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Influence of Photosynthetic Photon Flux Density on UV-BInduced Photoinhibition in Soybean Leaves: Comparison of Preconditioning and Concomitant Light Treatments

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INFLUENCE OF PHOTOSYNTHETIC PHOTON FLUX DENSITY ON

UV-B-INDUCED PHOTOINHIBITION IN SOYBEAN LEAVES:

COMPARISON OF PRECONDITIONING AND

CONCOMITANT LIGHT TREATMENTS

by

Charles W. Warner

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

1982

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Dr. Martyn M. Caldwell for gererously supplying suggestions and encouragement throughout this study and preparation of this thesis. I would also like to extend appreciation to Drs. Frank Salisbury, Herman Wiebe and Niel Van Alfen for their ideas and suggestions on this study. The assistance of Steve Flint and Alan Carpenter is also sincerely appreciated. I would like to thank my wife Nancy for her support and understanding over the two years this study required.

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ABSTRACT

Influence of Photosynthetic Photon Flux Density on UV-B-
Induced Photoinhibition in Soybean Leaves: Comparison of
Preconditioning and Concomitant Light Treatments

by

Charles W. Warner, Master of Science

Utah State University, 1982

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Department: Range Science

Previous studies indicate that the degree of UV-B-
induced photosynthetic inhibition may be highly dependent
upon the photosynthetic photon flux density (PPFD, total
quantum flux in the waveband 400-700 nm) incident on a
plant. This study illustrates that Essex soybean leaves
(Glycine max) preconditioned under high PPFD suffered
less UV-B-induced photoinhibition than when preconditioned
under low PPFD. However, sensitivity to UV-B increased
when soybean leaves received high-PPFD as a concomitant
treatment.

The relative magnitude of UV-B-induced damage was
similar for both light-limited and light-saturated
photosynthesis. This probably indicates that UV-B is

inhibiting fundamentally different photosynthetic processes.

Soybean leaves preconditioned under high PPFD had greater specific-leaf-weight, chlorophyll a/b ratio, and crude flavonoid content. The total chlorophyll concentration of soybean leaves preconditioned under high PPFD increased slightly over a UV-B irradiation period of five hours. Total chlorophyll concentration of leaves preconditioned under low PPFD decreased slightly in response to the same irradiation period. (41 Pages)

INTRODUCTION

Ultraviolet radiation has been shown to be effective in reducing growth and photosynthesis in a number of sensitive but unrelated plant species (Caldwell, 1971; Sisson and Caldwell, 1977; Van et al., 1977; Allen et al., 1977; Biggs et al., 1975). The ultraviolet-B (UV-B) portion of the electromagnetic spectrum (280-320 nm) is especially important in this respect because it includes those wavelengths that occur in the terrestrial solar spectrum and are readily absorbed by plants (Brandle et al., 1977; National Academy of Sciences, 1979). Essentially all solar radiation of wavelength shorter than 290 nm is absorbed prior to reaching the Earth's surface by stratospheric ozone (Caldwell, 1979).

Current predictions suggest that stratospheric ozone may decrease. This decrease is primarily the result of anthropogenic perturbations (Hudson and Reed, 1979). This would result in both an increase in the level of UV and an extension of the terrestrial solar spectrum to include somewhat shorter wavelengths than presently reach the Earth's surface. Because UV-B radiation is effective in inducing photochemical reactions, such an increase in solar ultraviolet irradiation might have important biological consequences.

Recent studies indicate that the degree of UV-B-induced photosynthetic inhibition may be highly dependent upon the photosynthetic photon flux density (PPFD, total quantum flux in the waveband 400-700 nm) incident on a plant. A number of researchers have concluded that plants grown in greenhouses suffer less photoinhibition by UV-B than plants grown in growth chambers (Sisson and Caldwell, 1976; Bennett, 1981). It has been suggested that this might be because of the higher levels of PPFD in greenhouse studies (Sisson and Caldwell, 1976; Van et al., 1976; Bennett, 1981).

In studies where two or more levels of PPFD have been used, there has been a general observation that higher PPFD has an ameliorating effect on UV-B-induced photoinhibition (Sisson and Caldwell, 1976; Teramura, 1980; Teramura et al., 1980; Teramura and Caldwell, 1981). From these experiments, it is not possible to determine if the reduction in photoinhibition results from the use of high PPFD given concomitantly with UV-B, or as the result of preconditioning the plant at high PPFD.

The objectives of this study were to: 1) assess the relative importance of PPFD, given either concomitantly or as a preconditioning treatment, in alleviating the supression of photosynthesis by UV-B, 2) determine if the

effect of UV-B is more pronounced on light-limited or light-saturated photosynthesis, and 3) examine the extent to which total chlorophyll, chlorophyll a/b ratio, and UV-B absorbing pigments are influenced by PPFD and UV-B.

MATERIALS AND METHODS

Plant materials and growth conditions

Essex soybean (Glycine max) was grown from seed in pots containing 700 cm^3 of a 3:2 mixture (V:V) of potting soil and vermiculite. Seedlings were placed in a controlled-environment growth chamber (Mallory Co.) equipped with a 6000-W xenon arc lamp. One layer of Mylar Type D (DuPont Corp.) plastic filter was used to absorb all radiation shorter than 320 nm produced by the lamp. In addition, all plants were mounted on a conveyor type rotator system within the growth chamber to insure homogenous irradiation. The plants were watered daily with half-strength Hoagland's solution, temperature was kept at 25 $\pm$ 1.5 °C, and a 12-hour daily photoperiod was maintained throughout the experiment.

Preconditioning treatment

Following full development of the first trifoliate leaf, as described by Fehr and Caviness (1977), each plant was assigned to one of two groups (Appendix). One of the groups was maintained at 750 umol quanta m^{-2}s^{-1} PPFD, as measured with a quantum sensor (Li-Cor Co. model LI-190).

Neutral density shading cloth was suspended over the remaining group to reduce the incident PPFD to 70 umol quanta $m^{-2}s^{-1}$. The second and third trifoliate leaves expanded completely under these PPFD levels. These groups constituted the high- and low-light-preconditioned plants and are analogous to sun and shade-adapted plants. Other environmental conditions were maintained as closely as possible to the values obtained before separation. For this study, only the second trifoliate leaf of each plant was used. Since photosynthetic capacity is closely associated with leaf age and stage of development (Sestak, 1977; Teramura and Caldwell, 1981), a plant was considered no longer usable when the third trifoliate leaf was fully developed. This method assured that the maximum age difference between any two leaves utilized in the study was five days.

Concomitant PPFD and UV-B treatment

The system used for the concomitant PPFD and UV treatment is shown schematically in Figure 1. In order to initiate the concomitant treatment, a preconditioned soybean plant was removed from the growth chamber. A single leaflet from the second trifoliate leaf was removed for chlorophyll and crude flavonoid analysis (described later). The remaining two leaflets were placed, still intact, in a plant gas exchange cuvette that was adapted with a modified

Fig 1.   Schematic representation of the system used

for the concomitant PPFD and UV-B treatment.

PPFD was supplied from a 1000-W multivapor

lamp and filtered through a water bath heat

trap to remove excessive infrared radiation

also produced by the lamp. Ultraviolet

radiation was supplied by four FS-20 sunlamps

arranged in a square, and placed parallel to

the leaf surface. Cellulose acetate film was

placed between the lamps and the plant leaves

to remove radiation shorter than 290 nm. A

Peltier heat exchanger was used to control

leaf temperature within the gas exchange

cuvette.

H2O Bath
Heat Trap

UV Lamps

Cellulose
Acetate Film

Gas Exchange
Cuvette

Heat
Exchanger

Multivapor
Lamp

1000-W

Scale 1:5

9
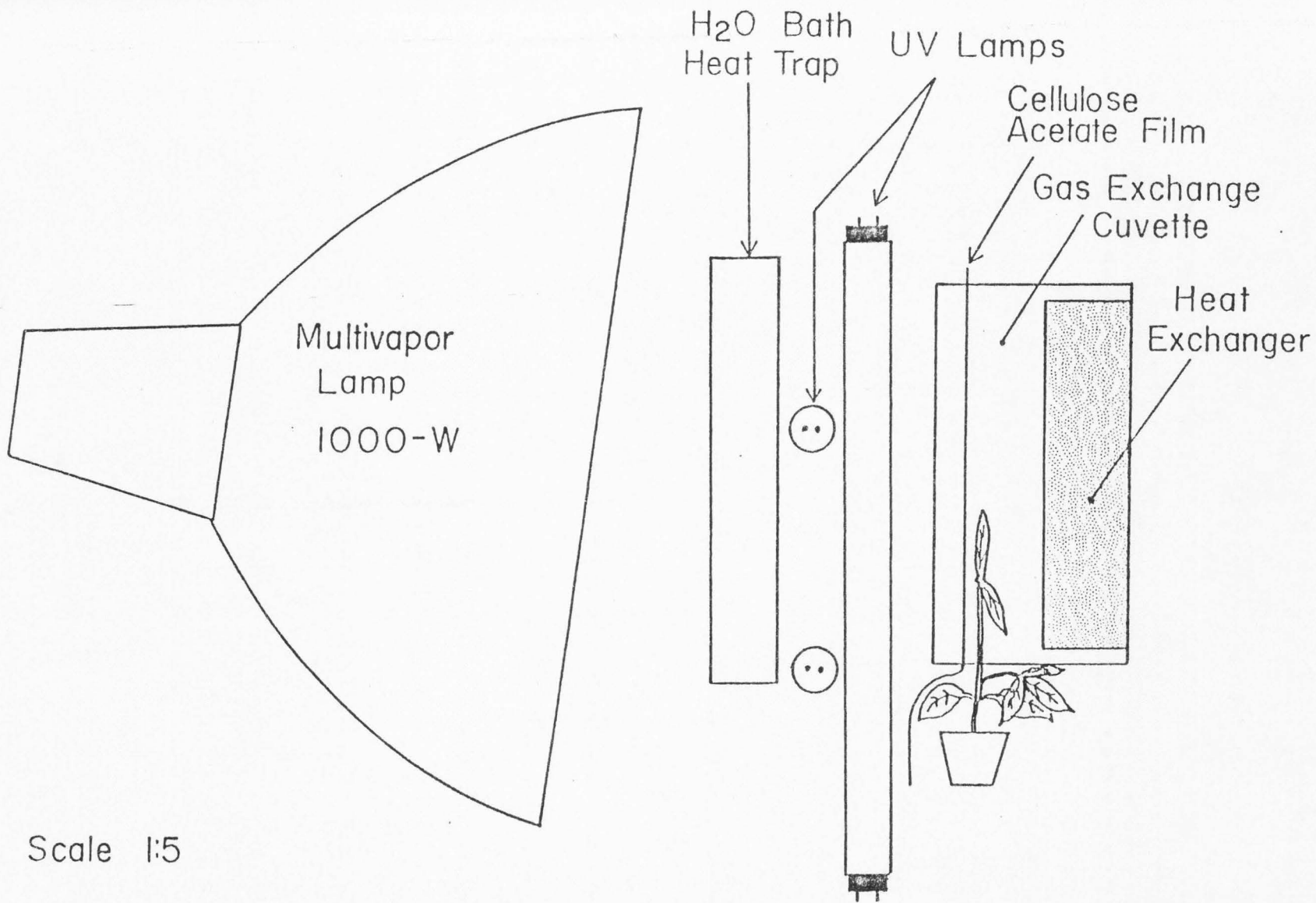
cover. The cover consisted of cellulose acetate film, which transmits essentially no UV shorter than 290 nm. Because cellulose acetate photodegrades when exposed to UV, the cover film was replaced daily.

While in the gas exchange cuvette, the leaf was irradiated with PPFD from a 1000-W multivapor lamp (General Electric Corp.). In order to remove the excessive infrared radiation produced by the lamp, the light was filtered through 4.5 cm of circulating water. For the high-light-concomitant treatment, the PPFD incident on the leaf was adjusted to 850 umol quanta $m^{-2}s^{-1}$. This value was chosen because we determined in a preliminary study that 850 umol $m^{-2}s^{-1}$ is sufficient PPFD to saturate photosynthesis of both groups, yet not high enough to cause direct photoinhibition of either. Neutral density shading cloth was used to reduce the PPFD to 150 umol $m^{-2}s^{-1}$ for the low-light-concomitant treatment.

Ultraviolet radiation was supplied by four FS-20 sunlamps (Westinghouse Co.) arranged in a square and placed parallel to the leaf surface (Fig 1). This configuration provided a reasonably uniform radiation field without obstructing the visible light beam. Spectral UV-B flux density was measured with a double grating Optronic model 742 spectroradiometer coupled to a Hewlett Packard HP-85 data acquisition system. The spectral distribution of the

lamps, as measured at a distance of 16 cm and filtered through the cellulose acetate cuvette cover, is shown in Figure 2.

Because the spectral composition of the irradiance from the lamps does not perfectly simulate the natural solar spectrum, a direct comparison of total UV-B flux between the two sources is not particularly meaningful. Biological photoreactions have a pronounced wavelength dependence in the UV-B waveband. For this reason, the spectral irradiance was multiplied by weighting functions that describe its potential to effect biological photoreactions. The weighting functions are based on experimentally determined action spectra. For comparative purposes, UV-B radiation was weighted with two action spectra. One is based on a generalized plant UV-B response function (Caldwell, 1971) and the other on a DNA damage action spectrum (Setlow, 1974). The irradiance used in this study was equivalent to 290 effective mW m^{-2} when weighted with the generalized plant action spectrum, or 41 mM m^{-2} when weighted according to the DNA-damage spectrum. On a daily dose basis (5-hour irradiation period), the plant effective UV-B irradiance was approximately 5200 J $m^{-2}d^{-1}$. The leaves were exposed to this high flux density over this relatively short time interval in an effort to avoid confounding the results with leaf aging. Control groups were also run, using Mylar Type D film filters over

Fig 2.   The spectral distribution of four FS-20
          sunlamps measured at a distance of 16 cm and
          filtered through cellulose acetate film,
          compared with normal global UV irradaition

          at solar noon.
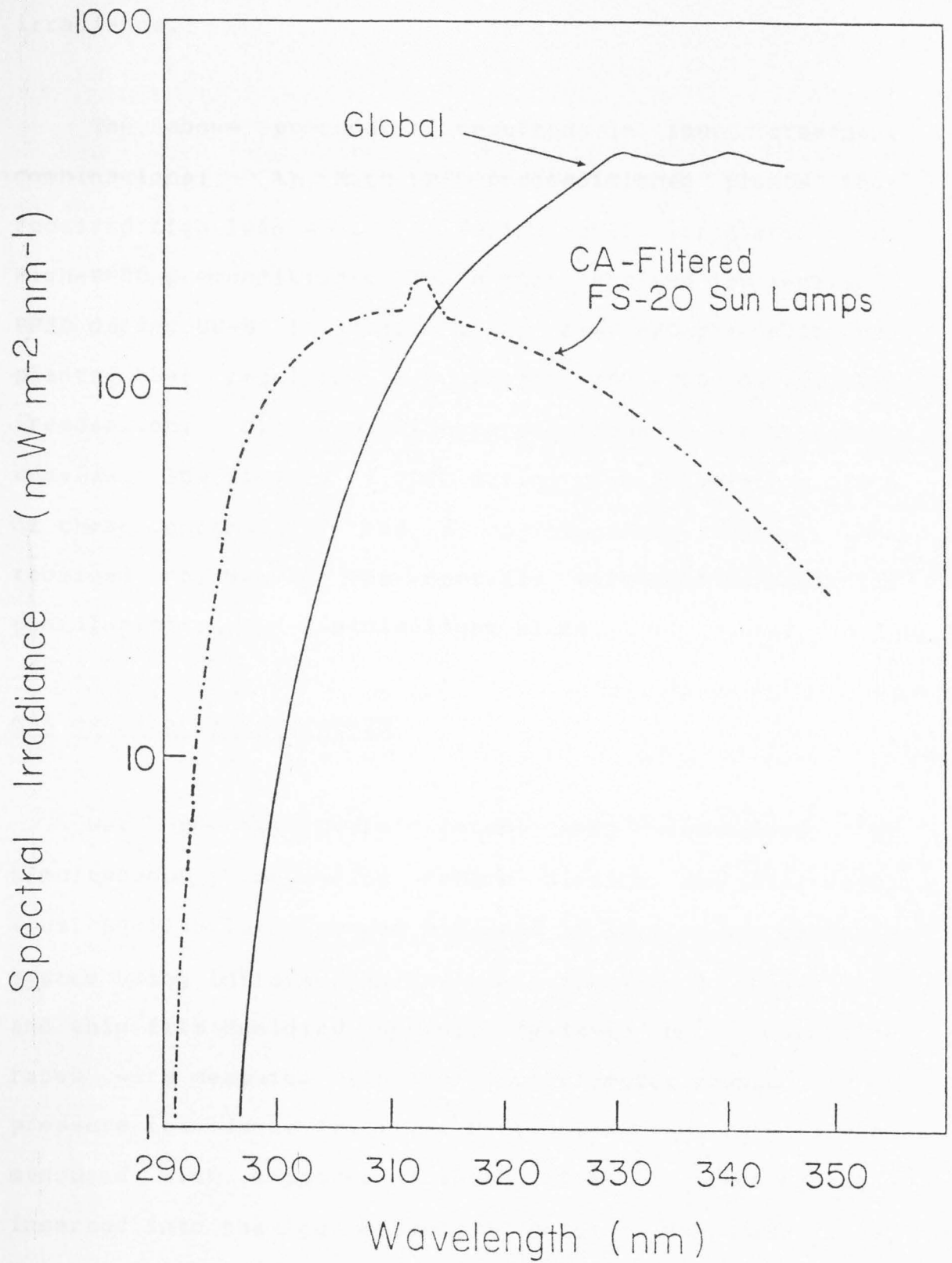
the UV lamps to remove the UV-B portion of their irradiance.

The above procedures resulted in four treatment combinations: A) High-PPFD-preconditioned plants that received high levels of PPFD during UV-B irradiation. B) High-PPFD-preconditioned plants that received low levels of PPFD during UV-B irradiation. C) Low-PPFD-preconditioned plants that received high levels of PPFD during UV-B irradiation. D) Low-PPFD-preconditioned plants that received low levels of PPFD during UV-B irradiation. Each of these combinations had a corresponding control that received no UV-B. The controls were used to check for photoinhibition by visible light alone.

Gas exchange measurements

Net photosynthetic rates were determined by simultaneously measuring carbon dioxide and water vapor flux. Equilibrium flux was measured in an open gas exchange system using infrared gas analyzers (Beckman Co. model 215) and thin film humidity sensors (Vaisala Co.). Gas flow rates were measured with a pneumotachometer coupled with a pressure transducer (Validyne Co.). Leaf temperatures were measured with copper-constantan fine wire thermocouples inserted into the leaf tissue. A Peltier heat exchanger, located behind the leaf, was used to maintain leaf

temperatures between 20° and 22°C. Intercellular CO_2 concentrations were maintained at 220 $\pm$ 10 ppm by adjusting incoming CO_2 concentrations and flow rate. All photosynthetic measurements were taken at equilibrium flux of CO_2 and water vapor.

Photosynthetic rates were determined both before and after the concomitant treatment period. Any deviation from the initial photosynthetic rate was assumed to result from the UV irradiation. Each of the treatment combinations was subdivided into two groups. Photosynthesis in one group was measured under conditions of limiting light (150 umol $m^{-2}s^{-1}$ PPFD), and in the other group measured under saturating light (850 umol $m^{-2}s^{-1}$ PPFD). The data were subjected to analysis of variance (Ostle and Mensing, 1975).

Crude flavonoid and chlorophyll extract

Extracts containing UV absorbing pigments were obtained by the method described by Caldwell (1968). The pigments included in these extracts were primarily of the flavonoid group, although a few other UV-B absorbing compounds would be included (Caldwell et al., 1982). In this procedure, a tissue sample was removed from the leaflet that had been severed from the trifoliate leaf before UV-B irradiation. The sample was ground with a hand

homogenizer in 1 ml of a methanol-water-HCl solution (70:29:1 v/v) and diluted to a total of 10 ml. The sample was then centrifuged for 10 minutes, and an aliquot of the resulting supernatant was examined in a double-beam spectrophotometer at 305 nm (Beckman model 35). This process was repeated using a tissue sample taken from a leaflet after the 5-hour irradiation treatment.

The total chlorophyll and chlorophyll a/b ratios were determined using a modification of the method described by Arnon (1949). This procedure uses 80% acetone to extract the chlorophyll, which is then expressed on a leaf area basis. As with the flavonoid analysis, tissue samples were taken from both UV irradiated and nonirradiated leaflets. The remaining leaflet was used in determining the specific leaf weight (dry weight/leaf area).

RESULTS

Soybean adaptation to different PPFD

Even though Essex soybean leaves can exhibit many of
the characteristics of higher plant leaves grown under
low-light and high-light conditions, the photosynthetic
response of soybean leaves from the high- or low-light
environments was the same (Fig 3). The lack of difference
in photosynthetic response of sun and shade leaves to light
has been shown for other varieties of soybean as well
(Burnside and Bohning, 1957; Lugg, 1978; Gauhl, 1976).
Atriplex patula (Fig 4, from Boardman, 1977) exemplifies
the typical photosynthetic response curves for sun and
shade leaves of many higher plants. Unlike soybean,
Atriplex patula has both a higher light saturated rate of
photosynthesis and higher light compensation point
(photosynthesis = respiration), when grown under a
high-light environment. The reason why soybean
photosynthetic rates do not differ according to light
treatment in a manner similar to Atriplex patula is not
clear. However, Jurik et al. (1979) state that the
photosynthetic response shown by soybean may be common to
plants that are genetically adapted to environments of
reduced PPFD. The high- and low-light-preconditioning
treatments produced mean specific-leaf-weights (SLW) of
39.6 g m^{-2} and 17.1 g m^{-2} repectively, which is a 2.3 fold

Fig 3.   Photosynthetic light saturation curves for
         high-PPFD-preconditioned soybean leaves
         (dashed line) and low-PPFD-preconditioned
         soybean leaves (solid line). There are no
         statistically significant differences
         between the two curves even though the
         Specific Leaf Weight (SLW = grams dry weight
         per meter^2) was 2.3 times greater in leaves
         preconditioned under high-PPFD.
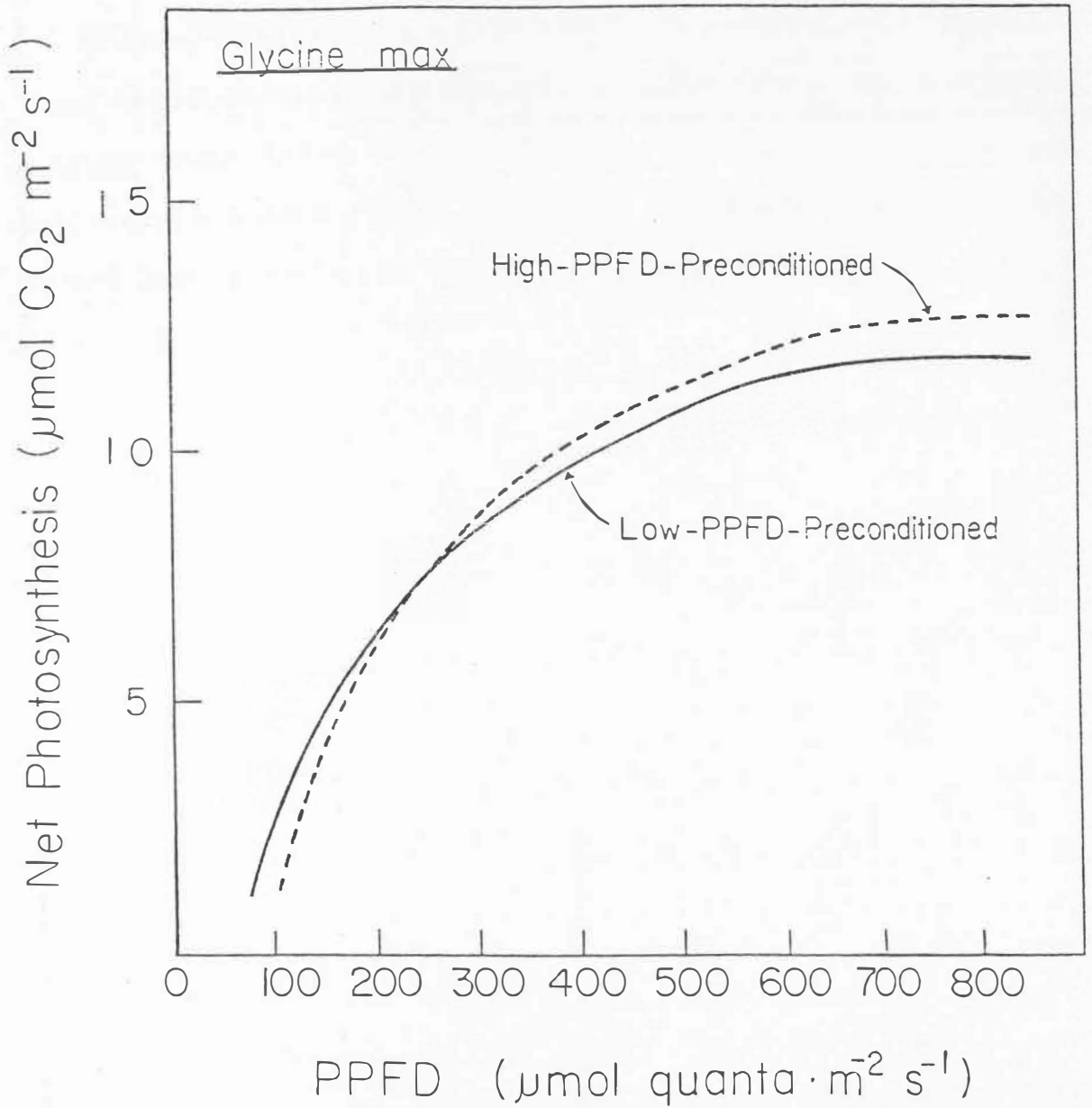
Fig 4.   Photosynthetic light saturation curves for

high-PPFD-preconditioned (dashed line) and

low-PPFD-preconditioned (solid line) Atriplex

patula. A. patula showes both a higher light

saturated rate of photosynthesis, and light

compensation point when grown in a high light

environment.

Atriplex patula

High-PPFD-Preconditioned

Low-PPFD-Preconditioned
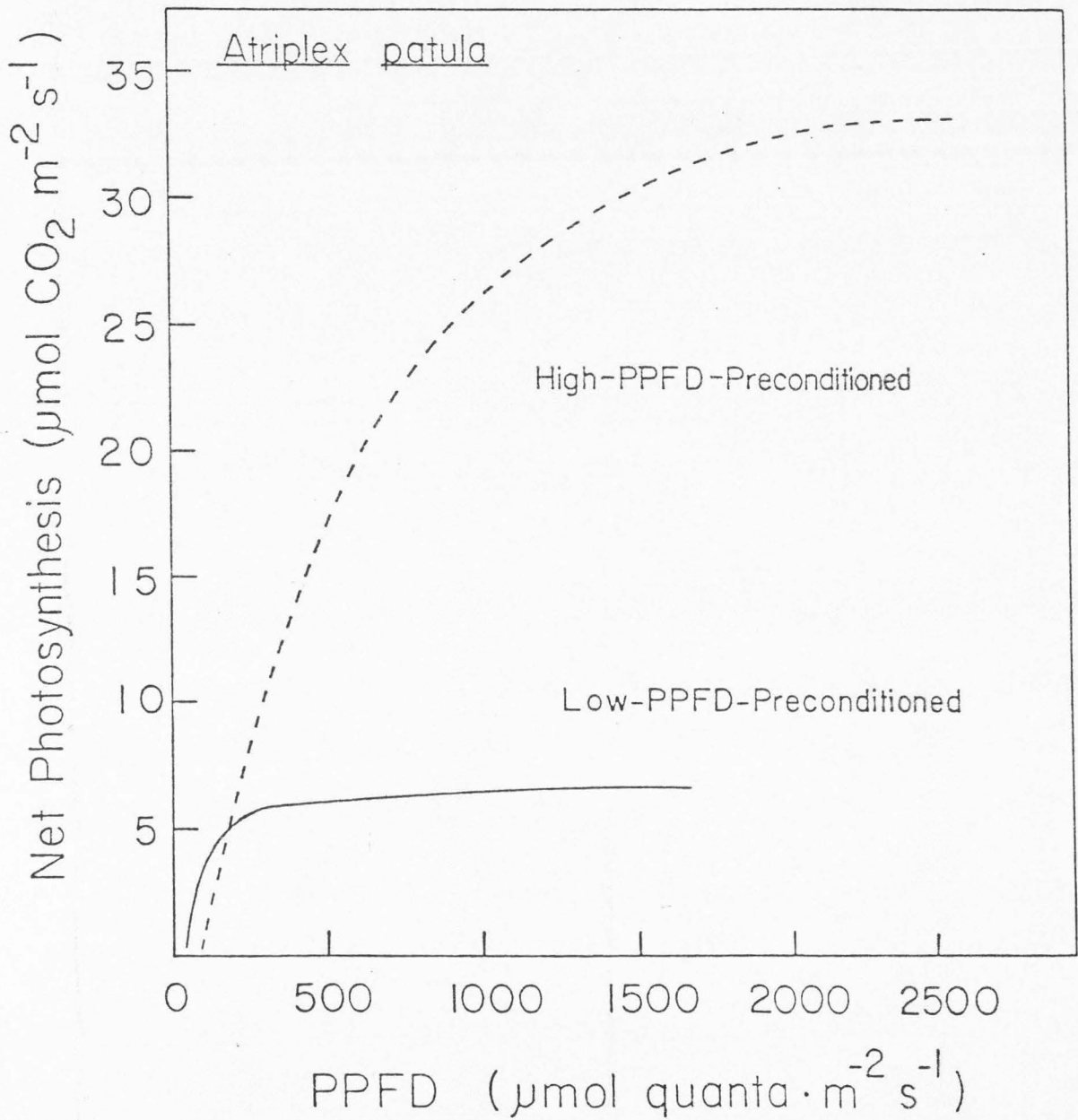
Adapted from Boardman 1977

difference. Less internodel elongation and higher crude flavonoid content (expressed as extract absorbance at 305 nm, Fig 5) resulted from preconditioning the soybean plants under high-light. Although no significant difference in total chlorophyll concentration was observed (Fig 6), the chlorophyll a/b ratio was significantly greater (P =.99) in leaves preconditioned under high-light (Fig 7).

Response to UV-B radiation

Of the leaf pigments measured (crude flavonoids, chlorophyll a/b, and total chlorophyll), only the total chlorophyll concentration appeared to be altered by UV-B. This change in concentration by UV-B was dependent on the preconditioning-light treatment (Fig 6). Following UV-B irradiation, the total chlorophyll concentration from leaves preconditioned under high-PPFD was significantly greater (P = .99) than from leaves preconditioned under low-PPFD. Both preconditioning and concomitant PPFD treatments had highly significant effects (P =.99) on UV-B induced photoinhibition (Fig 8). There were no significant interactions between PPFD treatments. When soybean plants were grown under high-light intensity, the inhibition of photosynthesis by UV-B radiation was less than for leaves that had been grown under low-light intensity. There was also a less pronounced but stastically significant reduction in photoinhibition when low-light was used as a

Fig 5.   Crude flavonoid absorbance per unit leaf area
          (305 nm) of soybean leaf extracts from four
          Preconditioning- and concomitant-PPFD-treatment
          combinations. Vertical lines represent $\pm$ 1
          standard error (n = 80). Open bars represent
          absorbance before UV-B treatment, hatched bars
          represent absorbance after a five hour UV-B
          irradiation period.
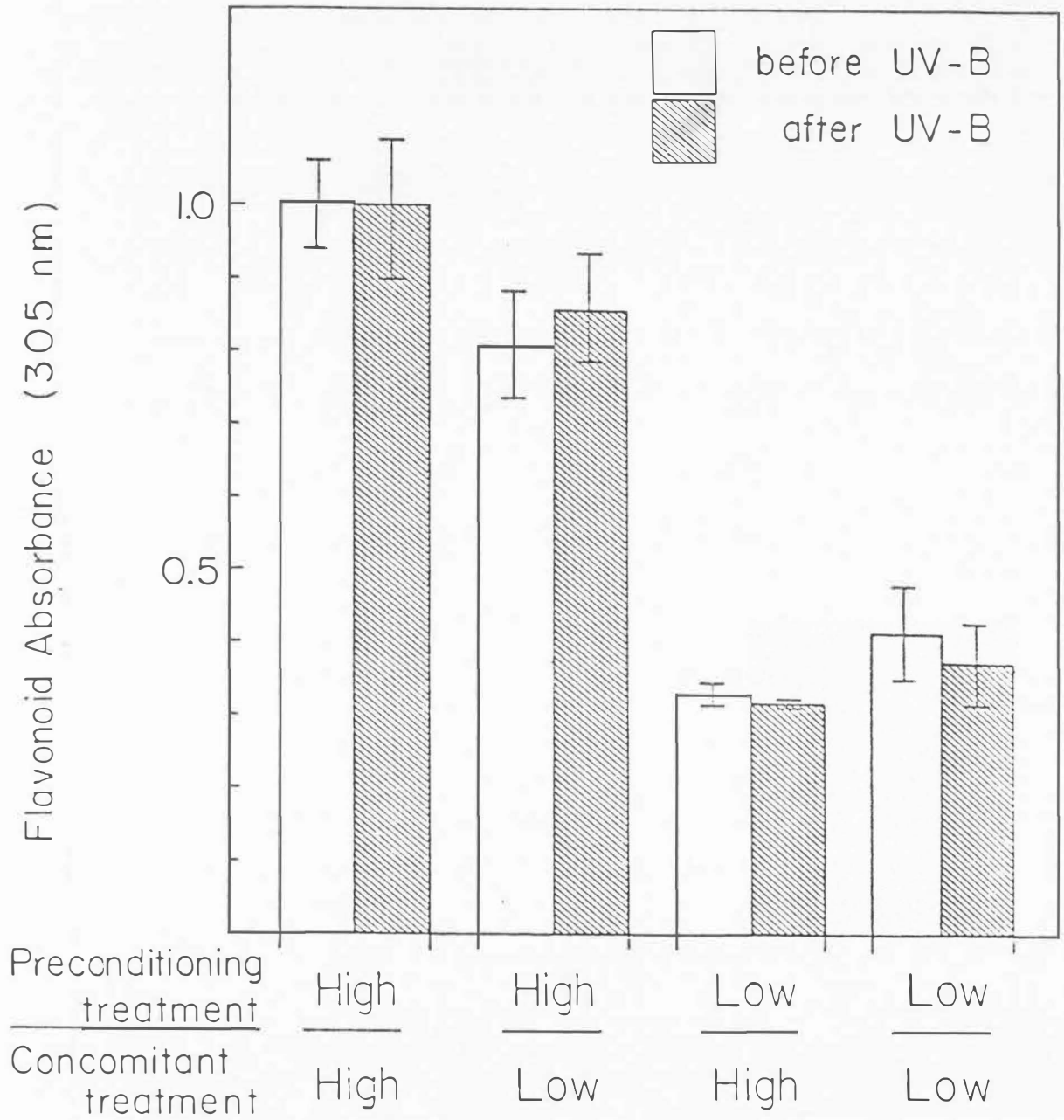
Fig 6.   Total chlorophyll concentration of soybean

         leaf extracts from four preconditioning- and

         concomitant-PPFD-treatment combinations.

         Vertical lines represent $\pm$ 1 standard error

         (n = 80).   Open bars represent concentration

         before UV-B treatment, and hatched bars

         represent concentration after the five hour

         irradiation period.
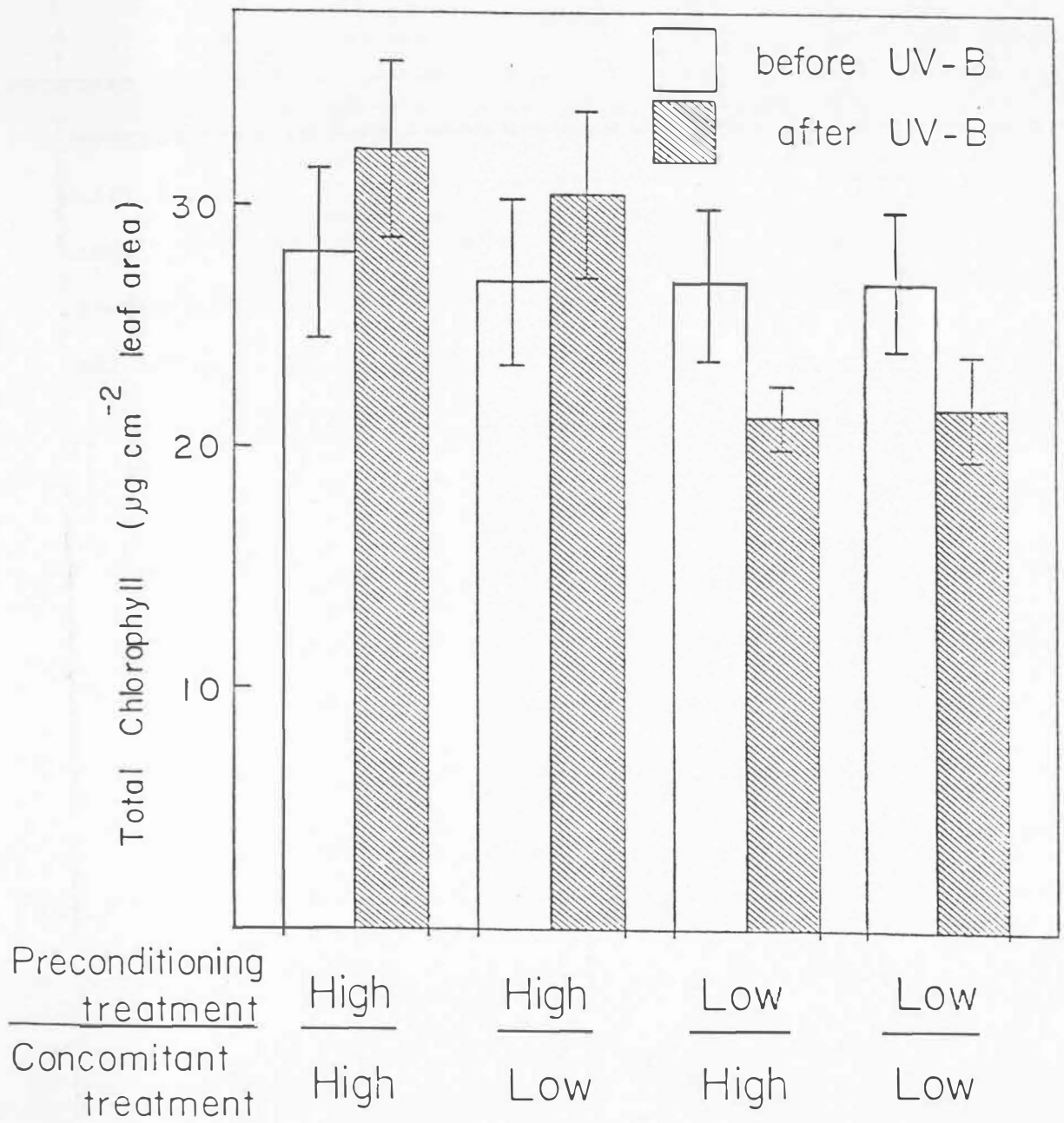
Fig 7.   Chlorophyll a/b ratios of soybean leaf extracts
from four preconditioning- and concomitant-
PPFD-treatment combinations.   Vertical lines
represent ± 1 standard error (n = 80). Open
bars represent ratios before UV-B treatment,
and hatched bars represent ratios after the
five hour UV-B irradiation period.
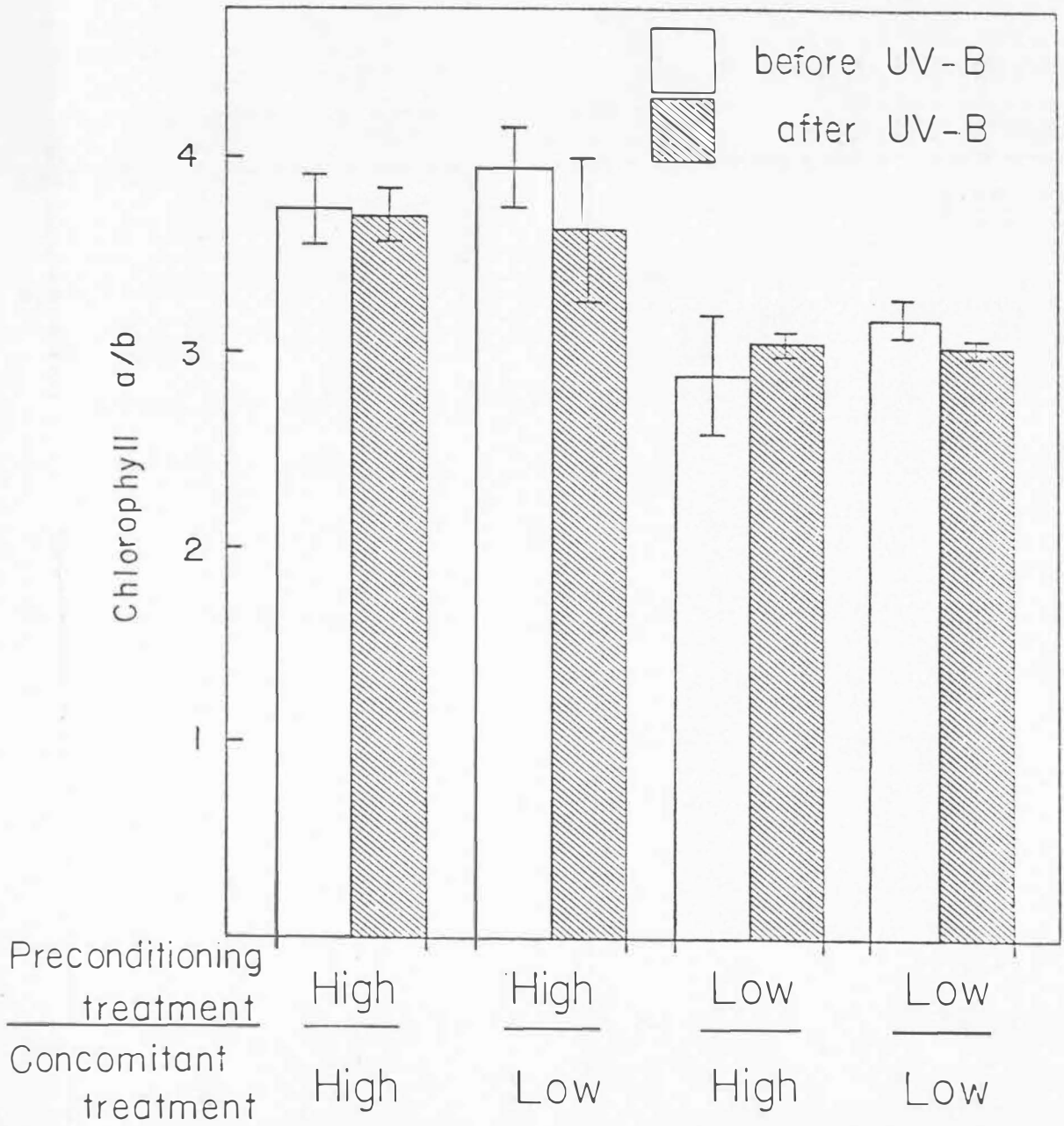
Fig 8.   Relative effect of UV-B irradiation on

photosynthesis of leaves from each of the four

PPFD-treatment combinations. Vertical bars

represent $\pm$ 1 standard error (n = 80). Points

below below the horizontal line represent

enhanced photosynthesis. Points above the

line represent photosynthetic inhibition.
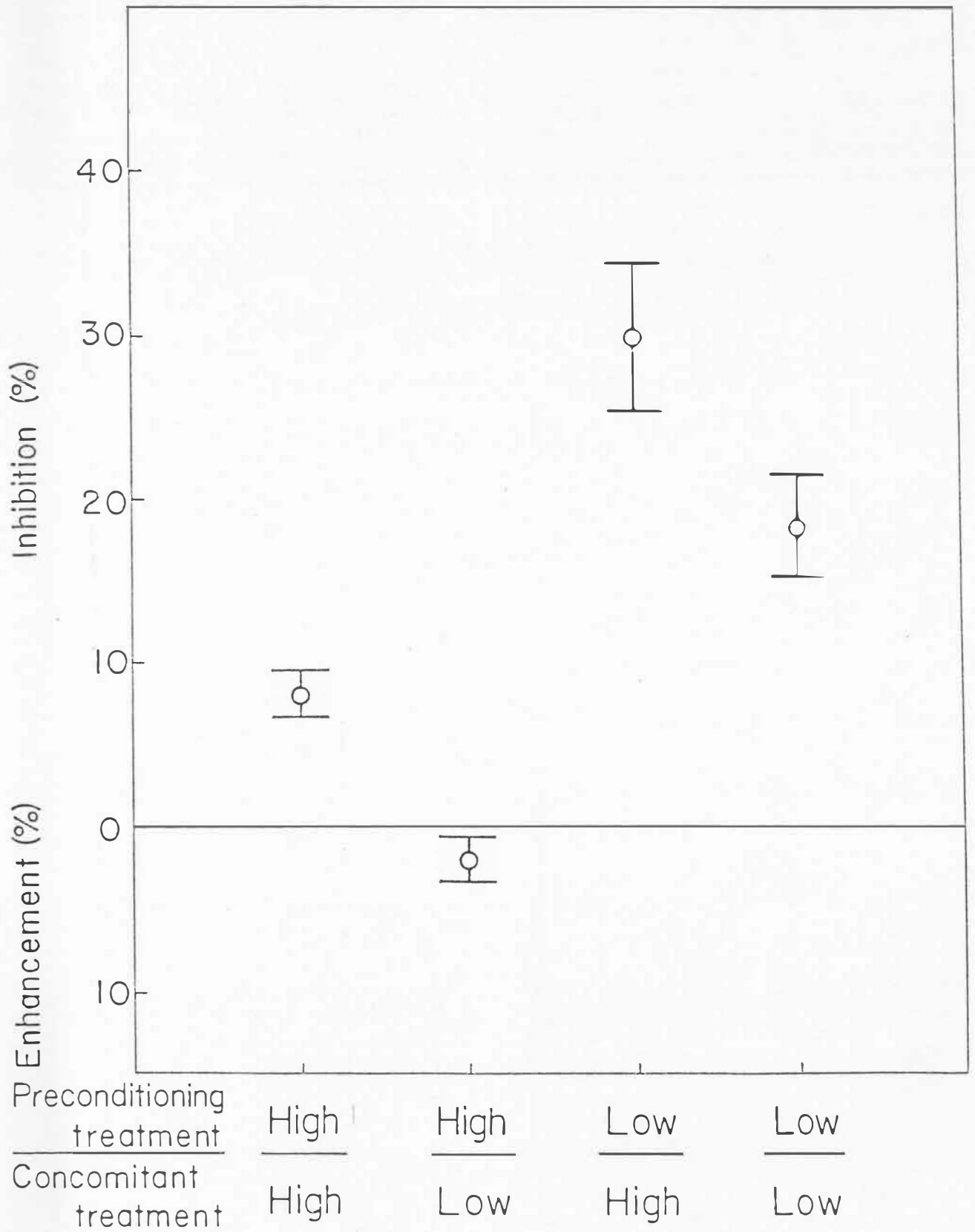
concomitant treatment. The least UV-B-induced photoinhibition was observed from those leaves that were preconditioned under high-light and received low-light as a concomitant treatment. Maximum photoinhibition by UV-B resulted from the combination of low-preconditioning light and high-concomitant light. No photoinhibition was observed in any of the control group combinations. Thus, the photoinhibition observed in the UV-B irradiated groups resulted from either the UV-B alone, or possibly a combination of UV-B and PPFD.

The results from the experiment indicated in Figure 9 suggest that the relative magnitude of UV-B-induced damage was similar for both light-limited and light-saturated photosynthesis. The relative inhibition for light-limited versus light-saturated photosynthesis was not significantly different within any treatment combination.

Fig 9.   Relative effects of UV-B irradiation on light-
         limited and light-saturated photosynthesis of
         leaves from each of the four PPFD-treatment
         combinations. Vertical bars represent $\pm$ 1
         standard error (n = 80). Points below the
         horizontal line represent photosynthetic
         enhancement. Points above the line the line
         represent photosynthetic inhibition.

○ = Light Limited Ps.
○ = Light Saturated Ps

| Preconditioning treatment | High | High | Low | Low |
| Concomitant treatment | High | Low | High | Low |

DISCUSSION

Flavonoids and related phenolic compounds in the outer leaf tissues are considered important in absorbing UV-B before it reaches sensitive photosynthetic targets (Van et al., 1977; Robberecht et al., 1980). Robberecht and Caldwell (1978) found that epidermal UV transmittance of plants grown under full sun tended to be less than other species grown under more shaded conditions. This corresponds with the increased flavonoid concentration observed in the high-PPFD-preconditioned soybeans from this study. Unfortunately, because of the difficulty involved in peeling the epidermal layer from soybean leaves, we were unable to measure their epidermal UV transmittance. It does seem plausible, however, that the reduced photoinhibition of plants grown at high PPFD may be the result of greater protection by UV screening compounds in the epidermis.

Radiation that penetrates the epidermis must be absorbed by sensitive chromophores in order to have an effect on photosynthesis. The UV chromophores for photosynthetic inhibition are not precisely known but suggestions include: plastoquinone (Mantai and Bishop, 1967), photosystem II reaction centers, points along the electron transport chain (Brandle et al., 1977), and thylakoid membranes (Mantai et al., 1970).

Photoreactivation is an enzyme-mediated, light-dependent repair of DNA damaged by UV (Cline et al., 1969; Sutherland, 1981; Setlow, 1966). Caldwell (1981), however, suggests that nucleic acids are not likely primary chromophores for photosynthetic damage. Thus, DNA damage and photosynthetic inhibition are, probably, fundamentally different photobiological reactions, and photoreactivation cannot be directly involved in the reduction of photosynthetic inhibition by UV-B. The possibility of any immediate repair or protective processes driven by visible irradiance is unlikely in view of the increased, rather than decreased, photoinhibition observed in soybean leaves that received high-PPFD as a concomitant treatment.

There are several changes in plant leaves that occur when plants are grown in high-PPFD environments (Boardman, 1977; Bjorkman et al., 1972). Some of these changes may be related to the decreased UV-B sensitivity observed in the sun-adapted soybean leaves. The two-fold increase in crude flavonoid concentration found in high-PPFD-preconditioned leaves may be partially responsible for the corresponding decrease of photoinhibition. Because of the UV absorption properties of flavonoid compounds, their presence in significant quantities in leaf tissue could provide an effective UV filter. Ultraviolet-B-induced damage to the thylakoid membrane system could be more pronounced in

shade-adapted leaves and result in greater photosynthetic inhibition than in sun-adapted leaves. A light harvesting protein (LHP) is thought to bind thylakoid membranes together into stacks (grana). Shade grown plants have a higher concentration of this protein, which may increase their energy transfer efficiency to both photosystem I and photosystem II (Boardman, 1977). If photosynthetic efficiency of shade-adapted leaves is more dependent on grana stacking, disruption of this LHP would limit photosynthetic rates to a greater extent.

While the pattern of response to UV-B demonstrated in this study indicates that the preconditioning treatment is of primary importance in determining photosynthetic sensitivity, the highly significant concomitant treatment effect should not be overlooked. It is unlikely that differences in gross morphological features between sun- and shade-adapted leaves account for the increased sensitivity to UV-B that occurs when soybean leaves concomitantly receive high-PPFD. The data presented here support the conclusions of Caldwell et al. (1982) that differences in sensitivity must involve more than simple differences in radiant flux reaching physiological targets in the chloroplasts. A mechanism through which the low-PPFD-concomitant treatment might reduce photoinhibition is presently unknown. The importance of concomitant PPFD suggests the need to investigate compounds whose absorbance

in the UV is altered as a function of incident PPFD. Machler and Nosberger (1980) have shown that PPFD has a strong influence on the activity of rapidly extracted ribulosebisphosphate carboxylase (RuBPcase). If the activated form of RuBPcase is more sensitive to UV-B irradiation, greater photosynthetic damage to leaves concomitantly exposed to high-PPFD would be expected. Additional research is needed to resolve this problem.

The reduction of both light-limited and light-saturated rates of photosynthesis could indicate that UV-B is simultaneously inhibiting fundamentally different parts of the photosynthetic process. At low-PPFD, the primary photosynthetic processes (light absorption and conversion into chemical energy) are rate-limiting. Light-limited photosynthetic rates are thought to depend on the number of chlorophyll molecules per photosynthetic unit, the number of functional reaction centers, and activity of the carboxylase system (von Cammerer, 1981; Bjorkman and Holmgren, 1963; Buchanan, 1980). Light-saturated photosynthesis in normal air is thought to be limited by the regeneration of RuBP, which in turn depends on the supply of ATP and NADPH and thus on electron transport capacity (Bjorkman, 1968; Gauhl, 1976; von Cammerer, 1981; von Cammerer and Farquhar, 1931). It is possible, however, that damage done to primary photoreactions could lead to inhibition of photosynthesis

in both low-light and saturating-light (Gauhl, 1976). Temperature, CO_2 concentration, and stomatal resistance also influence photosynthetic rates, but as these factors were kept relatively constant, they were probably not significant in this study.

This study emphasizes the need to carefully monitor PPFD when attempting to determine the effects of UV-B on photosynthesis. Since the soybeans used in this study were grown under artificial conditions, special care should be taken in predicting quantitative responses to UV-B radiation of plants that are grown under field conditions. Controlled field studies are probably essential to critically evaluate the effects of enhanced UV-B radiation on plants grown under more natural conditions.

LITERATURE CITED

Allen L. H., C. V. Vu, R. H. Burg, L. A. Garrard. 1977. Impact of solar UV-B radiation on crops and crop canopies. Prepared for Environmental Protection Agency BACER program. 100 pp. Available from: EPA, Washington, DC; EPA-IAG-D6-0168.

Arnon D. I. 1949. Copper enzymes in isolated chloroplasts: polyphenol oxidase in Beta vulgaris. Plant Physiol. 24: 1-15.

Bennet, J. H. 1981. Photosynthesis and gas diffusion in leaves of selected crop plants exposed to ultraviolet-B radiation. Journal Environ. Quality 10: 271-275.

Biggs, R. H., W. B. Sisson, M. M. Caldwell. 1975. Response of higher terrestrial plants to elevated UV-B irradiance. Pages 4-34 to 4-50 in D. S. Nachtwey, M. M. Caldwell, R. H. Biggs, eds. Impacts of climatic change on the biosphere. Part 1: ultraviolet radiation effects. U. S. Dept. Transportation, Washington, DC. Available from National technical Information Service, Springfield, VA. DOT-TST-75-55.

Bjorkman, O. 1968. Further studies on differentiation of photosynthetic properties in sun and shade ecotypes of Solidago virgaurea. Physiol. Plant. 21: 84-99

Bjorkman, O. and P. Holmgren. 1963. Adaptability of the photosynthetic apparatus to light intensity in ecotypes from exposed and shaded habitats Physiol. Plant. 16: 889-914

Bjorkman, J., N. K. Boardman, J. M. Anderson, S. W. Thorne, D. J. Goodchild and N. A. Pyliotis. 1972. Effects of light intensity during growth of Atriplex patula on the capacity of photosynthetic reactions, chloroplast components and structure. Carnegie Institute of Washington Year Book 71: 115-135.

Boardman, N. K. 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. 28: 355-377

Brandle, J. R., W. F. Campbell, W. B. Sisson, and M. M. Caldwell. 1977. Net photosynthesis, electron transport capacity, and ultrastructure of Pisum sativum L. exposed to ultraviolet-B radiation. Plant Physiol. 60: 165-169

Buchanan, B. B. 1980. Role of light in the regulation of chloroplast enzymes. Ann. Rev. Plant Physiol. 31: 341-374

Burnside, C. A. and R. H. Bohning. 1957. The effect of prolonged shading on the light saturation curves of apparent photosynthesis in sun plants. Plant Physiol. 32: 61-63.

Caldwell, M. M. 1968. Solar ultraviolet radiation as an ecological factor for alpine plants. Ecological Monographs 38: 243-268

Caldwell, M. M. 1971. Solar UV irradiation and the growth and development of higher plants. Pages 131-177 in A. C. Giese, ed. Photophysiology Vol. 6, Academic Press, New York, New York

Caldwell, M. M. 1979. Plant life and ultraviolet radiation: Some perspective in the history of the Earth's UV climate. BioScience 29: 519-525.

Caldwell, M. M. 1981. Plant response to solar ultraviolet radiation. Pages 169-198. in O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, eds. Encyclopedia of Plant Physiology, New Series, Vol. 12A. Physiological plant ecology: Responses to the physical environment. Springer-Verlag, Berlin.

Caldwell, M. M., R. Robberecht, R. Nowak, W. D. Billings. 1982. Differential photosynthetic inhibition by ultraviolet radiation in species from the arctic-alpine life zone. Arctic and Alpine Research 14:195-202

Fehr, W. R. and C. E. Caviness. 1977. Stages of soybean development. Agriculture and Home Economics Experiment Station. Available from: Iowa State University, Ames, Iowa. CODEN: IWSRBC (80) 1-12

Cline, M., G. I. Conner, F. B. Salisbury. 1969. Simultanious reactivation of ultraviolet damage in Xanthium leaves. Plant Physiology 44:1674-1678

Gauhl, E. 1976. Photosynthetic response to varying light intensity in ecotypes of Solanum dulcamara L. from shaded and exposed habitats Oecologia 22: 275-286

Hudson, R. D. and E. I. Reed. 1979. The stratosphere: Present and future. Chlorofluoromethanes and the stratosphere. NASA Ref. Publ. 1049 Sci. and Techn. Info. Serv., Springfield, VA.

Jurik, T. W., J. F. Chabot and B. F. Chabot. 1979. Ontogeny of photosynthetic performance in Fragaria virginiana under changing light regimes. Plant Physiol. 63: 542-547

Lugg, D. G. 1978. Dynamic changes in leaf characteristics and their relationships with photosynthesis in field-grown soybeans. PhD Dissertation, Cornell University, Ithica, NY.

Machler, F. and J. Nosberger. 1980. Regulation of ribulose biphosphate carboxylase activity in intact wheat leaves by light, CO_2, and temperature. Journal of Experimental Botany 31(125): 1485-1491

Mantai, K. E., and N. I. Bishop. 1967. Studies on the effects of ultraviolet irradiation on photosynthesis and on the 520-nm light-dark difference spectra in green algae and isolated chloroplasts. Biochem. Biophys. Acta 131:350-356

Mantai, K. E., J. Wong and N.I. Bishop. 1970. Comparison studies of the effects of ultraviolet irradiation on photosynthesis. Biochem. Biophys. Acta 197: 257-266

National Academy of Sciences. 1979. Protection against depletion of stratospheric ozone by chlorofluorocarbons. pp 392. Washington, D. C. Available from Office of Publications, National Academy of Sciences, Washington, DC.

Ostle, B., and R. Mensing. 1975. Statistics in research. Iowa State University Press, Ames, Iowa. 596 pp.

Robberecht, R. and M. M. Caldwell. 1978. Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. Oecologia 32: 277-287

Robberecht, R. M. M. Caldwell and W. D. Billings. 1980. Leaf ultraviolet optical properties along a latitudinal gradient in the arctic-alpine life zone. Ecology 61: 612-619

Sestak, Z. 1977. Photosynthetic characteristics during ontogenesis of leaves 1. chlorophylls. Photosynthetica 11: 367-448

Setlow, R. B. 1966. Cyclobutane-type pyrimidine dimers in polynucleotides. Science 153: 379-386

Setlow, R. B. 1974. The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proceedings of the National Academy of Sciences, USA 71: 3363-3366

Sisson, W. B. and M. M. Caldwell. 1976. Photosynthesis, dark respiration, and growth of Rumex patentiaL. exposed to ultraviolet irradiance (288-315 nm) simulating a reduced atmospheric ozone column. Plant Physiol. 58: 563-568

Sisson, W. B. and M. M. Caldwell. 1977. Atmospheric ozone depletion: reduction of photosynthesis and growth of a sensitive higher plant exposed to enhanced UV-B radiation. Journal Experimental Botany 28: 691-705

Sutherland, B. M. 1981. Photoreactivation. BioScience 31(6):439-444

Teramura, A. H. 1980. Effects of ultraviolet-B irradiances on soybean. I. Importance of photosynthetically active radiation in evaluating ultraviolet-B irradiance effects on soybean and wheat growth. Physiol. Plant. 48: 333-339

Teramura, A. H. and M. M. Caldwell. 1981. Effects of ultraviolet-B irradiance on soybean. IV. Leaf ontogeny as a factor in evaluating ultraviolet-B irradiance effects on net photosynthesis. Amer. J. Bot. 68: 934-941

Teramura, A. H., R. H. Biggs and S. Kossuth. 1980. Effects of ultraviolet-B irradiances on soybean. II. Interaction between ultraviolet- B and photosynthetically active radiation on net photosynthesis, dark respiration, and transpiration. Plant Physiol. 65: 483-488

Van, T. K., L. A. Garrard and S. H. West. 1976. Effects of UV-B radiation on net photosynthesis of some crop plants. Crop Science 16: 715-718

Van, T. K., L. A. Garrard and S. H. West. 1977. Effects of 298-nm radiation on photosynthetic reactions of leaf disks and chloroplast preparations of some crop species. Environmental and Experimental Botany 17: 107-112

von Caemmerer, S. 1981. On the relationship between chloroplast biochemistry and gas exchange of leaves. PhD Thesis, 189 pp. Australian National University, Canberra, Auatralia.

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

APPENDIX

APPENDIX

Determination of soybean leaf maturity requires identification of the leaf immediately above the one in question (Fehr and Caviness, 1977). As the seedling develops, the cotyledons emerge from the soil and remain directly opposite each other on the lower part of the main stem (Fig 1). Two unifoliate leaves, also located directly opposite each other, develop immediately above the cotyledons. The leaves that develop above the unifoliate leaves are trifoliate, and successively alternate from one side of the stem to the other. Young leaves have three leaflets that resemble cylinders. As leaf development progresses, each leaflet unrolls until the edges separate and the leaflet flattens. A leaf is considered fully developed when the leaf at the node above it has unrolled to the point that the edges of each leaflet are not touching.

Leaflet edges
not touching

Leaflet edges
touching

X-Section