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CHLOROPHYLL FLUORESCENCE PROBE OF ULTRAVIOLET-B PHOTOINHIBITION
OF PRIMARY PHOTOREACTIONS IN INTACT LEAVES

by

Robert S. Nowak

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Range Ecology

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1980

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Robert S. Nowak

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ABSTRACT

Chlorophyll Fluorescence Probe of Ultraviolet-B Photoinhibition
of Primary Photoreactions in Intact Leaves

by

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Utah State University, 1980

Major Professor: Martyn M. Caldwell
Department: Range Science

Damage to primary photosynthetic reactions caused by environmental stress can be assessed by measurement of chlorophyll fluorescence induction in intact leaves. This approach was applied in studies of ultraviolet-B photoinhibition of photosynthesis in Pisum sativum L. and Rumex patientia L. leaves. At ultraviolet-B dose rates insufficient to cause inhibition of net photosynthesis, changes in the magnitude of fluorescence transients did occur, which suggested direct effects on chloroplast pigments in Pisum and inhibition of photosynthetic electron transport between the photosystems in both species. Leaves of these two species subjected to a much higher dose rate had a significant reduction of net photosynthesis and changes in the magnitude of fluorescence transients that indicated partial loss of water-splitting capability and direct effects on chloroplast pigments. Ultraviolet radiation-induced changes of photosynthetic thylakoid membranes may be ultimately responsible for these disruptions of the primary photosynthetic reactions. (41 pages)

INTRODUCTION

Damage to primary photosynthetic reactions caused by environmental stress can be assessed by measurement of chlorophyll fluorescence induction (Kautsky effect) in intact leaves (Schreiber et al. 1978, Wiltens et al. 1978). Ultraviolet (UV) irradiation of higher plants can affect several morphological and physiological features of sensitive plants, including photosynthesis (Caldwell 1971, 1977). Sisson and Caldwell (1976) reported decreases in net photosynthetic rates after as little as two hours of UV-B (280-320 nm) irradiation. This immediate photosynthetic damage suggests a need to examine the nature of UV photoinhibition of primary photosynthetic reactions. Previous research with isolated chloroplasts has indicated several sites of UV photoinhibition of photosynthesis, including the thylakoid membrane and photosystem II (PSII) photoreactions. The primary site of UV photoinhibition of photosynthesis in intact leaves is not known. Measurement of chlorophyll fluorescence induction at physiological temperatures has been used in this study to examine the effects of UV-B radiation on the primary photosynthetic reactions in intact leaves.

Not all of the light energy absorbed by a leaf is used to fix CO_2 . Absorbed light energy is distributed into photosynthetic electron transport through the photosystems to the Calvin cycle, heat dissipation, chlorophyll a (chl a) fluorescence, and transfer of the excitation energy between chlorophylls. Exciton transfer between light harvesting chlorophyll (chl LH) and P680, the reaction center chlorophyll of PS II, is tightly coupled and reversible.

However, energy transfer from P680 and chl LH to P700, the reaction center chlorophyll of photosystem I (PSI), is not reversible (Butler 1978).

In the PSII photoreaction, light energy absorbed by the antennae chlorophyll is transferred to P680, which causes a charge separation oxidizing P680 and reducing the primary electron acceptor for PSII, Q (Amesz and Duysens 1977). A second electron acceptor, R, is thought to interact with Q, mediating electron transfer from P680 to the intersystem electron carrier pool, A, in pairs of electrons (Sauer 1979). The paired electrons travel through A to P700, where they ultimately are used to reduce the primary electron acceptor for PSI, X (Golbeck et al. 1977). The Calvin cycle is coupled with X through NADP and other intermediates (see Appendix).

Oxidized P680 from the PSII photoreaction is reduced by electrons from the primary electron donor, Z, and undergoes four photo-induced oxidations before water is split. In dark-incubated chloroplasts, S_n is in the ground and lowest oxidized states, S_0 and S_1 . During continuous illumination, S_n undergoes a gradual redistribution to the ground and three lower oxidized states, S_0 , S_1 , S_2 , and S_3 . The highest oxidized state, S_4 , does not accumulate since it is thought to rapidly react with water, which releases a pair of electrons, and returns to the ground state (Kok and Velthuys 1977, Radmer and Cheniae 1977).

At physiological temperatures, fluorescence from higher plant chloroplasts emanates predominantly from chl a678 of PSII with a main band width maximum at 685 nm. Chlorophylls associated with PSI

fluoresce only very weakly, and their fluorescence does not appear to be affected by the photochemical activity of P700 (Papageorgiou 1975). Other pigments of photosynthetic importance as well as the reaction center chromophores do not have measureable fluorescence (Govindjee and Papageorgiou 1971, Butler 1978). Dark-adapted plants exhibit a characteristic, biphasic induction of chlorophyll fluorescence known as the Kautsky effect (Fig. 1). Upon illumination, fluorescence from a dark-adapted plant rises instantly to an initial level, O. Fluorescence rapidly increases to an intermediate level, I, and then to a maximum, P. Fluorescence rapidly decreases to a quasi-steady state, S, then slowly rises to a plateau, M, before slowly decreasing to a terminal level, T. This biphasic induction is distinguished into a fast change (O-I-P-S) and a slow change (S-M-T) (Wassink 1951, Rabinowitch 1956), which occur in a few seconds and a few minutes, respectively. The Kautsky effect reflects the change of the photosynthetic apparatus from a dark-adapted state, where photosystem and intersystem components are in their ground states, to a light-adapted state, where the photosynthetic apparatus is in an active, steady state. Further changes can occur in the terminal (T) transient with long, continued irradiation (Papageorgiou 1975).

By studying the changes in fluorescence transients during UV irradiation, changes in the primary photosynthetic reactions induced by UV radiation may be elucidated. This technique has been used in studies with other stresses. For example, Schreiber et al. (1978) examined ozone injury to the primary photosynthetic reactions.

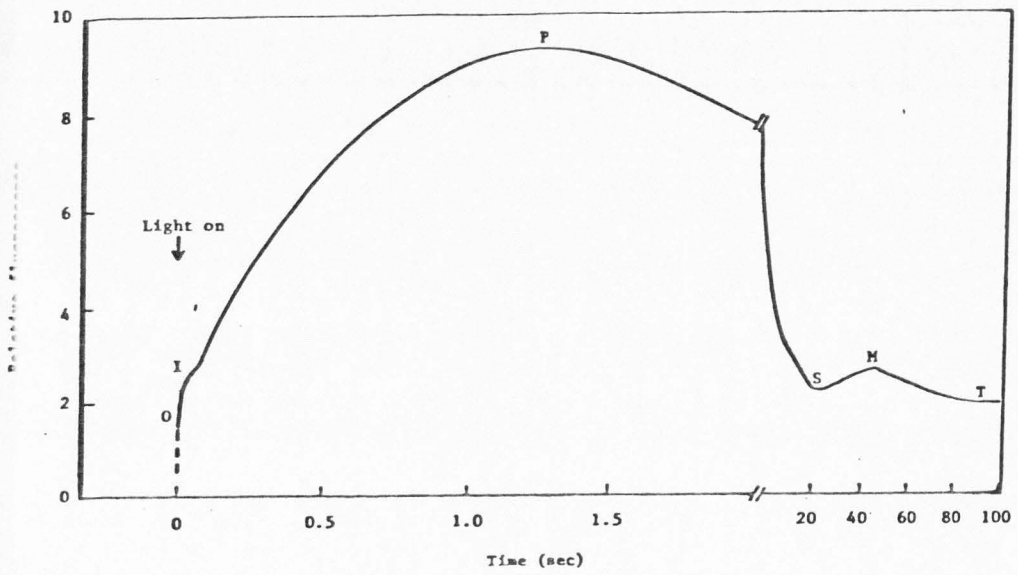


Figure 1. Schematic of the biphasic induction of chlorophyll fluorescence (Kautsky effect) for a dark-adapted higher plant leaf.

Through interpretations of changes in the fluorescence transients, they concluded that water-splitting capacity is the first process affected by ozone treatment, followed by a decrease in electron transport between the photosystems. Thus, measurements of relative chlorophyll fluorescence from intact leaves may be useful in probing changes in the primary photosynthetic reactions during exposure to UV radiation.

This study examined the effects of UV-B irradiation on the primary photosynthetic reactions of intact leaves through interpretations of changes in fluorescence transients. At doses insufficient to affect net photosynthetic rates, UV-B irradiation affected photosynthetic electron transport. However, at higher UV-B dose rates, net photosynthetic rates were decreased significantly. In addition, a decrease of water-splitting capacity and direct effects on the PSII reaction center were observed.

METHODS

Pisum sativum L. cv. Early Alaska and Rumex patientia L. were grown from seed in growth chambers in 600 cm³ of a sterilized 3:2 mixture of potting soil to sand. Both before and during supplemental UV-B irradiation, environmental conditions within the growth chamber were maintained at a constant 25°C air temperature and a 16-hour photoperiod of 500 μ Einsteins \cdot m⁻² \cdot s⁻¹ photosynthetically active radiation (400-700 nm) (PAR), measured with a Lambda Instruments Co. LI-190 quantum sensor. The chamber light source was a 6000-W xenon arc filtered by 2-mm Schott Co. WG-320 glass filters (Fig. 2).

Four Westinghouse Co. FS-40 Sunlamps were used for supplemental UV-B irradiation during the middle eight hours of the photoperiod, and two UV-B dose rates were used. For the lower UV-B dose rate, plants were placed under either a 13-mm cellulose acetate film or a 13-mm "Mylar D" film (DuPont Corp.) supported by a semi-cylindrical frame, except for the Rumex L3 experiment. Plants used in the Rumex L3 experiment were grown under 13-mm "LLumar" film (Martin Processing, Inc.), which absorbs all UV radiation, and the control leaves were placed under a LLumar film rather than a Mylar D film. Our experiments were primarily designed to examine UV-B photoinhibition of photosynthesis, since only irradiance in this portion of the solar spectrum would be increased if the stratospheric ozone layer was depleted. However, there is evidence that UV-A (320-400 nm) irradiance may also inhibit photosynthesis (Jones and Kok 1966a), and the Rumex L3 experiment was conducted to determine if there is a significant contribution of UV-A irradiance to photoinhibition of

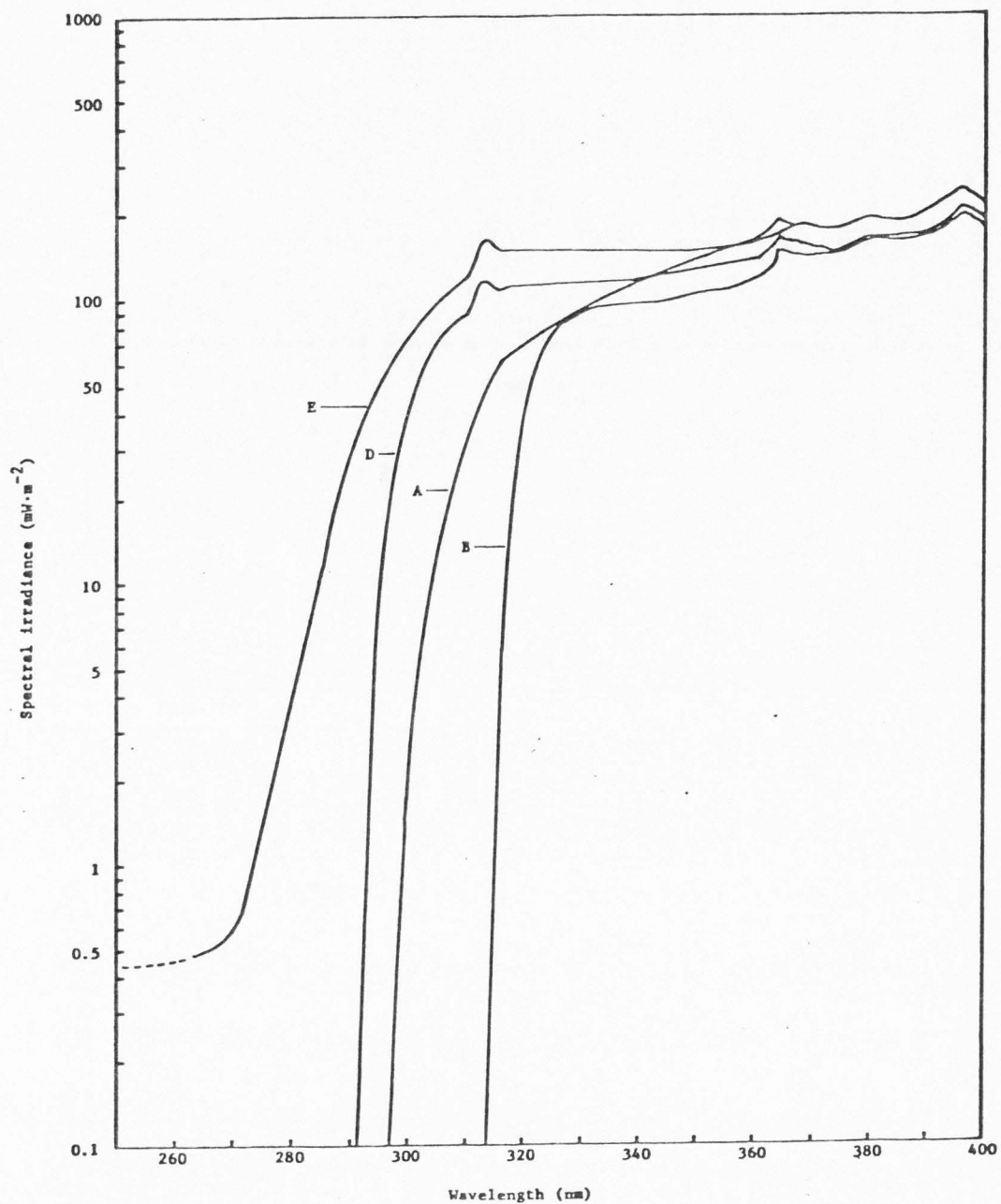


Figure 2. Spectral UV irradiance in growth chambers for the different lamp/filter systems. Letters correspond to the lamp/filter systems in Table 1.

photosynthesis. Spectral UV irradiance under each lamp/filter system is shown in Fig. 2. Since cellulose acetate solarizes, cellulose acetate films were replaced every third day. A single leaf or leaflet pair from each plant were held parallel to the lamps by monofilament nylon. Seven or eight replicates were involved in each experiment, and were exposed to supplemental UV-B irradiance for eight to fourteen days. Leaves were approximately the same age within any experiment, although leaves in the Pisum L2 experiment were about one week younger than the leaves in the Pisum L1 experiment at the start of supplemental UV-B irradiation. Plants were rotated within the chamber because the UV-B and visible radiation fields were not completely homogeneous within the growth chambers. For the higher UV-B dose rate, leaves held parallel to the FS-40 Sunlamps were exposed to unfiltered UV radiation from the sunlamps during the middle eight hours of a single photoperiod (Fig. 2). For these experiments, four to eight uniform leaves were selected, and measurements of net photosynthesis and fluorescence before and after supplemental UV-B irradiation were compared for each leaf. Two of the higher dose rate experiments, Pisum H3 and Rumex H4, were conducted without any visible irradiance during supplemental UV-B irradiation.

Spectral UV irradiance was measured with a modified Gamma Scientific Corp. grating spectroradiometer (Model 2900 Photometer and Model 700-31 Monochrometer). Because plant responses to UV irradiation are usually very wavelength-specific, total UV irradiance may not be biologically meaningful. For comparative purposes, UV spectral irradiance was weighted with two biological action spectra. The gen-

eralized plant action spectrum (Caldwell 1971) was used for one biologically effective UV-B integration ($UV-B_{BE}$). The action spectrum from Jones and Kok (1966a) for non-substrate-limited photoinhibition of isolated spinach chloroplast reactions was used to calculate an integrated UV-B irradiance which relates specifically to photoinhibition of photosynthesis (UV_{PSN}). Plant sensitivity increases exponentially in both action spectra with decreasing wavelength. There are large numerical differences between daily integrated UV irradiance under any one lamp/filter system using the different weighting functions. These differences are partly a result of the action spectra characteristics, but are largely due to the arbitrary normalization of these spectra at different wavelengths (280 nm for $UV-B_{BE}$, 250 nm for UV_{PSN}). Therefore, only comparisons among lamp/filter systems using a single weighting function are meaningful. Although neither action spectrum may be appropriate for UV photoinhibition of photosynthesis in intact leaves, they provide a weighted comparison between control and supplemental UV treatments. Average, weighted UV flux densities are given in Table 1. The total UV irradiance varied with 15% of the average values because of filter aging and a nonhomogeneous radiation environment. Pretreatment growth conditions correspond with "A" except for the Rumex L3 experiment which was "C". Control leaves for the lower UV-B dose rate experiments were exposed to the "B" dose rate, except for the Rumex L3 experiment which was "C". Supplemental UV-B radiation for the lower UV-B dose rate experiments correspond to "D", and for the higher UV-B dose rate experiments, "E".

Table 1. Daily integrated irradiance for the lamp/filter systems. Unweighted integrated irradiance, irradiance weighted with the generalized plant action spectrum of Caldwell (1971) (UV_{BE}), and irradiance weighted using the action spectrum of chloroplast reaction photoinhibition of Jones and Kok (1966a) (UV_{PSN}) are listed for each lamp/filter system. A dashed line indicates that the weighting function does not apply in this waveband.

Irradiating regime	Daily integrated irradiance ($\text{kJ}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)				Weighting function	Lamp/filter system
	UV-A	UV-B	UV-C	Total UV		
A	633	32	0	665	unweighted	6000-W xenon arc with 2-mm Schott Co. WG-320 glass filters.
	----	0.72	----	0.72	UV_{BE}	
	81	14	0	95	UV_{PSN}	
B	507	10	0	517	unweighted	6000-W xenon arc with 2-mm Schott Co. WG-320 glass filters and four FS-40 lamps. Plant bed filtered by Mylar D film.
	----	0	----	0	UV_{BE}	
	63	1	0	64	UV_{PSN}	
C	0	0	0	0	unweighted	6000-W xenon arc with 2-mm Schott Co. WG-320 glass filters and four FS-40 lamps. Plant bed filtered by LLumar film.
	----	0	----	0	UV_{BE}	
	0	0	0	0	UV_{PSN}	
D	546	57	0	603	unweighted	6000-W xenon arc with 2-mm Schott Co. WG-320 glass filters and four FS-40 lamps. Plant bed filtered by cellulose acetate film.
	----	3.22	----	3.22	UV_{BE}	
	72	26	0	98	UV_{PSN}	
E	669	99	1	769	unweighted	6000-W xenon arc with 2-mm Schott Co. WG-320 glass filters and four FS-40 lamps. Plant bed unfiltered.
	----	10.16	----	10.16	UV_{BE}	
	92	49	1	142	UV_{PSN}	

Net photosynthetic rates were measured in an open gas exchange system using Beckman Co. 215 infrared gas analyzers. Water vapor flux was measured with Vaisala Co. thin film humidity sensors. Gas flow rates were controlled and measured with a pneumotachometer coupled with a Validyne Co. pressure transducer. Gas exchange measurements were conducted at $1000 \mu\text{Einstein} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR, which exceeded light saturation of photosynthesis, with leaf temperature between 21° and 23°C , measured with fine wire thermocouples inserted into the leaf. Net photosynthetic rates were corrected to the same intercellular CO_2 concentrations (Caldwell et al. 1977). Determinations of net photosynthetic rates were made periodically during the lower UV-B dose rate experiments, and before and after supplemental UV-B irradiance for the higher UV-B dose rate experiments. Measurements of relative chlorophyll fluorescence from intact leaves were made after a 60- to 90-minute dark incubation period following the end of the photoperiod with a leaf fluorescence probe manufactured by R. Brancker Res., Ltd., Ottawa, Canada (Plant Productivity Fluorometer, Model SF-10) which is based on the design of Schreiber et al. (1975). The fluorescence induction curve was measured at room temperature and was recorded on an oscillograph (Statos 1 Recorder, Varian Graphics Co.). Measurement of fluorescence induction was made both before and during supplemental UV-B irradiation in the lower UV-B dose rate experiments, and before and after supplemental UV-B irradiation in the higher UV-B dose rate experiments.

An analysis of variance and F test using a design for a split-plot in time experiment was used for analysis of the lower UV-B dose rate photosynthetic and fluorescence data. A significant ($p < 0.05$) treatment-day interaction was interpreted as indicating UV-B photo-inhibition. Duncan's multiple range test was used for planned comparisons of control and treatment means if the F test was significant, except in the Pisum L2 experiment. The treatment-day interaction of the I-P fluorescence transient in the Pisum L2 experiment was not significant, but Duncan's multiple range test was used to determine significant differences ($p < 0.05$) between the means. A paired t test was used to test for significant differences ($p < 0.05$) between means of photosynthetic rates and fluorescence transients in the higher UV-B dose rate experiments. Statistical tests were not done among experiments.

RESULTS

The instantaneous, initial O fluorescence level is insensitive to the photochemical processes which occur in photosynthesis, since it originates from the bulk chlorophyll of the photosystems before energy is trapped at the reaction center (Govindjee and Papageorgiou 1971, Papageorgiou 1975). For stressed plants, a decrease in the O transient relative to control plants indicates effects on chloroplast pigments, which can include direct effects on the PSII reaction center and chlorophyll destruction (Schreiber et al. 1978, Wiltens et al. 1978). The O transient in *Pisum* leaves exposed to the lower UV-B dose rate was depressed after 5 to 7 days (Fig. 3A), and the treatment-day interaction of the O transient was significant ($p < 0.05$). For the O fluorescence transient of *Rumex* leaves exposed to the lower UV-B dose rate, treatment-day interactions were not significant when compared with control leaves (Fig. 3B).

The magnitude of the O-I rise is determined by the relative abundance of reduced Q in redox equilibrium with A. During the O-I rise, Q is photoreduced by PSII (Wiltens et al. 1978). At I, fluorescence levels slightly due to a rapid reoxidation of reduced Q by R and A. These secondary electron carriers, R and A, are reoxidized by PSI and X (Govindjee and Papageorgiou 1971, Papageorgiou 1975, Wiltens et al. 1978). For stressed plants, a relative increase in magnitude of this transient would indicate accumulation of reduced Q due to inhibition of photosynthetic electron transport (Schreiber et al. 1978). Leaves of *Rumex* exposed to the lower UV-B

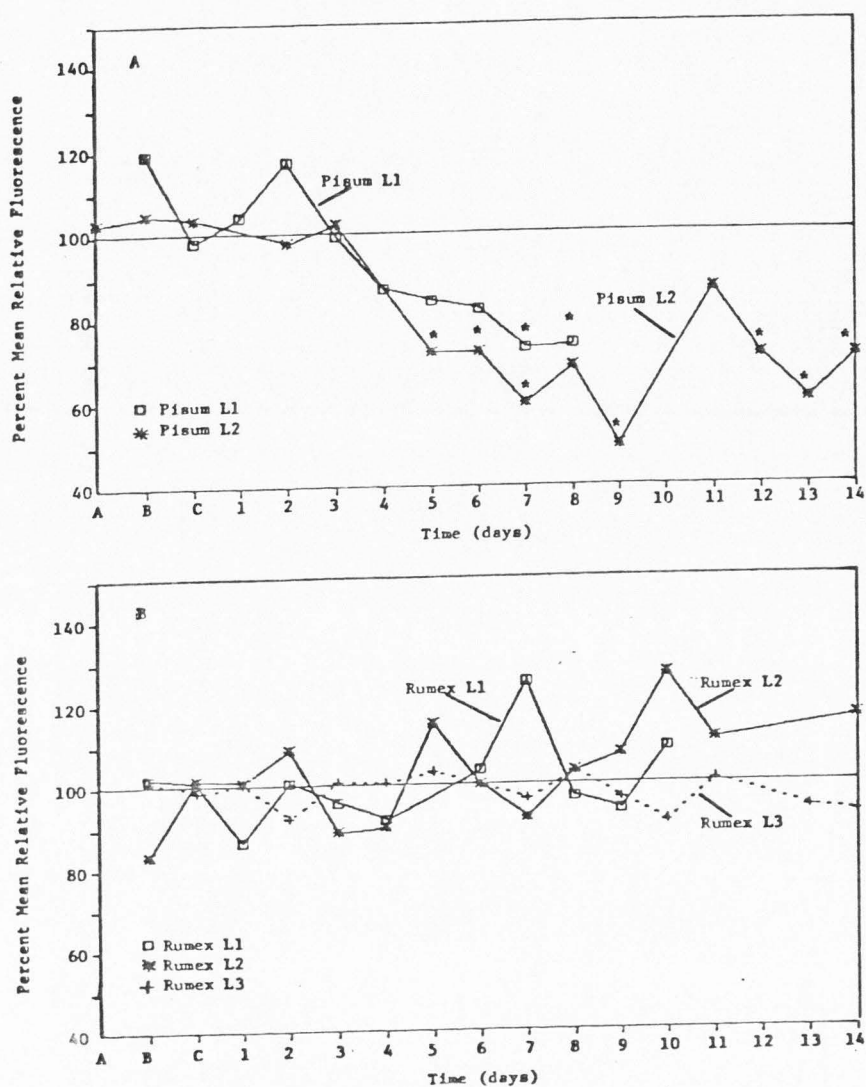


Figure 3. Changes in the O fluorescence transient of Pisum (A) and Rumex (B) leaves exposed to the lower UV-B dose rate. Mean relative fluorescence of UV-B treatment leaves expressed as a percent of mean relative fluorescence of control leaves is plotted versus days of supplemental UV-B irradiation. Lettered days represent pretreatment measurements. Treatment-day interactions were significant ($p < 0.05$) in both Pisum experiments. Days on which there was a significant difference between means are indicated by an asterisk. Seven to eight replicates per treatment per experiment. The Rumex L3 experiment is not replicated.

dose rate exhibited a significant increase ($p < 0.05$) in the O-I transient (Fig. 4B). The treatment-day interaction for the O-I transient of *Pisum* leaves exposed to the lower UV-B dose rate was not significantly different from control leaves (Fig. 4A).

Changes in the magnitude of the I-P transient can indicate either disruption of photosynthetic electron transport between the photosystems or inhibition of the water-splitting mechanism. The rise from I to P corresponds with further reduction of A as the pool of oxidized X is exhausted (Govindjee and Papageorgiou 1971, Wiltens et al. 1978). Water-splitting supplies the electrons necessary to reduce the electron carriers, but since a pool of oxidized X is not immediately regenerated by the Calvin cycle, reduced Q, A, and X accumulate as fluorescence peaks at P. As with the O-I transient, an increase in the magnitude of the I-P rise would suggest disruption of photosynthetic electron transport between the photosystems. A decrease in the I-P rise would be caused by a reduction of water-splitting capacity; electron pressure from water-splitting would be insufficient to produce the high, transitory reduction level of Q at the fluorescence peak, P (Schreiber et al. 1978). The statistical significance of the treatment-day interactions for the I-P rise differed among experiments for each species. In the *Pisum* L1 experiment, the I-P transient of leaves exposed to the lower UV-B dose rate increased after 6 days of enhanced UV-B irradiation. Although the treatment-day interaction for the *Pisum* L2 experiment was not significant with an analysis of variance, the I-P fluorescence magnitude of leaves exposed to enhanced UV-B radiation was significantly

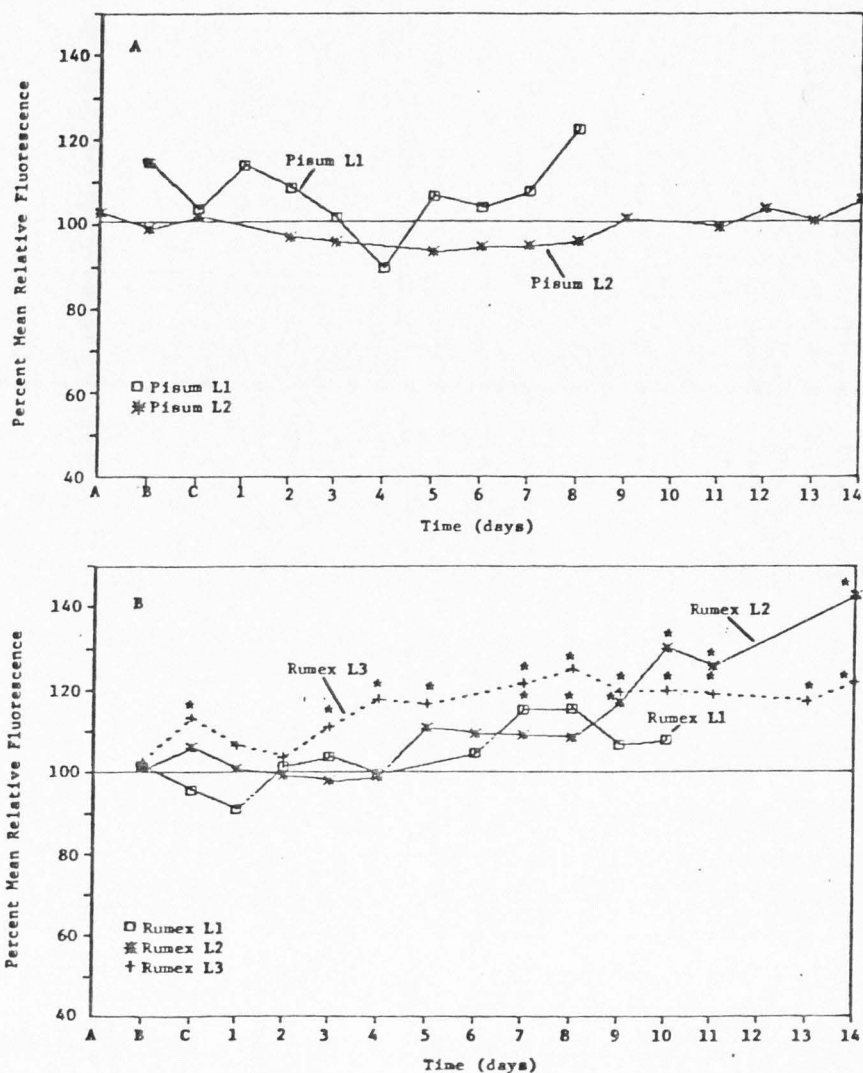


Figure 4. Changes in the magnitude of the O-I fluorescence transient of *Pisum* (A) and *Rumex* (B) leaves exposed to the lower UV-B dose rate. Mean relative fluorescence of the UV-B treatment leaves expressed as a percent of mean relative fluorescence of control leaves is plotted versus days of supplemental UV-B irradiation. Lettered days represent pretreatment measurements. Treatment-day interactions were significant ($p < 0.05$) in all three *Rumex* experiments. Days on which there was a significant difference between means are indicated by an asterisk. Seven to eight replicates per treatment per experiment. The *Rumex* L3 experiment is not replicated.

greater than control leaves after 13 days of treatment using Duncan's multiple mean comparison (Fig. 5A). The leaves of *Pisum* plants in the *Pisum* L1 experiment were one week older than the leaves in the *Pisum* L2 experiment when enhanced UV-B irradiation began. Since the increases in the I-P magnitude were seven days apart, the differences observed between the two *Pisum* experiments may be due to an interaction of leaf age with UV-B radiation. For *Rumex* leaves, treatment-day interactions were significant only in the *Rumex* L3 experiment. Leaves exposed to the lower UV-B dose rate exhibited a decrease in the magnitude of the I-P rise at the end of enhanced UV-B irradiation (Fig. 5B). The control leaves for this experiment were not exposed to any UV radiation, whereas control leaves in the other two experiments were exposed to UV-A both before and during the experiments, which may explain the differences in statistical significance of the treatment-day interaction in the *Rumex* experiments.

Apparently the difference in UV-B irradiance between control and UV-B irradiated leaves at the lower UV-B dose rate is insufficient to cause changes in fluorescence transients, even though it is insufficient to cause a significant decrease in net photosynthesis. Analysis of variance of the net photosynthetic rates of control leaves and leaves exposed to the lower UV-B dose rate did not show a significant treatment-day interaction in either species. A representative comparison of net photosynthetic rates of control leaves and leaves exposed at the lower UV-B dose rate as they changed during the course of an experiment are shown in Fig. 6.

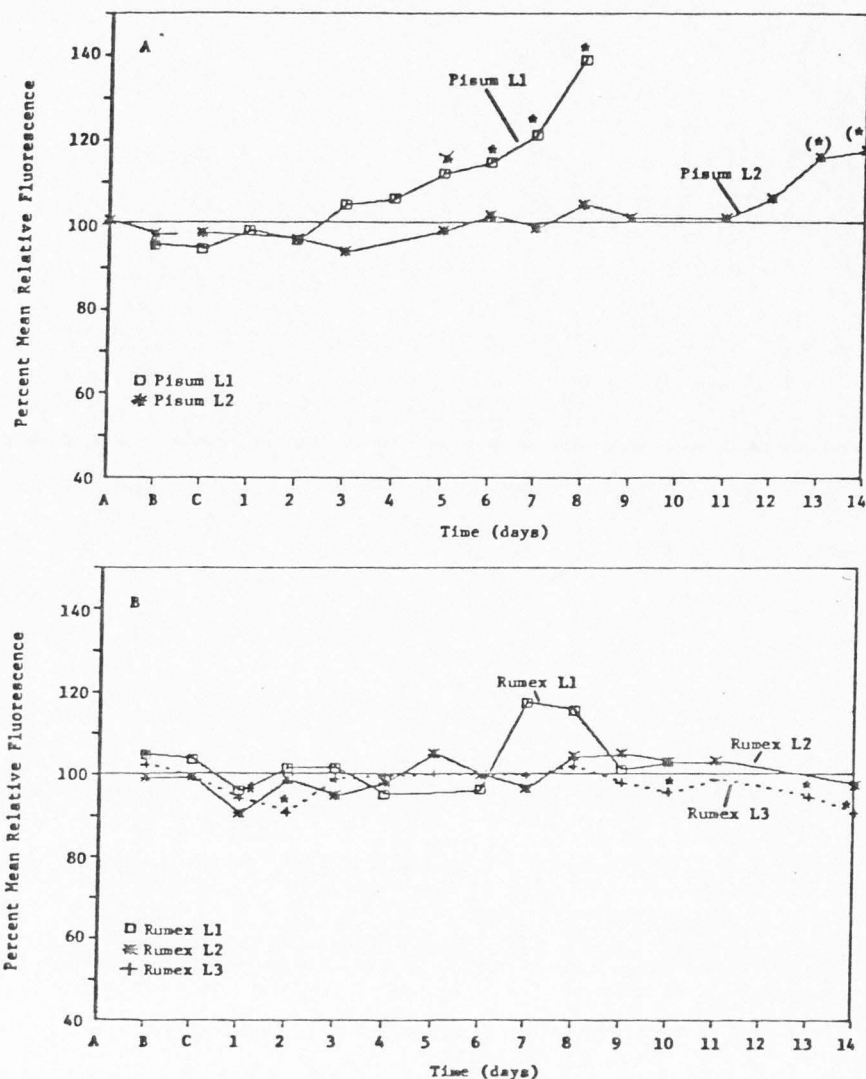


Figure 5. Changes in the magnitude of the I-P fluorescence transient of *Pisum* (A) and *Rumex* (B) leaves exposed to the lower UV-B dose rate. Mean relative fluorescence of the UV-B treatment leaves expressed as a percent of the mean relative fluorescence of control leaves is plotted versus days of supplemental UV-B irradiation. Lettered days represent pretreatment measurements. Treatment-day interactions were significant ($p < 0.05$) only in the *Pisum* L1 and *Rumex* L3 experiments. Days on which there was a significant difference between means are indicated by an asterisk. An asterisk in parenthesis indicates that the F test for treatment-day interaction was not significant, but significant with Duncan's multiple range test. Seven to eight replicates per treatment group per experiment. The *Rumex* L3 experiment is not replicated.

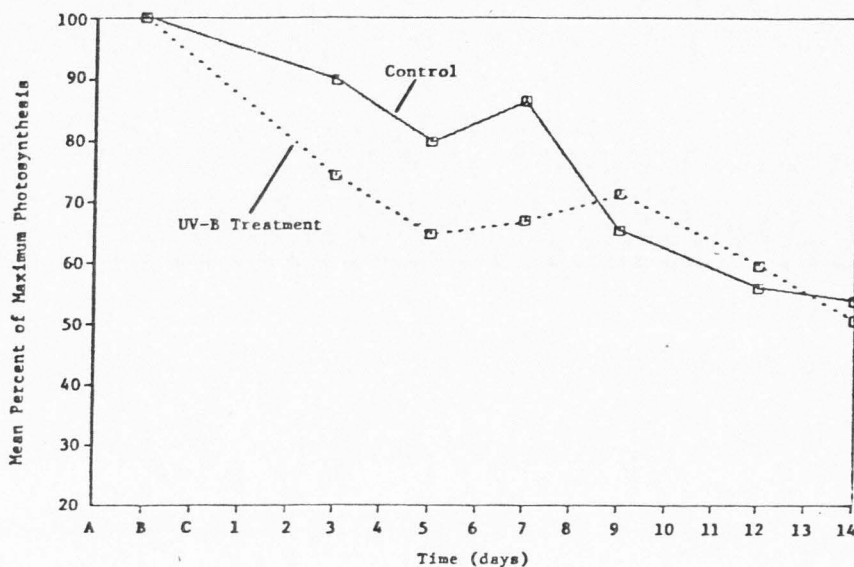


Figure 6. Changes in net photosynthetic rates of control leaves (solid line) and UV-B treatment leaves (dashed line) exposed to the lower UV-B dose rate. These results from the *Pisum* L2 experiment are representative of the results of the other lower UV-B dose rate experiments. Net photosynthetic rates, corrected for intercellular CO_2 concentrations, are expressed as a mean percent of maximum photosynthetic rate and plotted versus days of supplemental UV-B irradiation. Lettered days indicate pretreatment measurements. Treatment-day interactions were not significant in any of the lower UV-B dose rate experiments ($p > 0.05$). Three replicates per treatment group.

With a single eight-hour exposure to a high UV-B dose, which included a small proportion of UV-C (see Fig. 2), the net photosynthetic rates of both species were significantly depressed (Table 2). The differences in percent reduction of net photosynthesis in the Pisum H3 and Rumex H4 experiments may be due to UV-B irradiation without the presence of visible irradiance.

The changes in fluorescence transients that accompanied the significant reduction of net photosynthesis when leaves were irradiated with the higher UV-B dose rate are shown in Table 2. The O fluorescence transient of both species was not changed in a consistent manner when leaves were exposed to this high UV-B dose. In two Pisum experiments the O transient was significantly depressed, but in the third, it increased significantly. The O transient increased significantly in only one Rumex experiment, and decreased in three other Rumex experiments, though not significantly. As discussed earlier, a decrease in the O transient in stressed plants relative to control plants would indicate effects on the chloroplast pigments such as chlorophyll destruction or direct effects on the PSII reaction center. A relative increase would suggest a functional dissociation of the light harvesting and antennae chlorophyll complexes from the reaction center (Armond et al. 1979).

The O-I transient decreased after the high dose of UV-B radiation in both species, though not significantly in all experiments (Table 2). A decrease in the O-I transient of stressed plants relative to control plants may indicate disruption of the PSII reaction centers. The oxidized, quenching form of Q would predominate when

Table 2. Percent changes in fluorescence transient magnitudes and in net photosynthetic rates of *Pisum* and *Rumex* leaves after a single eight-hour exposure to the high UV-B dose rate. A negative percent change indicates a reduction and a positive percent change indicates an increase of the posttreatment measurement relative to the pretreatment measurement. Significant differences ($p < 0.05$) between mean pretreatment and mean posttreatment measurements are indicated by an asterisk. The number of replicates for each experiment (n) are indicated. The *Pisum* H3 and *Rumex* H4 experiments are not replicated.

Experiment	Percent change in relative fluorescence			Percent change in net photosynthesis	n
	0	0-I	I-P		
<i>Pisum</i> H1	-32*	-54*	-83*	----	8
<i>Pisum</i> H2	+93*	-15	-69*	-39*	6
<i>Pisum</i> H3	-85*	-67*	-91*	-73*	4
<i>Rumex</i> H1	-22	-17*	-16*	----	8
<i>Rumex</i> H2	-11	-29*	-41*	-39*	6
<i>Rumex</i> H3	-6	-27*	-40*	-24*	4
<i>Rumex</i> H4	+135*	-16	-58*	-74*	6

the O-I transient is decreased, since the secondary electron acceptors would be able to reoxidize Q when it was reduced by P680. Thus, at these higher UV-B dose rates, the PSII reaction center apparently is directly affected by UV-B radiation.

The I-P fluorescence transient was significantly reduced in both species in the higher UV-B dose rate experiments (Table 2). As discussed earlier, a relative decrease in the I-P transient would indicate partial loss of water-splitting capacity.

DISCUSSION

The mechanism of UV photoinhibition of photosynthesis has not been completely resolved, and may involve more than one site or pathway. The water-splitting side of PSII (Yamashita and Butler 1968), the photosystem reaction centers (Okada et al. 1976), and the chloroplast thylakoid membrane (Mantai et al. 1970) have been suggested as sites of UV photoinhibition. Brandle et al. (1977) attributed partial loss of photosynthetic capacity to dilation of the thylakoids and associated disruption of electron transport, but also suggested direct effects on PSII and, to a lesser extent, PSI. The protein and lipid components of plant membranes, including the photosynthetic membranes, may be primary sites of UV absorption (Wright 1978). Disruption of the thylakoid membrane by various stresses has been shown to inhibit oxygen evolution (Arntzen 1978). Photosynthetic pigment and electron transport systems are integrated components of the thylakoid membrane (Muhlethaler 1977). Dilation of the thylakoid membrane by UV may interfere with normal light-induced conformational changes of the membrane components, and may be the primary mechanism of UV photoinhibition of photosynthesis. Decreases in photosynthetic electron transport capacity and oxygen evolution as well as disruption of photosystem reaction centers could be secondary effects.

The evidence suggests that UV radiation, especially UV-B and UV-C radiation, is generally detrimental to photosynthesis. However, plant sensitivity to UV photoinhibition is highly wavelength depen-

dent. The action spectra of Caldwell (1971) and Jones and Kok (1966a) exhibit exponential increases in plant sensitivity with decreasing wavelength. These action spectra were employed as weighting functions to provide a biologically meaningful comparison of UV-B irradiance used in these experiments. However, UV photoinhibition may operate through different pathways at different wavelengths. Therefore, results of studies using 254-nm radiation may not always yield comparable results to those using UV-B radiation. Further, polychromatic UV-B irradiation may elicit different effects than monochromatic UV-B irradiation.

Ultraviolet-B radiation has been shown to affect photosynthetic capacity to different degrees depending on the visible irradiance to which the plant is exposed. Sisson and Caldwell (1976) found greater depressions of net photosynthetic rates in *Rumex* plants grown in the presence of low visible irradiance than in higher visible irradiance. Teramura et al. (1980) also found increased sensitivity of plants grown in the presence of low visible irradiance to UV-B photoinhibition of photosynthesis. Because of this difference in sensitivity and the goal to conduct this study in an ecological context, polychromatic UV-B irradiance and a moderate intensity of visible irradiance ($500 \mu\text{Einstein} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR) were used in our experiment.

The effects of UV-B radiation of the PSII primary photosynthetic reactions of intact leaves of *Pisum* and *Rumex* were examined by measuring changes in the magnitude of fluorescence transients. Two dose rates were employed. At the lower UV-B dose rate, evidence for

a decline in the photosynthetic electron transport capacity was found in both species. In addition, direct effects on photosynthetic pigments, including the PSII reaction center, were implicated by a decrease of the O transient in Pisum. At this lower UV-B dose rate, net photosynthesis of leaves exposed to UV-B radiation was not significantly depressed. At the higher UV-B dose rate, changes in the O and O-I transients indicated disruption of the PSII reaction centers, and the decrease in the magnitude of the I-P fluorescence rise indicated a reduction of water-splitting capability. Net photosynthetic rates were also significantly reduced.

The changes in the magnitude of fluorescence transients of leaves exposed to supplemental UV-B irradiance, relative to control leaves, were attributed to inhibition of the primary photosynthetic reactions. However, over a period of days, the pigments in the epidermis of stressed leaves may change. For example, anthocyanin production is common in plant leaves exposed to environmental stress. Thus, differential absorption of the fluorescence radiation by pigments in the epidermis of treatment and control leaves may represent a possible confounding factor in the measurement of chlorophyll fluorescence induction, especially for the lower UV-B dose rate experiments. If there was a differential absorption of fluorescence radiation, then one would expect uniform decreases in the magnitudes of all the fluorescence transients. Since none of the lower UV-B dose rate experiments had uniform changes in the magnitude of all the fluorescence transients, differential absorption of fluorescence radiation by pigments in control and treatment leaves probably is not a significant confounding factor.

Evidence from fluorescence transient changes for UV-B photo-inhibition of photosynthetic electron transport between the photosystems occurred in the lower UV-B dose rate experiments (Fig. 4B and 5A). This result contrasts with the experiments of Jones and Kok (1966b) which showed very little effect of monochromatic UV radiation between 230 and 400 nm on photosynthetic electron transport in isolated spinach chloroplasts. However, Brandle et al. (1977) found a 34% decrease in the photosynthetic electron transport capacity of chloroplasts isolated from Pisum leaves exposed to only 20 hours of polychromatic UV-B irradiation in the presence of low visible irradiance. This UV-B irradiation was similar to that of our lower UV-B dose rate experiments. Plastoquinone destruction was observed in isolated swiss chard and spinach chloroplasts exposed to 254-nm radiation (Shavit and Avron 1963, Mantai and Bishop 1967). However, Mantai and Bishop (1967) concluded that plastoquinone destruction was not the major cause of 254-nm photoinhibition of photosynthesis, and Mantai et al. (1970) suggested that the photoinhibition may be most likely due to disruption of the structural integrity of the thylakoid membrane, which in turn could cause a decrease in photosynthetic electron transport capacity. Since the disruptions of photosynthetic electron transport suggested by changes in fluorescence transients occurred in leaves which did not exhibit significant depressions of net photosynthesis, inhibition of photosynthetic electron transport between the photosystems may be one of the first primary photosynthetic reactions affected by UV-B irradiation.

Both the I-P transient and net photosynthetic rates of *Pisum* and *Rumex* leaves exposed to a single, high dose of UV-B decreased significantly (Table 2). The decrease of the I-P transient suggests inhibition of water-splitting activity. Mantai et al. (1970) also observed a decrease in the variable fluorescence of isolated spinach chloroplasts in experiments with 254-nm radiation, which would implicate a reduction of water-splitting capacity. Decreases in the Hill reaction activity of chloroplasts isolated from *Pisum* leaves exposed to polychromatic UV-B, corresponding to the lower UV-B dose rates used in this study, but in the presence of low visible radiation flux were noted by Brandle et al. (1977). Garrard et al. (1976) also found decreases in Hill reaction activity of chloroplasts isolated from bean and cabbage leaves exposed to UV-B and visible irradiances similar to that used by Brandle et al. (1977). Decreases in Hill reaction activity have been observed in isolated chloroplasts from swiss chard (Shavit and Avron 1963) and spinach (Jones and Kok 1966b, Mantai et al. 1970) which were exposed to 254-nm radiation, and in isolated chloroplasts from pea, peanut and collard which were exposed to 298-nm radiation (Van et al. 1977). Yamashita and Butler (1968) postulated that the site of 254-nm inhibition of photosynthesis is on the water-splitting side of PSII.

The relative changes in the O transient suggest direct effects in the PSII reaction center and chloroplast pigments (Fig. 3A, Table 2). The effects of UV-B radiation on the O fluorescence transient appear to be dose related as well as species specific. Jones and Kok (1966a) reported that photoinhibition of isolated spinach chloro-

plast reactions exposed to monochromatic UV radiation between 230 and 400 nm was independent of oxygen concentration, which would eliminate chlorophyll bleaching as the mechanism of photoinhibition. Chlorophyll concentrations of *Rumex* leaves exposed to UV-B and visible irradiances similar to our experiments were not reduced relative to control leaves (Sisson and Caldwell 1976). However, significant reductions of chlorophyll concentrations in leaves exposed to about the same UV-B flux but in the presence of lower visible irradiance have been found (Garrard et al. 1976, Vu et al. 1980). Disruption of the PSII reaction center has been exhibited in isolated spinach chloroplasts exposed to 254-nm radiation (Okada et al. 1976). The fluorescence transient experiments suggest that UV-B radiation-induced disruption of the PSII reaction center and associated pigments may occur at doses insufficient to affect net photosynthetic rates in some species, such as *Pisum*. At higher UV-B doses, which included some shorter wavelength (UV-C) radiation, direct effects on the PSII reaction center and chloroplast pigments induced by UV-B radiation appeared to be quite evident in both species.

Other effects on the primary photosynthetic reactions have been noted in studies with isolated chloroplasts. Cyclic photophosphorylation of chloroplasts exposed to 254-nm and 298-nm radiation has been shown to decrease (Shavit and Avron 1963, Jones and Kok 1966b, Mantai et al. 1970, Van et al. 1977), but this was generally less sensitive to UV photoinhibition than Hill reaction activity. Okada et al. (1976) reported inactivation of P700 and disruption of the antennae chlorophyll of PSI by 254-nm radiation, although again these UV radiation-induced disruptions were not as pronounced as

those associated with PSII. These effects of monochromatic UV radiation on the primary photosynthetic processes were not examined in our experiments.

The reaction centers and associated antennae chlorophyll, the water-splitting process, and the photosynthetic electron transport system, which are all integrated into the thylakoid membrane, are affected by UV irradiation. Membrane proteins may be an important UV chromophore (Wright 1978), and dilation of the thylakoid membrane may be the primary mechanism of UV photoinhibition of photosynthesis (Mantai et al. 1970, Brandle et al. 1977). Our results are consistent with this hypothesis, although fluorescence induction measurements do not provide direct indications of membrane damage.

If thylakoid membrane proteins are important UV chromophores, then this mechanism of UV photoinhibition of photosynthesis may explain the differences in sensitivity of species exposed to UV-B radiation in the presence of high and of low visible intensities reported by Sisson and Caldwell (1976) and Teramura et al. (1980). There is more protein associated with the light harvesting chlorophyll in plants grown in the presence of low visible irradiance than in plants grown in the presence of high visible irradiance. This light harvesting protein is thought to bind the thylakoid membranes together into stacks, or grana, which may increase the efficiency of energy transfer to the photosystems in plants grown in the presence of low visible irradiance (Arntzen 1978, Bennett 1979). Thus UV radiation-induced dilation of the thylakoid may be more im-

portant in the inhibition of photosynthesis for plants grown in the presence of low visible irradiance, but this may be comparatively unimportant in leaves grown in the presence of high visible irradiance.

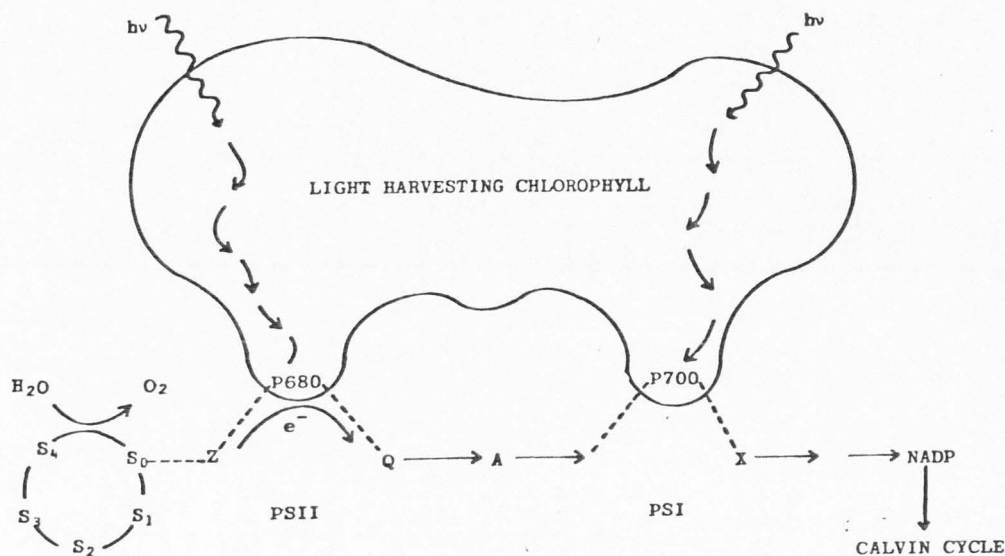
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APPENDIX



Appendix. Schematic of the primary photosynthetic reactions. Light energy ($h\nu$), absorbed by the light harvesting chlorophyll complex, is funneled to the reaction center chlorophylls, P680 for photosystem II (PSII) and P700 for photosystem I (PSI). In the PSII photoreaction, electrons (e^-) are generated from water-splitting after a four stage oxidation process. The water-splitting enzyme (S_n) is coupled through the primary electron donor for PSII (Z) to the reaction center. Electrons are transferred to PSI by means of the intersystem electron transport chain, which consists of the primary electron acceptor for PSII (Q) and various other electron carriers (A). The intersystem electron carrier pool includes plastoquinone, cytochrome f, and plastocyanin. The PSI reaction center accepts electrons from the pool A, and uses absorbed light energy to increase the energy of the electrons. The primary electron acceptor for PSI (X) is then coupled to the Calvin cycle.