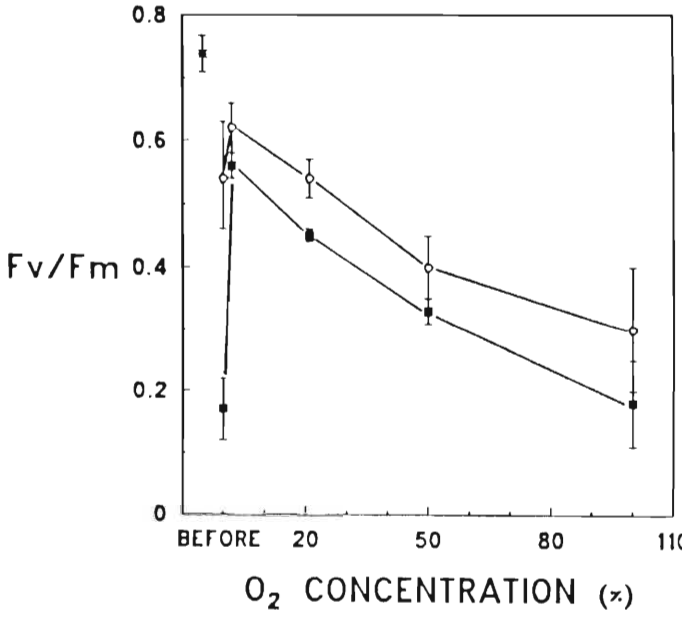


Figure 3.2.7 Effect of O_2 supplied during photoinhibitory treatments on the Fv/Fm ratio, 5 (■) a 80 (o) minutes after leaves were returned to am bient conditions. Fv/Fm before photoinhibitor treatment is shown by the symbol (*). (N=3)

centration supplied during the inhibition (Figure 3.2.7). The F_v/F_m ratio, measured 5 minutes after leaves were returned to ambient conditions, was reduced to 25% of initial values if 0% O_2 was supplied during inhibitory treatments. The ratio recovered to 77% of pre-inhibitory value over the 80 minute recovery period. When O_2 was supplied during inhibitory treatments the extent of the decrease in F_v/F_m at 5 minutes was linearly related to increasing O_2 concentration, but the extent of the recovery over the subsequent 80 minutes remained fairly constant. The least reduction in F_v/F_m occurred at 2% rather than in the absence of O_2.

The changes in the rate constants for non-radiative (K_D) and photochemical energy dissipation (K_P) offer some explanation of the observed changes in PSII photochemical efficiency (Figures 3.2.8 and 9). K_D and K_P were calculated from the mean F_o and F_m values according to Björkman (1987) and Greer (1988) (see 2.5). K_D was increased by inhibitory treatments and is approximately linearly related to the oxygen concentration supplied during the inhibition; this trend was found even after leaves had been returned to ambient conditions for 80 minutes (Figure 3.2.8). K_P was decreased considerably by the inhibitory treatments but the decrease was not strongly dependent on the O_2 supplied during the inhibition (Figure 3.2.9). K_P showed little recovery once plants were returned to ambient conditions. Hence the observed changes in the PSII photochemical efficiency (F_v/F_m) of leaves that were inhibited at various oxygen concentrations could be attributed mainly to changes in non-radiative energy dissipation and to a lesser extent to the decrease in K_P with increasing O_2 concentration.

3.3) Effect of supplying CO2 in the absence of O2.

It is proposed that photoinhibition occurs when the capacity of plant biochemistry to utilize reductant is limited and excess reductant is produced (Osmond, 1981). Under normal conditions the major sink for reductant is the fixation of CO_2. If ambient CO_2 is limiting, the role of the carboxylation processes and the utilization of reductant may be assessed.

The concentration of CO_2 supplied during the inhibitory treatment effected the extent to which assimilation rate was decreased but did not effect the subsequent recovery. CO_2 supplied at 10 or 19 μmol mol^{-1} offered little protection from photoinhibition and 10 minutes after the leaves were returned to ambient conditions

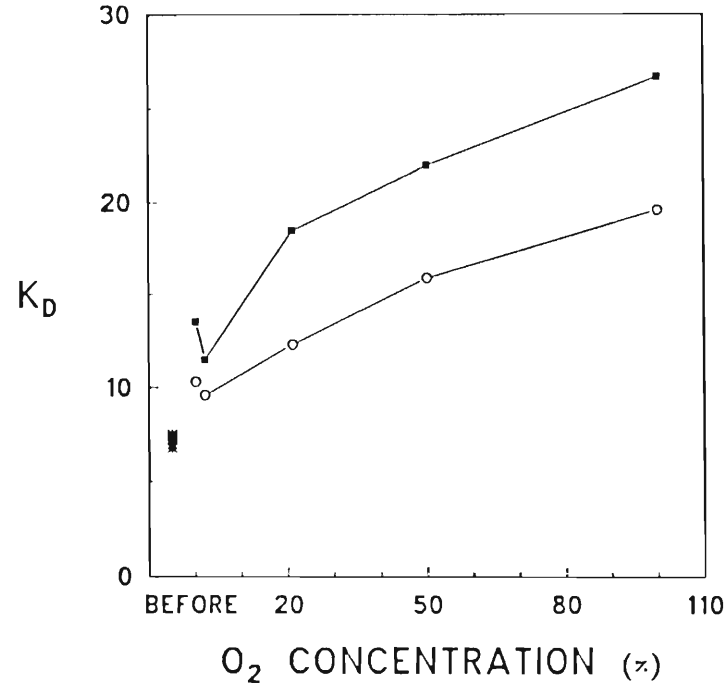


Figure 3.2.8 Effect of O_2 supplied during photoinhibitory treatments on the rate constants for non-radiative energy dissipation (K_D), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. K_D values measured before photoinhibitory treatment are shown by the symbols (*).

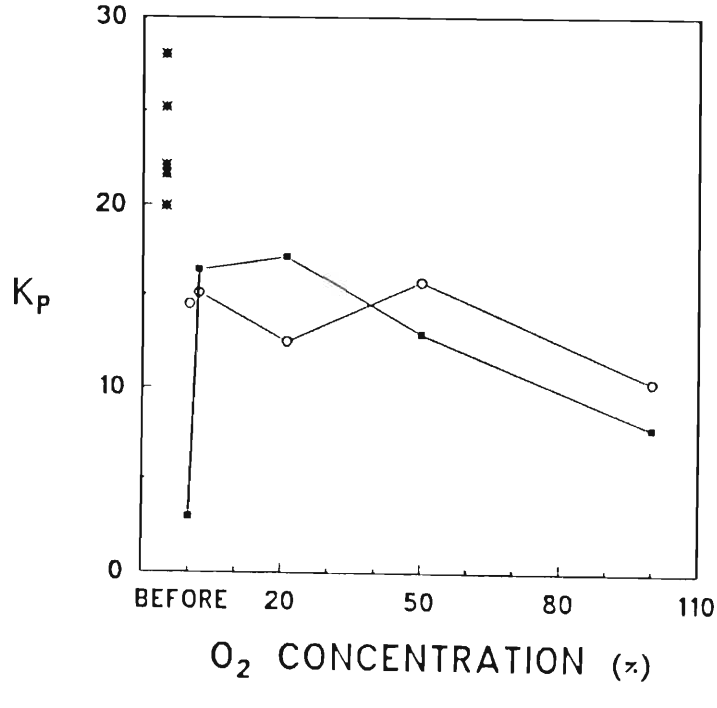


Figure 3.2.9 Effect of O_2 supplied during photoinhibitory treatments on the rate constants for photochemical energy dissipation (K_P), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. K_P values measured before the photoin-

assimilation rates were 40% of the pre-inhibitory rates (Figure 3.3.1 A and B). This result is comparable with the rate measured in leaves after 10 minutes of recovery following inhibition in the absence of CO_2 (Figure 3.3.1 E). Assimilation rates of leaves inhibited in the presence of 10 or 19 μmol mol^{-1} were not significantly different from leaves inhibited in the absence of CO_2 (Table 3.3.1). CO_2 supplied at concentrations of 36 or 50 μmol mol^{-1} decreased the extent of the photoinhibition and after 10 minutes of recovery, rates were 90% of pre-inhibitory rates (Figure 3.3.1 B and C) These rates were significantly different from rates obtained for leaves inhibited in the absence of CO_2 (Table 3.3.1).

CO_2 CONCENTRATION (%)	SIGNIFICANT DIFFFERENCE BETWEEN TREATMENT LEAVES AND LEAVES INHIBITED WITH 0 % O_2 AND CO_2 (TREATMENT E)
10	P> 0.05
19	P> 0.05
36	P= 0
50	P= 0

Table 3.3.1 Significant differences between leaves inhibited in the absence of CO_2 and in the presence of different CO_2 concentrations.

Irrespective of the concentration supplied during the inhibition the assimilation rate was approximately 90% of the pre-inhibitory rate 80 minutes after leaves were returned to ambient conditions (Figure 3.3.1 A, B, C and D).

To summarise the effect of the various CO_2 concentrations supplied during the photoinhibitory treatments mean normalised assimilation rates 10 and 80 minutes after the treatment was plotted against CO_2 concentration (Figure 3.3.2). The protective effect of CO_2 supplied at concentrations of 36 and 50 μmol mol^{-1} suggests that only limited reductant utilization and carbon cycling is necessary to protect against photoinhibition. Other workers (Krause and Cornic, 1987; Powles, Cornic and Louasson, 1984) have recorded that plants supplied CO_2 at the CO_2 compensation point concentration showed little photoinhibition . The incomplete (90%)

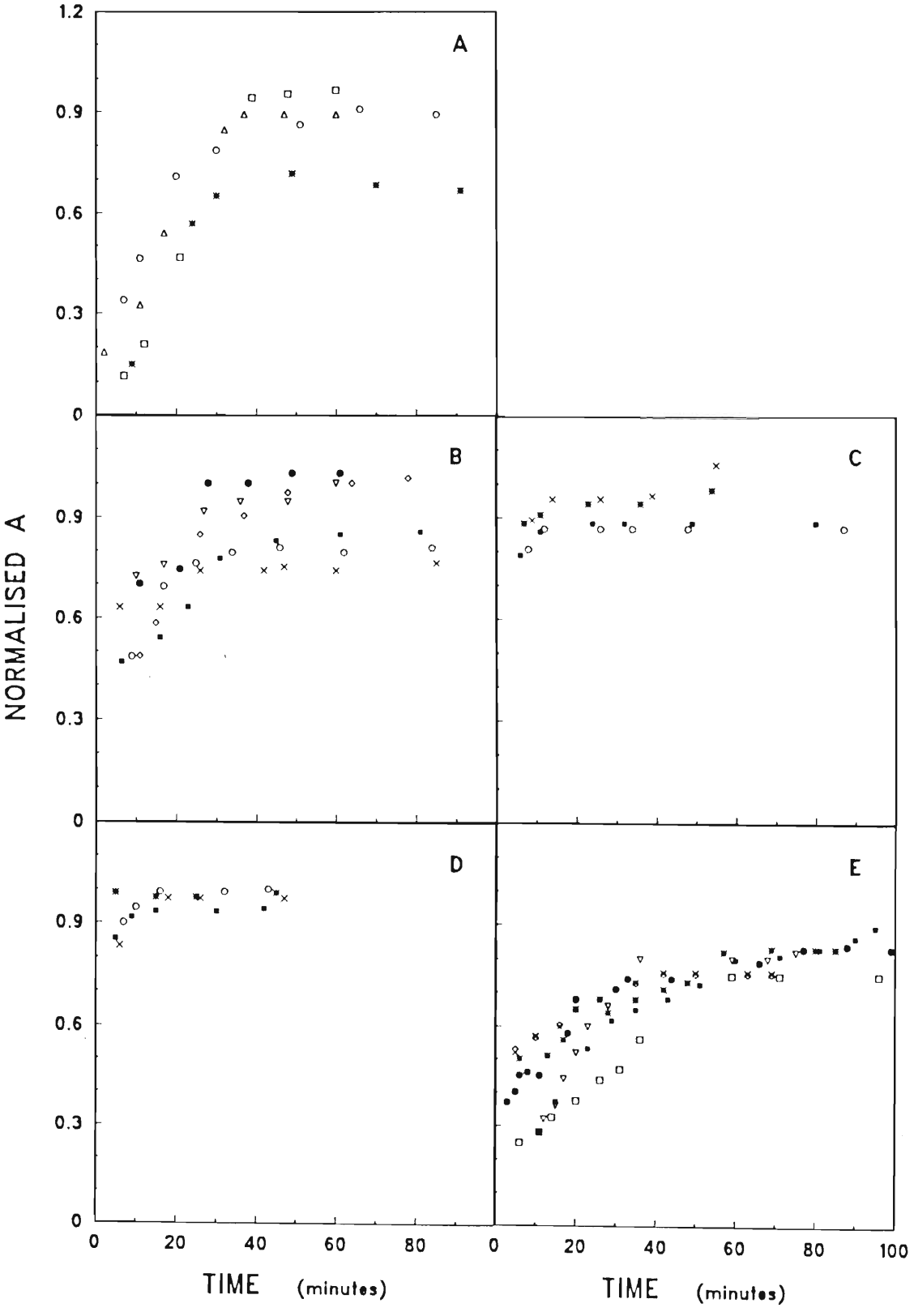


Figure 3.3.1 Recovery of normalised CO_2 assimilation following inhibitory treatments in the presence of CO_2 at the concentrations 10 (A), 19 (B), 36 (C), 50 (D) and 0 μmol mol^{-1} (E). Different symbols represent replicates with individual leaves.(N=3, 7, 4, 4 and 6 for A, B, C, D and E, respectively)

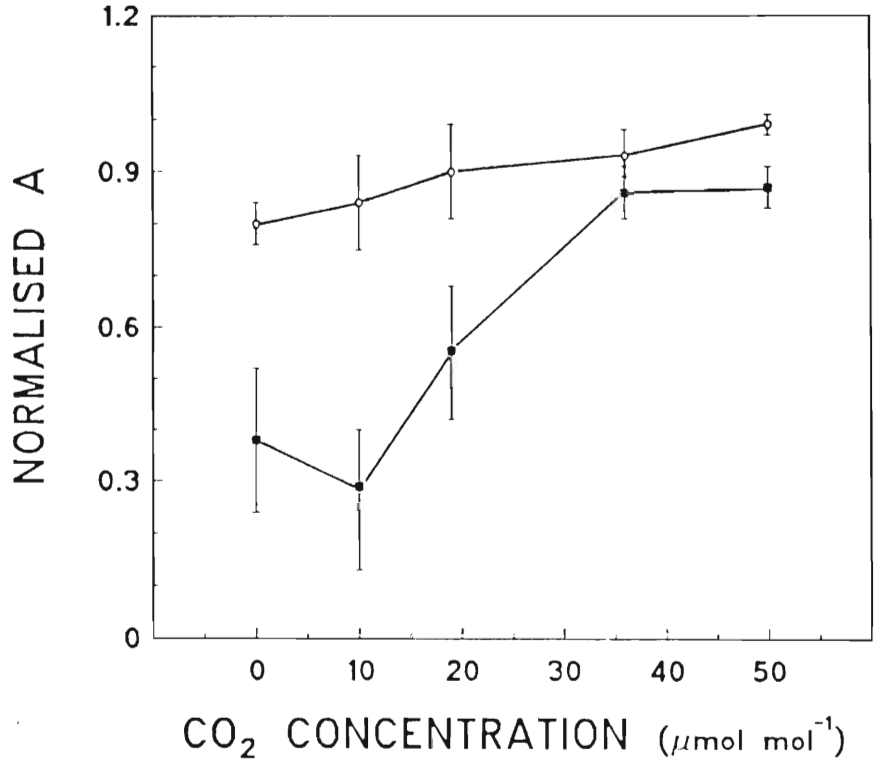


Figure 3.3.2 Summary graph of the effect of CO_2 supplied during photoinhibitory treatments on the normalised assimilation rates 10 (■) and 80 (o) minutes after leaves were returned to ambient conditions.

recovery at all supplied CO_2 concentrations, as with all O_2 concentrations, indicated that a certain component of photoinhibition was irreversible over 80 minutes.

The effect of supplying CO_2 during inhibitory treatments on the PSII photochemistry was investigated using chlorophyll fluorescence. F_m was reduced by the inhibitory treatments at all CO_2 concentrations but the reduction in F_m was less at 49 μmol mol^{-1} than at 0, 21 or 36 μmol mol^{-1} (Figure 3.3.3). F_m recovered to the same extent irrespective of the CO_2 concentration supplied during the photoinhibition, but was approximately 26% lower than pre-inhibitory values.

F_o was increased by inhibitory treatments and this increase was approximately linearly related to the decrease in CO_2 concentration supplied during inhibition (Figure 3.3.4). F_o recovered and although complete for leaves supplied with 49 μmol mol^{-1} CO_2, was incomplete for all concentrations below this. The response of F_o at 49 μmol mol^{-1} was similar to the response noted for leaves supplied with O_2 during photoinhibition.

Both F_v and the F_v/F_m ratio were decreased by the photoinhibition and this decrease was approximately linearly related to the decrease in CO_2 concentration (Figure 3.3.5 and 6). F_v and the F_v/F_m ratio of leaves inhibited in the presence of 0, 12 and 36 μmol mol^{-1} of CO_2, all recovered to approximately 63% and 73% of the pre-inhibitory values, respectively (Figures 3.3.5 and 6). F_v and F_v/F_m of leaves supplied 49 μmol mol^{-1} exhibited greater recovery and recovered to within 78% and 87% of pre-inhibitory values, respectively. Evidently CO_2 concentrations of approximately 49 μmol mol^{-1} were sufficient to substantially reduce the inhibition of PSII electron transport, yet even at this concentration, as was found in CO_2 assimilation experiments, a certain component of the photoinhibition remained irreversible.

The observed changes in rate constants K_D and K_P for leaves inhibited in the presence of CO_2 was different from the effects noted for leaves supplied O_2. K_D, although increased by the inhibition, showed no dependency on the CO_2 concentration at which leaves were inhibited, except at 49 μmol mol^{-1} (Figure 3.3.7). K_D recovered to the same extent irrespective of the CO_2 concentration supplied during the inhibition. K_P was decreased by the photoinhibitory treatments and this decrease was strongly dependent on the supplied CO_2 concentration, as was the subsequent recovery once plants were returned to ambient conditions (Figure 3.3.8). The changes in the F_v/F_m ratio of plants photoinhibited in the presence of CO_2

could be attributed mainly to changes in the rate constant for photochemistry rather than to changes in non-radiative energy dissipation.

3.4) Effect of supplying glycolate and phosphoglycerate to leaves photoinhibited in the absence of CO_2 and O_2.

Supplying both CO_2 and O_2 have been shown to reduce photoinhibition. It has been proposed that this effect is because CO_2 and O_2 allow both the utilization of reductant and the cycling of carbon metabolism to occur. It has been proposed that supplying intermediates of the Benson Calvin cycle and photorespiration may have similar protective effects. Cornic, Woo and Osmond (1982) showed that the addition of Benson Calvin cycle intermediates protected against photoinhibition.

The assimilation rate of non-photoinhibited (control) leaves fed PGA via the transpiration stream decreased such that after 60 minutes following the commencement of the feeding (a length of time which would coincide with 30 minutes of pre-inhibitory feeding and 30 minutes of inhibitory treatment), assimilation rate had decreased by 50% (Figure 3.4.1). Because of this decline in the assimilation rate of the controls no further CO_2 exchange experiments were performed on leaves fed PGA. In contrast to this, glycolate fed to leaves for up to 145 minutes did not effect the assimilation rate (Figure 3.4.2). Glycolate was metabolised as was evident by the incorporation of C^{14} into organic acids, sugars, amino acids and PGA in leaves that were fed labeled glycolate (Figure 3.4.3).

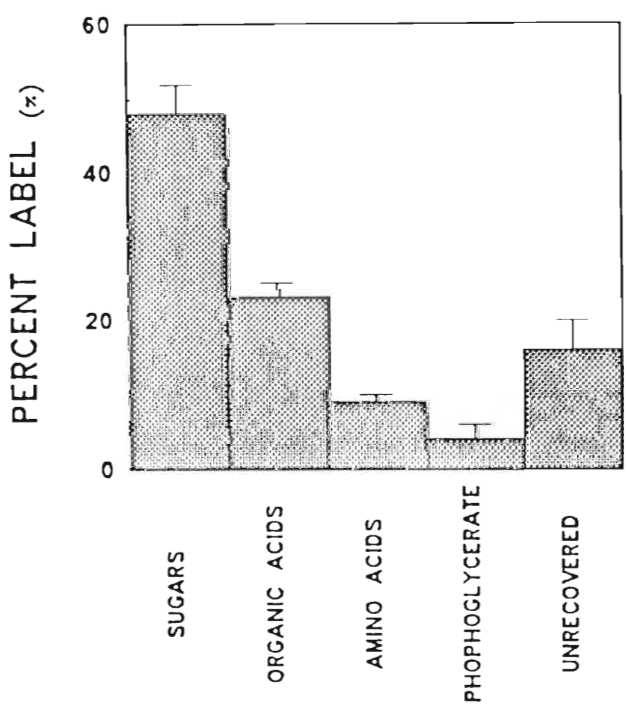
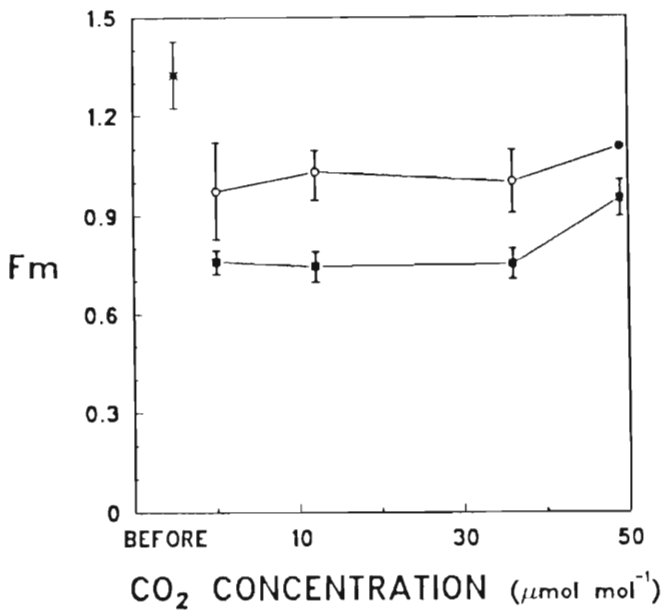


Figure 3.4.3 Incorporation of C^{14} into sugars, organic acids, amino acids and PGA of leaves fed labeled glycolate, via the transpiration stream. (N=3)



gure 3.3.3 Effect of CO_2 supplied during photoinhibitory treatments on the maximum fluorescence yield (Fm), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. Fm before photoinhibitory treatment is shown by the symbol (*). (N=3)

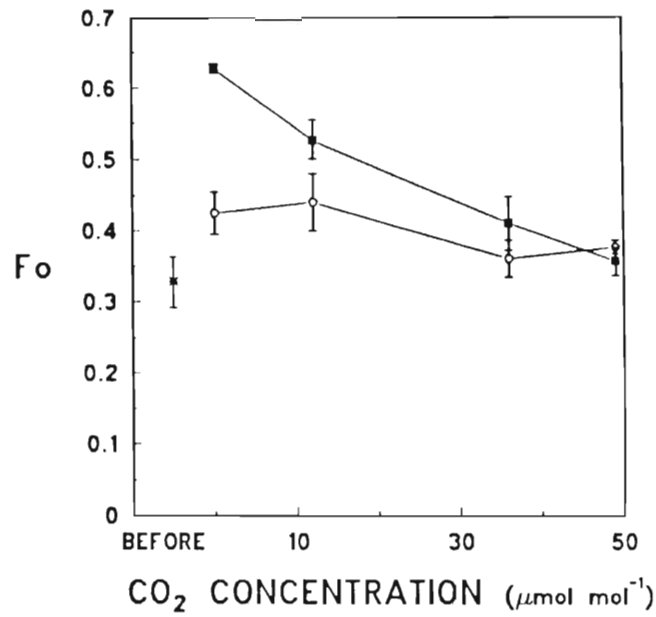
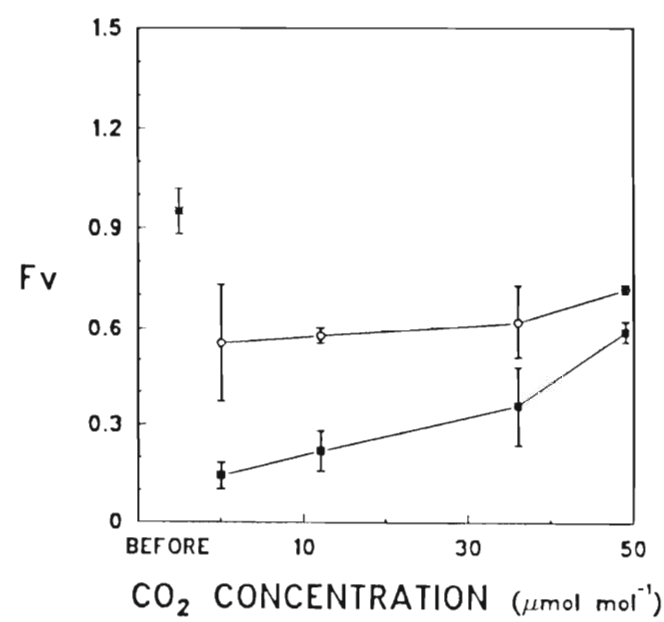


Figure 3.3.4 Effect of CO_2 supplied during photoinhibitory treatments on the initial fluorescence yield (Fo), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. Fo before photoinhibitory treatment is shown by the symbol (*). (N=3)



igure 3.3.5 Effect of CO_2 supplied during photoinhibitory treatments on the variable fluorescence (Fv), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. Fm before photoinhibitory treatment is shown by the symbol (*). (N=3)

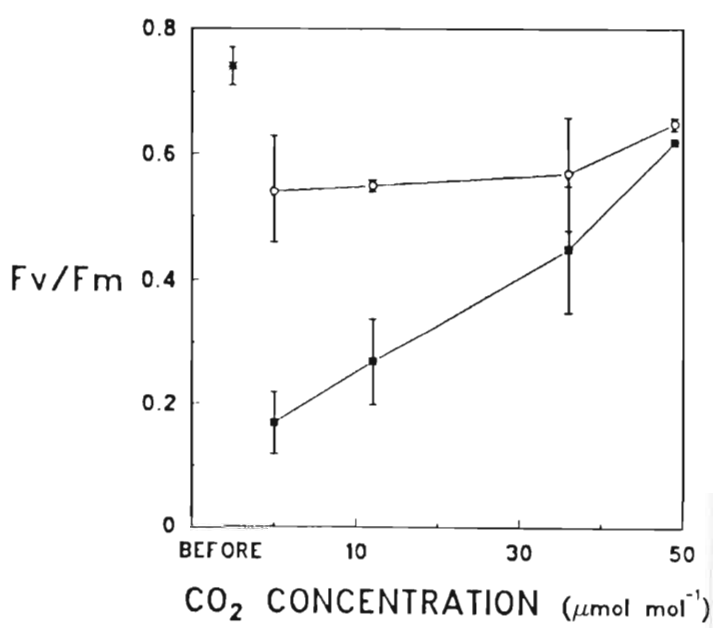


Figure 3.3.6 Effect of CO_2 supplied during photoinhibitory treatments on the Fv/Fm ratio, 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. Fm before photoinhibitory treatment is shown by the symbol (*). (N=3)

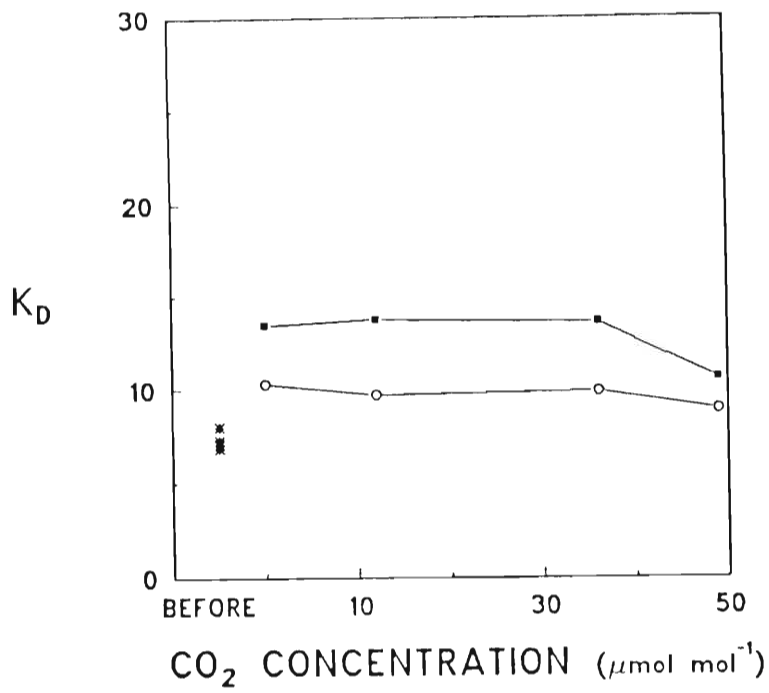


Figure 3.3.7 Effect of CO_2 supplied during photoinhibitory treatment on the rate constant for non-radiative energy dissipation (K_D), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. K_D before photoinhibitory treatment is shown by the symbols (*).

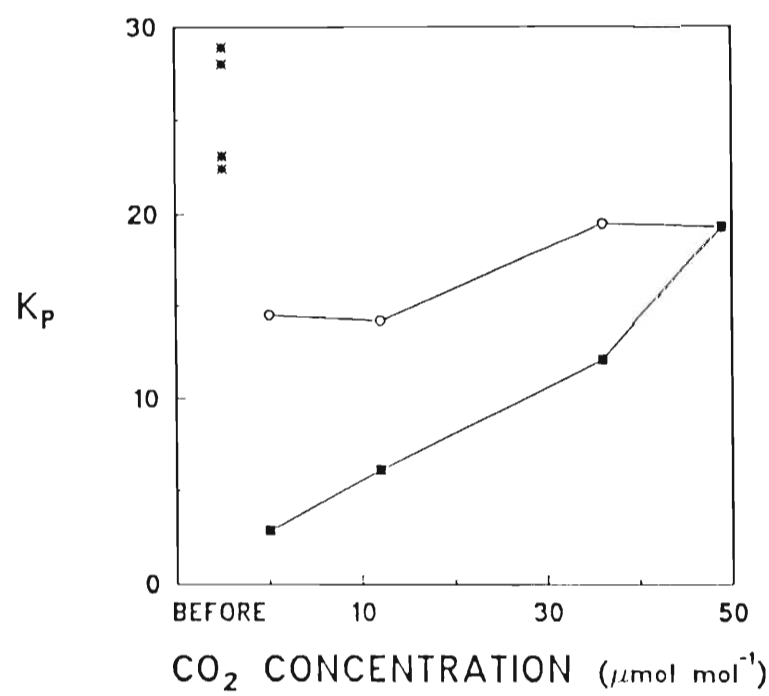


Figure 3.3.8 Effect of CO_2 supplied during photoinhibitory treatments on the rate constant for photochemical energy dissipation (K_P), 5 (■) and 80 (o) minutes after leaves were returned to ambient conditions. K_P before photoinhibitory treatment is shown by the symbols (*).

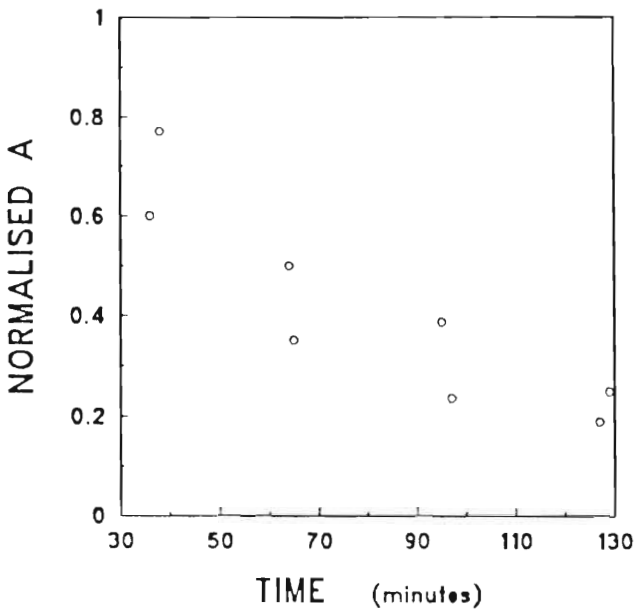


Figure 3.4.1 Effect of feeding phosphoglycerate (10 mM), via the transpiration stream, on the normalised CO_2 assimilation rate of non-photoinhibited leaves. (N=2)

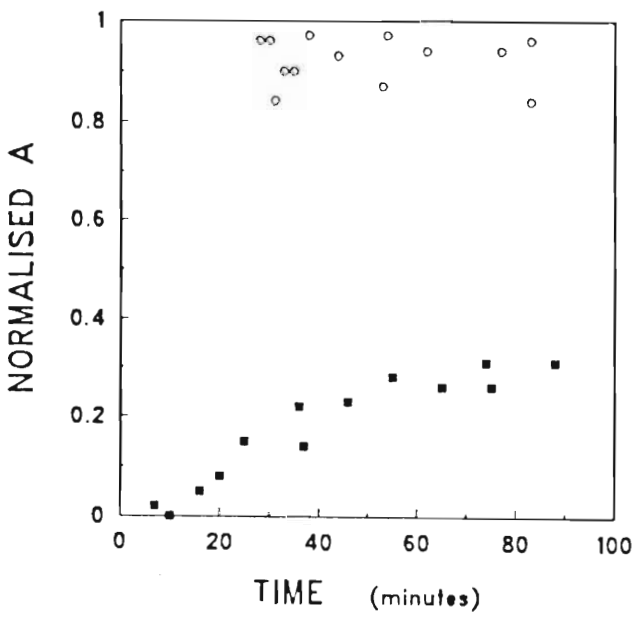


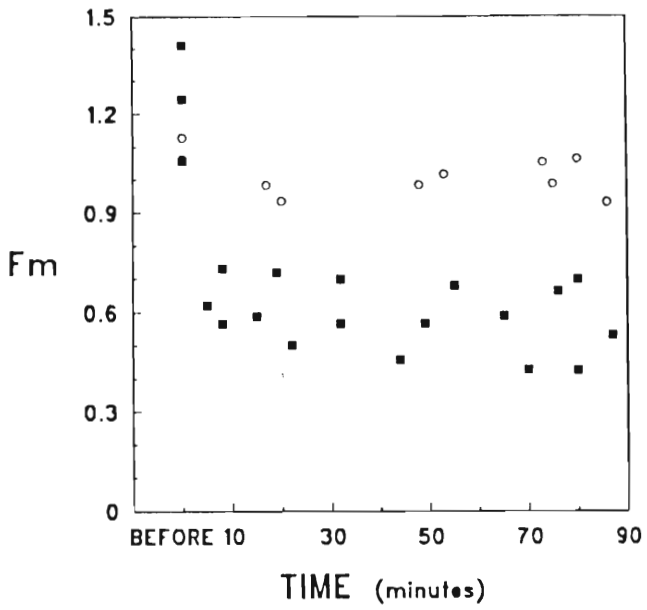
Figure 3.4.2 Effect of feeding phosphoglycerate (10 mM), via the transpiration stream, on the normalised CO_2 assimilation rate of non-photoinhibited (o) and photoinhibited (■) leaves. (N=3)

Feeding glycolate offered no protection from photoinhibition but actually enhanced the inhibition. CO_2 assimilation rate 10 minutes after leaves were returned to ambient conditions was 0% and recovered to only 30% after 80 minutes (Figure 3.4.2). This result suggests that photorespiration is unimportant in C_4 plants. However, the interpretation of the result is complicated because of the two photosynthetic cell types found in this species and because of the energy transfer between these cell types. Recovery of assimilation rate of photoinhibited leaves fed glycolate was less than that of unfed photoinhibited leaves, suggesting that glycolate metabolism may have utilized energy necessary for the recovery from photoinhibition.

Neither PGA nor glycolate fed to control leaves effected the light reactions as was indicated by unchanged F_m, F_o, F_v and F_v/F_m between non-photoinhibited leaves fed or not fed the intermediates (Figures 3.4.4 to 3.4.10). The F_m values of leaves 5 minutes after they were returned to ambient conditions were 44 and 52% of pre-inhibitory values for PGA and glycolate, respectively. Neither F_m values of leaves fed PGA or glycolate increased over the 80 minutes recovery period and leaves fed PGA showed a slight decrease in F_m during the 80 minute recovery period (Figures 3.4.4 and 3.4.5). F_o values for leaves fed PGA or glycolate were increased by photoinhibitory treatments and showed little subsequent recovery (Figures 3.4.6 and 3.4.7). Owing to the changes in F_m and F_o the values of F_v were markedly reduced by the inhibitory treatments as was evident by the values recorded 5 minutes after leaves had been returned to ambient conditions (Figures 3.4.8 and 3.4.9). F_v values from leaves that had been fed PGA showed no recovery and F_v values from leaves fed glycolate recovered to approximately 25% of pre-inhibitory values. Similar trends were observed in the efficiency of PSII photochemistry of leaves fed intermediates prior to photoinhibitory treatments (Figures 3.4.10 and 3.4.11). Although the intermediates did not effect the light reactions prior to photoinhibition, they did appear to effect the metabolism such that the severity of the photoinhibition was enhanced.

3.5) Recovery of the carboxylation processes and the level of RuBP regeneration following photoinhibition.

In C_3 plants the intitial slope and CO_2 saturation level of $A:c_i$ curves are measures of the carboxylation efficiency and RuBP regeneration rate respectively (assuming a



gure 3.4.4 Effect of feeding phosphoglycerate (10 mM), via the transpiration stream, on the Fm of non-photoinhibited (o) and photoinhibited (■) leaves. Fm measured before the photoinhibitory treatment is indicated at time 0. (N=3)

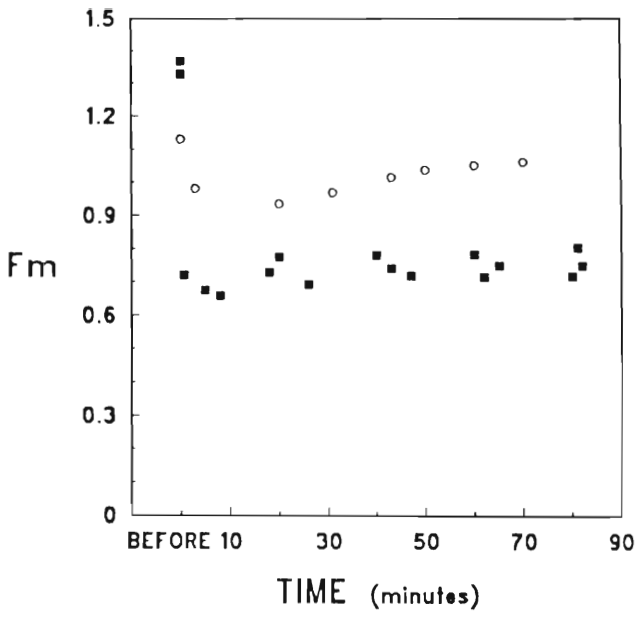
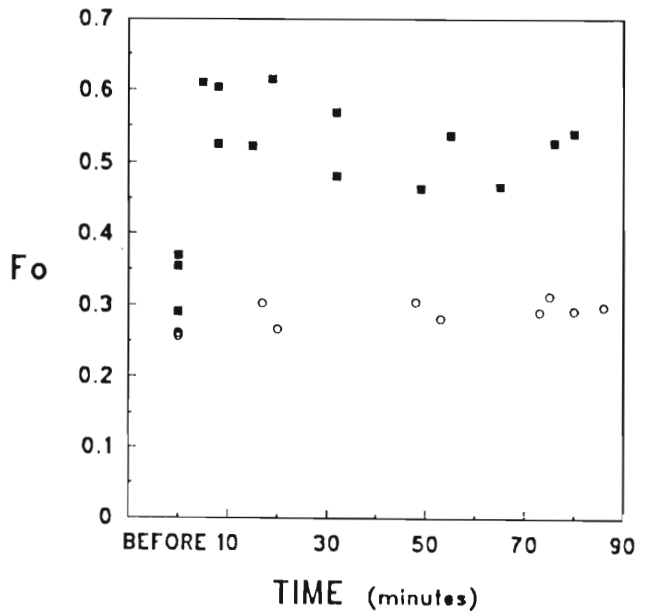


Figure 3.4.5 Effect of feeding glycolate (10 mM), via the transpiration stream, on the Fm of non-photoinhibited (o) and photoinhibited (■) leaves. Fm measured before the photoinhibitory treatment is indicated at time 0. (N=3)



re 3.4.6 Effect of feeding phosphoglycerate (10 mM), via the transpiration stream, on the Fo of non-photoinhibited (o) and photoinhibited (■) leaves. Fo measured before the photoinhibitory treatment is indicated at time 0. (N=3)

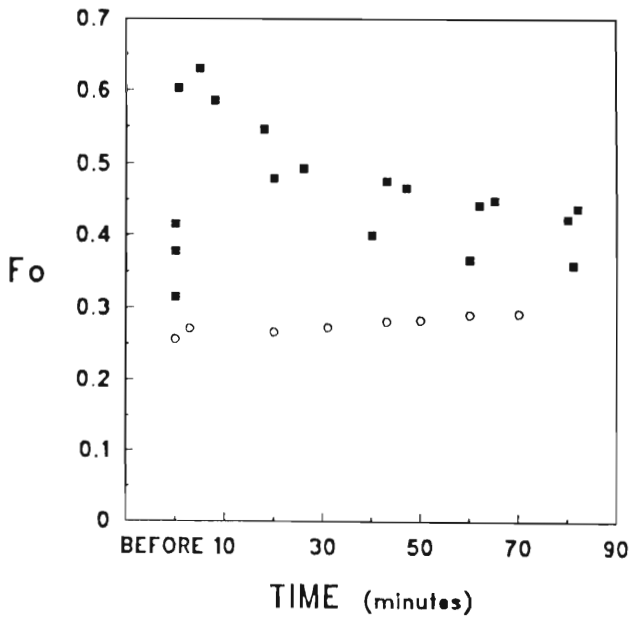
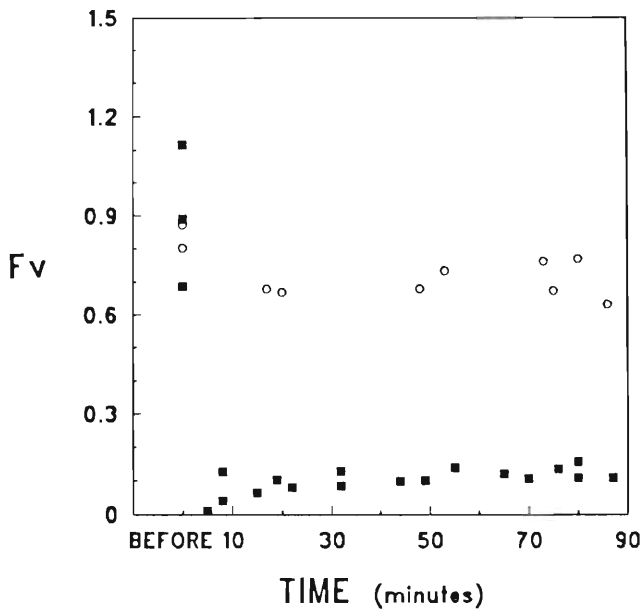


Figure 3.4.7 Effect of feeding glycolate (10 mM), via the transpiration stream, on the Fo of non-photoinhibited (o) and photoinhibited (■) leaves. Fo measured before the photoinhibitory treatmen is indicated at time 0. (N=3)



gure 3.4.8 Effect of feeding phosphoglycerate (10 mM), via the transpiration stream, on the Fv of non-photoinhibited (o) and photoinhibited (■) leaves. Fv measured before the photoinhibitory treatment is indicated at time 0. (N=3)

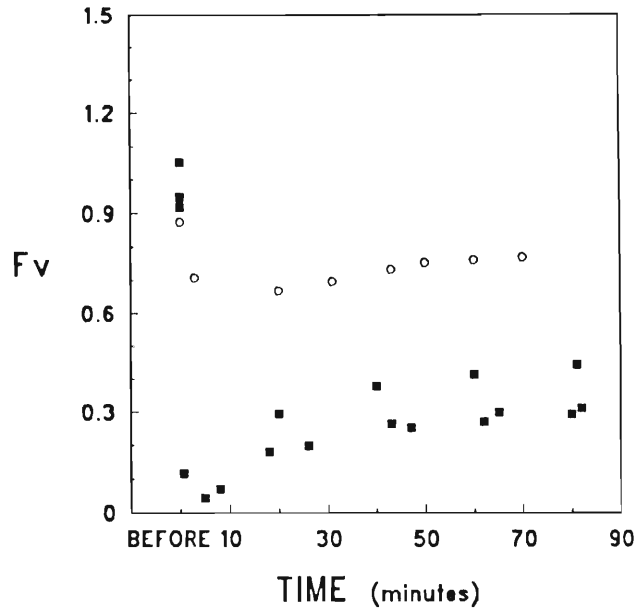
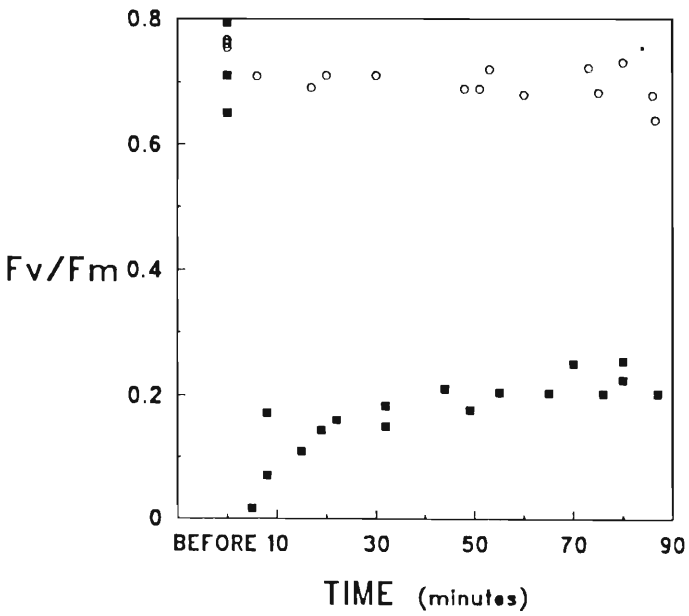


Figure 3.4.9 Effect of feeding glycolate (10 mM), via the transpiration stream, on the Fv of non-photoinhibited (o) and photoinhibited (■) leaves. Fv measured before the photoinhibitory treatmen is indicated at time 0. (N=3)



igure 3.4.10 Effect of feeding phosphoglycerate (10 mM), via the transpiration stream, on the Fv/Fm ratio of non-photoinhibited (o) and photoinhibited (■) leaves. Fv/Fm measured before the photoinhibitory treatment is indicated at time 0. (N=3)

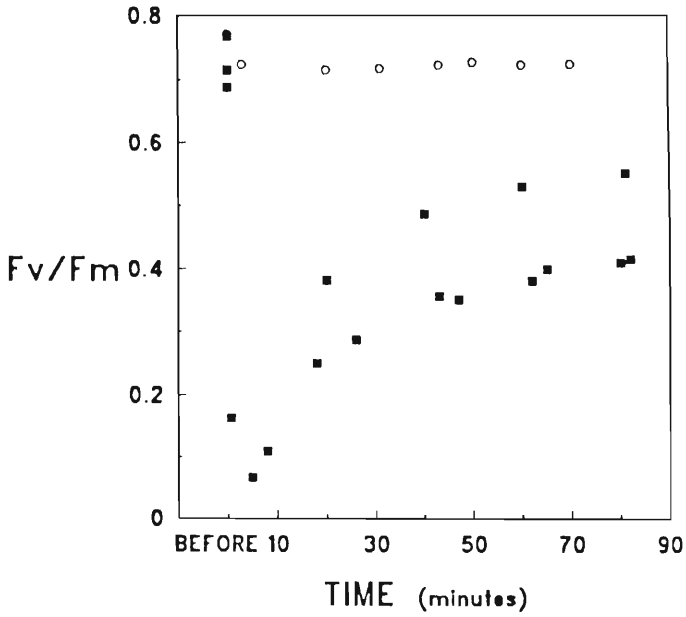


Figure 3.4.11 Effect of feeding glycolate (10 mM), via the transpiration stream, on the Fv/Fm ratio of non-photoinhibited (o) and photoinhibited (■) leaves. Fv/Fm measured before the photoinhibitory treatment is indicated at time 0. (N=3)

uniform stomatal response (Sharkey and Seeman, 1987). In C_4 plants, because the K_m of the primary CO_2 fixing enzyme is lower than that of Rubisco, the initial slope of the A:c_i curve is much steeper. It is not known which processes limit carbon assimilation at CO_2 saturation in C_4 plants, although it is likely to be some component of carbon cycling. Photoinhibitory treatment of maize decreased both the initial slope and CO_2 saturation level of the A:c_i curve. On returning the plants to a normal PPFD (550 mol m^{-2} s^{-1}) there was a fairly rapid recovery of the initial slope, but by 80 minutes the CO_2 saturated rate had not completely recovered (Figure 3.5.1). A:c_i curves constructed 20, 40 and 80 minutes after plants were returned to ambient PPFD were significantly different from curves constructed from control leaves (Table 3.5.1). The recovery of the light reactions could account for the partial recovery of the CO_2 saturation rate over time as the carbon cycling processes in C_4, as in C_3 photosynthesis, are energy dependent.

TIME OF RECOVERY (MINS)	SIGNIFICANT DIFFFERENCE BETWEEN TREATMENT LEAVES AND LEAVES INHIBITED WITH 0 % O_2 AND CO_2
20	P< 0.001
40	P< 0.001
60	P< 0.001

Table 3.5.1 Significant differences in A:c_i curves from non-photoinhibited leaves and from inhibited leaves after various recovery times.

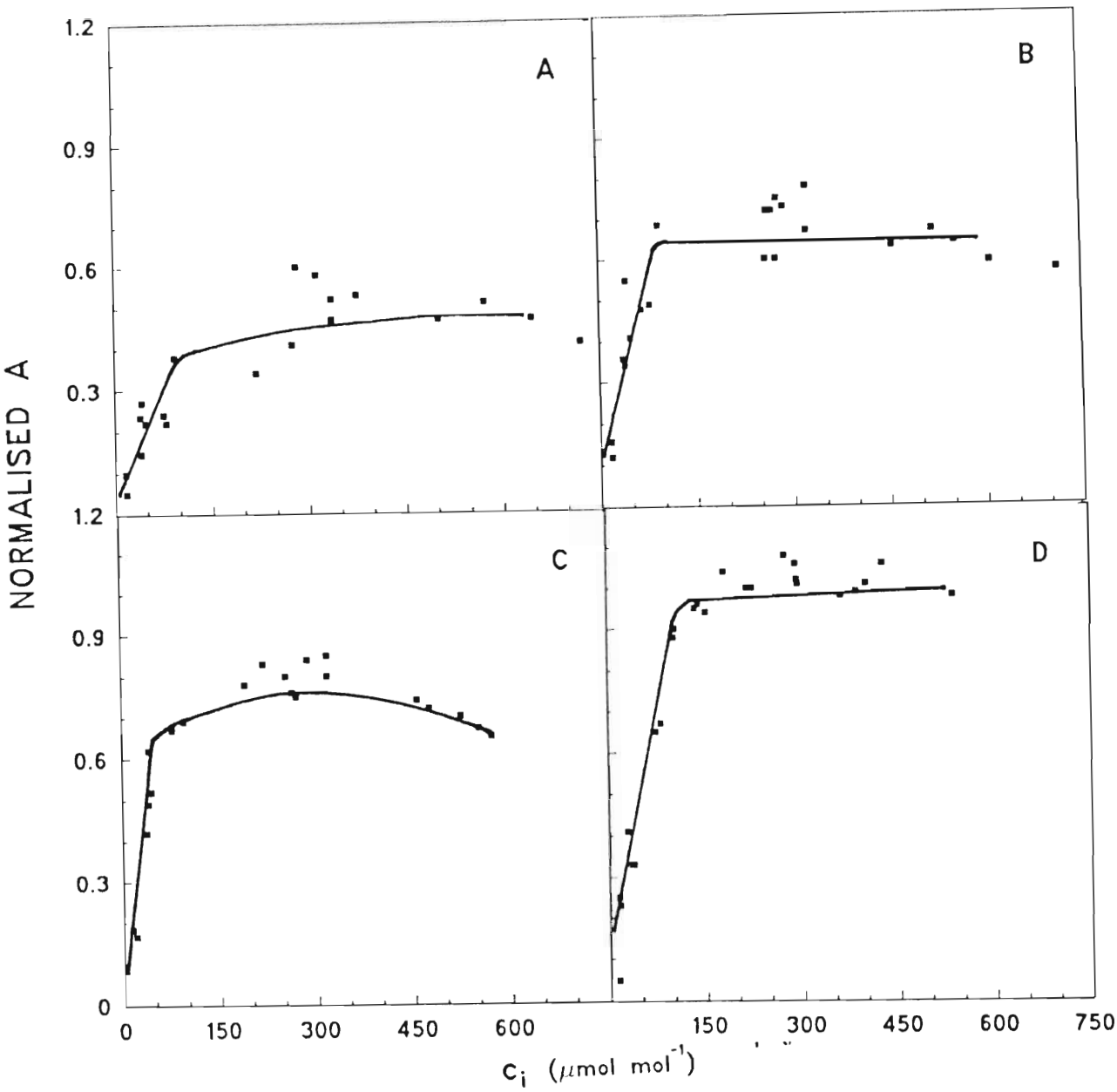


Figure 3.5.1 Effect of increasing internal CO_2 concentration (c_i) on the normalised CO_2 assimilation rate 20 (A), 40 (B) and 80 (C) minutes after leaves were returned to ambient PPFD. Control CO_2 response curves were constructed from non-photoinhibited leaves (D).

(4) DISCUSSION

Photoinhibition is a complex phenomenon involving many processes including irreversible damage, reversible processes, protective mechanisms and repair processes. This final chapter attempts to interrelate these processes with the findings of this investigation to give an insight into the phenomenon of photoinhibition with special reference to C_4 plants.

The second leaves of nine day old maize seedlings showed a decreased capacity for CO_2 fixation and PSII electron transport when exposed to high light intensities. This photoinhibition was exacerbated when O_2 and CO_2 were removed during the high light treatment, a phenomenon that has been noted in many other plant species, isolated chloroplasts and unicellular algae (Powles, 1984; Powles, Cornic and Louason, 1984).

It has been proposed that under conditions of reduced ambient CO_2 or O_2, carbon metabolism and the utilization of reductant is restricted (Osmond,1981; Chatervedi and Nielsen, 1987). The electron transport chain becomes fully reduced leading to configurational alterations to PSII (Powles, 1984). Such alterations include both irreversible damage and reversible changes to the photosystems (Greer, 1988; Critchley, 1988). Protection against photoinihibition by the reversible components appears to be limited. This is supported by the finding that when leaves were exposed to inhibitory treatments in excess of one hour in the absence of CO_2 and O_2, the CO_2 assimilation was still completely inhibited after 80 minutes of recovery (Figure 3.1.5). Similar results have been demonstrated in other species (Powles and Osmond, 1979; Powles and Thorne, 1981). Although reversible photoinhibition is viewed by many workers (Krause, 1988; Demmig, Winter, Kruger and Czygan, 1987) as a protective mechanism, several mechanisms have been proposed which may act to prevent or reduce photoinihibition (Powles and Osmond, 1979; Powles, Chapman and Osmond, 1980).

Under normal conditions the major path of carbon flow, and the sink of reductant, is the Benson-Calvin cycle in C_3 plants and the integrated carbon pathway in C_4 plants. The concentration of CO_2 required by carbon metabolism to prevent photoinhibition is remarkably low. Concentrations of ambient CO_2 concentrations of 36 or 49 μmol mol^{-1} markedly reduced the photoinhibition of maize leaves (Figure 3.3.2). CO_2 at or approximating the CO_2 compensation point has been shown to

prevent photoinhibition in other species (Powles and Osmond, 1979; Powles, Chapman and Osmond, 1980; Powles, Cornic and Louasson, 1984) and in isolated chloroplasts (Cornic, Woo and Osmond, 1982). In view of the low concentration required to prevent photoinihibition it is unlikely that the quantitative utilization of reductant protects against inhibition (Krause and Cornic, 1987) and it may be the cycling of carbon that offers the protection.

Supplying C_3 spinach chloroplasts with intermediates of the Benson-Calvin cycle has been shown to prevent photoinhibition (Cornic, Woo and Osmond, 1982). These workers found that of all the Benson-Calvin cycle intermediates supplied, PGA offered the most protection. In the present study PGA fed via the transpiration stream exacerbated the photoinhibition of PSII (Figure 3.4.8) and inhibited CO_2 assimilation of non-photoinhibited leaves (Figure 3.4.1). This result may be explained in terms of the C_4 pathway. In NADP-malic enzyme species the Benson-Calvin cycle is restricted to the bundle sheath cells which produce little reductant. The dependence of maize bundle sheath cells on mesophyll cells for reductant was demonstrated by Farineau (1975) who showed that the CO_2 assimilation of bundle sheath cells fed PGA was decreased and that this decrease was due to an insufficient supply of reductant which is required for RuBP regeneration. NADPH and CO_2 are imported into the bundle sheath cells via the decarboxylation of malate. The production of malate from hydroxy-pyruvate requires CO_2 and hence the shuttle of CO_2 and reductant to the bundle sheath cells may only operate in the presence of CO_2. As maize leaves were inhibited in the absence of both O_2 and CO_2 this shuttle would not have been operated and the Benson-Calvin cycle would have been deprived of both the substrate CO_2 and reductant. The intermediates were, however, fed to leaves in ambient air for a 30 to 45 minute period prior to the photoinhibitory treatment and are likely to have had boosted the carbon pools. As this resulted in no protection it is likely that the integrated carbon pathway is required to prevent photoinhibitory damage.

Supplying oxygen at various concentrations, to leaves during photoinhibitory treatments results in a more complex response in the extent of the photoinhibition of CO_2 assimilation than did supplying CO_2. This may have been because several "protective" systems are effected by ambient O_2 concentration. These include photorespiration (Powles, 1984), the Mehler reaction and associated glutathione reactions (anti-oxidant system)(Radmer and Kok, 1976) and non-radiative energy dissipation (Krause, 1988).

It has been proposed that photorespiration protects C_3 plants against photoinhibition (Powles and Osmond, 1979). Supplying O_2 to C_3 plants has been shown to have a marked effect on the extent of photoinhibition and the subsequent recovery (Nilsen, Chaturvedi and Dons, 1984; Powles, Cornic and Louasson, 1984; Krause, Koster and Wong, 1985). In contrast to this the photoinhibition of C_4 plants, which have low rates of photorespiration (Chollet and Ogren, 1975), were little effected by the O_2 concentration supplied during the inhibition (Brown and Morgan, 1980; Powles, Chapman and Osmond, 1980; Powles, Cornic and Louasson, 1984). In the above mentioned investigations photoinhibited C_4 leaves were allowed to recover to a saturated level before post-inhibitory measurements were made, hence the extent of the photoinhibition immediately following the inhibition was not recorded. In the present investigation it was found that the extent of the photoinhibition, measured immediately following the inhibition was effected by the concentration of O_2 supplied during the photoinhibition. The effect of O_2 concentration on the subsequent recovery was less marked (Figure 3.2.2). This was not an artifact of the short inhibitory treatment used as leaves supplied 21% O_2 during 0.5 or 2 hour inhibitory treatments were protected to the same extent (Figure 3.2.3).

The O_2 concentrations of 2% and 21% had the greatest and similar protective effects (Figure 3.2.2). The lack of difference in effect between the two O_2 concentrations suggests that photorespiration was not the system responsible for the protection. Photorespiration is thought to be inoperative, or much reduced, at O_2 concentrations as low as 2% (Krause and Cornic, 1987). Morot-Gaudry (1980) showed that the incorporation of ^{14}C from labeled CO_2 into the photorespiratory intermediates of maize leaves, although low, was reduced by 90% if leaves were transferred from 21% to 1% ambient oxygen.

Although C_4 species are capable of photorespiration (Volk and Jackson, 1972), the process is confined to the bundle sheath cells due to the location of Rubisco. Chollet and Ogren (1972) demonstrated that CO_2 assimilation of isolated bundle sheath cells, and not mesophyll cells, was inhibited by O_2. Several authors have suggested that in C_4 species the major sites of photoinhibition is the mesophyll chloroplasts (Powles, Chapman and Osmond, 1980). This may be because PSII has been shown to be the major site of photoinhibition (Powles, 1984; Krause, 1988) and mesophyll chloroplasts of NADP-malic species have a larger component of PSII than do bundle sheath chloroplasts (Powles, Chapman and Osmond, 1980; Li and Noth-

nagel, 1989). If bundle sheath photorespiration protected against photoinhibition, reductant and CO_2 would need to be shuttled to bundle sheath cells via malate decarboxylation. Malate production can occur only in the presence of CO_2 and as leaves in the experiments reported here were inhibited in the absence of CO_2, it is unlikely that the observed protection was due to photorespiration.

Zelitch (1966) found that the photorespiratory intermediate, glycolate, was metabolised when fed to maize leaf discs. Similarly in this study, it was found that labeled glycolate was metabolized when fed to maize leaves via the transpiration stream (Figure 3.4.3). Glycolate would be expected to boost the photorespiratory intermediates, increasing carbon flow and the potential to utilize reductant. Glycolate, however, offered no protection against photoinhibition (Figure 3.4.2); emphasizing that the observed protection was unlikely to have been due to photorespiration.

The anti-oxidant system is found in both cell types in C_4 species (Nakano and Edwards, 1987) and has the potential to offer protection against photoinhibition under conditions of reduced CO_2. The anti-oxidant system may be assessed by monitoring the pools sizes of reduced and oxidized glutathione by the technique of Smith (1985). This assay, however, proved to be ineffective as the colorimetric reaction was inhibited by maize leaf extract (results not shown). Krause and Cornic (1987) suggest that in view of the considerable protective effect noted when low O_2 (1%) is supplied to leaves during photoinhibition, it is unlikely that the anti-oxidant system could account for the quantitative energy utilization at such low O_2 concentrations and the system may function to poise electron transfer (Krause, Koster and Wong, 1985).

The enhanced photoinhibition noted at high O_2 concentrations (Figure 3.2.2) may be due to enhanced oxygen radical production, which is considered the main contributor to PSII oxidative damage (Asada and Takahashi, 1987). If oxygen radical production exceeds the capacity of the anti-oxidant system, superoxide dismutase, catalase and the carotenoids to metabolize radicals, damage is thought to ensue. Similarly, decreased assimilation rates have been recorded in bean leaves exposed to 100% O_2 for 30 minutes (Poskuta, Wroblewska and Mikulska, 1976). These workers showed that there was a conversion of Benson-Calvin cycle intermediates to photorespiratory intermediates which may have reduced the capacity of the Benson-Calvin cycle to assimilate CO_2 once the substrate was reintroduced into the system and this led to decreased assimilation rates. Mesophyll cells isolated from maize

showed a decreased capacity for CO_2 fixation at superatmospheric O_2 concentration and Chollet (1973) suggested that phosphoenol-pyruvate carboxylase (Pepcase) was directly inhibited by the oxygen. It is unlikely that either a conversion of Benson Calvin cycle intermediates or the inhibition of Pepcase occured in photoinhibited maize leaves, as both CO_2 assimilation and PSII efficiency were decreased. It is more likely that the observed damage was due to radical production, a result that supports the findings of Nakano and Asada (1987) who showed that photosynthetic O_2 evolution of *Euglena* cells exposed to 100% O_2 and high light were inhibited and attributed this to photooxidative damage.

Supplying CO_2 or O_2 to maize leaves during photoinhibitory treatments had similar effects on CO_2 assimilation and PSII photochemistry, and as the primary effect of photoinhibition is on PSII photochemistry (Powles, 1984), the response of CO_2 assimilation can be attributed to changes in electron transport. Interruption of the electron transport chain would effect the enzymes requiring reduction for activation, including NADP-malic dehydrogenase, pyruvate dikinase (Powles, 1984), as well as the energy dependent processes. RuBP regeneration has been shown to be the main factor limiting CO_2 assimilation following a photoinhibitory treatment (Krause and Cornic, 1987). The recovery of CO_2 assimilation observed in this investigation was shown to involve both an increase in the carboxylation efficiency and the level of substrate cycling - both of which may be attributed to the restoration of the light reactions and electron transport, increasing the energy production and activating enzymes (Figure 3.5.1). Those enzymes that are not activated by the light driven electron transport chain are not effected; suggesting that the enzymes are not directly effected by photoinhibition (Powles and Björkman, 1982).

Some differences in the response of PSII photochemisty and CO_2 assimilation to photoinhibition were observed. O_2 supplied at either 2% or 21% protected CO_2 assimilation to the same extent (Figures 3.2.2) but the efficiency of PSII of leaves supplied 21% was reduced in relation to leaves supplied 2% O_2 (Figure 3.2.7). This disparity in effect may well be because the capacity of electron transport exceeds the capacity for CO_2 assimilation (Krause and Cornic, 1987) and hence electron transport may be reduced to a certain extent without affecting CO_2 assimilation.

Photorespiration appears not to be an important protective mechanism in C_4 species (Powles, Cornic and Louasson, 1980). The role of the anti-oxidant system in photoinhibition is as yet unclear but is unlikely to be one of quantitative energy

utilization (Krause and Cornic, 1987). Observed changes in the PSII chlorophyll fluorescence suggest that the observed protective effects were due to changes in the light reactions. Observed changes in chlorophyll fluorescence were typical of photoinihibited leaves (Powles, 1984) and included decreased maximum and variable fluorescence yields such that F_v/F_m was decreased, indicating a reduction in PSII efficiency. The observed changes in PSII chlorophyll fluorescence of leaves supplied CO_2 or O_2 were, however, fundamentally different.

When CO_2 was supplied at concentrations less than 50 μmol mol^{-1} (in the absence of O_2) there was a marked decrease in PSII efficiency and this decrease was due mainly to a large increase in F_o (Figures 3.3.3 - 3.3.6). Large increases in F_o values of leaves inhibited in the absence of O_2 are well documented (Krause, Koster and Wong, 1985; Krause and Cornic, 1987). Similar increases in F_o have also been observed in leaves photoinhibited at low temperatures (Greer, Kipnis and Laing, 1988). Krause and Weis (1984) have attributed increases in F_o to photoinhibitory damage likely to involve the partial detachment of antennae pigments from PSII reaction centres, or to the destruction of the centres themselves, resulting in a decrease in the rate constant for photochemistry (K_P). The observed increases in F_o were reversible and after 80 minutes were within 20% of pre-inhibitory values (Figure 3.3.4). The recovery of F_o, and hence K_P, resulted in the increase in both PSII efficiency (Figure 3.2.7) and CO_2 assimilation (Figure 3.2.2). Greer, Berry and Björkman (1985) observed similar recovery of F_o, F_m and the photon yield once bean leaves were removed from photoinhibitory conditions. The result of which was the recovery of primary photochemistry.

The increase in F_o was suppressed only once CO_2 concentrations were increased such that the photoinhibition was substantially reduced. The mechanism by which F_o increases during photoinhibition and declines during recovery is not known, although *de novo* chloroplastic synthesis of the Q_B protein has been shown to be necessary for the recovery from photoinihibition (Ohad, Kyle and Arntzen, 1984). The recovery process has also been shown to require light (Greer, Berry and Björkman, 1986) suggesting that recovery requires energy. This may account for both glycolate and PGA fed leaves showing little recovery from photoinhibition as the metabolism of these intermediates would have created a considerable energy sink.

The m-RNA necessary for the synthesis of the Q_B protein is abundant and the

protein has a high turnover even under normal conditions (Ohad, Kyle and Arntzen, 1984; Kyle and Ohad, 1986). Due to the abundance of this particular m-RNA, the synthesis of Q_B can be initiated rapidly and this process may occur within 60 minutes, the time period in which the recovery of PSII photochemistry was observed.

The photoinhibitory treatment increased K_D only slightly and this increase was independent of the CO_2 concentration supplied during the inhibition (Figure 3.3.7). It appears that O_2 is necessary for the mechanism by which K_D increases. The recovery of both CO_2 assimilation and PSII efficiency, although not complete, were approximately the same irrespective of the CO_2 concentration supplied during the photoinhibition (Figure 3.2.2). On the other hand, the recovery of plants supplied O_2 was dependent on the O_2 concentration supplied during the inhibition. This suggests that when O_2 is removed irreversible inhibition is reduced because the potential for oxidative damage is removed.

The decrease in fluorescence yield of leaves supplied various concentrations of O_2 was due to a decrease in the maximum fluorescence rather than due to an increase in F_o (Figure 3.2.4 - 3.4.7). The F_o value was little effected by the photoinhibition or by the concentration of O_2 supplied during the inhibition (Figure 3.2.5). Hayden, Baker, Percival and Beckwith (1986) found similar changes in F_o, F_m and F_v/F_m ratio of stress treated maize mesophyll thylakoid membranes and were able to demonstrate that these changes were due to an interruption of the energy transfer from LHCII to PSII. Björkman (1987) demonstrated that such fluorescence changes are characteristic of an increase in non-radiative energy dissipation (K_D) and Havaux (1989), using a photoacoustic technique, confirmed that the decrease in fluorescence yield was associated with the dissipation of energy as heat. The observed increase in K_D of photoinhibited maize leaves was strongly dependent on the O_2 concentration supplied during the inhibition (Figure 3.2.8). Interestingly, the O_2 concentration (2%) that offered the most protection against photoinhibition resulted in the least increase in K_D. Photoinhibition at this concentration was markedly reduced by some unidentified mechanism.

Demmig and Winter (1988) have identified three components of non-radiative energy dissipation, one of which was shown to relax within 10 to 30 seconds upon darkening (Demmig and Winter, 1988). As maize leaves in this investigation were incubated for five minutes in complete darkness before chlorophyll fluorescence

measurements were made, would have allowed this rapidly reversible component to have relaxed, and it would thus have been unrecorded. It may have been this mechanism which offered the protection during the photoinhibtion, particularly at the low O_2 concentrations. This requires further investigation. Why such a mechanism should be more prevalent at low O_2 concentrations but not in the absence of O_2, is as yet inexplicable. It is possible that as O_2 is increased the longer term quenching mechanisms are favoured.

It has been suggested that under conditions of limiting CO_2 or in the absence of CO_2, O_2 may act as the terminal electron acceptor (Mehler reaction). Rather than functioning primarily as an energy dissipating mechanism, the anti-oxidant system may allow the formation of the trans-thylakoidal pH gradient which has been found to be associated with non-radiative energy dissipation (Krause and Cornic, 1987). The pH gradient is thought to result in ultrastructural changes of the photosystems (Krause and Cornic, 1987). The activities of several of the enzymes involved in the anti-oxidant system have been shown to increase when leaves are incubated at supra-atmospheric O_2 concentrations ($F_o$ster and Hess, 1980; Nakano and Edwards, 1987) suggesting that this pathway is favoured at higher O_2 concentrations. This implies that higher O_2 concentrations should be more effective in maintaining the trans-thylakoidal pH gradient and consequently increasing non-radiative energy dissipation. However, this is not necessarily so as the Mehler reaction has a high affinity for O_2 (Asada and Takahashi, 1987) and an optimal pH gradient may be established at low O_2 concentrations. Results of this investigation indicated that the observed protection decreased with increasing O_2 concentration and non-radiative energy dissipation processes with longer relaxation kinetics became more important. It is quite feasible that the quenching associated with the pH gradient offered protection at low O_2 concentrations but as the O_2 concentration was increased the production of damaging oxygen species exceeded the protective function. Krause and Cornic (1987) consider the Mehler reaction to have three possible roles in photoinhibition and these include the utilization of reductant and a poising of cyclic electron transfer, the build-up of a high pH gradient, and the production of potentially damaging oxygen species. The effect of ambient O_2 concentration on these processes is not known and it is possible that increasing O_2 concentration may affect these mechanisms in a dissimilar manner.

Photochemical energy dissipation (K_P) of leaves supplied O_2 was also affected by the photoinhibition but the extent of the decrease in K_P was not as strongly depen-

dent on the O_2 concentration as was the case for K_D. Hence the decrease in the PSII efficiency of leaves inhibited in the presence of O_2 can be attributed mainly to an increase in K_D rather than a decrease in K_P. The rate constant K_P decreased and recovered little once leaves were returned to ambient conditions (Figure 3.2.9) showing that the photoinhibition involved both reversible "protective" mechanisms and damage of PSII photochemistry.

(5) CONCLUSIONS

The hypothesis that photoinhibition occurs when reductant production exceeds its utilization and that photoinhibition could be prevented by supplying low concentrations of CO_2 was supported by this investigation. The protective effect of the low O_2 concentration suggests that quantitative energy utilization was not important and that the maintenance of carbon flow may have offered the protection.

Although it has been shown that energy utilization by the Benson-Calvin cycle prevents photoinhibition in C_3 plants, in C_4 plants it appears that integrated carbon metabolism is required for protection.

Photorespiration appears to be an unimportant protective mechanisms in C_4 species. The maximal protection offered by 2% O_2 could not be attributed to an increase in the rate constant for non-radiative energy dissipation. The five minute dark incubation of leaves prior to fluorescence measurements does mean that the changes in the fast relaxing component of non-radiative energy dissipation would not have been recorded. Although the possibility exists that this mechanism offered the observed protection, other unknown processes may have been involved.

The role of the anti-oxidant system could not be assessed but the observed protection at low O_2 suggests that it is unlikely to be due to a quantitative energy utilization and may involve the poising of the electron transport reactions and the establishment of a trans-thylakoidal pH gradient. The damage observed at higher O_2 concentrations was probably due to excessive oxygen radical production.

The mechanisms of non-radiative energy dissipation are largely unknown but are likely to involve ultrastructural changes. The zeaxanthin cycle, Q_B inactivation and a high trans-thylakoidal pH gradient have all been implicated in photoinhibition and may be responsible for the ultrastructural changes. Most of these mechanisms would be effected by O_2 concentration which may account for the observed differences in K_D at the various O_2 concentrations supplied to maize leaves.

The photoinhibition of leaves in the presence and absence of O_2 was due to different mechanisms as charaterised by fluorescence measurements. Irrespective of whether O_2 or CO_2 were supplied during the inhibition, a certain component of the inhibition was reversible within 80 minutes, and this component decreased particu-

larly at high O_2 concentrations, where a larger component of the photoinhibition remained irreversible. The irreversible component was probably due to photo-oxidative damage. Photoinhibition is a complicated phenomenon that involves protective mechanisms, irreversible inhibition and photoinhibitory damage. Presumably when the capacity of the protective systems and reversible components are exceeded, more permanent damage results.

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