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Protective mechanisms and acclimation to solar ultraviolet-B radiation in *Oenothera stricta*

by

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INTRODUCTION

Solar ultraviolet-B radiation (UV-B, 280-320 nm) may represent a significant, although subtle, environmental stress in the life history of terrestrial plant species (Caldwell 1968, Bogenrieder and Klein 1977, Caldwell et al. 1980, Robberecht et al. 1980). The shortwave solar UV radiation flux that prevailed during the development of early land plants has been suggested to have been an important factor in higher plant evolution, particularly in regard to the development of UV protection and repair mechanisms (McClure 1976, Caldwell 1979, Lee and Lowry 1980, Lowry et al. 1980). Although this intense shortwave UV radiation is no longer a factor in the terrestrial environment, solar UV-B radiation is sufficiently actinic to damage plant tissues and physiological processes of sensitive plants (Biggs et al. 1975, Sisson and Caldwell 1976, 1977, Brandle et al. 1977). As will be discussed in this paper, there are several plant adaptations that can either ameliorate or repair the damaging effects of UV-B radiation in plant tissue; adaptations that are integral to plant UV-B acclimation. The study centered

on those mechanisms that have the potential for protecting plant processes from injury and their role in plant acclimation to UV-B radiation. Furthermore, the degree of phenotypic plasticity in UV protective mechanisms and acclimation in view of the natural solar UV-B radiation flux and in an enhanced UV-B irradiance environment in the future was examined.

The effect of UV radiation on biological systems is highly wavelength dependent and comprises a relatively small proportion of the energy received from the total solar spectrum. Its capability to induce photochemical reactions in organisms can increase logarithmically with decreasing wavelength (Giese 1964, Setlow 1974, Caldwell et al. 1980). This is significant in regard to the absorption spectra of nucleic acids and proteins, which extend into the UV-B radiation spectrum and increase rapidly with decreasing wavelength. Upon absorption of UV radiation, changes in the structure of these molecules, as well as injury to plant tissues, may occur (Murphy 1975). Pyrimidine dimer formation in nucleic acids can interfere with normal DNA replication, and thus there is a significant interaction between these biologically important molecules and UV-B radiation.

The penetration of solar UV radiation through the atmosphere is restricted by stratospheric ozone absorption, which effectively prevents the penetration of radiation of wavelengths shorter than approximately 295 nm into the lower atmosphere. As illustrated by Caldwell et al. (1980), absorption of radiation in the atmosphere and UV effects in

OVERVIEW

This final report of our contract, which was initiated in October, 1975, is presented in the form of several sections pertaining to various facets of this research. Most of these reports are in the form of articles which have been published in the peer-reviewed scientific literature. All of these articles for reviews have been accomplished under the full or partial support of this contract. In addition to the published articles, three recent reports which have not yet been submitted for publication are included. These report results of work completed during the last six months of this contract. Studies of S. D. Flint concerning pollen sensitivity to ultraviolet radiation and ultraviolet optical properties of reproductive plant organs, the final study of R. Robberecht concerning acclimation potential of plants to UV-B radiation, and the final report of an action spectrum for inhibition of photosynthesis by UV radiation (Caldwell, Flint and Camp) constitute these three reports. The segments of this report are organized in chronological order.

Research accomplished in this contract is the result of several researchers which include: W. D. Billings, James Brandle, William Campbell, Judith Dickson, Stephan Flint, Fred Fox, Steven Holman, Susan Lindoo, Robert Nowak, Ronald Robberecht, William Sisson. The technical research assistance of L. B. Camp and Harvey Neuber and the electronic engineering assistance of Gary Harris and Charles Ashurst have been instrumental in this research. We are very pleased with the

results and productivity of research resulting from this contract.

We wish to express our appreciation to the National Aeronautics and Space Administration, both for the financial assistance provided and the continuity of this funding over the six year period of this contract. We also express our appreciation to D. S. Nachtwey, the Technical Monitor for this contract, for his continuing review of this research, the encouragement he has provided, and the stimulating discussion which have often led to new developments in our research program.

FINAL REPORT

TO

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

ON CONTRACT NAS-9-14871

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PROJECT LEADER

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The Ultraviolet Radiation Environment of Pollen and its Effect on Pollen Germination

DESTRUCTION of atmospheric ozone by chlorofluorocarbons is currently predicted to reach an equilibrium value of 6 to 16% depletion. Ultraviolet radiation, particularly at the shorter wavelengths of the UV-B region (280 - 320 nm), will increase considerably. For example, at a 16% ozone depletion under temperate, sea level conditions at the summer solstice, the model of Green et al. (1980) predicts an increase of 2.4 times current levels at 296 nm, but only an increase of 1.3 times the current level at 306 nm. The degree to which this radiation will damage terrestrial plants depends upon how they respond to different wavelengths in the UV waveband. Plant functions directly mediated by nucleic acids (as are some aspects of pollen germination) will be damaged more by this increase at shorter wavelengths than plant processes (such as photosynthesis) which are inhibited by longer wavelengths than those likely to interfere with DNA mediated processes (Caldwell 1981).

It is clear that radiation of short wavelengths (UV-C) can damage pollen to the extent of reduced viable seed set. Plant breeders, using UV-C (primarily the actinic peak at 254 nm produced by mercury lamps), have experienced an increase in the percent of shrunken kernels when irradiating maize pollen (Pfahler and Linskens 1977, Stadler and Uber 1942). Shorter duration treatments of wheat pollen (Faberge 1957)

produced no reduction in seed set although a number of seed were not viable (Steinitz-Sears and Sears 1957). However, due to the low penetrance of UV to the nuclei of pollen grains, and the variation in this penetrance due to the excentric position of the nuclei (Stadler and Uber 1942, Figure 2), this method has not gained widespread acceptance. Werfft (1951) treated ten species with UV-C for 3 hours and found in vitro pollen germination was decreased by 28% to 100%, depending on the species. Extrapolating the results of 254 nm treatments to UV-B induced damage may be tenuous, however (Nachtwey 1975).

Attempts to examine natural levels of solar UV radiation on pollen have been limited. Werfft (1951) found a reduction in the germination of pollen that had previously been placed in cellophane envelopes and exposed to sunlight; unfortunately it is uncertain whether this reduction in germinability is due to UV, heating of the envelope, or other factors. This work is, however, used as evidence that UV is injurious to plant pollen (Linskens 1964, Proctor and Yeo 1972), as is correlative evidence such as the increase in pollen pigments with increases in elevation (Neamtu and Boda 1970, as cited by Stanley and Linskens 1974). Recently, Pfahler (1981) found only a slight inhibition of germination in maize pollen treated during in vitro germination with UV-B from a filtered sunlamp, but a substantial decrease in the growth of the pollen tubes. Chang and Campbell (1976) found both an inhibition of pollen germination and a decrease in

pollen tube length in two clones of Tradescantia after 1.5h of UV-B irradiation simulating a somewhat more substantial decrease in atmospheric ozone than is currently predicted.

Before it is possible to accurately assess the significance of ultraviolet damage to pollen, one must have an idea of the radiation environment experienced by the pollen. This will be determined by the interaction of a number of events: the diurnal timing of flower opening and anthesis; the duration of pollen presentation prior to the activity of the pollen vector, the distance and time of transport, and by the optical properties and geometrical configuration of the flower.

To determine whether pollen receives any appreciable dose of UV before it is shed, we examined the UV transmittance of anthers and corollas. Since may flowers open only briefly, the corolla may play a substantial role in shielding the pollen before anthesis, and the anther wall provides protection between anthesis and dehiscence.

Once the pollen is shed from the anthers, it may not only be subjected to incoming solar UV, but also to UV reflected from the corollas. Since the geometry of some flowers may direct a portion of this reflected radiation toward the reproductive organs (Kevan 1975), we felt it was necessary to determine whether this reflection also occurred in the UV-B waveband. Though pollination biologists have

accumulated much UV reflectance data, it is often photographic and thus determined for a broad band in the UV-A region (Clark 1979, Abrahamson and McCrea 1977, Horovitz and Cohen 1972) with 350 nm the approximate lower bound (Hill 1977).

Tabulation of the abundant literature data on the diurnal timing of various aspects of pollination permits us to generalize further about the ultraviolet radiation environment of pollen, since UV intensity differs dramatically at different times of day.

Pollen germination is a time of intense physiological activity which is easily disrupted by chemicals (Stanley and Linskens 1974, Pfahler et al. 1980, Pfahler 1981) and has even been examined as a bioassay (Dashek et al. 1981). As pollen of many species is exposed to sunlight during germination on the stigma, UV-B radiation could also be physiologically damaging. The steadily accumulating literature contrasting the development and physiology of pollen from binucleate and trinucleate species (Hoekstra and Bruinsma 1979,1980) suggests the potential for differences in UV sensitivity and possible mechanisms to explain these differences.

METHODS AND MATERIALS- Timing of anthesis, pollination, and other life history parameters- Literature data, primarily from temperate latitudes, was tabulated to give a representative view of the time of pollen shed in wind pollinated species and times of pollen presentation in insect pollinated species. When only ranges of times were presented, the midpoints of the ranges were taken as the peaks. Histograms or additional data were available to clarify the time of peak pollen availability for some species. This data is depicted in histograms below a curve of the diurnal variation in DNA damaging UV radiation (Setlow 1974) striking a flat surface as calculated from the irradiance values predicted by the model of Green et al. (1980).

For statistical analysis the frequency of peak pollen availability was tabulated for the hour centering on solar noon (1130-1229), the periods one hour removed from solar noon (1030-1129 and 1230-1329), two hours removed, etc., resulting in seven periods. Insect and wind pollinated species were tabulated separately. The chi-square test was used to determine whether the number of occurrences in each time period was significantly different than if the species peaks were equally distributed from solar noon to 6 hours distant from solar noon. For insect pollinated species, the data was retabulated on the basis of time of first pollen shed, and the chi-squared test was applied to the time periods between 0530 and 1229. The proportion of species

possessing binucleate or trinucleate pollen in each time category was tabulated for the species attractive to insects using Brewbaker's (1967) list.

Optical properties of corollas and anthers- All material was obtained from fresh flowers grown in the greenhouse or collected from the field. Sources of material are indicated for each species in the figure legends. Flowers either were recently opened or about to open in the near future.

Transmittance was determined between 290 and 400 nm using the integrating sphere system of Robberecht and Caldwell (1978). The regular 2 mm diam circular sample port was used for all corolla tissue except the inner corolla of Lupinus meridanus where it was necessary to use a micro-sample port (0.8 X 1.2 mm slit) in order to examine a relatively flat corolla section. As this species has two layers of corolla tissue covering the floral parts, transmittance values of the inner and outer corollas were multiplied together. For all other species, only single layers of corolla tissue were used, even though the corolla may be heavily imbricated before the flower opens. Samples were oriented so that transmittance was measured from the exterior to the interior of the corolla, which is representative of flowers ready to open or corollas shading the reproductive organs.

Anther transmittance was determined with the micro-sample port described earlier. All anthers were collected in the morning before dehiscence and kept at sufficient humidity to prevent dehiscence while they were transported to the lab. Dissecting needles were used to break the anther open, remove pollen, and obtain a single thickness of the anther wall.

Corolla reflectance between 290 and 400 nm was measured using the integrating sphere with a modification to allow reflectance measurements to be made on intact disks of tissue (Robberecht et al. 1980). Fresh, recently opened flowers were used for all measurements.

Ultraviolet treatment of dry pollen- Pollen was treated in dry petri plates with filters covering the plates as is subsequently described for the pollen germination tests. Pollen viability was determined with a fluorescein diacetate stain (Heslop-Harrison and Heslop-Harrison 1970).

Ultraviolet irradiation during pollen germination- Variability in pollen germination between replicates of the same treatment has been a continual source of frustration for many workers in experiments where statistical analyses of pollen germination is necessary. Some workers have minimized this problem by switching from an agar medium, which often induces different percentages of pollen to germinate due to differing water-restricting conditions on the surface, to a

solution of sucrose and nutrients. These liquid media produce a more uniform (repeatable) germination percentage between replicates (Hoekstra and Bruinsma 1975a). For our purposes, where we are examining germination during ultraviolet irradiation, a liquid media is unacceptable since the radiation could be partly attenuated before it reached the pollen grain. Thus, we have found it necessary to minimize other sources of variation.

In all our germination experiments, pollen was sprinkled onto a solidified medium containing sucrose (15% for all species except Geranium viscosissimum which required 34% sucrose), 0.6% agar, 0.03% $CaNO_3$ (to minimize the effects of sowing pollen at varying densities (Linskens 1964)), and 0.01% Boric acid (a germination promoter whose exact metabolic role has yet to be elucidated (Lewis 1980)). This medium was applied to a microscope slide (3 ml per slide) which was placed into a shallow petri plate. Filter paper under the slide was moistened with a salt solution to keep the humidity at 97% (excess humidity causes condensation atop the media) and the plastic filter (0.13 mm thick Mylar D for the control or 0.13 mm thick cellulose acetate for the UV treatment) was secured over the dish and sealed with petroleum jelly to achieve the desired humidity. The plates (3 to 5 replicates per experiment) were assembled alternatingly between the treatment and control and were immediately placed under FS-20 lamps in a constant 25C incubator. No other illumination was provided.

Germination was terminated with a killing and preservative solution (Pfahler 1981). Percent germination was determined by microscopic examination (40X or 100X) of the slides. Data was analyzed with a paired t-test (after using the Arcsin (proportion)$^{-2}$ transformation). The paired t-test was most appropriate as it permitted a comparison of slides sown with pollen sequentially, and thus usually similar in their inherent pollen viability.

During the initial stages of this research, the ultraviolet irradiation of the lamp and filter system was determined at 2 nm intervals with a modified Gamma Corp. high resolution, grating spectroradiometer (Model 2900 Photometer and Model 700-3 Monochromator). Later a double grating Optronic monochromator (Model 742), which measured at 1 nm intervals, was used in all experiments. The particular instrument used is designated in Tables 1 and 2. All irradiance values were weighted with the DNA-damage action spectrum of Setlow (1974).

In all species used, the stigmatic surface would naturally be exposed to sunlight during pollination. Papaver rhoeas cv. shirley single (Pappaveraceae) is a strain of the common European field poppy (Bailey and Bailey 1976). Scrophularia peregrina (Scrophulariaceae) is a European annual capable of self pollination (Shaw 1962). The annual Cleome lutea (Capparidaceae) and the perennial Geranium

viscosissimum var. nervosum (Geraniaceae) occur in the Great Basin of the western U. S. Brewbaker (1967) reports the genera Papaver, Cleome, and Scrophularia possess binucleate pollen while taxa in the Geraniaceae are trinucleate.

RESULTS-Timing of anthesis- The presentation of pollen in relation to the diurnal fluctuation of DNA damaging UV radiation is shown in Figures 1 and 2. Figure 1 presents data on peak pollen shed from wind pollinated species while Figure 2 presents the times of peak and initial pollen availability in species the various investigators considered to be attractive to insects. The data used for peak times of pollen availability are presented in Appendices 1 and 2.

The patterns of pollen presentation differ substantially between wind and insect pollinated species. When compared with a scenario of equal peak pollen presentation over the 0530-1830 period, species attractive to insects show significantly more reports of pollen presentation between 1030 and 1330 ($P<0.01$) and significantly less in the periods 5 and 6 hours away from solar noon ($P<0.005$). The proportion of trinucleate species in this group does not change over the course of the day. If one looks at the time of first presentation of pollen instead of the peak or midpoint of presentation, a vastly different picture emerges. Between 0630 and 0929, the number of species presenting pollen is significantly greater ($P<0.025$) than expected, while between 0930 and 1229 it is significantly ($P<0.025$) lower than

Figure 1. Diurnal times of peak pollen shed from plants with wind dispersed pollen, depicted with the relative intensity of DNA damaging UV radiation throughout the day. Data used to compile the pollen histogram are presented in Appendix 1.

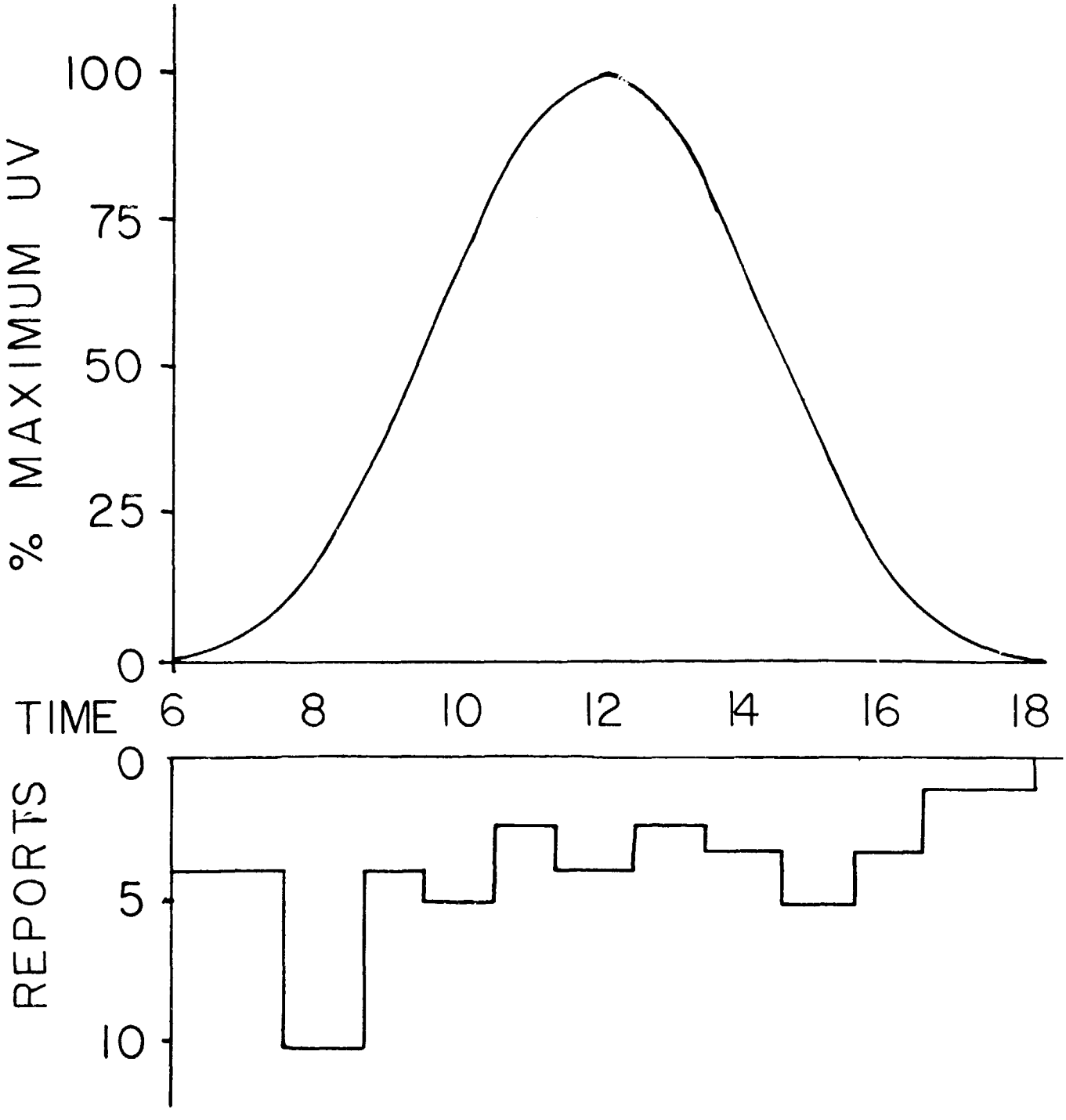
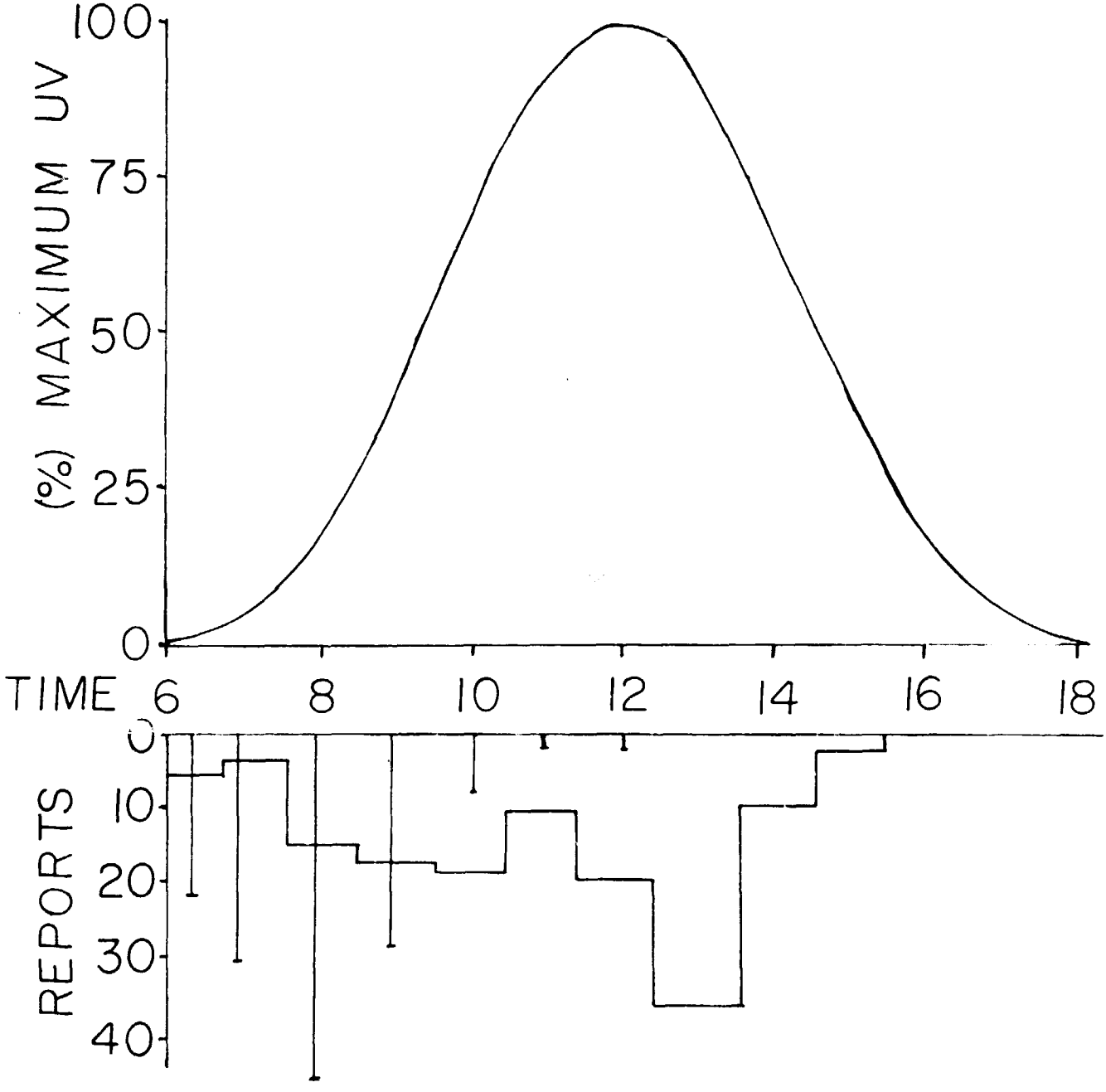


Figure 2. Diurnal times of pollen presentation in plants whose pollen is attractive to insects, depicted with the relative intensity of DNA damaging UV radiation throughout the day. Peaks or midpoints of pollen presentation are represented by the bar histogram while times of first pollen presentation are represented by the narrow lines. Data used to compile the peak of pollen presentation histogram are given in Appendix 2.

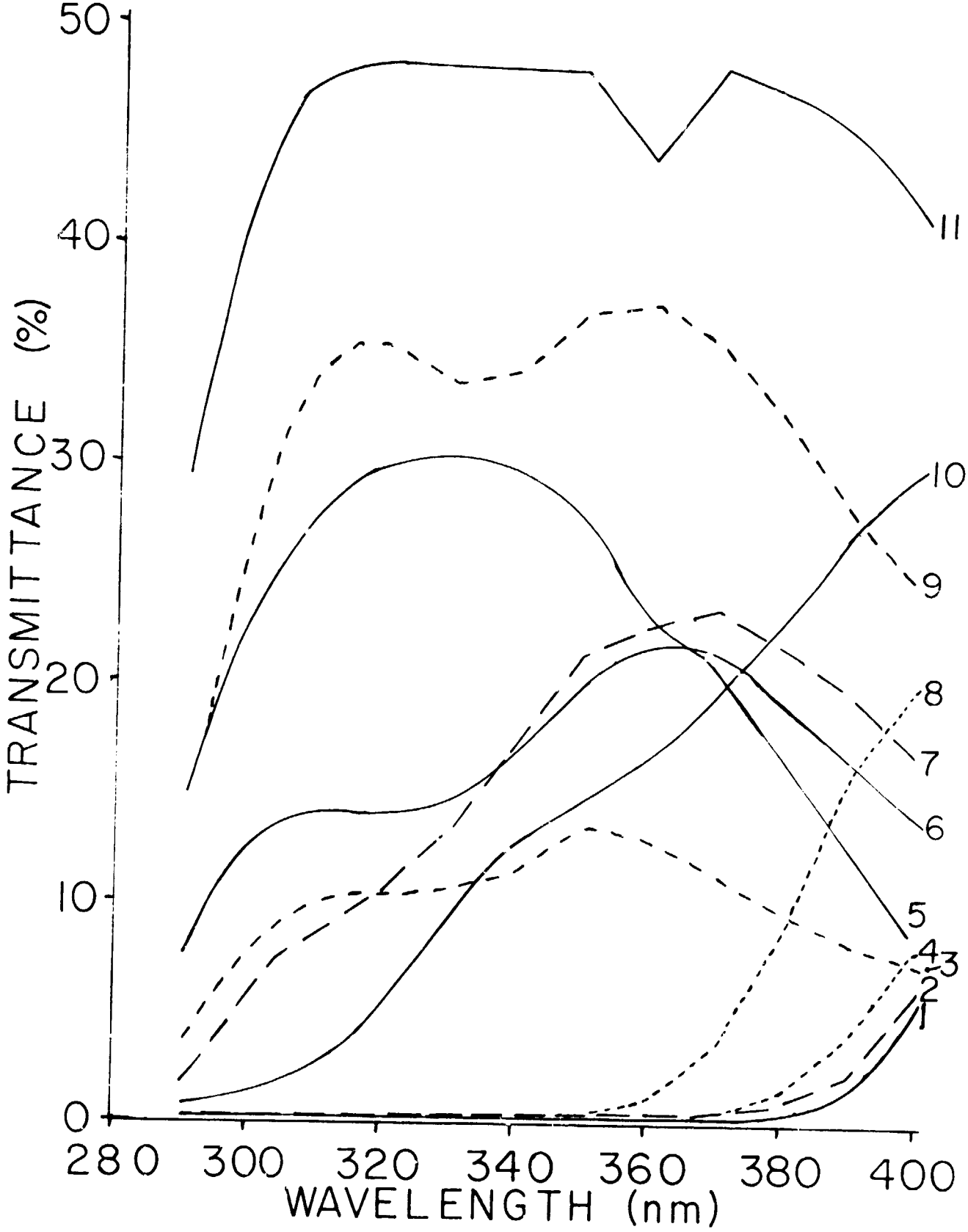


expected. Wind pollinated species show a much more uniform spread of pollen presentation with only one significant ($P<0.05$) increase over the expected frequency, at four hours away from solar noon. This was primarily due to the 0730-0830 period.

Differences in altitude, albedo of adjacent surfaces, atmospheric aerosols, and ozone concentration have little effect on the proportional distribution of UV throughout the day. The UV data depicted in Figures 1 and 2 represent global irradiation (dirrect + diffuse) for midsummer conditions in temperate latitudes. At other times of year, when daylengths are shorter, the curve will show an even narrower peak. At different latitudes only slight changes will be seen in the shape of the curve. If irradiance is calculated incident on a surface normal to a line between the surface and the sun, rather than incident on a horizontal surface, again only slight changes will be seen in the shape of the curve.

Corolla transmittance- Figure 3 shows the UV transmittance through the corollas of ten species. In general, corollas severely attenuate UV-B, although there are marked differences between species and even between corolla colors within a cultivar. Corollas of Sphaeralcea emoryi (curve 9) and Portulaca grandiflora (curves 5 and 11) transmit substantially more UV-B than any of the other species examined. Data are not shown for Petuniaxhybrida and Hibiscus trionum corollas, as their UV transmittance was so

Figure 3. Transmittance of ultraviolet radiation through a single layer of corolla material. 1.*Sidalcea neo-mexicana*, 2.*Geranium viscosissimum* var. *nervosum*, 3.*Brassica juncea*, 4.*Lupinus meridanus*, 5.*Portulaca grandiflora* cv. double moss rose (gold corolla), 6.*Cleome lutea*, 7.*Colutea arborescens*, 8.*Chicorium intybus*, 9.*Sphaeralcea emoryi*, 10.*Erodium cicutarium*, 11.*Portulaca grandiflora* cv. double moss rose (cream corolla). All corollas except those from *Colutea* and *Chicorium* were from greenhouse grown plants.


50
40
30
20
10
0
TRANSMITTANCE (%)
280
300
320
340
360
380
400
WAVELENGTH (nm)
11
10
9
8
7
6
5
4
3
2
1

low it would fall below all the other curves in Figure 3.

Anther transmittance- Figure 4 shows the very low level of UV transmittance through the anthers of five species. One additional species, Tulipa gesneriana, is not shown in the figure as its anthers were essentially opaque to all wavelengths between 290 and 400 nm.

Corolla reflectance- In most of the 20 species examined, UV-B reflectance was rather low. Six species (Geranium visvosissimum var. nervosum, Grindelia squarrosa, weedy Helianthus annuus, Eschscholzia californica, the white portion of Hibiscus trionum and the light yellow portion of Abelmoschus esculentus (okra) corollas) had UV reflectance values that were about one percent or less, and thus are not shown in Figure 5. Six species showed low reflectance values across the entire waveband (Figure 5A.), while another six species showed increasing reflectance at wavelengths approaching the visible waveband (Figure 5B.). Reflectance of four species peaked in the UV-A waveband (Figure 5C.). This data is summarized in Figure 6 and compared with a tabulation of Macior's (1978) data for the visible waveband. Sphaeralcea emoryi does not conform to the generalization of low UV reflectance, just as it did not conform to the generalization concerning UV transmittance through the corolla. At 320 nm, its reflectance was 30%.

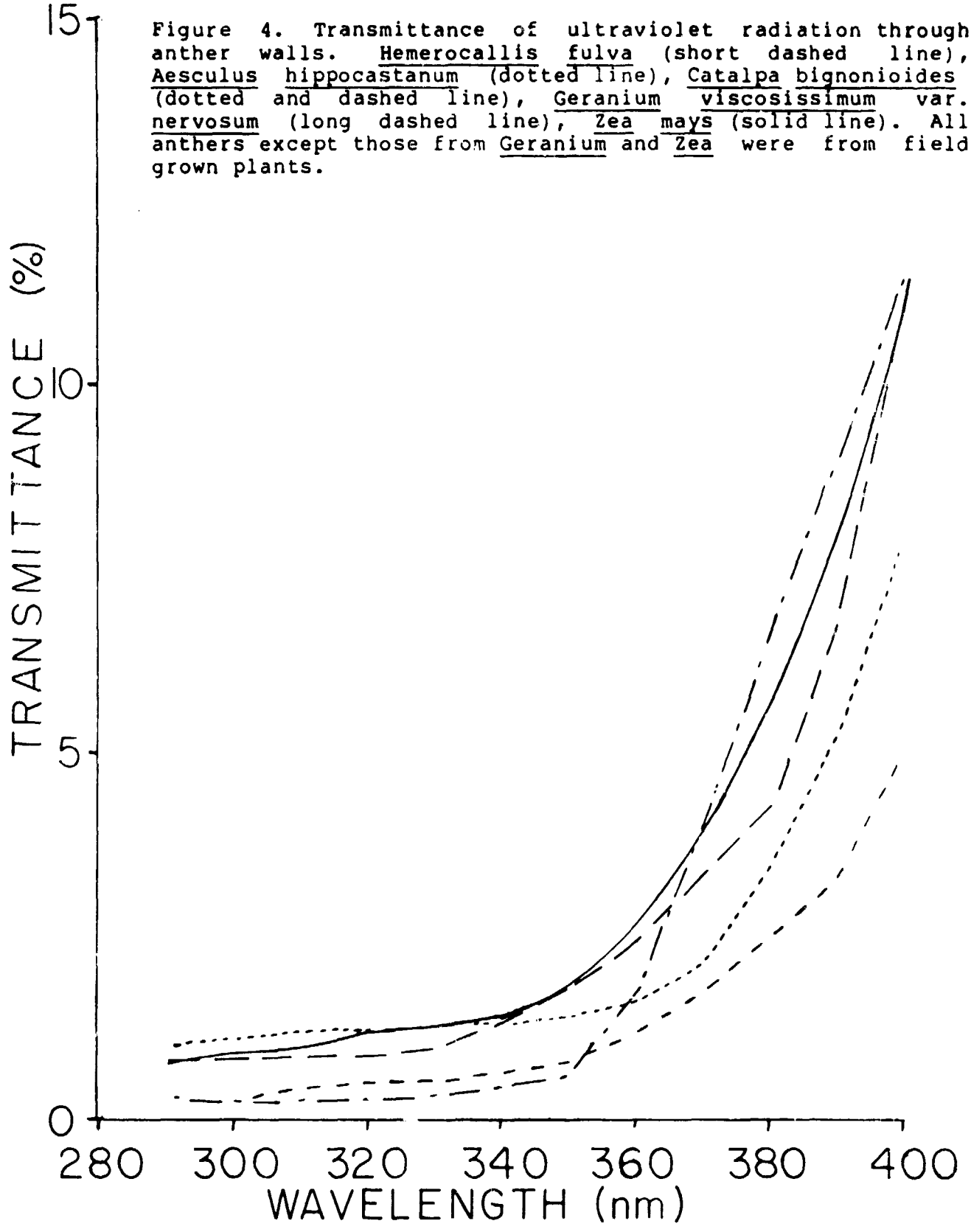
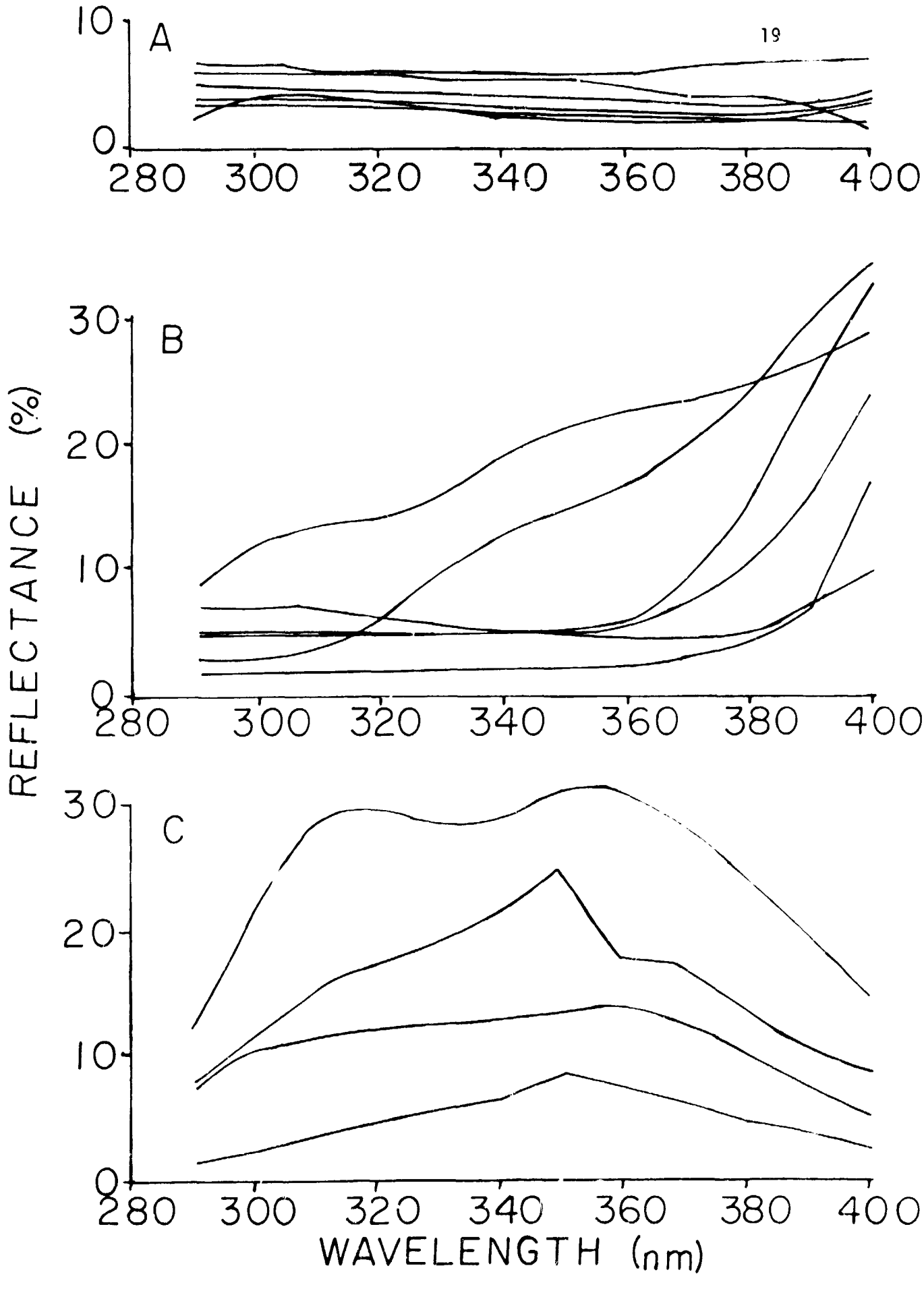


Figure 4. Transmittance of ultraviolet radiation through anther walls. Hemerocallis fulva (short dashed line), Aesculus hippocastanum (dotted line), Catalpa bignonioides (dotted and dashed line), Geranium viscosissimum var. nervosum (long dashed line), Zea mays (solid line). All anthers except those from Geranium and Zea were from field grown plants.

Figure 5. Reflectance of corolla material in the UV-A and UV-B wavebands. Greenhouse and field grown plants are marked (G) and (F), respectively. 5A.: Species with low reflectance, arranged in order of decreasing reflectance at 350 nm: Hibiscus trionum (purple portion of corolla) (F), Abelmoschus esculentus (maroon portion of corolla) (F), Sidalcea neo-mexicana (G), Tulipa gesneriana (F), Convolvulus arvensis (F), Brassica fimbriata (F). 5B.: Species whose reflectance increases at longer wavelengths, arranged in order of decreasing reflectance at 400 nm: Erodium cicutarium (G), Chicorium intybus (F), Campanula rapunculoides (F), Malva neglecta (F), Rubus idaeus (F), Papaver rhoeas (G). 5C.: Species whose reflectance peaks in the UV-A waveband, arranged in order of decreasing reflectance at 350 nm: Sphaeralcea emoryi (G), Oenothera stricta (G), Verbascum thapsus(F), Brassica juncea (G).


A
B
C
10
0
30
20
10
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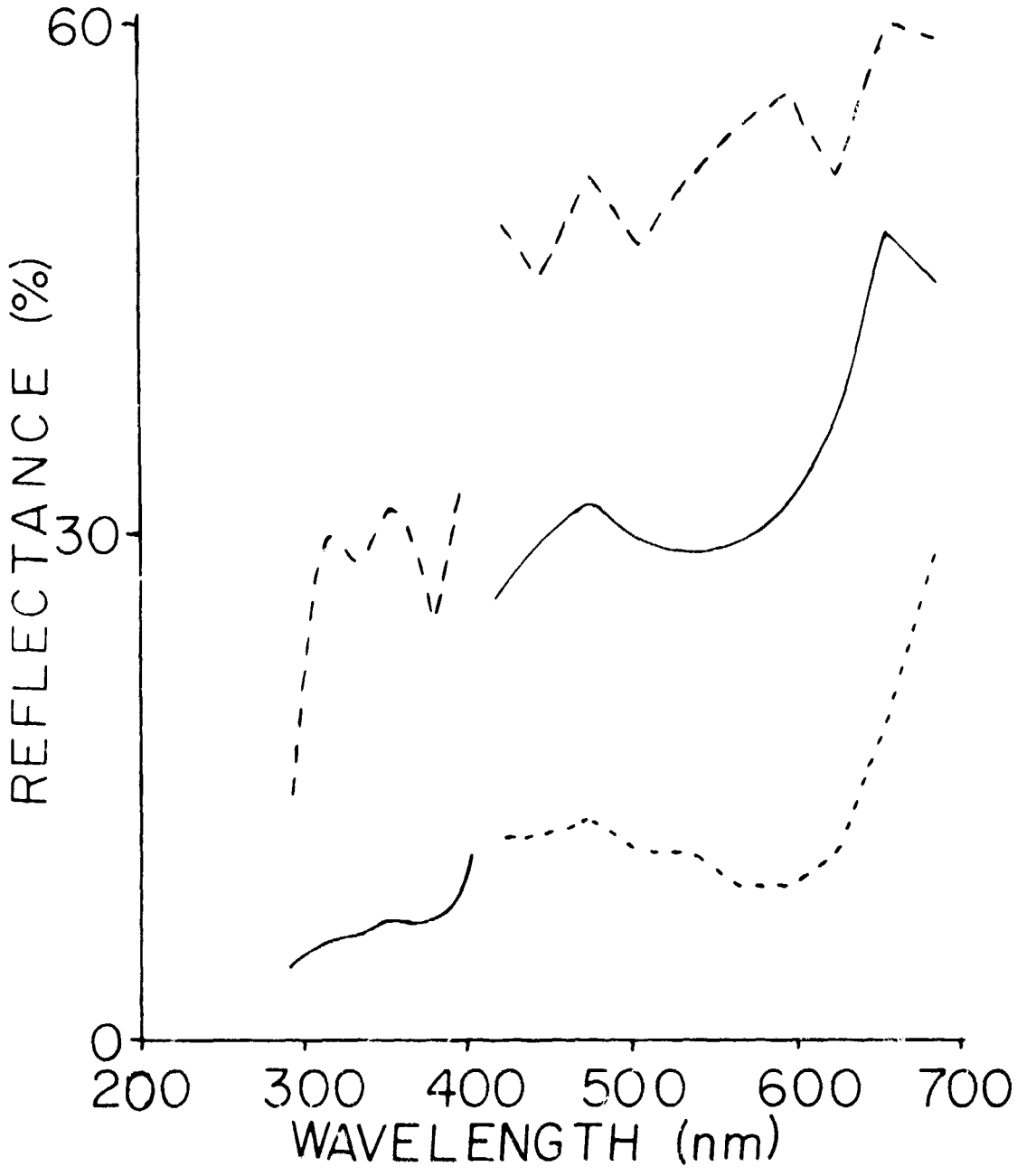
280
300
320
340
360
380
400
REFLECTANCE (%)
WAVELENGTH (nm)

Figure 6. Corolla reflectance. Summary of our data (290-400 nm) in the UV waveband and Macior's (1978) data (415-685 nm) in the visible waveband. Maximum (dashed line), mean (solid line), and minimum values are shown, except for minimum UV reflectance values, which approached zero.



Pollen viability and germination- Substantial doses of UV-B caused no dramatic mortality in dry pollen (Table 1) as indicated by the fluorescein diacetate stain but caused substantial decreases in germination of some species when the irradiation was supplied during germination (Table 2). The three binucleate species showed much greater sensitivity to UV-B irradiation during germination than the trinucleate Geranium pollen, whose germination was not generally inhibited. Data which could explain possible mechanisms for this difference are given in Table 3. Considerable unexplained variation was seen between replicated experiments of both Papaver and Geranium. Dose rates approaching current levels, such as the first experiment with Cleome, failed to produce significant inhibition, however, extremely high dose rates which are several times current levels never completely prevented germination in Papaver.

DISCUSSION- Timing of anthesis- Among wind pollinated species there is only one significant increase (0730-0829) over an equal distribution of times of peak pollen shed throughout the day. Still, the majority of reports of pollen shed do not occur during the 5 hours of most intense UV centered on solar noon as only 33% fall within this period. The remaining peaks occur in the other 8 hours of the survey.

This situation is more complex for plants whose pollen is attractive to insects. Sixty-nine percent of these reports have their peak pollen presentation times during the

Table 1. Viability following irradiation of dry pollen (as indicated by the fluorescein diacetate stain). Dose rates measured with the Gamma monochromator.

Species	Dose rate[1.]	Sample size[2.]	Control viability	% inhibition
Portulaca grandiflora cv. double moss rose	8.8	270	77%	1
Plantago lanceolata	23.5	850	75%	7

1. $mW\ m^{-2}$ DNA effective irradiation.
2. Number of pollen grains sampled.

Table 2. Inhibition of pollen germination by UV-B radiation during the germination period. All treatments were for 3 hours except the first Papaver treatment (which was 2 hours treatment followed by 1 hour of darkness) and the second Cleome treatment which was 3.3 hours.

Species	Dose rate[1]	Sample size[2]	Control germination (percent)	Percent inhibition	P level
Binucleate					
Papaver rhoeas (G)	10.9	2400	38.5	26.0	P<0.10
	10.9	2400	60.1	14.0	NS
	9.6	2400	51.3	35.0	P<0.05
	22.0	1800	50.4	23.0	P<0.05
	22.0	2400	60.1	35.0	P<0.05
	27.3	1800	36.7	52.0	P<0.10
Cleome lutea (G)	6.5	2400	84.5	2.4	NS
	9.6	2400	29.9	33.0	P<0.10
Scrophularia peregrina (O)	11.7	2660	60.0	53.0	P<0.025
Trinucleate					
Geranium viscosissimum var. nervosum (O)	11.7	2350	17.0	(12.0)[3]	NS
	11.7	2150	16.6	7.0	NS
	11.7	2900	24.4	20.0	P<0.10
	11.7	4050	14.2	7.0	NS

1. mW m^{-2} of DNA effective radiation. (G) = Gamma monochromator, (O) = Optronic monochromator.

2. Number of pollen grains sampled.

3. Enhancement.

5 hours of most intense mid-day UV, yet 93% of the reports show that pollen shed is initiated before 0930. Thus, data showing the time of the actual pollination event are critical to estimating the degree to which solar UV radiation could potentially interfere with plant reproduction. This type of data is, unfortunately, not generally available. Bagging experiments conducted in the field with summer squash have shown pollination (as represented by fruit set) to be nearly 90% complete by 0800 (Tepedino 1981), yet this may not be representative of most higher plants.

The proportion of pollinators carrying nectar rather than pollen over the course of a day may indicate the depletion of pollen supplies and thus suggest when pollination takes place. Brassica (Free and Nuttall 1968), Taraxacum (Free 1968b), and sometimes apple (Free 1968b) show trends suggesting a depletion of pollen supplies later in the day. Bee loads of strawberry pollen peaked at 1100 (Free 1968a), which corresponded to the morning pollen presentation peak in this species (Percival 1955). Raspberry pollen, which is shed more equally throughout the day (Percival 1955) did not show a similar peak (Free 1968a). This suggests pollen removal may occur fairly rapidly and that an examination of times of pollinator activity alone would contribute little to an understanding of when pollination takes place.

Anther transmittance- Despite the use of the microsample port, only particularly large anthers could be examined with this system. While these four species include both monocots and dicots and binucleate and trinucleate species, it is possible that our use of only large anthers biases our sample. The largest anthers examined (Tulipa gesneriana) were sufficiently thick that they were totally opaque to UV. Perhaps smaller anthers are somewhat more translucent to UV. Still, even if there was a substantial increase in UV transmission in smaller anthers, possibly due to thinner walls, our data suggests the anther walls would still offer substantial protection from UV-B.

Corolla transmittance- If one considers the relatively low UV-B transmittance of most corollas, the manner in which they are imbricated before the flower opens, and the low UV-B transmittance of anthers, pollen will be found to be nearly totally protected from potential UV-B damage before the flower opens and still well protected until anthesis. Thereafter its exposure may vary greatly from species to species depending upon flower geometry, time and method of pollination, etc.

Flowers whose geometry never exposes pollen to solar UV, such as the legumes Colutea and Lupinus which we sampled, provide a low UV-B environment for pollen at all times except during dispersal. Exposure to sunlight during dispersal in insect pollinated plants may be very brief as a large percent

of pollen is redeposited onto the next several flowers visited (Waddington 1981). This often occurs within very short distances (a few meters with many even less than a meter) (Hodges and Miller 1981, Schmidtt 1980, Waddington 1981).

Corolla reflectance- Kevan (1979) states that UV reflectance from flowers is usually less than 40%. In an examination of Arctic flowers, he found that, by 360 nm, reflectance of whole flowers was often minimal (Kevan 1972). When analyzed as component parts, he found even highly reflective areas never exceeded 40% reflectance at 360 nm. Thompson et al. (1972) show similar data for supposedly highly reflective nectar guides; peak reflectance was only 20% at about 350-360 nm.

In a qualitative survey of 300 species, Guldberg and Atsatt (1975) were able to correlate UV reflectance with corolla color. Among yellow and violet flowers, which most frequently reflect UV, only about half the flowers were classified as UV reflectors. Ultraviolet reflectance levels thus seem to be sufficiently low that it is doubtful this reflected UV significantly increases the radiation environment of the pollen of most species. It is also interesting to note that the species Kevan (1975) found to be "solar furnaces", Dryas integrifolia and Papaver radicatum have very low reflectance values at 360 nm (Kevan 1972, Figures 12 and 13). It is doubtful this reflectance level

would increase in the UV-B as we never saw any marked increases in the UV-B regions of our spectral scans.

Pollen viability- Assessment of pollen viability with the fluorescein diacetate stain is considered to be a valid predictor of germinability (Shivanna and Heslop-Harrison 1981, Ockendon and Gates 1976). As dry pollen treated with high dose rates of UV radiation showed little decrease in viability, we believe solar UV will be only a minor factor in the mortality of dry pollen. It is still possible that more subtle damage could interfere with processes such as seed set, however.

Pollen germination during ultraviolet irradiation- Though many of the dose rates we used are in excess of what is expected under current scenarios of ozone depletion, they clearly show that germination of binucleate pollen may be inhibited by radiation in the UV-B waveband. Species shedding trinucleate pollen, which are estimated to comprise 30% of all taxa (Brewbaker 1967), may be much less susceptible. Dose rates which caused inhibition of binucleate pollen germination failed to consistently do so in trinucleate pollen of Geranium. When inhibition was seen, it was much less than was found in binucleate pollen and seldom statistically significant. A similar situation is seen in the trinucleate pollen of maize where a high dose rate produced 4% enhancement and a very high dose rate produced 7% inhibition of germination (Pfahler 1981).

Though we did not quantify time to germination, occasional observations suggested that Geranium pollen germinated earlier than the binucleate species being used. It is possible that lag time to germination differed among samples of the same species, accounting for some of the variability.

The species we germinated under UV-B irradiation were insect pollinated. Stanley and Linskens (1974) claim wind pollinated species are less sensitive to UV radiation. However, as Whitehead (1969) suggests that pollen grains with thin exines would be dispersed by wind more readily than those with thick exines, and the exine (at least in maize) is the location of UV absorbing flavonoids (Pfahler 1978), we would expect wind pollinated species to be more sensitive to UV radiation.

The presence of two types of pollen, that shed with two nuclei as compared to three nuclei, has been well known since Brewbaker's (1967) tabulation of 265 families. It is only recently, however, that extensive physiological comparisons have been made between the two pollen types and a third category, the intermediate or "advanced" binucleate type (Hoekstra and Bruinsma 1979).

Table 3. Comparison of binucleate, "advanced binucleate", and trinucleate grains with regards to physiological, developmental, and protective factors which may confer differential sensitivity to UV-B radiation.

Factor	Binucleate	"Advanced Binucleate"	Trinucleate	References
Physiological development:				
Presynthesized RNA	yes	yes	?	Mascarenhas (1975,1978) Frankis and Mascarenhas (1980)
Photoreactivation enzymes	?	?	yes (maize)	Ikenaga et al. (1974)
Second mitosis	arrested at prophase of mitosis when shed		≥ 1 day before shed	Brewbaker and Emery (1962) Mascarenhas (1975)
Ribosomes	monoribosomes	polyribosomes	Asteraceae: few maize: many polysomes	Hoekstra and Bruinsma (1979)
Mitochondrial development at dehiscence	undeveloped	nearly complete at dehiscence		Hoekstra (1979)
Alternative oxidase pathway	no	no	yes	Hoekstra and Bruinsma (1980)
Respiration rate	low	low	high	Hoekstra (1979) Hoeksta and Bruinsma (1975b, 1979)

Table 3 (continued)

Factor	Binucleate	"Advanced Binucleate"	Trinucleate	References
Developmental times:				
Germination (min. till start)	(5-70 min.) n=14 md.=28 min.	(2-6 min.) n=2	(2-30 min.) n=11 md=10 min.	2.
Protein synthesis (min. till start)	10 min.	2 min.	never any substantial level after dehiscence	Hoekstra and Bruinsma (1979)
Stigma penetration (min.)	75 min. n=1	--	(7-30 min.) n=3 md=15 min.	Heslop-Harrison (1977) Hoekstra and Bruinsma (1978) Sastri and Shivanna (1979) Wilms (1980)
Fertilization	(3.5 h.-5 days) n=13 md=3 days	--	(30 min.-24 h.) n=3 md=6 h.	3.
Rate of tube growth (mm h^{-1})	(0.1-1.5 mm h^{-1}) n=11 md=0.2 mm h^{-1}	--	(6-20 mm h^{-1}) n=2	4.
Protection:				
Diffusable screening compounds	high	?	low	Kirby and Smith (1974)
Stigma surface	both wet and dry stigmas present	dry	tend to have dry stigmas	Heslop-Harrison and Shivanna (1977)

Table 3 (continued)

1. md.=mode

2. Cass and Peteya (1979), Cresti et al. (1980), Heslop-Harrison (1977), Hoekstra and Bruinsma (1978,1979), Lehman and Puri (1967), Mascarehhas (1978), Nakamura(1978), Sastri and Shivanna (1979), Sedgley (1977), Sedgley and Buttrose (1978), Wilms (1980).

3. Ascher and Peloquin (1966), Heslop-Harrison (1977), Hoekstra and Bruinsma (1978), Hopping and Jerram (1979), Kroh et al. (1979), Lee (1980), Sastri and Shivanna (1979), Sedgley (1977), Sedgley and Scholefield (1980), Socias i Company et al. (1976), Stott (1972), Thompson and Liu (1973).

4. Ascher and Peloquin (1966), Facteau et al. (1973), Gilissen and Branties (1978), Heslop-Harrison (1977), Hoekstra and Bruinsma (1978), Hopping and Jerram (1979), Kroh et al. (1979), Lee (1980), Lehman and Puri (1967), Nakamura (1978), Redalen (1980), Sedgley and Buttrose (1978), Williams and Maier (1977).

Table 3 presents a summary of the extensive physiological, developmental, and structural differences which may influence the UV sensitivity of these three pollen types. For brevity, references for the statements in the following discussion are not repeated it they have already been cited in Table 3.

Before binucleate grains germinate, mitochondria must first complete their development. Protein synthesis (measured as leucine incorporation) does not begin till about 10 minutes, as only monoribosomes are present at dehiscence. As there is a considerable lag time to germination (in comparison to advanced binucleate and trinucleate species), UV could potentially interfere with these metabolic processes as the pollen grain prepares to germinate. It is likely that, if physiological data were available, some of the rapidly germinating species tabulated as binucleate would be reclassified as "advanced binucleate" and the modal germination lag time would actually be greater than our 28 minute value.

Advanced binucleate grains begin protein synthesis within 2 minutes, as their mitochondria are highly organized at dehiscence and polyribosomes are present rather than monoribosomes. Thus, there is less potential for UV damage, both due to the decreased lag time before germination and fewer processes subject to damage.

Trinucleate grains (generally low in ribosomes) stand no chance of suffering a decrease in germination because of UV damage to protein synthesis since there is never any substantial synthesis in most trinucleate species after dehiscence. The presence of many poly-ribosomes in some trinucleate grains (e.g., maize) is attributed to the need for the pollen tubes to grow through the long style. These grains germinate rapidly and stigma penetration occurs more quickly than in binucleate species, although data on times to stigma penetration are sparse for all types of pollen.

When incubated under humid conditions, trinucleate grains exhibit respiration rates several times greater than binucleate grains. The two types of pollen also differ in the biochemistry of their respiratory systems; the alternative oxidase system is significant only in trinucleate species. While it is difficult to speculate on how these two respiratory systems could contribute directly to their UV-B sensitivity, Hoekstra and Bruinsma (1979, 1980) hypothesize that trinucleate grains conduct "nonsense biosynthesis" and are in imminent danger of losing their germination ability when ATP synthesis fails to meet the demand. Thus, any UV damage which impairs ATP synthesis could have much more severe consequences for trinucleate grains.

Nucleic acids and proteins may serve as possible chromophores for UV-B damage (Caldwell 1979, Figure 1). While RNA is usually present in cells in such large quantities that UV damage to RNA is considered to be negligible, large differences in RNA content could influence UV damage. Frankis and Mascarenhas (1980) have demonstrated the presence of presynthesized messenger RNA in dry pollen of Tradescantia paludosa, an "advanced" binucleate species. While Mascarenhas (1975) stated that all pollens tested appear to have stored RNA, it is uncertain whether any trinucleate species have been examined. In the trinucleate Asteraceae, where germination is independent of protein synthesis and few ribosomes are present, it would appear there is little need for RNA. Thus, if germination is sufficiently rapid that UV damage to proteins is not significant, pollen of these species should be relatively immune to UV-B inhibition of germination. It is interesting to note, however, that our analysis of peak pollen presentation times for species attractive to insects did not show any increase in the proportion of trinucleate species as solar noon was approached.

DNA is likely a chromophore for UV-B damage in pollen and DNA condition was the original basis for the distinction between the two pollen types over 50 years ago. When pollen is shed in the binucleate condition, a diffuse vegetative nucleus and a condensed generative nucleus (arrested in prophase of mitosis, with the DNA already duplicated) are

present in the grain (Mascarenhas 1975). Thus UV may interfere with the completion of mitosis during tube growth. Trinucleate pollen is less susceptible to this type of damage as generative cell division usually takes place at least a day before the pollen is shed (Brewbaker and Emery 1964) and it may (at least in maize) possess enzymes which can photoreactivate DNA damage. Whether binculeate grains can do this is uncertain. Furthermore, the two sperm nuclei of trinucleate pollen present "multiple targets" for UV irradiation. If one is damaged, perhaps it will fertilize the triploid endosperm, causing less damage than if it fertilized the embryo (Pfahler and Linskens 1977).

During the migration of the generative nucleus through the pollen tube, there may be a period of exposure to UV as the pollen tube frequently grows along the surface for a short distance (Cass and Peteya 1979, Heslop-Harrison and Shivanna 1977, Kroh et al. 1979, Sedgley and Scholefield 1980, Wilms 1980) before penetrating into the stigmatic tissue, where it will likely be more protected. Though the rate of tube growth is slower and thus time to fertilization much longer in binucleate pollen, stigma characteristics may provide more protection. Stigmatic exudates (more often found on species with binucleate pollen) contain UV absorbing phenolic compounds (Martin 1970) which could possibly protect pollen during germination as it is immersed in this fluid upon landing on the stigma. This protection may be important as binucleate (Chang and Campbell 1976) and trinucleate (at

least maize) (Pfahler 1981) pollen tube growth is inhibited by UV-B.

Environmental (eg. low humidity (Henny 1980), low temperature (Thompson and Liu 1973)), pathogenic (Hodgkin and Lyon 1979), or anthropogenic (eg. fungicide (Redalen 1980), SO_2 (Facteau and Rowe 1981)) stress factors which decrease pollen germination or tube growth may act either in an additive or synergistic manner with UV radiation effects and exacerbate the consequences of ozone depletion. As plant reproductive systems and their efficiency may be responsible for the differences between higher categories (genera, families, etc.) in the angiosperms (Stebbins 1970), we can expect a multitude of diverse physiological systems in pollen and possibly diverse response to UV. Thus we should not expect all trinucleate grains to be without ribosomes since maize, as previously noted, has many to facilitate tube growth through the long style. Furthermore, protein synthesis in trinucleate Brassica pollen occurs as part of the regulation of its incompatibility response (Ferrari and Wallace 1977). Because of this diversity in physiological mechanisms, and the differences in diurnal timing of pollination, the effects of solar ultraviolet on plant reproduction will be much more complex and variable than its effects on the physiology of the vegetative organs.

LITERATURE CITED

Abrahamson, W. G., and K. D. McCrea. 1977. Ultraviolet light reflection and absorption patterns in populations of Rudbeckia (Compositae). Rhodora 79:269-277.

Adams, D. E., W. E. Perkins, and J. R. Estes. 1981. Pollination systems in Paspalum dilatum Poir. (Poaceae): An example of insect pollination in a cemperate grass. Amer. J. Bot. 68: 389-394.

Ascher, P. D., and S. J. Peloquin. 1966. Effect of floral aging on the growth of compatible and incompatible pollen tubes in Lilium longiflorum. Amer J. Bot. 53:99-102.

Bailey, L. H., and E. Z. Bailey. 1976. Hortus third. MacMillan Publishing Co., New York.

Brewbaker, J. L. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. Amer. J. Bot. 54:1069-1083.

Brewbaker, J. L. and G. C. Emery. 1962. Pollen Radiobotany. Rad. Bot. 1:101-154.

Caldwell, M. M. 1979. Plant life and ultraviolet radiation: Some perspective in the history of the Earth's UV climate. Bioscience 29:520-525.

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.

Cass, D.D., and D. J. Peteya. 1979. Growth of barley pollen tubes in vivo I. Ultrastructural aspects of early tube growth in the stigmatic hair. Can. J. Bot. 57:386-396.

Chang, D. C. N., and W. F. Campbell. 1976. Response of Tradescantia stamen hairs and pollen to UV-B

irradiation. Environ. and Exp. Bot. 16:195-199.

Clark, C. 1979. Ultraviolet absorption by flowers of the Eschscholzioideae (Papaveraceae). Madrono 26:22-25.

Cresti, M., F. Ciampolini, and G. Sarfatti. 1980. Ultrastructural investigations on Lycopersicum peruvianum pollen activation and pollen tube organization after self- and cross-pollination. Planta 150:211-217.

Dashek, W. V., R. L. Harmon, L. B. Adlestein, et al. 1981. Use of Lilium longiflorum cv ace pollen germination and tube elongation as a bioassay for the hepatocarcinogens, aflotoxins. Environ. Health Perspectives 40:267-278.

Emecz, T. I. 1962. The effect of meteorological conditions on anthesis in agricultural grasses. Ann. Bot. 26: 159-172.

Estes, J. R., and R. W. Thorp. 1975. Pollination ecology of Pyrrhopappus carolinianus (Compositae). Amer. J. Bot. 62: 148-159.

Faberge, A. C. 1957. A method for treating wheat pollen with ultraviolet radiation for genetic experiments. Genetics 42:618-622.

Facteau, T. J., and K. E. Rowe. 1981. Response of sweet cherry and apricot pollen tube growth to high levels of sulfur dioxide. J. Am. Soc. for Hort. Sci. 106:77-79.

Facteau, T. J., S. Y. Wang, and K. E. Rowe. 1973. The effect of hydrogen fluoride on pollen germination and pollen tube growth in Prunus avium L. cv. 'Royal Ann'. J. Am. Soc. for Hort. Sci. 98:234-236.

Ferrari, T. E., and D. H. Wallace. 1977. A model for self-recognition and regulation of the incompatibility response of pollen. Theor. and Appl. Genet. 50:211-225.

Frankis, R., and J. P. Mascarenhas. 1980. Messenger RNA in the ungerminated pollen grain: a direct demonstration of its presence. Ann. Bot. 45:595-599.

Free, J. B. 1968a. The foraging behaviour of honeybees (Apis mellifera) and bumblebees (Bombus spp.) on blackcurrant (Ribes nigrum) , raspberry (Rubus idaeus) and strawberry (FragariaxAnanassa) flowers. J. Appl. Ecol. 5:157-168.

Free, J. B. 1968b. Dandelion as a competitor to fruit trees for bee visits. J. Appl. Ecol. 5: 169-178.

Free, J. B., and P. M. Nuttall. 1968. The pollination of oilseed rape (Brassica napus) and the behaviour of bees on the crop. J. Agric. Sci. (Camb.) 71: 91-94.

Gilissen, L. J. W., and N. B. M. Brantjes. 1978. Function of the pollen coat in different stages of the fertilization process. Acta Bot. Neerl. 27:205-212.

Green, A. E. S., K. R. Cross, and L. A. Smith. 1980. Improved analytic characterization of ultraviolet skylight. Photochem. Photobiol. 31: 59-65.

Guldberg, L. D., and P. R. Atsatt. 1975. Frequency of reflection and absorption of ultraviolet light in flowering plants. Am. Midl. Nat. 93:35-43.

Henny, R. J. 1980. Relative humidity affects in vivo pollen germination and seed production in Dieffenbachia maculata "Perfection". J. Amer. Soc. Hort. Sci. 105:546-548.

Heslop-Harrison, Y. 1977. The pollen-stigma interation: pollen-tube penetration in Crocus. Ann. Bot. 41:913-922.

Heslop-Harrison, J., and Y. Heslop-Harrison. 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Stain Technol. 45:115-120.

Heslop-Harrison, Y. and K. R. Shivanna. 1977. The receptive surface of angiosperm stigma. Ann. Bot. 41:1233-1258.

Hill, R. J. 1977. Technical note: ultraviolet reflectance-absorbance photography; an easy inexpensive research tool. Brittonia 29:382-390.

Hodges, C. M., and R. B. Miller. 1981. Pollinator flight directionality and the assessment of pollen returns. Oecologia 50:376-379.

Hodgkin, T., and G. D. Lyon. 1979. Inhibition of Solanum pollen germination in vitro by the phytoalexin rishitin. Ann. Bot. 44:253-255.

Hoekstra, F. A. 1979. Mitochondrial development and activity of binucleate and trinucleate pollen during germination in vitro. Planta 45:25-36.

Hoekstra, F. A., and J. Bruinsma. 1975a. Viability of Compositae pollen: germination in vitro and influences of climatic conditions during dehiscence. Z. Pflanzenphysiol. 76:36-43.

Hoekstra, F. A., and J. Bruinsma. 1975b. Respiration and vitality of binucleate and trinucleate pollen. Physiol. Plant. 34:221-225.

Hoekstra, F. A., and J. Bruinsma. 1978. Reduced independence of the male gametophyte in angiosperm evolution. Ann. Bot. 42:759-762.

Hoekstra, F. A., and J. Bruinsma. 1979. Protein synthesis of binucleate and trinucleate pollen and its relationship to tube emergence and growth. Planta. 146:559-566.

Hoekstra, F. A., and J. Bruinsma. 1980. Control of respiration of binucleate and trinucleate pollen under humid conditions. Physiol. Plant. 48:71-77.

Hopping, M. E., and E. M. Jerram. 1979. Pollination of Kiwifruit (Actinidia chinensis Planch.): stigma-style structure and pollen tube growth. New Zealand Journal of Botany 17:233-240.

Horovitz, A., and Y. Cohen. 1972. Ultraviolet reflectance characteristics in flowers of crucifers. Amer. J. Bot.

59:706-713.

Hoshino, T., K. Ujihara, and S. Shikata. 1980. Time and distance of pollen dispersal in grain sorghum. Jap. J. Breed. 30: 246-250.

Hyde, H. A., and D. A. Williams. 1945. Studies in atmospheric pollen. II. Diurnal variation in the incidence of grass pollen. New Phytol. 44: 83-94.

Ikenaga, M., S. Kondo, and T. Fujii. 1974. Action spectrum for enzymatic photoreactivation in maize. Photochem. Photobiol. 19:109-113.

Jones, M. D., and J. G. Brown. 1951. Pollination cycles of some grasses in Oklahoma. Agron. J. 43: 218-222.

Kevan, P. G. 1972. Floral colors in the high arctic with reference to insect-flower relations and pollination. Can. J. Bot. 50:2289-2316.

Kevan, P. G. 1975. Sun-tracking solar furnaces in high arctic flowers: Significance for pollination and insects. Science 189:723-726.

Kevan, P. G. 1979. Vegetation and floral colors revealed by ultraviolet light: interpretational difficulties for functional significance. Amer. J. Bot. 66:749-751.

Kirby, E. G., and J. E. Smith. 1974. Elutable substances of pollen grain walls. p. 127-130 in H. F. Linskens (ed.). Fertilization in higher plants. North Holland Publishing co., Amsterdam.

Kroh, M., M. H. Gorissen, and P. L. Pfahler. 1979. Ultrastructural studies on styles and pollen tubes of Zea mays L.: General survey of pollen tube growth in vivo. Acta Bot. Neerl. 28:513-518.

Lee, C. L. 1980. Pollenkeimung, Pollenschlauchwachstum und Befruchtungsverhaltnisse bei Prunus domestica. II. Pollenschlauchwachstum im Griffel. Die Gartenbauwissenschaft 45:241-248.

Lehman, W. F., and Y. P. Puri. 1967. Rates of germination and tube growth of stored and fresh alfalfa (Medicago sativa L.) pollen on agar medium. Crop Sci. 7:272-273.

Lewis, D. H. 1980. Are there inter-relations between the metabolic role of boron, synthesis of phenolic phytoalexins, and the germination of pollen? New Phytol. 84:261-270.

Linskens, H. F. 1964. Pollen Physiology. Annual Review of Plant Physiology 15:255-270.

Macior, L. W. 1978. Pollination ecology of vernal angiosperms. Oikos 30: 452-460.

Martin, F. W. 1970. The ultraviolet absorption profile of stigmatic extracts. New Phytol. 69:425-430.

Mascarenhas, J. P. 1975. The biochemistry of angiosperm pollen development. Bot. Rev. 41:259-314.

Mascarenhas, J. P. 1978. Ribonucleic acids and proteins in pollen germination. Pages 400-406 in Proceedings of the fourth international palynological conference, Vol. 1. Lucknow, India.

Nachtwey, D. S. 1975. Linking photobiological studies at 254 nm with UV-B. Pages 3-50 to 3-75 in D. S. Nachtwey, M. M. Caldwell, and R. H. Biggs, editors. Impacts of climatic change on the biosphere. Climatic impact assessment program monograph 5. Report DOT-TST-75-55. U.S.D.O.T., Springfield, Virginia.

Nakamura, N. 1978. Physiological studies on the pollen growth of Camellia japonica L. in vitro. J. of Yokohama City Univ. 5(1):1-100.

Neamtu, G., and C. Boda. 1970. Stud. Cerc. Biochem. 13:307. (Original not seen, cited by Stanley and Linskens (1974)).

Nishiyama, I., and L. Blanco. 1980. Avoidance of high temperature sterility by flower opening in the early morning. Japan Ag. Res. Quart. 14: 116-117.

Ockendon, D. J., and P. J. Gates. 1976. Reduced pollen viability in the onion (Allium cepa). New Phytol. 76:511-517.

Ogden, E. C., J. V. Hayes, and G. S. Raynor. 1969. Diurnal patterns of pollen emission in Ambrosia, Phleum, Zea, and Ricinus. Amer. J. Bot. 56: 16-21.

Parker, R. L. 1926. The collection and utilization of pollen by the honeybee. Memoir 98 of the Cornell University Agricultural Experiment Station.

Percival, M. S. 1955. The presentation of pollen in certain angiosperms and its collection by Apis mellifera. New Phytol. 54: 353-368.

Pfahler, P. L. 1978. Biology of the maize male gametophyte. p. 517-530 in D. B. Walden (ed.). Maize Breeding and Genetics. John Wiley and sons, New York.

Pfahler, P. L. 1981. In vitro germination charactristics of maize pollen to detect biological activity of environmental pollutants. Environ. Health Perspectives 37:125-132.

Pfahler, P. L., and H. F. Linskens. 1977. Ultraviolet irradiation of maize (Zea mays L.) pollen grains. I. Pollen genotype effects on kernel development. Theor. Appl. Genet. 49:253-258.

Pfahler, P. L., H. F. Linskens, and M. Wilcox. 1980. In vitro germination and pollen tube growth of maize (Zea mays) pollen. IX. Pollen source genotype and nonionic surfactant interactions. Can. J. Bot. 58:557-561.

Proctor, M., and P. Yeo. 1972. The pollination of flowers. Taplinger, New York.

Redalen, G. 1980. Effects of fungicides on pollen germination and fruit set in raspberries. Die

Gartenbauwissenschaft 45:248-251.

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.

Sanchez, R. L., and D. G. Smeltzer. 1965. Sorghum pollen viability. Crop Sci. 5: 111-113.

Sastri, D. C., and K. R. Shivanna. 1979. Role of pollen wall proteins in intraspecific imcompatibility in Saccharum bengalense. Phytomorphology 29:324-330.

Schmidtt, J. 1980. Pollinator foraging behavior and gene dispersal in Senecio (Compositae). Evolution 34:934-943.

Sedgley, M. 1977. The effect of temperature on floral behaviour, pollen tube growth, and fruit set in the avocado. J. Hort. Sci. 52:135-141.

Sedgley, M., and M. S. Buttrose. 1978. Some effects of light intensity, daylength, and temperature on flowering and pollen tube growth in the watermellon (Citrullus lanatus). Ann. Bot. 42:609-616.

Sedgley, M., and P. B. Scholefield. 1980. Stigma secretion in the watermelon before and after pollination. Bot. Gaz. 141:428-434.

Setlow, R. B. 1974. The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc. Natl. Acad. Sci. USA 71:3363-3366.

Shaw, R. J. 1962. The biosystematics of Scrophularia in western North America. Aliso 5:147-178.

Shivanna, K. R., and J. Heslop-Harrison. 1981. Membrane state and pollen viability. Ann. Bot. 47:759-770.

Socias i Company, R., D. E. Kester, and M. V. Bradley. 1976. Effects of temperature and genotype on pollen tube growth in some self-incompatible and self-compatible almond cultivars. J. Amer. Soc. Hort. Sci. 101:490-493.

Stadler, L. J., and F. M. Uber. 1942. Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. Genetics 27:84-118.

Stanley, R. G., and H. F. Linskens. 1974. Pollen: Biology, biochemistry, management. Springer-Verlag, New York.

Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in the angiosperms, I. Pollination Mechanisms. Ann. Rev. Ecol. Syst. 1:307-326.

Steinitz-Sears, L. M., and E. R. Sears. 1957. Ultraviolet and X-ray induced chromosomal aberrations in wheat. Genetics 42:623-630.

Stephens, J. C., and J. R. Quinby. 1934. Anthesis, pollination, and fertilization in sorghum. J. Agric. Res. 49: 123-136.

Stott, K. G. 1972. Pollen germination and pollen-tube characteristics in a range of apple cultivars. J. Hort. Sci. 47:191-198.

Synge, A. D. 1947. Pollen collection by honeybees (Apis mellifera). J. Animal Ecol. 16: 122-138.

Tepedino, V. J. 1981. The pollination efficiency of the squash bee (Peponapis pruinosa) and the honeybee (Apis mellifera) on summer squash (Cucurbita pepo). J. Kansas Ent. Soc. 54:359-377.

Thompson, M. M., and L. J. Liu. 1973. Temperature, fruit set, and embryo sac development in 'Italian' prune. J. Amer. Soc. Hort. Sci. 98:193-197.

Thompson, W. R., J. Meinwald, D. Aneshansley, and T. Eisner. 1972. Flavonols: Pigments responsible for ultraviolet absorption in nectar guide of flower. Science 177:528-530.

Waddington, K. D. 1981. Factors influencing pollen flow in bumblebee-pollinated Delphinium virescens. Oikos 37:153-159.

Werfft, R. 1951. Uber die Lebensdauer der Pollenkorner in der freien Atmosphare. Biologisches Zentralblatt 70:354-367.

Whitehead, D. R. 1969. Wind pollination in the angiosperms: evolutionary and environmental considerations. Evolution 23:28-35.

Williams, R. R., and M. Maier. 1977. Pseudocompatibility after self-pollination of the apple Cox's Orange Pippin. J. Hort. Sci. 52:475-483.

Wilms, H. J. 1980. Ultrastructure of the stigma and style of spinach in relation to pollen germination and pollen tube growth. Acta Bot. Neerl. 29:33-47.

Appendix 1. Peak times of pollen shed in wind pollinated species.

0530-0629 Sorghum vulgare (11 varieties) (Stephens and Quinby, 1934); Cynosurus cristatus, Lolium perenne (Hyde and Williams, 1945); Eragrostis curvula (Jones and Brown, 1951)

0630-0729 Phleum pratense (Ogden et al., 1969), var. Bd 2308 (Emecz, 1962); P. nodosum, Dactylis glomerata S37 (Emecz, 1962); Ambrosia trifida (Ogden et al., 1969)

0730-0829 Paspalum dilatatum (Adams et al., 1981); Oryza glaberrima (Nishiyama and Blanco, 1980); Ambrosia artemisiifolia (Ogden et al., 1969); Sorghum vulgare (Hoshino et al., 1980); Leymus (Elymus) cinereus, L. (E.) triticoides, L. (E.) salina (Dewey, personal communication); Arrhenatherum avenaceum, Trisetum flavescens (Hyde and Williams, 1945); Zea mays (Jones and Brown, 1951)

0830-0929 Alopecurus pratensis, Dactylis glomerata S143, Phleum pratense S51 (Emecz, 1962); Sorghum vulgare (Sanchez and Smeltzer, 1965)

0930-1029 Ricinus communis, Zea mays (3 varieties) (Ogden et al., 1969); Dactylis glomerata S26, Phleum pratense S48 (Emecz, 1962); Secale cereale (Jones and Brown, 1951)

1030-1129 Oryza sativa (Nishiyama and Blanco, 1980); Festuca pratensis (Emecz, 1962; Hyde and Williams, 1945)

1130-1229 Festuca ovina (Emecz, 1962); Lolium perenne (Hyde and Williams, 1945); Sorghum halepense, Panicum virgatum (Jones and Brown, 1951)

1230-1329 Lolium perenne S24 (Emecz, 1962); Zea mays (2 varieties) (Ogden et al., 1969)

1330-1429 Lolium perenne (2 varieties) (Emecz, 1962); Hordeum nodosum (Hyde and Williams, 1945); Festuca elatior var. arundinaceae (Jones and Brown, 1951)

1430-1529 *Agropyron cristatum*, *Elytrigia* (*Agropyron*) *spicata*, *E.* (*A.*) *intermedia*, *E.* (*A.*) *elongata*, *E.* (*A.*) *dasystachya* (Dewey, personal communication)

1530-1629 *Lolium perenne* ex France, *Festuca arundinaceae* (Emecz, 1962); *Holcus lanatus* (Hyde and Williams, 1945)

1630-1729 *Festuca rubra* (Hyde and Williams, 1945)

1730-1829 *Bromis inermis* (Jones and Brown, 1951)

Appendix 2. Peaks of pollen presentation in insect pollinated plants.

0530-0629 Calystegia sylvestris, Papaver dubium, Hypericum perforatum, Fuchsia magellanica, Aesculus hippocastanum (Percival, 1955)

0630-0729 Ligustrum vulgare, Sarothamnus scoparius (Percival, 1955); Pyrrhopappus carlinianus (Estes and Thorp, 1975)

0730-0829 Capsicum sp. (5 varieties in August), Papaver somniferum, Verbascum thapsiformae, V. phlomoides (Stanley and Linskens, 1974); Brassica alba (Synge, 1947); Papaver rhoeas (Stanley and Linskens, 1974, Synge, 1947); Paspalum dilatatum (Adams et al., 1981); Convolvulus minor, Sinapis arvensis, Rosa spinosissima, Centaurea montana, Tradescantia virginiana, Helianthemum chamaecistus, Raphanus raphanistrum, Aucuba japonica (Percival, 1955); Epilobium angustifolium (Percival, 1950, Synge, 1947)

0830-0929 Rosa arvensis, R. multiflora, Verbena officinalis (Stanley and Linskens, 1974); Ambrosia trifida, Rosa blanda, R. rugosa, R. setigera, Zea mays (Parker, 1926); Sonchus oleraceus, Clematis vitalba, Cirsium palustre, Ribes sanguineum, Layia elegans, Plantago lanceolata, Brassica oleracea, Deutzia gracilis (Percival, 1955); Papaver orientale (Percival, 1955)

0930-1029 Capsicum sp. (June) (Stanley and Linskens, 1974); Trifolium repens, Ranunculus acris (Synge, 1947); Rosa xanthinia (Parker, 1926); Buddleia variabilis, Crocosmia pottsii, Sambucus nigra, Ranunculus bulbosus, Helianthus annuus, Epilobium montanum, E. hirsutum, Ulex gallii, U. europaeus, Cheiranthusx allionii, Ribes nigrum, Prunus cerasus, Impatiens roylei, Berberis darwinii (Percival, 1955)

1030-1129 Convolvulus tricolor, Capsicum sp. (1 variety in August) (Stanley and Linskens, 1974); Ranunculus repens, Taraxacum officinale, Zea mays, Senecio jacobea, Epilobium parviflorum x E. adenocaulon, Eschscholzia californica, Petroselinum crispum, Ilex aquifolium, Aubrieta deltoidea (Percival, 1955)

1130-1229 Trifolium pratense (Synge, 1947); Fragaria chiloensis, Prunus americana, Taraxacum officinale (Parker, 1926); Anemone apennina, A. nemorosa, Ranunculus ficaria, Epilobium adenocaulon, E. parviflorum, Cardamine pratensis, Limnanthes douglasii, Centranthus ruber, Alisma plantago-aquatica, Crataegus monogyna, Cheiranthus cheiri, Allium porrum, Bartonia aurea, Phacelia tanacetifolia, FragariaxAnanassa (Percival, 1955)

1230-1329 Cercis canadensis, Eupatorium urticaefolium,Lonicera morrowi, L. tatarica, Melilotus alba, M. officinalis, Prunus cerasus, Pyrus communis, P. ioensis, Rubus nigrobaccus, Rudbeckia laciniata, Symphoricarpos orbiculatus, S. racemosus laevigatus, Tilia americana, Trifolium hybridum, T. repens, (Parker, 1926); Endymion non-scriptus, Scilla sibirica, Allium ursinum, Rubus idaeus, R. fruticosus, R.loganobaccus, Arabis albida, Lunaria annua, Leucojum aestivum, Magnolia stellata, Reseda odorata, Trapaeolum majus, Digitalis purpurea, Prunus laurocerasus, P. persica, Scilla hispanica, Arctium vulgare, Crocus aureus (Percival, 1955)

1330-1429 Lycium vulgare, Melilotus alba, Rubus occidentalis, R. strigosus, Salix fragilis (Parker, 1926); Pyrus malus (Parker, 1926, Percival, 1955); Vicia fava (Synge, 1947, Percival, 1955); Pyrus communis, Tussilago farfara (Percival, 1955)

1430-1529 Asparagus officinalis (Parker, 1926); Magnolia x soulangeana (Percival, 1955)

Protective mechanisms and acclimation to solar ultraviolet-B radiation in *Oenothera stricta*

by

Ronald Robberecht and Martyn M. Caldwell

INTRODUCTION

Solar ultraviolet-B radiation (UV-B, 280-320 nm) may represent a significant, although subtle, environmental stress in the life history of terrestrial plant species (Caldwell 1968, Bogenrieder and Klein 1977, Caldwell et al. 1980, Robberecht et al. 1980). The shortwave solar UV radiation flux that prevailed during the development of early land plants has been suggested to have been an important factor in higher plant evolution, particularly in regard to the development of UV protection and repair mechanisms (McClure 1976, Caldwell 1979, Lee and Lowry 1980, Lowry et al. 1980). Although this intense shortwave UV radiation is no longer a factor in the terrestrial environment, solar UV-B radiation is sufficiently actinic to damage plant tissues and physiological processes of sensitive plants (Biggs et al. 1975, Sisson and Caldwell 1976, 1977, Brandle et al. 1977). As will be discussed in this paper, there are several plant adaptations that can either ameliorate or repair the damaging effects of UV-B radiation in plant tissue; adaptations that are integral to plant UV-B acclimation. The study centered

on those mechanisms that have the potential for protecting plant processes from injury and their role in plant acclimation to UV-B radiation. Furthermore, the degree of phenotypic plasticity in UV protective mechanisms and acclimation in view of the natural solar UV-B radiation flux and in an enhanced UV-B irradiance environment in the future was examined.

The effect of UV radiation on biological systems is highly wavelength dependent and comprises a relatively small proportion of the energy received from the total solar spectrum. Its capability to induce photochemical reactions in organisms can increase logarithmically with decreasing wavelength (Giese 1964, Setlow 1974, Caldwell et al. 1980). This is significant in regard to the absorption spectra of nucleic acids and proteins, which extend into the UV-B radiation spectrum and increase rapidly with decreasing wavelength. Upon absorption of UV radiation, changes in the structure of these molecules, as well as injury to plant tissues, may occur (Murphy 1975). Pyrimidine dimer formation in nucleic acids can interfere with normal DNA replication, and thus there is a significant interaction between these biologically important molecules and UV-B radiation.

The penetration of solar UV radiation through the atmosphere is restricted by stratospheric ozone absorption, which effectively prevents the penetration of radiation of wavelengths shorter than approximately 295 nm into the lower atmosphere. As illustrated by Caldwell et al. (1980), absorption of radiation in the atmosphere and UV effects in

biological systems are highly wavelength dependent. When this is coupled with the latitudinal ozone gradient, a pronounced natural gradient in the biological effectiveness of UV-B radiation results. Under natural conditions ozone formation and destruction are balanced, thereby maintaining the stratospheric ozone layer at a relatively stable concentration (Griggs 1966, Hidalgo 1975). However, chemical reactions between stratospheric pollutants such as chlorofluoromethanes are predicted to shift this balance toward greater ozone destruction (National Academy of Sciences 1979a, b). A reduction in the ozone layer will increase the UV-B irradiance on Earth, and shift the terrestrial solar spectrum somewhat toward shorter wavelengths. Such enhancement of UV-B radiation could present a potential stress for plant species because of its photochemical potency and increased overlap with the absorption spectra of nucleic acids and proteins. The study of plant response to the natural solar UV radiation flux therefore has particular significance to predicting the consequences of an enhanced UV-B climate on plant species.

Plant adaptations that temper the effects of UV-B radiation can be classified into mechanisms by which plants avoid radiation, adaptations that alter the path of radiation incident on the leaf, and repair processes. Highly inclined leaves are of limited value for the avoidance of solar UV-B radiation, since a relatively large proportion of UV-B radiation is diffuse radiation (Caldwell et al. 1980). Repair processes can act on damaged nucleic acids. While these mechanisms can repair certain

types of damage caused by UV-B radiation and thereby protect the plant from permanent damage, the rate of repair may not be able to keep pace with the damage sustained in enhanced or high UV-B irradiance environments. Plant adaptations that attenuate UV-B radiation in the outer layers of the leaf, thereby reducing UV-B injuries in the leaf, are considered protective mechanisms. The considerable differences observed in the UV sensitivity of plant species (Cline and Salisbury 1966, Biggs, et al. 1975, and Van and Garrard 1975) may be explained by the combined efficiency of these three mechanisms.

In addition to differences in species sensitivity, some plants appear to have the capability to acclimate to changes in the UV radiation regime. Studies of the effects on leaves of intensified UV-B radiaion resulting from events such as plant emergence near alpine snowbanks (Caldwell 1968), sudden exposure to UV radiation as in the case of greenhouse-grown plants transferred to the field (Bogenrieder and Klein 1977), and UV-irradiation of plants in controlled experiments (Robberecht and Caldwell 1978), suggest that some plant species acclimate to an intensified radiation regime through physiological changes.

Plant acclimation may involve the synthesis of UV-absorbing pigments such as flavonoid and related compounds, possibly related to the capacity of the epidermis to attenuate radiation. These compounds absorb UV radiation. Their role as UV-B radiation filters in the leaf epidermis has been suggested by several investigators (Caldwell 1968, Gausman et al. 1975,

Wellmann 1975a, Robberecht and Caldwell 1978, Murphy et al. 1979, Lee and Lowry 1980) and may be responsible for attenuating a major portion of the UV-B radiation incident on a leaf.

In general, over 90% of the UV-B radiation incident on a leaf is attenuated in the epidermis. Reflectance of UV-B radiation from glabrous leaves is generally less than 10% (Gausman et al. 1975, Robberecht et al. 1980). In some species, a certain degree of phenotypic plasticity in the degree of epidermal UV-B attenuation has been demonstrated (Robberecht and Caldwell 1978). The capacity to decrease epidermal UV-B transmittance appears to vary among species and may partially explain the degree of differential sensitivity of species to UV-B radiation. If flavonoid and related phenolic compounds function as major UV-filters in the epidermis and mesophyll layers and are increased in response to UV-B irradiation, this could be an important mechanism by which plants acclimate to UV-B radiation. This mechanism would, of course, operate in conjunction with plant repair processes and other mechanisms of radiation avoidance. Thus, the dynamic balance between these adaptations ultimately determines the degree of plant sensitivity to UV-B radiation.

These mechanisms of UV-B acclimation may be most pronounced in species that occur in high UV-B irradiance environments typical of low latitude, high elevation sites (Robberecht et al. 1980, Caldwell 1981). The investigation of species that have recently invaded these habitats, such as Oenothera stricta

Ledeb., may be particularly useful in the elucidation of a UV-B acclimation mechanism. Although indigenous to temperate South America, O. stricta is adventive in parts of the low latitude Andes (Dietric 1977) and recently in high elevation, high UV-B irradiance, sites on the Hawaiian Islands. The ability of this species to colonize and survive in these environments and its particular leaf characteristics provided a species well suited to experimentation and the study of UV-B acclimation in plants. The relatively horizontal orientation of mature leaves, along with minimal self-shading of leaves, simplified the dosimetry. Furthermore, the epidermal characteristics of O. stricta allowed for consistent separation of the leaf into epidermal and mesophyll layers for an analysis of leaf optical properties.

The study initially focused on the penetration of UV-B radiation into the leaf and secondly on its effects on photosynthesis. Attenuation of damaging UV-B radiation in the upper leaf epidermis would be expected to significantly reduce the UV-B flux incident at the mesophyll cell layer and the extent of damage to photosynthesis. Moreover, a mechanism for increasing UV-B attenuation in the outer leaf layers would be critical for survival in high natural UV-B irradiance environments as well as for intensified UV-B irradiance in the event of ozone reduction. Absorption of UV-B radiation by flavonoid and related phenolic compounds in the leaf appears to be responsible for the wavelength selectivity and effectiveness of epidermal attenuation. Although additional attenuation in the mesophyll may occur, UV-B incident on sensitive targets in the

mesophyll in some cases can be sufficient to result in significant photosynthetic depression.

METHODS

Instrumentation, UV-B milieu and measurement

Ultraviolet radiation was measured with a modified Gamma Corp. spectroradiometer, which consisted of a high-resolution grating monochromator (Model 700-31) and an autoranging photometer (Model 2900). This specific characteristics of this instrument, its calibration and computer refinement of the data are discussed in detail by Caldwell et al. (1980).

The UV-B radiation data in this study are presented on the basis of the efficacy of these wavelengths in biological systems. This so called "effective" UV-B radiation provides a more biologically relevant measure of the radiation to which plants are exposed, because UV-B radiation is related to its effect on plants by an action spectrum. The generalized plant action spectrum developed by Caldwell (1971), which describes the effectiveness of UV-B to induce damage in plants, was employed here as the weighting function for UV-B irradiance. In accordance with this action spectrum, the effect of UV-B radiation is highly wavelength dependent and increases nearly three orders of magnitude from 313 to 280 nm (Caldwell et al. 1980). The equation presented in their paper was used to relate spectral UV-B irradiance and the weighting function for integrated biologically effective UV-B irradiance in units of mW/m^2. On a daily dose basis, units of effective J/m^2 were used.

Greenhouse UV-B radiation study

In the greenhouse, solar UV-B radiation was simulated with filtered sunlamps (Westinghouse Corp. Model FS-40). When filtered with a 0.13-mm cellulose acetate (CA) filter, a plastic film that is opaque below approximately 288 nm (Fig. 1), the spectral distribution approximates the UV-B spectral distribution found in solar radiation. This is not a true simultion of solar UV-B radiation, however. As is illustrated in this figure, the CA-filtered sunlamps transmit wavelengths as short as 288 nm, whereas there is essentially no radiation below 295 nm in the field. Furthermore, these fluorescent sunlamps have several emission lines in the UV-A waveband (320-400 nm), and thus a filtered sunlamps control is required. A Mylar plastic film (0.13 mm, DuPont Co.) was used as a control filter, since it is opaque to UV radiation below approximately 315 nm (see Fig. 1). Because the spectral distribution of these filtered sunlamps provides an imperfect simulation of the UV-B radiation regime found in nature, the action spectrum described above was used to relate sunlamp and solar UV-B spectral irradiance.

Two sets of filtered sunlamps suspended above the plant canopy comprised the control and UV-B radiation treatments in the greenhouse experiments. The lamps were individually wrapped with either a CA or Mylar filter. The treatment group was thus exposed to UV-B radiation while the control group received only radiation of wavelengths longer than 315 nm. The spectral

distribution of filtered-sunlamp radiation for representative dose rates of 0 and 90 biologically effective mW/m^{-2} is shown in Fig. 1. In most experiments, plants were exposed to this UV-B dose rate for 6 hours per day, resulting in approximately 2050 biologically effective $J.m^{-2}.d^{-1}$. This corresponds to the daily effective UV-B irradiance experienced at the summer solstice and an ozone thickness of 0.272 cm (at standard temperature and pressure), at 42 degrees N latitude. Such an ozone concentration represents a reduction of 15% from that normally prevailing during June (approximately 0.320 atm . cm). A lower UV-B dose rate of 45 effective mW/m^2 (970 $J.m^{-2}.d^{-1}$) was used for a pretreatment in some experiments and higher dose rates of up to 155 effective mW/m^2 (4470 $J.m^{-m}.d^{-1}$) were used for the determination of threshold UV-B sensitivity levels.

Plant sensitivity and UV attenuation measurements

Measurements of photosynthesis were made with a modified Siemens Corp. gas exchange system. This system allowed the measurement of photosynthesis and transpiration under controlled CO_2 concentrations, temperature and humidity. A cuvette similar to that described by Patterson et al. (1977) was incorporated into the gas exchange system. A computerized data acquisition system permitted the rapid evaluation of intercellular CO_2 concentration, leaf temperature, cuvette humidity and temperature, and net photosynthesis. Photosynthesis was measured under photosynthetically active quantum flux (400 to 700 nm) of 1100 $umol/.m^{-2}.s^{-1}$. The range in leaf temperature was 22 to 25°C.

Intercellular CO_2 concentration was calculated as the difference between CO_2 concentration outside the leaf and the product of the photosynthetic rate and leaf CO_2 diffusion resistance. The computer data acquisition system allowed the determination of intercellular CO_2 concentration during measurements of photosynthesis, thereby permitting appropriate corrections during the examination period. Intercellular CO_2 concentrations were maintained at similar levels for all plants within each experiment for accurate comparisons between plants within an experiment.

Epidermal transmittance of UV and visible radiation was measured with an integrating sphere coupled to the Gamma spectroradiometer. This system has been previously described by Robberecht and Caldwell (1978). Briefly, measurement of transmitted radiation with an integrating sphere insures that radiation scattered at oblique angles as it penetrates through the tissue is detected by the photomultiplier tube. The intergrating sphere was coated with "Halon" (Diano Corp.), a nearly perfect reflector and diffuser of UV radiation (Venable and Kostkowski 1975). This sphere was designed to accommodate epidermal tissue as small as 2 x 3 mm. Transmitted radiation was measured at 5- and 10-nm intervals with the spectroradiometer throughout the UV waveband, and at intervals of 5-, 10, and 50-nm in the visible waveband (400-650 nm).

Leaf epidermal transmittance was measured on fresh, turgid leaves. Epidermal tissue was mechanically isolated, without the use of chemical isolating agents. Only epidermal tissue of at least 3 mm in diameter and free of perforations and mesophyll debris, as determined by microscopic inspection, was used. The tissue was placed in contact with moistened filter paper and immediately positioned over the entrance port of the integrating sphere for the determination of UV transmittance. The same tissue was used for subsequent measurements of visible transmittance. Measurements of UV transmittance in whole leaves and the mesophyll layer were also made.

Epidermal, meshophyll, and whole-leaf tissue was examined for flavonoid and related phenolic compounds. Tissue extractions were made with a methanol-water HCL solution (70:29:1 v/v) as described by Caldwell (1968) and Ribereau-Gayon (1972). This solution is effective in extracting UV- absorbing pigments such as flavonoids from plant tissue. Leaf tissue was ground in 3- to 5-ml of this solution and centrifuged for 10 minutes. The resulting supernatant was then examined in a Beckman double-beam spectrophotometer (Model 35). Abosrbance was recorded over the 250 to 360-nm waveband. Leaf extract absorbance is presented on a dry tissue weight basis (mg). In some cases, such as with the extraction of epidermal tissue, fresh or dry weight could not be obtained, so UV absorbance was expressed on a leaf area basis. The epidermal tissue area was 2 x 3 mm in size. The plants were

grown under greenhouse conditions and thus received no UV-B radiation during the early stages of growth. The midday intensity of photosynthetically active photon flux in the greenhouse ranged between 500-1000 $umol.m^{-2}.s^{-1}$ in the winter and 1000-2000 $umol.m^{-2}.s^{-1}$ in the summer. Seeds from Oenothera stricta were collected on Haleakala Crater (Maui, Hawaii), and cultivated under greenhouse conditions. Plants with mature leaves of similar size and development were selected for experimentation. In experiments where photosynthesis was measured, plants initially selected by the latter criteria were secondarily culled on the basis of similar pretreatment photosynthetic rates. The plants were then randomly assigned to either a control group, receiving no UV-B irradiation, or a UV-B treatment group. The range in sample sizes was 3 to 10 plants per treatment. In general, photosynthesis was examined during the irradiation period, while epidermal transmittance and flavonoid absorbance were determined at the conclusion of the irradiation period. These latter measurements could not be made without destruction of the leaf. Measurements of photosynthesis, transmittance and absorbance were generally made on the same leaf of each plant.

The results were compared for the statistically significant effects of UV-B irradiation on transmittance and absorbance with a one-way Student's "t" analysis. For the analysis of UV-B effects on photosynthesis, an analysis of variance test was used. Significant reductions in photosynthesis and epidermal transmittance or significant increases in flavonoid absorbance

were determined at a probability level of $p<0.05$. In the case of epidermal transmittance, integrated values of transmittance in the UV-B (biologically effective), UV-A, and visible (400-430-nm and 450-650 nm) wavebands were used for statistical comparisons.

RESULTS

UV-B attenuation in the leaf

The degree of UV attenuation by the epidermis of Oenothera stricta leaves was not uniform between 290 and 400 nm. Rather, epidermal UV transmittance was low in the UV-B region and increased in the longer wavelength UV-A region (Fig. 2). As illustrated by the epidermal transmittance spectra of field- and greenhouse-grown plants, the spectral distribution of transmittance between 290 and 400 nm appears to be characteristic for a species.

Additional UV attenuation occurs in the mesophyll. Essentially all UV radiation that penetrates through the epidermis is attenuated in the mesophyll cell layer. Less than 1% of the UV-B, and less than 1% to4% of the UV-A radiation incident on a leaf penetrates through the mesophyll to the lower epidermis. This final small amount of UV radiation is attenuated by the lower epidermis so that all UV-B radiation incident on a leaf is completely attenuated within the leaf. Similar results were found for other species (Robberecht, unpublished). Further stratification of the mesophyll layer to locate particular zones of UV attenuation within this tissue was not possible.

A certain degree of phenotypic plasticity in the degree of epidermal UV attenuation is possible, as observed in greenhouse-grown and field-grown plants (see Fig. 2). Although direct evidence for a specific cause of this plasticity was not apparent because of the differences in environmental conditions between the two locations, the data show that epidermal transmittance is rather plastic. The results of experiments in this study, however, demonstrate that a significant reduction of UV transmittance through the epidermis is caused by UV-B irradiation. This response is evident after 11 to 15 days of UV-B irradiation at a mean dose rate of 2050J.m^{-2}.d^{-1} (see Fig. 2 and Table 1). Significant reductions of 19 to 33% were observed in mature, fully expanded leaves exposed to UV-B radiation. The relative differences between transmittance in UV-B irradiated and nonirradiated leaves are based on the integral of transmittance over each waveband, indicated in Table 1. Alhough UV-B transmittance slightly increased in one experiment, all other experiments showed either a trend of reduced UV transmittance of a significant reduction in this parameter. No significant reduction in epidermal UV transmittance was observed for irradiation periods of less than 11 days.

A reduction of epidermis transmittance was also observed in the UV-A waveband after UV-B irradiation (see Table 1). Significant reductions in epidermal UV-A transmittance of 22 to 39% were observed after 11 to 15 days of UV-B irradiation. In

addition to the reduction in magnitude, a spectral shift in transmittance was evident. This shift was expressed as a greater reduction in the transmittance of UV-A than UV-B radiation.

The range in epidermal transmittance for UV-B irradiated and nonirradiated leaves is summarized for six experiments in Fig. 3. This figure illustrates the maximum and minimum mean epidermal transmittance observed for four wavebands between 290 and 650 nm. The effect of UV-B irradiation on epidermal transmittance was most pronounced in the UV waveband, as shown by the high degree of plasticity in epidermal UV transmittance as compared to transmittance of visible radiation.

The effect of UV-B irradiation on epidermal transmittance may differ somewhat with the age of the leaf. In regard to UV-B radiation, epidermal tissue from young leaves was considerably less transparent than epidermal tissue from mature leaves. No reduction in epidermal UV-B transmittance was observed after 11 days of UV-B irradiation. However, transmittance in young leaves was quite low in both the UV-B irradiated and nonirradiated treatments.

Sources of UV attenuation

The mechanism for reduced UV attenuation in the epidermis appears to involve flavonoid and related phenolic compounds in the tissue. Flavonoid absorbance in epidermal and mesophyll leaf extracts generally increased after exposure of the leaf to UV-B radiation (Table 2). Although flavonoid and related compounds generally do not have maximal absorption in the UV-B waveband, absorbance is presented in this region so the extract absorbance and epidermal transmittance can be viewed in the same waveband. Absorbance is an exponent and is related to transmittance such that a linear decrease in absorbance results in a logarithmic increase in transmittance (Jagger 1967). Thus, small changes in absorbance can result in relatively large changes in transmittance. This relationship is specific to solutions. Absorbance at 305 nm, a wavelength selected as representative of the biological effectiveness of the UV-B waveband, significantly increased by 50 to 100% in the epidermis in four out of five experiments. Although epidermal extract absorbance decreased by 12% in one experiment, this decrease was not statistically significant. In the mesophyll layer, absorbance significantly increased by 28 to 35% in two out of five experiments. In all experiments, a trend of increased flavonoid absorbance was observed in mesophyll extracts after UV-B irradiation. No significant increases in absorbance were observed in either tissue layer when the duration of UV-B irradiation was less than seven days at a dose rate of approximately 2050 biologically effective $J.m^{-2}.d^{-1}$.

A comparison of flavonoid absorbance in young expanding leaves (2- to 3-cm in length) and mature fully expanded leaves (10- to 15-cm in length) showed that absorbance increased significantly in the epidermis of both leaf types following UV-B irradiation. In epidermal tissue from young leaves, flavonoid absorbance increased to a slightly greater degree than in mature leaves after UV-B irradiation. Although absorbance was similar in epidermal extracts of both age classes, mesophyll absorbance was higher in young than mature leaves. This difference in extract absorbance between young and mature leaves was considerably greater after UV-B irradiation.

The importance of these UV-absorbing pigments in epidermal tissue, and presumably in the mesophyll, is indicated in Fig. 2 by the transmittance spectrum of epidermal tissue from which flavonoid and related phenolic compounds have been removed. Epidermal transmittance throughout the UV waveband is substantially higher when these compounds are not present in the tissue.

Penetration of visible radiation

The reduction in epidermal transmittance of UV-B radiation appears to have some effect on the transmittance of visible radiation. Epidermal transmittance in one portion of the visible region was reduced after UV-B irradiation. The reduction in transmittance was found only at the shorter wavelengths of the visible region and was reduced to a lesser degree in the visible

than in the UV waveband (Fig. 4 and Table 1). Epidermal transmittance was significantly reduced by 6 to 12% in the 400- to 430-nm waveband after 11 to 15 days of UV-B irradiation. Ultraviolet-B irradiation had no substantial effect on epidermal transmittance of visible radiation between 450 and 650 nm. In this region, relative difference in visible transmittance were on the order of 0 to 2%. The range in mean epidermal transmittance of visible radiation for all experiments is shown in Fig. 3. The effect of UV-B radiation on epidermal transmittance of visible radiation is substantially less than its effect on the transmittance of the UV waveband.

The effect of UV-B irradiation on the epidermal transmittance of visible radiation did not appear to differ between young and mature leaves. Epidermal transmittance of visible radiation in young leaves followed a similar trend as in mature laves. Epidermal transmittance between 400 and 430 nm was significantly reduced by 14% in young leaves. However, exposure to UV-B radiation did not significantly affect the transmittance of radiation between 450 and 650 nm in these leaves.

Photosynthesis and plant sensitivity

The degree of photosynthetic depression in response to UV-B irradiation may be indicative of a plant's sensitivity to UV-B radiation. The effect of UV-B irradiation on the photosynthetic rate was examined in conjunction with measurements of epidermal transmittance and extract absorbance. Leaves exposed to UV-B

radiation generally showed no substantial or significant photosynthetic depression for irradiation periods of up to 15 days with a moderate dose rate of approximately 2050 biologicaly effective $J.m^{-2}.d^{-1}$ (Fig. 5). As shown in the previous sections, epidermal UV-B transmittance was significantly reduced within this exposure period, along with significantly increased epidermal and mesophyll extract absorbance. On the basis of this photosynthetic response, young and mature leaves of Oenothera stricta do not appear to be highly sensitive to this level of UV-B radiation.

The UV-B sensitivity of this species is, however, evident at higher UV-B irradiance (Fig. 5). The "moderately high" and "high" dose rates of 3110 and 4470 biologically effective $J.m^{-1}.d^{-1}$ are approximately 20 to 72% higher than the maximum daily effective UV-B flux present in the Hawaiian habitats of this species (Caldwell et al. 1980). The moderately high UV-B dose rate represents approximately a 20% reduction in ozone at this location. A significant depression in photosynthesis was observed after 1.5 days of UV-B irradiation at both these dose rates. Photosynthetic depression, however, was accentuated in the high UV-B dose rate. The threshold for photosynthetic depression thus appears to be between the moderate (2050 effective $J.m^{-2}.d^{-1}$) and the moerately high dose rate (3110 effective $J.m^{-2}.d^{-1}$). These results indicate that O. stricta may be near its capacity for UV-B acclimation in the high UV-B irradiance environment on the Hawaiian Islands, in which the daily effective UV-B irradiation can be as high as 2597 $J.m^{-2}$

(Caldwell et al. 1980). Measurements of epidermal UV transmittance and absorbance were not made at these higher dose rates because of the tendency for UV-B induced structural damage to the leaf at the intense UV-B flux.

DISCUSSION

UV-B attenuation in leaf tissue

Flavonoids are phenolic compounds that are common in the leaf epidermis and mesophyll of higher plants (McClure 1976, Nozzolillo 1972). As observed in the present study, flavonoid and related compounds were found in these two leaf tissue layers of Oenothera stricta. Flavonoids have also been observed in chloroplasts of some species (McClure 1976, Weissenbock et al. 1976, Plesser and Weissenbock 1977). Although these compounds have other important functions in plants, such as for flower coloration (Harborne 1975, Silberglied 1979) and antiherbivore agents (McCLure 1976), their presence in significant quantities in leaf tissue could provide a significant UV filter in view of the UV absorption properties of flavonoid compounds.

There is convincing evidence for a relationship between the induction of flavonoid synthesis and UV-B radiation. The studies of Wellmann (1974, 1975a, 1975b) have demonstrated that UV radiation (with a maximum wavelength effectiveness below 300 nm) induces flavonoid synthesis in both cell suspension cultures of parsley and intact parsley plants. Flavonoid induction, as indicated by the induction of phenylalanine ammonia-lyase (PAL), was found to be linearly related to the UV dose. This enzyme is

directly involved with the synthesis of flavonoid compounds. The involvement of flavonoid compounds in a mechanism of UV-attenuation in leaf tissue is also suggested by the results of the present study. Irradiation of leaves with UV-B generally resulted in significant increases in epidermal and mesophyll extract absorbance (see Table 2). This extract solution has been shown to remove flavonoid and related compounds from leaf tissue and exhibits a typical flavonoid absorption spectrum between 250 and 360nm (McClure 1976). An increase in extract absorbance after UV-B irradiation suggests an increase in absorbance by flavonoid compounds.

Further evidence of solar UV-stimulation of flavonoid production is found in the field studies of Caldwell (1968) and laboratory studies of Sisson (1981). Caldwell observed a gradient of increased epidermal extract absorbance in emergent alpine plants as distance from the edge of snowbanks decreased. Solar UV irradiance in the plant environment can be substantiially increased by the reflective properties of snow (Bener 1960). Furthermore, the exclusion of solar UV radiation from the plants environment, with the use of filters, and subsequent removal of the filter for UV exposure, increased extract absorbance. Sisson (1981) recently showed that extract absorbance in Curcurbita pepo L. leaves increased after exposure to UV-B radiation emitted from filtered sunlamps. The development of flavonoid compounds as filters of solar UV radiation in early terrestrial plants, has been suggested (Lowry et al. 1980, McClure 1976, Seigler 1977, Swain 1975, Caldwell

1979). During the period of colonization of the terrestrial environment, shortwave UV radiation may have been a significant selective factor in the survival of early land plants.

Alkaloids are compounds that also absorb in the UV waveband (Holubek and Strouf 1965). These compounds are far less ubiquitous in higher plants than the flavonoids, and have generally been thought to have allelopathic as well as antiherbivory functions in the plant (Ehrlich and Raven 1964, Whittaker and Feeny 1971). Absorption of UV by alkaloids is probably secondary to their function as antiherbivore agents. These compounds would not have been identified with the procedures used in this study.

The absorption of UV-B radiation by flavonoid and related phenolic compounds may be reflected in the measurements of epidermal and mesophyll UV-B attenuation. The present study and other work (Lautenschlager-Fleury 1955, Gausman et al. 1975, McCree and Keener 1974, Robberecht and Caldwell 1978, Robberecht et al. 1980), have shown that the epidermis is capable of attenuating more than 90% of the UV-B radiation incident on the leaf. Furthermore, increase flavonoid absorbance is conconmitant with altered epidermal optical properties after UV-B irradiation, and thus the attenuating capability of the epidermis is plastic. These observations suggest a mechanism of UV-B attenuation in O. stricta that involves the biosynthesis of UV-absorbing flavonoid compounds in the epidermis and mesophyll in response to solar UV-B radiation. Although the UV-filtration

capacity of the epidermis of this species generally increased under UV-B irradiation (see Table 1), this plasticity appears to vary among species (see Robberecht and Caldwell 1978).

Increased epidermal UV-B attenuation results in reduced UV-B flux at the top of the mesophyll layer. Therefore, the UV-B dose rate incident on the leaf surface does not reflect the ultimate UV-B flux incident at the top of mesophyll. Further reduction of the residual UV-B radiation, not attenuated by the epidermis, occurs within the cell layer. This is shown by UV-B transmittance of less than 1% in tissue consisting of upper epidermis and mesophyll. The UV-B radiation flux incident on sensitive chromophores and physiological targets, such as nucleic acids and the photosynthetic apparatus, is therefore, a small portion of that striking the leaf and yet sufficient to cause damage.

Although UV absorbance generally increased and epidermal UV transmittance generally decreased in response to UV-B irradiation, the reduction in transmittance is less than would be expected from increases in absorbance. As mentioned previously, absorbance refers to radiation absorbed exponentially, and is related to transmittance on this basis (Jagger 1967). Thus, a twofold increase in absorbance can result in as much as a 10-fold decrease in transmittance. For solutions, this relationship between absorbance and transmittance conforms to theoretical expectations and is caused by the relatively homogeneous distribution of the dissolved

solute in the solvent. This is not the case in the leaf, where flavonoid compounds are highly localized in cell vacuoles and around plastids (McClure 1976). A strict exponential relationship between absorbance and transmittance in the epidermis is therefore not observed.

In addition to a reduction in the magnitude of epidermal UV-B and UV-A transmittance, a shift in the spectral distribution of transmittance was observed (see Fig. 2 and 4). This shift was expressed as greater reduction in UV-A transmittance than UV-B transmittance. This phenomenon may be related to the absorption characteristics of flavonoids. These compounds generally absorb more strongly in the UV-A than the UV-B portion of the solar spectrum. Thus, the greater reduction in UV-B transmittance may be due to greater flavonoid absorption in this region.

Cell wall constituents such as cellulose and hemicellulose are highly transparent to UV radiation and thus contribute relatively little to filtration capacity of the epidermis (Frey-Wyssling 1976). Cuticular waxes are composed of long chained parafins (or alkanes) or primary alcohols (Martins and Juniper 1970). These structures typically absorb below 200 nm, so no appreciable absorption of UV-B radiation is due to leaf waxes (Frey-Wyssling 1976, Crooks 1978). The attenuation of UV by culticular waxes was not appreciable in four species examined by Wuhrmann-Meyer and Wuhrmann-Meyer (1941). They measured extinction coefficients between 250 and 400 nm of the wax of

each species. Although some UV extinction was observed at 290 nm, the extinction coefficients were generally quite low.

Wavelength selectivity of epidermal tissue

In regard to solar radiation, the epidermis is a complex radiation filter with substantial differences in the attenuation capacity over the 290 to 700 nm waveband (see Fig. 4). Epidermal transmittance is typically less than 10% in the UV-B region, increases substantially with increasing wavelength between 320 and 400 nm, and becomes asymptotic at about 70 to 80% between 400 and 700 nm. These observations are very similar to the data presented by McCree and Keener (1974) on epidermal UV and visible transmittance in cabbage leaves. This pattern in the UV waveband has been demonstrated for 68 plant species by Robberecht et al. (1980). In addition to differences in the magnitude of transmittance between 290 and 700 nm, the spectral distribution of transmittance in the UV region appears to be relatively characteristic for each species. Since UV-B radiation is generally considered to be deleterious to plants (Caldwell 1971, 1981), effective filtration of UV-B by the epidermis would seem beneficial to the plant. In contrast, transmittance of visible radiation (400 to 700 nm) is essential for photosynthesis and therefore, the epidermis must be highly transparent to this radiation to maximize its penetration to the mesophyll.

The highly selective nature of epidermal attenuation may be a result of the combined optical properties of the structural and pigment components of the tissue. As mentioned above, cell wall constituents and cuticular waxes are, in general, relatively transparent to UV and visible radiation and, thus, do not substantially contribute to the attenuation capacity of the epidermis. Flavonoid compounds, however, absorb strongly in the UV region but not in the visible region. Anthocyanins, which absorb strongly between 520 and 560 nm, are the exception (McClure 1976). The presence of flavonoid compounds is thus a significnat factor determining the wavelength selectivity of the epidermis.

A limited amount of phenotypic plasticity in epidermal transmittance of visible radiation was evident. Transmittance of visible radiation between 400 and 430 nm was reduced similar to that of UV radiation, although to a lesser degree (see Table 1). The reduction in transmittance in the 400- to 430-nm waveband was between 6 and 14%. The effect of UV-B irradiation on epidermal transmittance in the 450- to 650-nm region was generally negligible.

Although the reductions in visible transmittance appear relatively small, slight reductions in photosynthetically active radiation incident at the mesophyll may affect the rate of photosynthesis. The small observed decreases in the penetration of visible radiation between 400 and 430 nm may be biologically significant, since chlorophyll absorbs in this region (Sestak et

al. 1971). The photosynthetically active radiation penetrating to the mesophyll would still most likely be sufficient to saturate photosynthesis at the high light intensities normally occurring at midday. However, at lower light intensities, occurring in the morning or late afternoon, a small reduction in epidermal transmittance of visible radiation could depress the rate of photosynthesis and reduce the total daily carbon gained by the plant. When epidermal transmittance between 400 and 650 nm is weighted on the basis of its effect on photosynthesis using data presented by McCree (1972) on the relative quantum yield for photosynthesis, the reductions in transmittance caused by UV-B irradiation are only 2 to 4%.

Plant UV-B sensitivity and acclimation

The photosynthetic rate of plants under the stress of UV-B radiation has been taken as an indicator of overall plant sensitivity by several investigators (Sisson and Caldwell 1976, 1977, Van et al. 1976, Brandle et al. 1977, Teramura et al. 1980, Sisson 1981). Although sensitivity to UV-B irradiation has been demonstrated in some species, the degree of UV-B sensitivity among species can be quite variable (Van et al. 1976).

The pattern of photosynthetic reduction under the three UV-B dose rates employed indicate that Oenothera stricta has a threshold for photosynthetic depression that can be related to

species is native or exotic. The moderate UV-B dose rate at which no substantial photosynthetic reduction was observed is approximately the maximal daily effective UV-B flux present in its native habitat of temperate South America. In the higher UV-B irradiance environments of the Hawaiian Islands, O. stricta may be near the threshold of its ability to tolerate UV-B irradiation. The moderately high UV-B daily dose of 3110 effective J/m^2 is approximately 20% higher than the daily effective dose in the Hawaiian Islands (Caldwell et al. 1981) and exceeds the tolerance threshold to UV-B irradiation in this species. Although the moderately high dose is 20% higher that the maximum daily effective dose in the Hawaiian Islands, it represents approximately a 14% decrease in stratospheric ozone. This decrease is within the predicted range of ozone depletion (National Academy of Sciences, 1979a and b). Thus O. stricta may not only be restricted in its range by the UV-B flux naturally present in its environment, its establishment in high irradiation habitats would be restricted in the event of a small reduction in ozone.

There is no apparent reciprocity in the effect of UV-B radiation on photosynthesis as was found by Sisson and Caldwell (1977). Short-term high UV-B irradiation results in greater photosynthetic depression than long-term low UV-B irradiation. Oenothera stricta can apparently acclimate to a moderate UV-B dose, but not to high UV-B irradiance. The latter exceeds the capacity of epidermal attenuation and repair processes, and is expressed in significant photosynthetic depression and eventual

physical damage to the leaf.

The evaluation of plant UV-B sensitivity and the processes leading to plant UV-B acclimation involves an interaction of several inportant factors. Although measurements of photosynthetic depression may be used as an index of plant sensitivity, it does not by itself indicate a mechanism of plant acclimation. Plant UV-B sensitivity and eventual acclimation is more likely a result of the dynamic balance between the UV-B irradiance penetrating the leaf, its absorption, and the efficiency of UV radiation repair mechanisms.

In order for UV-B radiation to elicit an effect on the photosynthetic apparatus, or any plant process, it must be absorbed. A high epidermal UV attenuation capacity, and the resulting low UV-B flux at the mesophyll layer, is thus the first factor that can influence plant sensitivity. The UV-B flux at the mesophyll (I_{mes}), weighted for its effectiveness for inducing damage in plants (Caldwell 1968), represents the effective UV-B irradiation with the potential to strike sensitive targets in the mesophyll. Figure 6 illustrates the effectiveness of leaf epidermal attenuation and I_{mes} of *O. stricta* leaves. The daily I_{mes} was calculated with equation given by Robberecht et al. (1980) and is shown for solar UV-B irradiance expected under natural conditions in June at 42° N latitude and under a 15% ozone depletion. These calculations illustrate that the penetration of UV-B radiation to the mesophyll layer is substantially reduced by the epidermis.

Increased epidermal UV attenuation in response to UV-B irradiation, and the resulting reduction of UV-B irradiance at the mesophyll, may represent a type of acclimation to UV-B radiation.

The phenotypic plasticity of epidermal transmittance, decreasing I_{mes}, is particularly important in view of the mechanisms of the UV repair processes that are present in plant. As described by Howland (1975) and Murphy (1975), the capacity of mechanisms that repair UV-B induced lesions in nucleic acids and proteins is limited. If the formation of pyrimidine dimers occurs at a rate that exceeds the repair capacity, damage to these biologically important molecules will acculmulate. Howland (1975) has demonstrated the phenomenon of repair capacity saturation in wild carrot protoplasts that were irradiated with UV-C radiation. While the effects of UV-C and UV-B are not always related, a similar repair mechanism may exist for the repair of UV-B induced damage.

CONCLUSIONS

The epidermis was found to be an effective filter of UV-B radiation and considerably limits the penetration of UV-B radiation into the leaf. Epidermal tissue of Oenothera stricta attenuated up to 95% of the UV-B radiation incident on the leaf. This represents a substantial reduction in the daily UV-B irradiance incident at the mesophyll layer, where the potential for UV-B induced damage is high.

The epidermis was not only found to be an effective UV-B filter, it appeared to have a high degree of wavelength selectivity. This tissue layer was highly transparent to visible radiation, with up to 80% of the visible radiation indcident on the leaf penetrating to the mesophyll, yet nearly opaque to UV-B radiation. The degree of UV-A attenuation was intermediate between attenuation of visible and UV-B radiation.

Some degree of phenotypic plasticity in the capacity of the epidermis to attenuate UV-B radiation was demonstrated. A reduction in the penetration of UV-B irradiance through the epidermis, and concomitant reduction of the UV-B flux at the mesophyll, may be one portion of the acclimation process of higher plants to UV-B radiation. Given this observed phenotypic plasticity, some predictions may be made in regard to UV-B transmittance in the epidermis of species in the plant community. Epidermal attenuation of solar UV-B radiation in Oenothera stricta would be expected to increase as solar UV-B irradiance increases over the growing season. In the event of an intensification of UV-B irradiance, due to a partial depletion of stratospheric ozone, epidermal UV-B attenuation may also become more effective in species with a high degree of plasticity in this parameter. This response in epidermal transmittance may be particularly important, since repair systems may not be able to keep pace with the rate of damage without some reduction in the UV-B flux incident at the mesophyll.

Effective UV-B attenuation and the wavelength selectivity in the epidermal layer may result from the presence of UV-absorbing compounds in this tissue. Flavonoid and related compounds are commonly found in leaf tissue, and strongly abosrb UV radiation. Their induction in response to UV irradiation has been demonstrated by Wellmann (1974). The increases in extract absorbance found in this study were apparently due to increased flavonoid absorbance in response to UV-B radiation. The ability for flavonoid synthesis in the leaf suggests a potentially responsive mechanism for altering the UV-B absorption properties of the epidermis under a changing UV-B irradiance environment.

The degree of epidermal transmittance in Oenothera stricta prior to UV-B exposure was rather low. The observed reduction in transmittance caused by UV-B irradiation may have been of a level sufficient to maintain any UV-B induced injury below a threshold point. This may account in part for the lack of plant sensitivity at moderate UV-B dose rates. When the attenuation capacity of the epidermis and mesophyll are exceeded, as at high UV-B dose rates, significant photosynthetic depression was observed.

The particular UV-B sensitivity of a species is, however, more likely the result of an interaction of several factors, rather than of epidermal UV-B attenuation alone. In addition to the UV-B attenuating capacity of the epidermis, factors such as UV-B absorption in the mesophyll and the efficiency of UV repair

systems may be important. The dynamic balance between the UV-B dose rae, the attenuation capacity in the epidermis and mesophyll, and repair systems perhaps determines the overall UV-B sensitivity of the plant.

Under field conditions, where plants are exposed to several different environmental stress factors simultaneously, the stress of UV-B radiation may be enhanced. This is suggested by the study of Fox and Caldwell (1978), where plants were simultaneously exposed to intensified UV-B radiation and the stress of interspcific competition. The results of this study showed that the same species may show different degrees of UV-B sensitivity, depending on the particular competitor species it encounters. Furthermore, the effect of UV-B irradiation was not expressed as a decrease in the total biomass of the two competing species, but as a decrease in the biomass or density of the more sensitive species.

The interaction of UV-B radiation with different environmental stress factors in the field has particular implications for plant community structure under an intensified UV-B radiation regime. Rather than an overall reduction in plant production in the community, intensified solar UV-B irradiance may result in slight changes in the competitive balance among species, which may eventually be expressed as subtle shifts in species composition and relative abundance.

Appendix 1

The following appendix summarizes the results of UV-B effects on Rumex obtusifolius and Rumex patientia for experiments similar to those described in the previous section. Epidermal UV transmittance of nonirradiated leaves is shown in Fig. 8. Transmittance differed significantly between the two Rumex species. The difficulty in obtaining fresh epidermal tissue of sufficient size for experimentation limited the study 'V optical properties in these species. Mesophyll transmittance of UV-B radiation in either species was negligible, similar to the attenuation found for Oenothera stricta.

Field UV-B study methods

The effect of solar UV-B radiation was examined in the field, using a UV transparent filter and a filter opaque to UV-B. Solar UV-B radiation could thus be removed from the plant's environment. These plastic filters were suspended on metal frames above a group of plants. A fan was positioned at the end of each frame to provide air circultion and convective cooling of the plants beneath these frames.

The frames were covered with either a 0.08-mm Aclar (Allied Chemical Corp.) or a 0.13-mm Mylar plastic filter. Aclar is highly transparent to UV radiation, whereas Mylar is opaque to wavelengths shorter than approximately 315 nm. The filter transmission characteristics are shown in Fig. 7. Plants

beneath the Aclar filter would not be exposed to the full solar UV-B flux because of the UV attenuation in this filter. Measurements of solar UV-B radiation beneath the filters were made three times daly during the experiment. These were made at approximately two hours before solar noon, at solar noon, and two hours after solar noon. Using this schedule, estimates of total daily solar UV-B, absolute and effective, could be determined by computer simulation modeling of solar UV-B irradiance (based on a model by Green et al. 1980). There were two major periods of field experimentation. Two experiments were conducted near the time of the summer solstice, between June 11th and July 7th. A second set of experiments was conducted between July 31st and August 8th. The mean daily effective UV-B irradiation beneath the filter-frames for the first experimentation period was estimated to be 1125 $J.m.^{-2}.d^{-1}$. The UV-B dose rate for the second period was estimated at 1151 $J.m^{-2}.d^{-1}$. Skies were generally clear during the periods of field experimentation.

The response of the two Rumex species to solar UV-B radiation in the field was similar to the trends observed for these species and Oenothera stricta under the simulated UV-B radiation regime in the greenhouse. Epidermal UV-B and UV-A transmittance of R. obtusifolis leaves were significantly reduced by 27% after eight days of exposure to solar UV-B radiation (Fig. 8). A similar trend in epidermal transmittance was also evident in a second experiment with R. obtusifolius, although in this case the decrease in transmittance after seven

days of solar UV-B irradition was not significant. No transmittance measurements of R. patientia leaves were possible because the UV-B irradiated epidermis could not be removed in samples of sufficient size for examination. The UV-B irradiated tissue was more prone to disintegration upon removal, as compared to the nonirradiated tissue. The measurement of epidermal transmittance from non-irradiated leaves was possible and shows a significant difference in the degree of transmittance between species (Fig. 9).

Although there was some difficulty in measuring epidermal transmittance in the two Rumex species, measurement of whole-leaf extract absorbance could be made consistently. Whole-leaf tissue abosrbance significantly increased in both species for field UV-B irradiation periods of more than five days. No significant increase in absorbance was observed in R. patientia leaves exposed to solar UV-B irradiation for three days (Table 3). The estimated daily dose of biologically and DNA effective UV-B for each experiment (see "Methods" section) was rel tively similar among experiments.

The effect of natural solar UV-B irradiance on the photosynthetic rates of these two Rumex species was also examined. The similarity of solar UV-B irradiance among the experiments allowed a reasonable degree of comparison between the replications. No clear short-term depression of photosynthesis was evident in either R. patientia or R. obtusifolius. The progression of photosynthesis during a three-

to eight-day exposure to solar UV-B radiation showed no significant depression in the photosynthetic rate. However, a trend of reduced photosynthesis is apparent in one experiment with each species. In these experiments, photosynthesis of the UV-B irradiated plants was depressed slightly below that of the nonirradiated plants after one day of exposure to solar UV-B radiation. Photosynthesis of the former remained lower than the plants not subjected to UV-B irradiation for the duration of the experiment. On the basis of the rate of photosynthesis in response to filtered solar UV-B irradiation, these species do not appear to be particularly sensitive to these moderate levels of solar UV-B radiation.

LITERATURE CITED

Bener, P. 1960. Investigation on the spectral intensity of ultraviolet sky and sun+sky radiation under different conditions of cloudless weather at 1590 m a.s.l. Contract AF 61(052)-54 Technical Summary Report Number 1, United States Air Force, Washington, District of Columbia, USA.

Bener, P. 1964. Investigation on the influence of clouds on ultraviolet sky radiation. Contract AF 61 (052)-618 Technical Note Number 3, United States Air Force. Washington, District of Columbia, USA.

Bener, P. 1972. Approximate values of intensity of natural ultraviolet radiation for different amounts of atmospheric ozone. Technical Report DAJA37-68-C-1017, United States Army, Washington, District of Columbia, USA.

Biggs, R.H., W.B. Sisson, and 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, and R.H. Biggs, editors. Impacts of climatic change on the biosphere. Climatic Impact Assessment Program Monograph 5. Report Number DOT-TST-75-55, United States Department of Transportation, Springfield, Virginia, USA.

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

Bolliger, R. 1959. Entwicklung und Strucktur der Epidermisaussewand bei einigen Angiospermenblattern. Journal of Ultrastructure Research 3:105-130.

Bogenrieder, A., and R. Klein. 1977. Die Rolle des UV-lichtes beim sog. Auspflanzungsschock von Gewachshaussetzlingen. Angewandte Botanik 51:99-107.

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, editor. Photophysiology Volume 6. Academic Press, New York, New York, USA.

Caldwell, M.M. 1977. The effects of solar UV-B radiation (280-315 nm) on higher plants: implications of stratospheric ozone reduction. Pages 597-607 in A. Castellani, editor. Research in Photobiology. Plenum, New York, New York, USA.

Caldwell, M.M. 1979. Plant life and ultraviolet radiation: some perspectives in the history of the Earth's UV climate. BioScience 29:520-525.

Caldwell, M.M. 1981, in press. Plant response to ultraviolet radiation. Encyclopedia of Plant Physiology.

Caldwell, M.M., R. Robberecht, and W.D. Billings. 1980. A steep latitudinal gradient of solar ultraviolet-B radiation in the arctic-alpine life zone. Ecology 61:600-611.

Cline, M.G., and F.B. Salisbury. 1966. Effects of ultraviolet radiation on the leaves of higher plants. Radiation Botany 6:151-163.

Crooks, J.E. 1978. The spectrum in chemistry. Academic Press, New York, New York, USA.

Dickson, J.G., and M.M. Caldwell. 1978. Leaf development of Rumex patientia L. (Polygonaceae) exposed to UV irradiation (280-320 nm). American Journal of Botany 65:857-863.

Dutsch, H.U. 1971. Photochemistry of atmospheric ozone. Pages 219-322 in H.E. Landsberg and J. Van Mieghem, editors. Advances in Geophysics Volume 15. Academic Press, New York, New York, USA.

Ehrlich, P.R. and P.H. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18:586-608.

Fox, F.M. and M.M. Caldwell. 1978. Competitive interaction in plant populations exposed to supplementary ultraviolet-B radiation. Oecologia 36:173-190.

Frey-Wyssling, A. 1976. The plant cell wall. Gebruder Borntraeger, Berlin, Germany.

Gast, P.R. 1965. Solar irradiance. Section 16-1 in S.L. Valley, editor. Handbook of Geophysics and Space Environments. Air Force Cambridge Research Laboratories, Office of Aerospace Research, United States Air Force, Hanscom Field, Bedford, Massachusetts, USA.

Gausman, H.W., R.R. Rodriquez, and D.E. Escobar. 1975. Ultraviolet radiation reflectance, transmittance, and absorptance by plant epidermises. Agronomy Journal 67:719-724.

Giese, A.C. 1964. Studies on ultraviolet radiation action upon animal cells. Pages 203-245 in A.C. Giese, editor. Photophysiology Volume 2. Academic Press, New York, New York, USA.

Green, A.E.S. 1966. The middle ultraviolet: its science and technology. John Wiley and Sons, New York, New York, USA.

Griggs, M. 1966. Atmospheric ozone. Pages 83-117 in A.E.S. Green, editor. The Middle Ultraviolet: Its Science and Technology. John Wiley and Sons, New York, New York, USA.

Harm, W. 1979. Relative effectiveness of the 300-320 nm spectral region of sunlight for the production of primary lethal damage in E. coli cells. Mutation Research 60:263-270.

Hidalgo, H. 1975. Potential increases in UV radiation from stratospheric flight. Pages 2-3 to 2-108 in D.S. Nacthwey, M.M. Caldwell, R.H. Biggs, editors. Impacts of climatic change on the biosphere. Climatic Impact Assessment Program Monograph 5. Report Number DOT-TST-75-55, United States Department of Transportation, Springfield, Virginia, USA.

C-2

Holubek, J. and O. Strouf. 1965. Spectral data and physical constants of alkaloids. Heyden and Son Limited, London, England.

Howland, G.P. 1975. Dark-repair of ultraviolet-induced pyrimidine dimers in the DNA of wild carrot protoplasts. Nature 254:160-161.

Jagger, J. 1967. Ultraviolet photobiology. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Koller, L.R. 1965. Ultraviolet radiation. John Wiley and Sons, New York, New York, USA.

Lautenschlager-Fleury, D. 1955. Uber die Ultraviolettdurchlassigkeit von Blattepidermen. Berichte der Schweizerischen Botanischen Gessellschaft 65:343-386.

Lee, D.W., and J.B. Lowry. 1980. Solar ultraviolet on tropical mountains: can it affect plant speciation? American Naturalist 115:880-883.

Lowry, B., D. Lee, and C. Henant. 1980. The origin of land plants: a new look at an old problem. Taxon 29:183-197.

Luckiesh, M. 1946. Applications of germicidal, erythemal and infrared energy. D. Van Nostrand, New York, New York, USA.

Mabry, T.J., K.R. Markham, and M.B. Thomas. 1970. The systematic indentification of flavonoids. Springer-Verlag, New York, New York, USA.

Martin, J.T. and B.E. Juniper. 1970. The cuticles of plants. St. Martin's Press, New York, New York, USA.

McClure, J.W. 1976. Secondary metabolism and coevolution. Nova Acta leopoldina. Supplement Number 7. Pages 463-496.

McCree, K.J. 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agricultural Meteorology 9:191-216.

McCree, K.J. and M.E. Keener. 1974. Effect of atmospheric turbidity on the photosynthetic rates of leaves. Agricultural Meteorology 13:349-357.

Murphy, T.M. 1975. Effects of UV radiation on nucleic acids. Pages 3-21 to 3-44 in D.S. Nachtwey, M.M. Caldwell, R.H. Biggs, editors. Impacts of climatic change on the biosphere. Climatic Impact Assessment Program Monograph 5. Report Number DOT-TST-75-55, United States Department of Transportation, Springfield, Virginia, USA.

Murphy, T.M., C.M. Hamilton, and H.E. Street. 1979. A strain of Rosa damascena cultured cells resistant to ultraviolet light. Plant Physiology. 64:936-941.

National Academy of Sciences. 1979a. Protection against depletion of stratospheric ozone by chlorofluorocarbons. Washington, District of Columbia, USA.

National Academy of Sciences. 1979b. Stratospheric ozone depletion by halocarbons: chemistry and transport. Washington, District of Columbia, USA.

Nozzolillo, C. 1972. The site and chemical nature of red pigmentation in seedlings. Canadian Journal of Botany 50:29-34.

Parrish, J.A., R.R. Anderson, F. Urbach, and D. Pitts. 1978. UV-B. Plenum Press, New York, New York, USA.

Patterson, D.T., J.A. Bunce, R.S. Alberte, and E. Van Volkenburgh. 1977. Photosynthesis in relation to leaf characteristics of cotton from controlled and field environments. Plant Physiology 59:384-387.

Plesser, A. and G. Weissenbock. 1977. Untersuchungen zur Lokalisation von Flavonoiden in Plastiden. IV.

Flavonoidigehalt intaker Chloroplasten aus Avena sativa L. Zeitschrift fur Pflanzenphysiologie 81:425-437.

Ribereau-Gayon, P. 1972. Plant Phenolics. Hafner, New York, New York, USA.

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 artic-alpine life zone. Ecology 61:612-619.

Seigler, D.S. 1977. Primary roles for secondary compounds. Biochemical Systematics and Ecology 5:195-199.

Sestak, Z., J. Catsky, P.G. Jarvis. 1971. Plant photosynthetic production. Dr. W. Junk N.V., The Hague, Netherlands.

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.

Silberglied, R.E. 1979. Communication in the ultraviolet. Annual Review of Ecology and Systematics 10:373-398.

Sisson, W.B. 1981. Photosynthesis, growth, and ultraviolet irradiance absorbance of Curcurbita pepo L. leaves exposed to ultraviolet-B radiation (280-315 nm). Plant Physiology 67:120-124.

Sisson, W.B., and M.M. Caldwell. 1976. Photosynthesis, dark respiration, and growth of Rumex patientia L. exposed to ultraviolet irradiance (288 to 315 nanometers) simulating a reduced atmospheric ozone column. Plant Physiology 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 u.v.-B radiation. Journal of Experimental Botany 28:691-705.

Swain, T. 1975. Evolution of flavonoid compounds. Pages 1096-1129 in J.B. Harborne, T.J. Mabry, and H. Mabry, editors. The Flavonoids Part 2. Academic Press, New York, New York, USA.

Teramura, A.H. 1980. Effects of ultraviolet-B irradiances on soybean. Plant Physiology 65:483-488.

Van, T.K., and L.A. Garrard. 1975. Effect of UV-B radiation on net photosynthesis of some C3 and C4 crop plants. Proceedings Soil and Crop Science Society, Florida, USA 35:1-3.

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

Venable, W.H., Jr., and H.J. Kostkowski. 1975. Reflectance of coatings made from Halon powder. Report, National Bureau of Standards, USA.

Weissenbock, G., A. Plesser, and K. Trinks. 1976. Flavonoidgehalt und Enzymaktivitaten isolieter Haferchloroplasten (Avena sativa L.). Berichte der Deutschen Botanischen Gessellschaft 89:457-472.

Wellmann, E. 1974. Regulation der Flavonoidbiosynthese durch ultraviolettes Licht und Phytochrom in Zellkulturen und Keimlingen von Petersilie (Petroselinum hortense Hoffm.) Berichte der Deutschen Botanischen Gessellschaft 87:267-273.

Wellmann, E. 1975a. UV dose-dependent induction of enzymes related to flavonoid biosynthesis in cell suspension cultures of parsley. FEBS Letters 51:105-107.

Wellmann, E. 1975b. Der Einfluss physiologischer UV-Dosen auf Wachstum und Pigmentierung von Umbelliferenkeimlingen. Pages 229-238 in E. Bancher, editor. Industrieller Pflanzenbau. Gessellschaft Forderung Industrieller Pflanzenbaues, Vienna, Austria.

Whittaker. R.H. and P.R. Feeney. 1971. Allelochemics: chemical interactions between species. Science 171:757-770.

Wuhrmann-Meyer, K. and M. Wuhrmann-Meyer. 1941. Untersuchungen uber die Absorption ultravioletter Strahlen durch Kutikular-und Wachsschichten von Blattern. I. Planta 32:43-50.

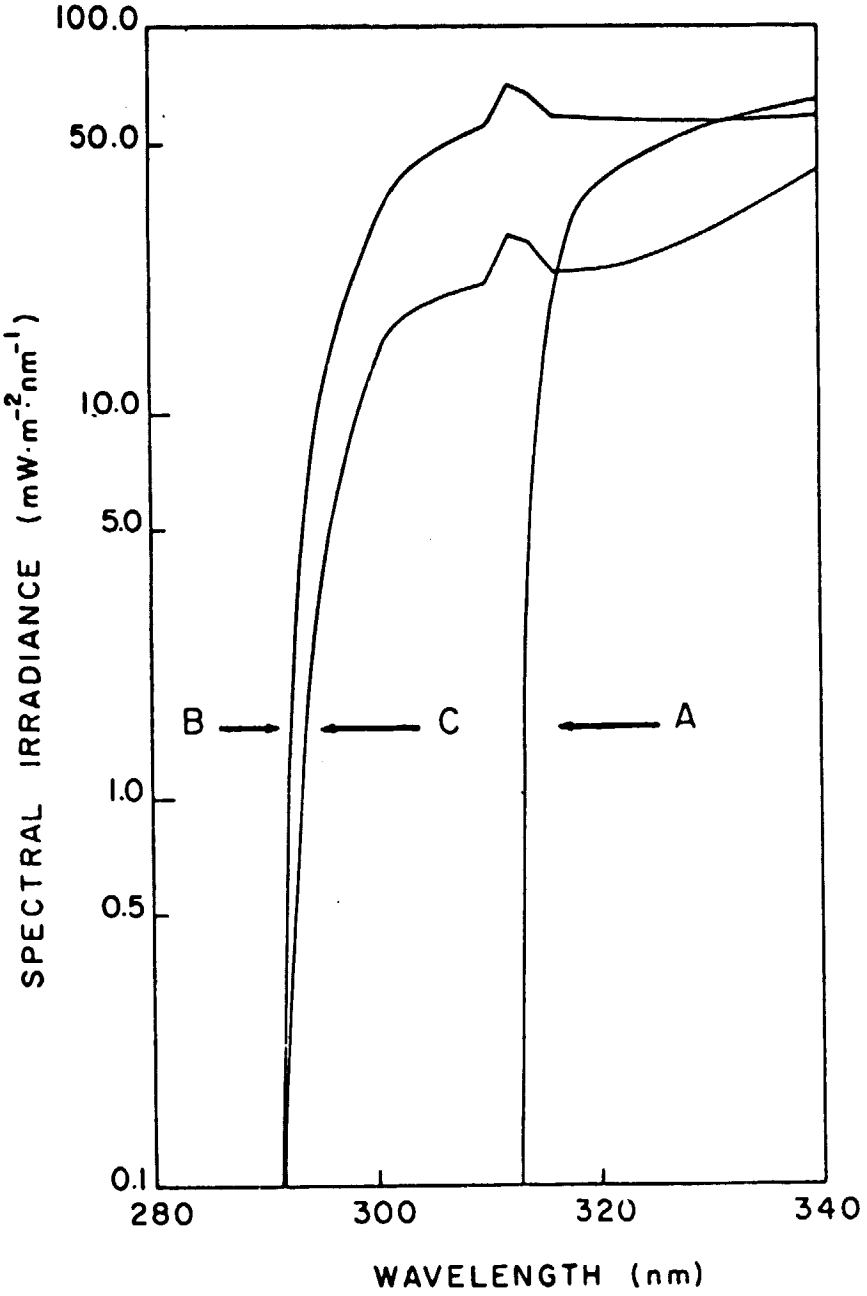


Fig. 1. The spectral distribution of fluorescent sunlamps filtered with Mylar (A) and cellulose acetate (B and C). A representative UV-B dose of 90 biologically effective mW/m^2 (12 DNA effective mW/m^2) is shown (B). Four layers of neutral density cloth reduced this dose to 37 biologically effective mW/m^2 (5 DNA effective mW/m^2) (C). Mylar-filtered sunlamps transmit essentially no effective UV-B radiation.

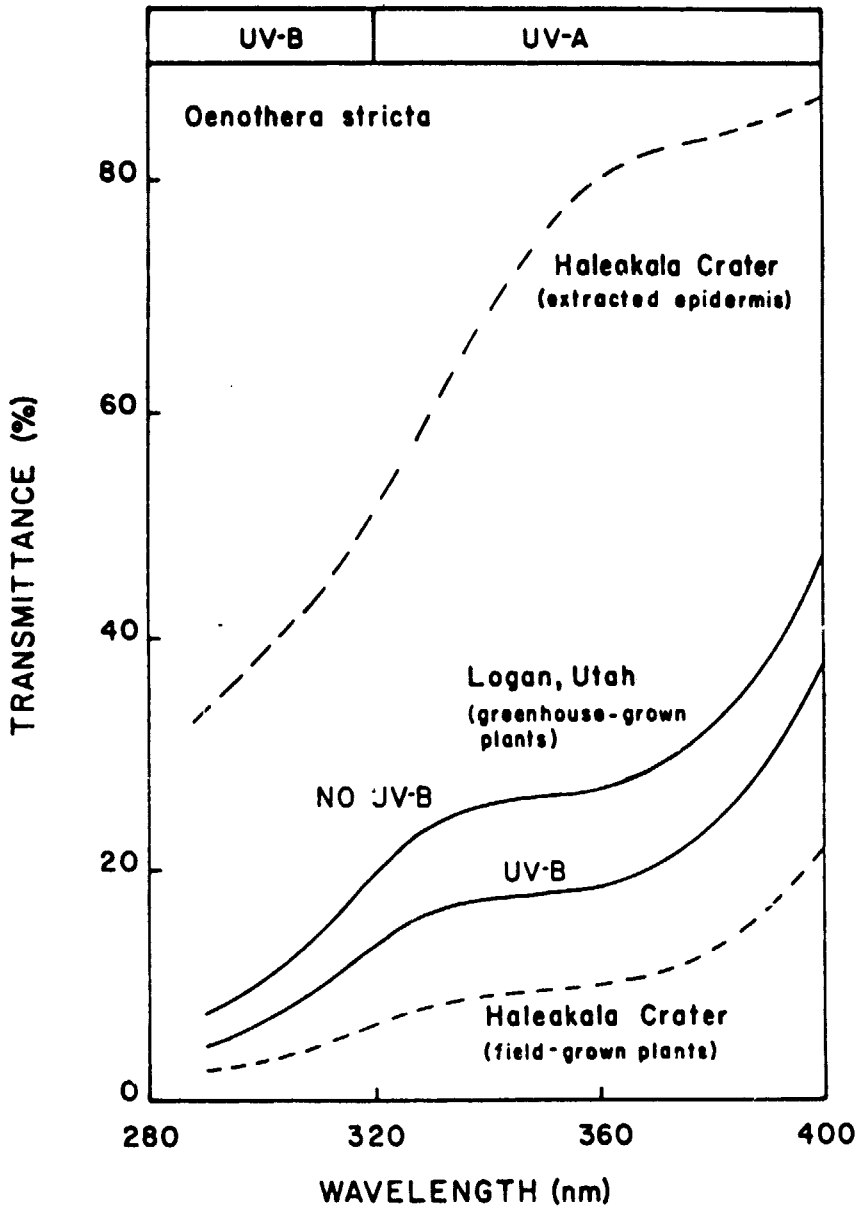


Fig. 2. Epidermal UV transmittance of Oenothera stricta from field-grown and greenhouse-grown plants. Exposure to UV-B irradiation (2050 effective $J.M^{-2}.d^{-1}$) resulted in a significant reduction in epidermal transmittance in greenhouse-grown plants. Extracted epidermis refers to tissue from which UV-absorbing pigments such as flavonoids have been removed.

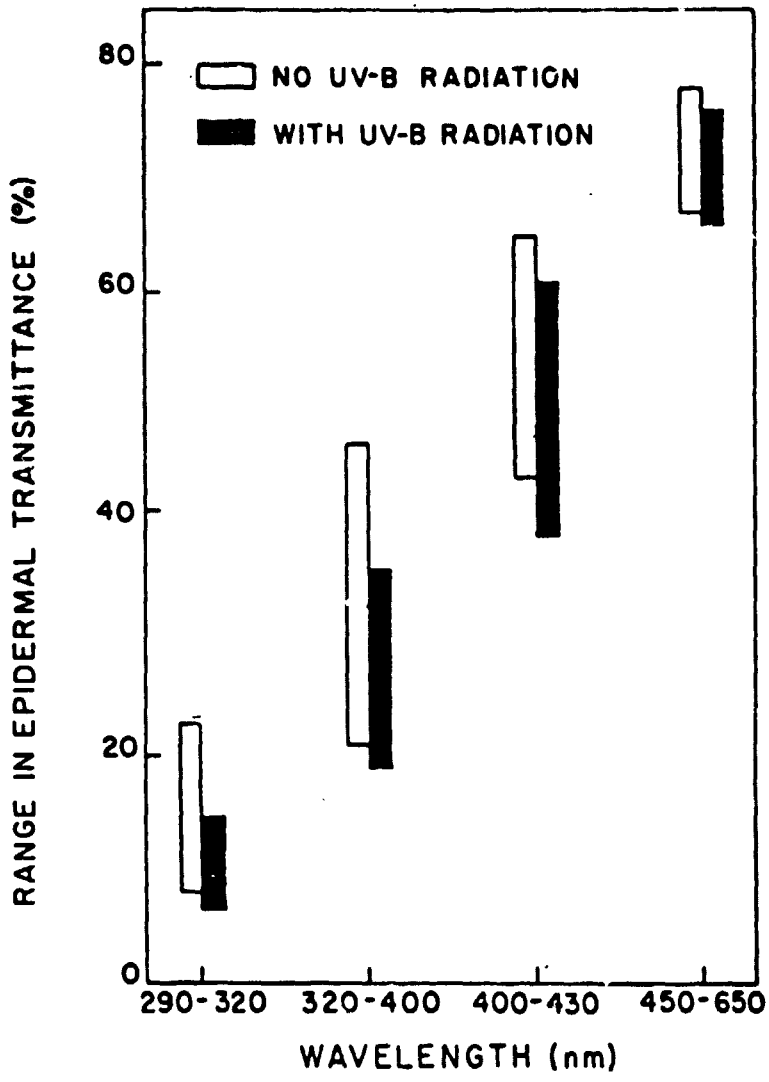


Fig. 3. Minimum and maximum mean epidermal transmittance of Oenothera stricta leaves of all experiments. Leaves exposed or unexposed to UV-B radiation are indicated by the solid and open bars, respectively.

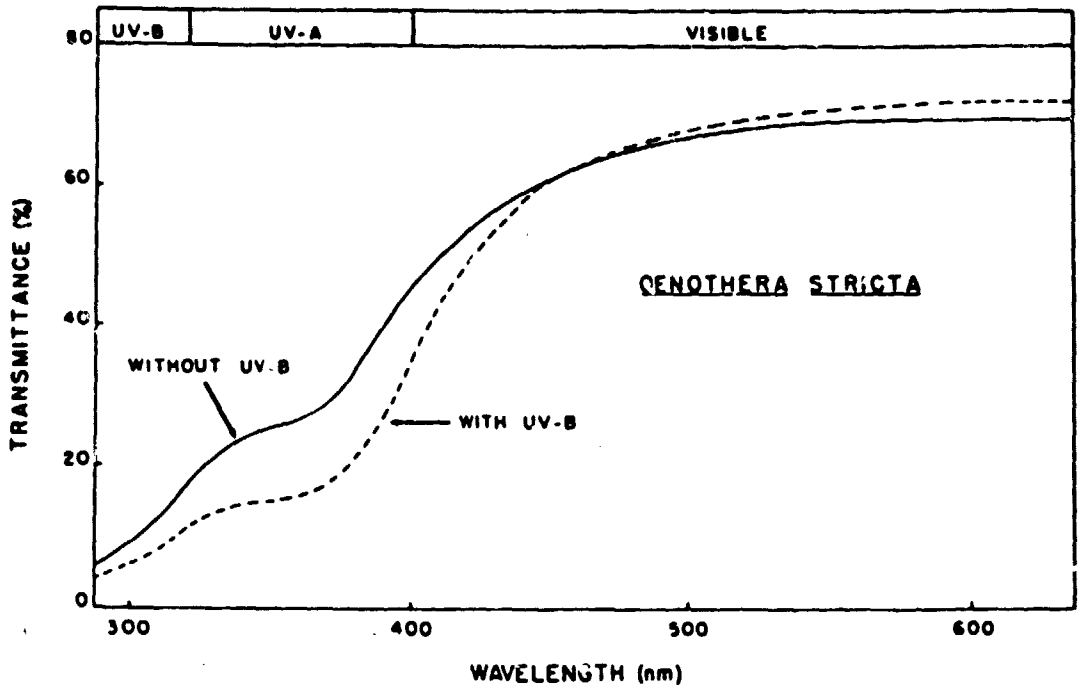


Fig. 4. Typical magnitude of transmittance of Oenothera stricta leaves with or without UV-B irradiation. The plants were exposed to 15 days of UV-B radiation at a mean dose rate of 2050 $J \cdot m^{-2} \cdot d^{-1}$, on a biologically effective basis. Epidermal transmittance of UV-B irradiated leaves are significantly lower ($p<0.05$) than the nonirradiated leaves between 290 and 430 nm. These spectra represent the mean of 10 samples with one standard error of the mean of less than 2% in the UV waveband and less than 5% in the visible waveband. The data correspond to the first experiment listed in Table 1.

Fig. 5. Photosynthesis in Oenothera stricta exposed to three levels of UV-B radiation. Significant ($p<0.05$) photosynthetic depression occurred under the moderately high and high UV-B dose rates.

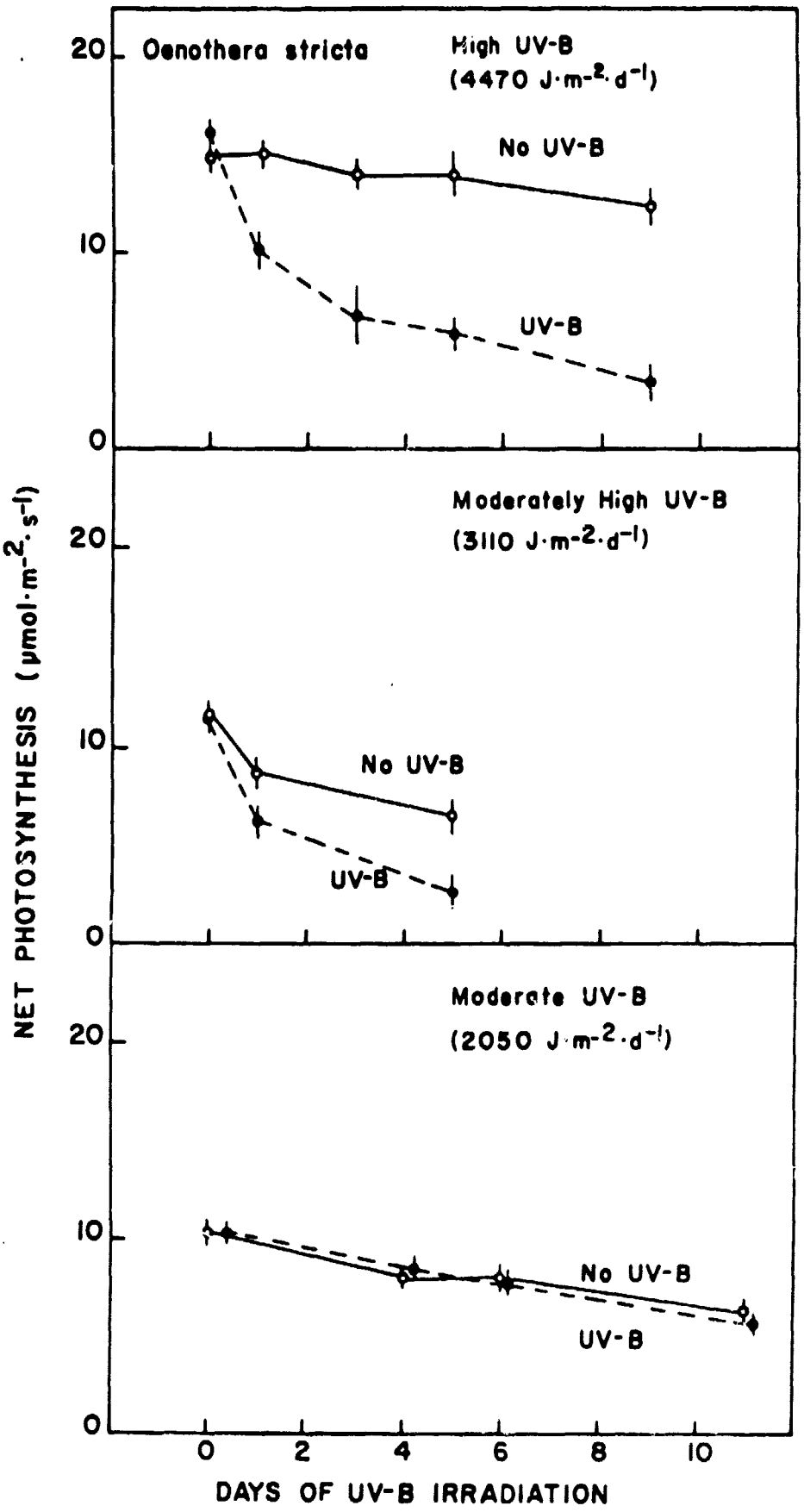

Oenothera stricta
High UV-B
(4470 J·m-2·d-1)
No UV-B
UV-B
Moderately High UV-B
(3110 J·m-2·d-1)
No UV-B
UV-B
Moderate UV-B
(2050 J·m-2·d-1)
No UV-B
UV-B
NET PHOTOSYNTHESIS (μmol·m-2·s-1)
DAYS OF UV-B IRRADIATION
0
10
20
0
2
4
6
8
10

Fig. 5.

Fig. 6. The mean daily Biologically effective UV-B flux at the mesophyll layer I(mes) for UV-B irradiated and nonirradiated leaves of Oenothera stricta under ambient and reduced stratospheric ozone concentrations. Incident UV-B irradiance was calculated using a computer simulation model developed by Green et al. (1980). Ultraviolet-B irradiance was calculated for an elevation of 1.5 km above sea level, 42 degrees N latitude, and mean ambient (0.320 atm.cm) and reduced (0.272 atm.cm) June ozone concentrations. The data correspond to the six separate experiments. The length of the UV-B irradiation periods are noted.

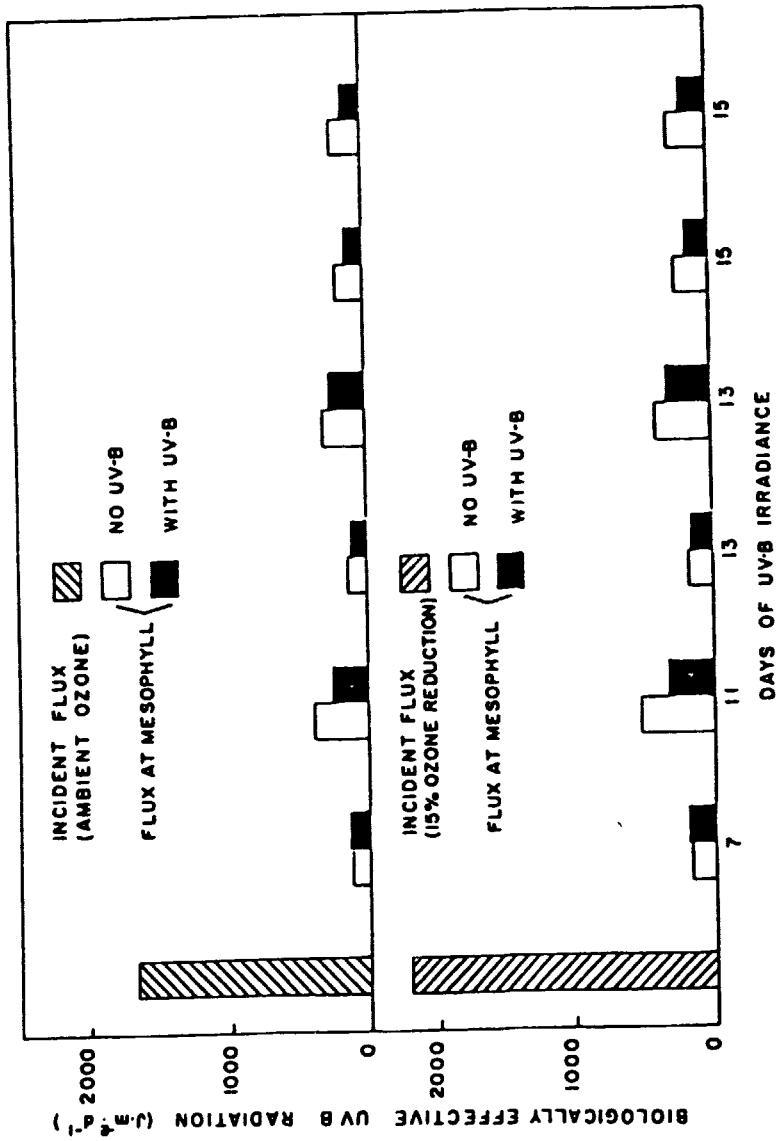

INCIDENT FLUX
(AMBIENT OZONE)
NO UV-B
FLUX AT MESOPHYLL
WITH UV-B
INCIDENT FLUX
(15% OZONE REDUCTION)
NO UV-B
FLUX AT MESOPHYLL
WITH UV-B
BIOLOGICALLY EFFECTIVE UV B RADIATION (J.m⁻².d⁻¹)
2000
1000
0
2000
1000
0
7
11
13
15
13
15
DAYS OF UV-B IRRADIANCE

Fig. 6.

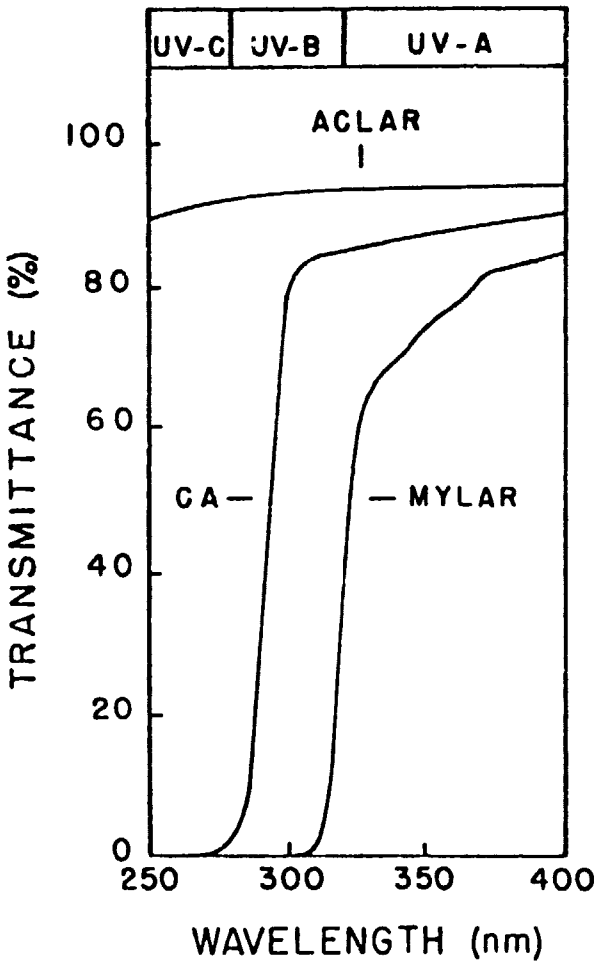


Fig. 7. Transmittance spectra of three filters used in experiments with Westinghouse FS-40 sunlamps or solar radiation.

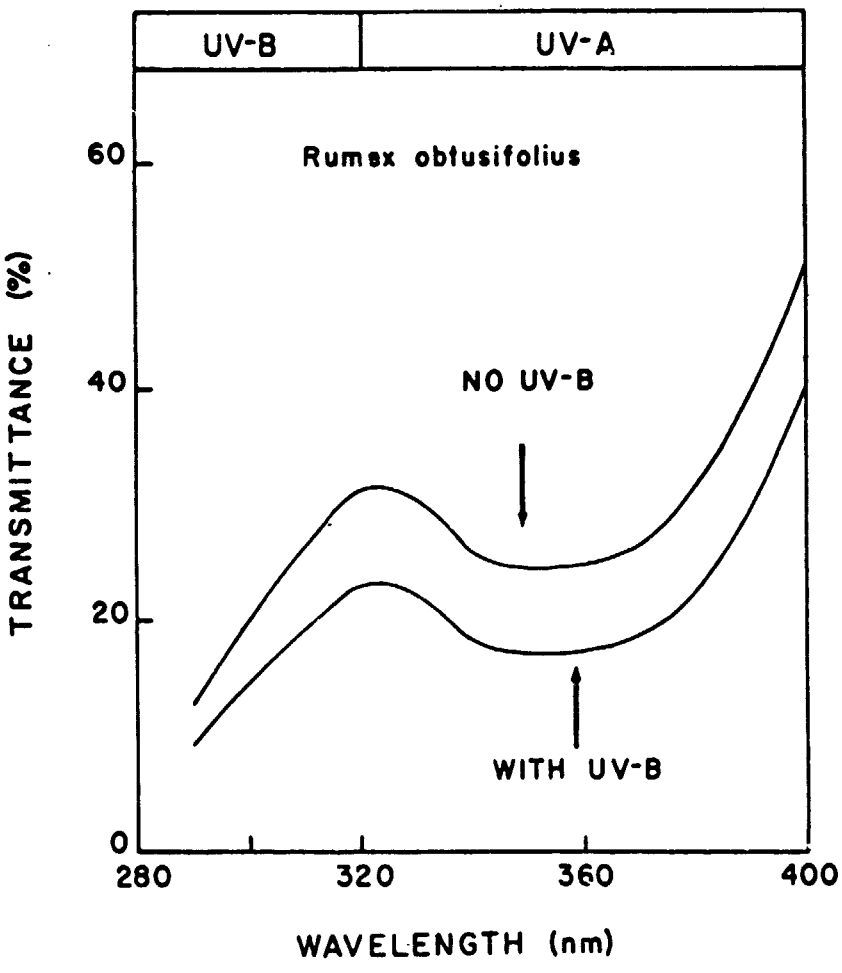


Fig. 8 . Epidermal UV transmittance of Rumex obtusifolius leaves with or without solar UV-B irradiation. The plants were exposed to lar UV-B radiation at a mean biologically effective dose rate of 1151 $J \cdot m^{-2} \cdot d^{-1}$ for eight days. These spectra represent the mean of nine samples per treatment. Epidermal transmittance of UV-B irradiated leaves was significantly lower ($p<0.05$) than that of nonirradiated leaves.

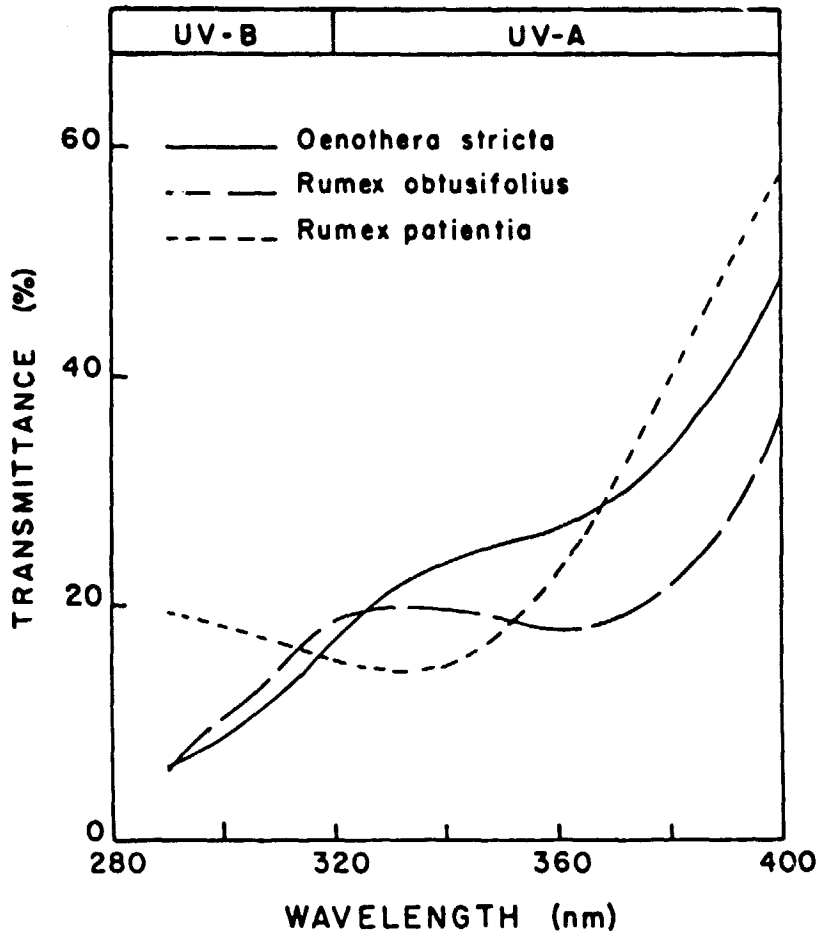


Fig. 9. Typical epidermal transmittance spectra for Oenothera stricta, Rumex obtusifolius, and R. patientia cultivated without exposure to UV-B radiation.

Table 1. The effect of UV-B irradiation on epidermal UV and visible transmittance in Oenothera stricta. The mean of each treatment for six separate experiments, representing seven to 10 samples per treatment, is presented. The mean UV-B dose rate was approximately 2050 biologically effective $J \cdot m^{-2} d^{-1}$. Significant reductions ($p<0.05$) in transmittance in each waveband are indicated by an asterisk.

Days of UV-B Irradiation	Mean Epidermal Transmittance (%) -- Waveband (nm)											
	290-320			320-400			400-430			450-650		
	No UV-B	UV-B	Relative Change (%)	No UV-B	UV-B	Relative Change (%)	No UV-B	UV-B	Relative Change (%)	No UV-B	UV-B	Relative Change (%)
15	11.1	7.4*	-33	30.9	18.9*	-39	44.5	39.1*	-12	67.9	69.4	2
15	13.1	8.8*	-33	29.9	22.0*	-26	--	--	--	71.2	71.2	0
13	18.0	14.5*	-19	42.5	33.3*	-22	--	--	--	--	--	--
13	7.6	6.6	-13	26.9	20.5*	-24	--	--	--	--	--	--
11	23.4	14.9*	-36	47.0	36.5*	-22	64.8	60.9*	-6	77.5	76.5	-1
7	7.7	8.6	12	21.4	21.7	1	--	--	--	67.0	65.6	-2

Table 2. Mesophyll and epidermal extract absorbance of UV-B irradiated and non-irradiated Oenothera stricta leaves. These values represent the mean of five to seven samples per treatment for each of five separate experiments. The mean UV-B dose rate was 2050 biologically effective $J \cdot m^{-2} \cdot d^{-1}$. Experiments in which absorbance was significantly increased ($p<0.05$) after UV-B irradiation are indicated by an asterisk.

Days of UV-B Irradiation	Mean Extract Absorbance (305 nm)					
	Mesophyll			Epidermis		
	No UV-B	UV-B	Relative Change (%)	No UV-B	UV-B	Relative Change (%)
15	0.50	0.64*	28	0.17	0.15	-12
15	0.26	0.30	15	0.05	0.08*	60
13	0.54	0.73*	35	0.03	0.06*	100
11	0.34	0.34	0	0.11	0.17*	55
7	0.54	0.57	6	0.04	0.06*	50

Table 3. Mean extract absorbance of whole-leaf tissue for two Rumex species exposed to solar UV-B radiation. The mean of three to five samples per treatment for each species is presented. Significant increases ($p < 0.05$) in absorbance are indicated by an asterisk.

Species	Days of Solar UV-B Irradiation	Mean Daily UV-B Dose ($J \cdot m^{-2}$)		Mean Extract Absorbance (305 nm)		
		Biologically Effective	DNA Effective	No UV-B	UV-B	Relative Difference (%)
Rumex patientia	3	1151	68	0.14	0.14	0
	5	1125	67	0.27	0.30 *	11
Rumex obtusifolius	8	1151	68	0.26	0.50 *	92

Action spectra for photosynthetic inhibition

M. Caldwell, S. Flint, L. Camp

Introduction

Traditionally, biological action spectra have been undertaken to elucidate photobiological mechanisms, and specifically to identify potential chromophores. Action spectra are usually assessed by evaluating biological responsiveness to monochromatic irradiation. In order to identify potential chromophores, there has been an emphasis on the fine structure of action spectra and much less attention to the tails of these spectra.

In assessment of the consequences of atmospheric ozone reduction, action spectra serve a very different role. Ultraviolet action spectra provide the basis for weighting functions to represent the relative biological effectiveness of spectral irradiance. For polychromatic irradiation, the weighted spectral irradiance can be integrated over a waveband of interest, thus:

$$\text{effective irradiance} = \int I_\lambda \; E_\lambda \; d\lambda$$

where I_λ is the spectral irradiance, and E_λ is the relative effectiveness of irradiance at wavelength$_\lambda$ to elicit a particular biological response. The limits to the integration

are prescribed by the wavelengths where either I_λ or E_λ approach zero. Irradiance can be expressed on either a photon or energy basis.

The utility of expressing biologically effective irradiance in the ozone reduction problem derives from the highly wavelength-specific absorption characteristics of atmospheric ozone and the wavelength specificity of biological action spectra in the UV-B waveband (National Academy of Sciences 1979, Nachtwey and Rundel 1981, Caldwell 1981). The expression of weighted effective irradiance is useful in addressing three basic issues:

(1) The increment of biologically effective irradiation anticipated with a given level of ozone reduction for a specific set of conditions, known as the radiation amplification factor, RAF, is very dependent on action spectrum characteristics (National Academy of Sciences 1979). Without calculation of "biologicaly effective" solar irradiation using a weighting function, the increment of total solar UV-B irradiation with ozone reduction is trivial, e.g., 1% increase of UV-B radiation for 16% ozone reduction for midday irradiance in the summer at temperate latitudes (Caldwell 1981). The increase of solar UV-B irradiation as a function of ozone reduction only becomes significant when the biological

effectiveness of this radiation is taken into account. By the same token, if the action spectrum of a particular biological phenomenon does not exhibit substantial specificity for the UV-B portion of the spectrum and a pronounced increase in effectiveness with decreasing wavelength, the RAF will be very small and this phenomenon can be eliminated from concern with respect to the consequences of ozone reduction without the necessity of dose-response studies.

(2) The steepness of the natural latitudinal gradient of solar UV-B that currently exists on the Earth's surface should also be evaluated in terms of potential biological effectiveness. The natural gradient of UV-B radiation serves as a basis for study of organism adaptation to solar UV-B radiation and can provide insight into potential consequences of ozone reduction. Yet, as with ozone reduction, without taking biological effectiveness of solar UV-B irradiation into account, the natural latitudinal gradient of solar UV-B radiation is virtually nonexistent (Caldwell 1981, Caldwell et al 1980).

(3) Since spectral irradiance received from commonly-used lamp systems for UV-B studies does not match that of solar irradiance, it is only possible to draw comparisons by calculating "biologicaly effective" radiation using action

spectra as weighting functions. Characteristics of action spectra will thus dictate the amount of radiant flux delivered by lamp systems in experiments designed to evaluate potential consequences of ozone reduction under different conditions.

With the ecological utility of action spectra in focus, different priorities with respect to UV-B action spectrum characteristics need to be emphasized. Since these spectra are to be applied in the evaluation of polychromatic irradiation, spectra derived from monochromatic irradiation studies may misrepresent the appropriate weighting function if radiation at different wavelengths causes interacting effects. Such interactions can occur in the case of nucleic acid lesions induced by UV-B irradiation (Natonal Academy of Sciences 1979). If such interacting effects are clearly understood, the net effect of polychromatic irradiation of a given spectral distribution could be calculated. However, if these are not well understood, the ecological utility of such action spectra can be severely diminished. An empirical approach to this problem involves the use of polychromatic radiation in the determination of action spectra. In this process, the biological responses to different combinations of polychromatic irradiation are determined and an action spectrum can be deconvoluted from this series of responses. With respect to the ozone reduction problem, the most logical combinations of

polychromatic irradiation involve a constant background of longwave UV-A and visible irradiance with increments of radiation at shorter wavelength intervals (see Methods). This process can sacrifice some of the fine structure of an action spectrum, but this is of less concern for the purpose of weighting functions.

This series of polychromatic irradiation distributions should be planned to account for the tail of the action spectrum into the UV-A, or visible waveband, as the case may be. Characteristics of the action spectrum below 280 nm are of no concern with respect to solar UV radiation changes because of the effectiveness of atmospheric ozone absorption even in the case of a severely depleted ozone layer (Caldwell 1979). Yet, if a particular lamp system emits shorter wavelengths, the weighting function should include these as well.

Inhibition of photosynthesis of higher plants by UV radiation is of potential concern with respect to ozone reduction. For some species under particular experimental conditions, solar UV at flux rates now received at temperate latitudes is sufficient to significantly reduce photosynthesis (Bogenrieder and Klein 1977, Sisson and Caldwell 1977). Yet to be resolved, however, is the extent to which plants experience photosynthetic inhibition under field conditions, or in the

event of ozone layer reduction. Nevertheless, there is sufficient impetus to select net photosynthesis for action spectrum analysis because of its potential susceptibility to solar UV radiation. Furthermore, net photosynthesis is an integrated physiological process which requires the integrity of membrane systems and the coordinated action of photochemical and involved enzymatic processes. Thus, it also serves as a useful indicator of plant response to stress.

Another plant growth process which might be influenced by UV-B irradiation is the expansion of leaves, especially cell division rates of leaves (Dickson and Caldwell 1978). Even though there is an adequate energy source for leaf expansion, UV irradiation could affect cell division processes thereby limiting leaf and plant growth. Thus, we have selected leaf disc expansion under conditions of adequate energy substrate as a phenomenon for action spectrum analysis.

Methods:

Assessment of photosynthetic depression resulting from UV irradiation involves the determination of net CO_2 uptake by plant leaves, exposure of the foliage to a particular polychromatic irradiation distribution, and then subsequent determination of CO_2 uptake capacity under identical

conditions. Ideally, the leaves would be exposed to the inactivating UV irradiation over a period of several days or weeks as would occur under field conditions. Unfortunately, this involves unreasonable biological and experimental complications. Leaf photosynthetic characteristics change appreciably with leaf age (e.g. Sisson 1981, Sisson and Caldwell 1977). These changes combined with the time and logistic requirements for such experiments render this approach infeasible. Thus, the irradiation periods ranged from one to 16 hours. Seven polychromatic radiation distribution were employed. For each a dose-response relationship was developed. Even with these irradiation periods, such experiments are extremely time consuming.

The spectral irradiance for the seven polychromatic irradiation distributions are shown in Figure 1. These are developed by using a 2.5-kW xenon high pressure lamp (Osram Co.). The lamp is contained in a housing consisting of an optics box, a shutter system and a series of filters. An aluminum-coated, front-surface mirror held by a cantilevered structure allows focusing and projection of the radiation beam onto the surface of a dichroic filter held at an angle of incidence of 45°. Approximately 80% of the visible and infrared radiation passes through the dichroic filter and is dissipated by a heat sink. The remaining 20%, and nearly all of

the radiation below 400 nm, are reflected toward the irradiation field used in the experiments. This allows experiments to be conducted without photoinhibition due to excessive visible irradiance or excessive leaf temperatures. Yet, there is sufficient visible flux (400 mole.m^{-1}.s^{-1} between 400 and 700 nm) to drive photosynthesis of the plants during irradiation. Before the radiant beam reaches the experimental irradiation field, it passes through a quartz diffuser and an absorption filter. The absorption filter is one of a series which provides the different polychromatic distributions shown in Figure 1. These filters are sharp cutoff absorption filters (Schott Co. WG series).

Leaves still intact with the remainder of the plant are placed on a slowly revolving stage in this radiation field to insure even irradiation of the foliage material.

Net photosynthetic rates of intact leaves are assessed by measurements of net CO_2 uptake under specific environmental conditions in a gas exchange cuvette. By measurement of simultaneous CO_2 and water vapor flux of the foliage as well other parameters including leaf temperature, CO_2 concentration in the cuvette air, and water vapor concentration in the cuvette, it is possible to calculate intercellular CO_2 concentrations within the leaf (e.g. Caldwell et al 1977).

Intercellular CO_2 is influenced primarily by photosynthetic rates, CO_2 in the cuvette airstream, and diffusion resistance provided by stomata. Since photosynthesis is normally substrate limited, it is important to manage the cuvette conditions to maintain a constant intercellular CO_2 concentration in the leaf before and after irradiation so that metabolic photosynthetic capacity of a leaf is being assessed uncomplicated by diffusion resistances. Direct coupling of the gas exchange system with a computer allows immediate assessment of these parameters so that adjustments in cuvette conditions can be made during the course of these measurements.

Inhibition of leaf disc expansion has been examined under the same high intensity xenon arc filter system. Leaf disc expansion was determined in a manner similar to that used earlier in our laboratory by Lindoo. Uniform leaf discs were excised from developing leaves and floated on a sucrose medium. After UV irradiation, the leaf discs were incubated on a sucrose, nutrient, and antibiotic medium for 65 hours. Leaf area was then determined with a leaf area meter.

A new system has been devised where the control discs are simultaneously treated under conditions identical to the UV irradiated leaf discs except for wavelength below 400 nm. This is achieved by floating the leaf discs atop a highly absorbant

sponge saturated with the sucrose medium. Half of the sponge is shielded with the plastic filter material Llumar, which removes all radiation below 400 nm. The other half is similarly covered with the plastic filter material Aclar, which transmits equally over all the UV wavelengths of interest. Thus, treatment and controls are in identical microenvironments. The entire assembly was placed on a rotating stage to assure uniform irradiation.

Using this system, we have attempted to develop a series of dose-response relationships for the four sharp-cut WG filters (280, 295, 305, and 320) showing inhibition of Rumex leaf disc expansion. A fifth filter (WG 335) produced no inhibition of leaf disc expansion up to 16 hours of treatment.

Results

The dose-response relationships for net photosynthetic inhibition of Rumex patientia leaves are shown in Figure 2 for different spectral irradiance distributions. Each data point represents a different leaf and was determined on a different date since these determiniations are quite time consuming. Nevertheless, these dose-response data reveal linear relationships when there is a significant response to the radiation. The coefficients of determination, R^2, range between

0.63 and 0.98 with an average of 0.87. The spectral irradiance regime provided by the filter with a cutoff at longer wavelengths (WG 360) did not result in inhibition of photosynthesis under these conditions even after 16 hours of exposure.

These photosynthetic inhibition data indicate that if there is sensitivity to a particular irradiation distribution, we are dealing with a linear portion of the dose-response relationship. This is important in developing action spectra. If a particular dose-response relationship exhibits diminished slope as with saturation, these data cannot be compared with a dose-response relationship which is in the initial, linear phase. The dose-response relationships presented here indicate that it is suitable to utilize the slopes of these linear regression relationships for biological response in developing these action spectra.

Dose-response relationships for inhibition of leaf disc expansion are shown in Figure 3. Despite the fact that dose rates for the leaf disc experiments were twice those depicted in Figure 1 for the photosynthetic inhibition study, inhibition of leaf disc expansion was negligible under radiation from the WG 355 filter. Thus, in Rumex the longer wavelengths capable of damaging photosynthesis do not interfere with leaf disc expansion.

In the extended duration treatments (WG filters 305, 320, and 335), we observed considerable reddening of the treatment leaf discs, which was never observed in either the prolonged Llumar treatments or the WG 280 or 295 treated leaf discs. This reddening response is apparently independent of the inhibition of expansion, as it was seen in the WG 335 leaf discs, which showed no inhibition of expansion.

Because of the differences in dose rate and handling of control leaf discs, the data in Figure 3 are not comparable to that in the 1980 Annual Report. We have not presented data for the 280 filter as we were unable to obtain a linear dose-response as we had achieved earlier (1980 Annual Report). We do not have an explanation for this, as the lower dose rate previously produced a very good linear dose-response relationship.

Discussion

The action spectrum for inhibition of photosynthesis falls between that of the general DNA action spectrum (Setlow 1974) and the generalized plant action spectrum (Caldwell 1971). The general characteristics of this action spectrum suggest that a

combination of pronounced increase in effectiveness with decreasing wavelength, substantial specificity for the UV-B waveband and very diminished response in the UV-A waveband will result in large radiation amplification factors when such action spectra are used as weighting functions. Thus, the potential increase in radiation with ozone reduction that may damage higher plant photosynthesis should receive attention. This is in contrast to conclusions that might be drawn from the action spectrum for a partial photosynthetic process, the Hill reaction, performed with monochromatic radiation on isolated spinach chloroplasts several years ago (Jones and Kok 1966).

Smith et al. (1980) tested different polychromatic UV radiation regimes in assessment of reduced capacity for $^{14}CO_2$ fixation by marine phytoplankton and found their data were in reasonable correspondence with the earlier Jones and Kok action spectrum. Thus, for phytoplankton such action spectrum characteristics suggest very small radiation amplification factors and that short-term inhibition of photosynthesis that might result from ozone reduction would be of negligible ecological consequence. Apparently, photosynthetic activity of intact higher plants responds quite differently to UV irradiation. The extent to which this action spectrum will apply to other higher plant species is now being tested.

Determination of dose-response relationships for leaf disc inhibition has been considerably more difficult than for inhibition of net photosynthesis. Deconvolution of an action spectrum from these data is not warranted at this time. However, comparison of the data with the DNA damage action spectrum suggests an action spectrum for inhibition of leaf disc expansion would fall off at longer wavelengths at least as steeply as the DNA damage spectrum. It would seem biologically plausible that inhibition of leaf expansion might be at least in part attributable to nucleic acid damage. As with photosynthetic inhibition, which is likely not immediately related to DNA damage (Caldwell, 1981), there should be large radiation amplification factors associated with an action spectrum for inhibition of leaf expansion. Thus, both photosynthesis and leaf expansion are biological phenomena which should receive continued study in the context of the ozone layer reduction problem.

Literature Cited

Bogenrieder, A., and R. Klein. 1977. Die Rolle des UV-Lichtes beim sog. Auspflanzungsschock von Gewachshaussetzlingen. Angewandte Botanik 51:99-107.

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.

Caldwell, M. M. 1979. Plant life and ultraviolet radiation: Some perspective in the history of the Earth's UV climate. BioScience 29:520-525.

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, Vol. 12A Physiological plant ecology I. Responses to the physical environments. Springer-Verlag, Berlin.

Caldwell, M. M., C. B. Osmond, and D. L. Nott. 1977. C_4 pathway photosynthesis at low temperature in cold-tolerant Atriplex species. Plant Physiology 60:157-164.

Caldwell, M. M., R. Robberecht, and W. D. Billings. 1980. A steep latitudinal gradient of solar ultraviolet-B radiation in the arctic-alpine life zone. Ecology 61:600-611.

Dickson, J. G., and M. M. Caldwell. 1978. Leaf Development of Rumex patientia L. (Polygonaceae) exposed to UV irradiation (280-320 nm). Amer. J. Bot. 65:857-863.

Jones, L. W., and R. Kok. 1966. Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. Plant Physiology 41:1037-1043.

Nachtwey, D. S., and R. D. Rundel. 1981. Ozone change: biological effects. in F. Bower and R. Ward eds. Man and stratospheric ozone. CRC Press, Inc., West Palm Beach, FL in press.

National Academy of Sciences 1979. Protection against depletion of stratospheric ozone by chlorofluorocarbons. Washington, D. C. 392 p.

Setlow, R. B. 1974. The wavelengths in sunlight effective in producing skin cancer; a theoretical anaysis. Proc. Nat. Acad. Sci. USA. 71:3363-3366.

Sisson, W. B. 1981. Photosynthesis, growth and ultraviolet irradiance absorbance of Cucurbita pepo L. leaves exposed to ultraviolet-B radiation (280-315 nm). Plant Physiology 67:120-124.

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. J. Exp. Bot. 28:691-705.

Smith, R. C., K. S. Baker, O. Holm-Hansen, and R. Olson. 1980. Photoinhibition of photosynthesis in natural waters. Photochem. and Photobiol. 31:585-592.

Figure Legends

1. Ultraviolet spectral irradiance provided by focused high pressure xenon lamp system with a series of sharp cutoff absorption filters of the Schott Co. WG series. The numbers by each spectral irradiance distribution indicate the designation for the particular WG filter employed in the irradiator system. These measurements were conducted with a double monochromator spectroradiometer.

2. Relative inhibition of net photosynthesis of Rumex patientia exposed for different periods of time to the spectral irradiance regimes portrayed in Figure 1.

3. Relative inhibition of leaf disc expansion for Rumex patientia exposed for different periods of time to spectral irradiance distributions twice the flux rates of those portrayed in Figure 1.

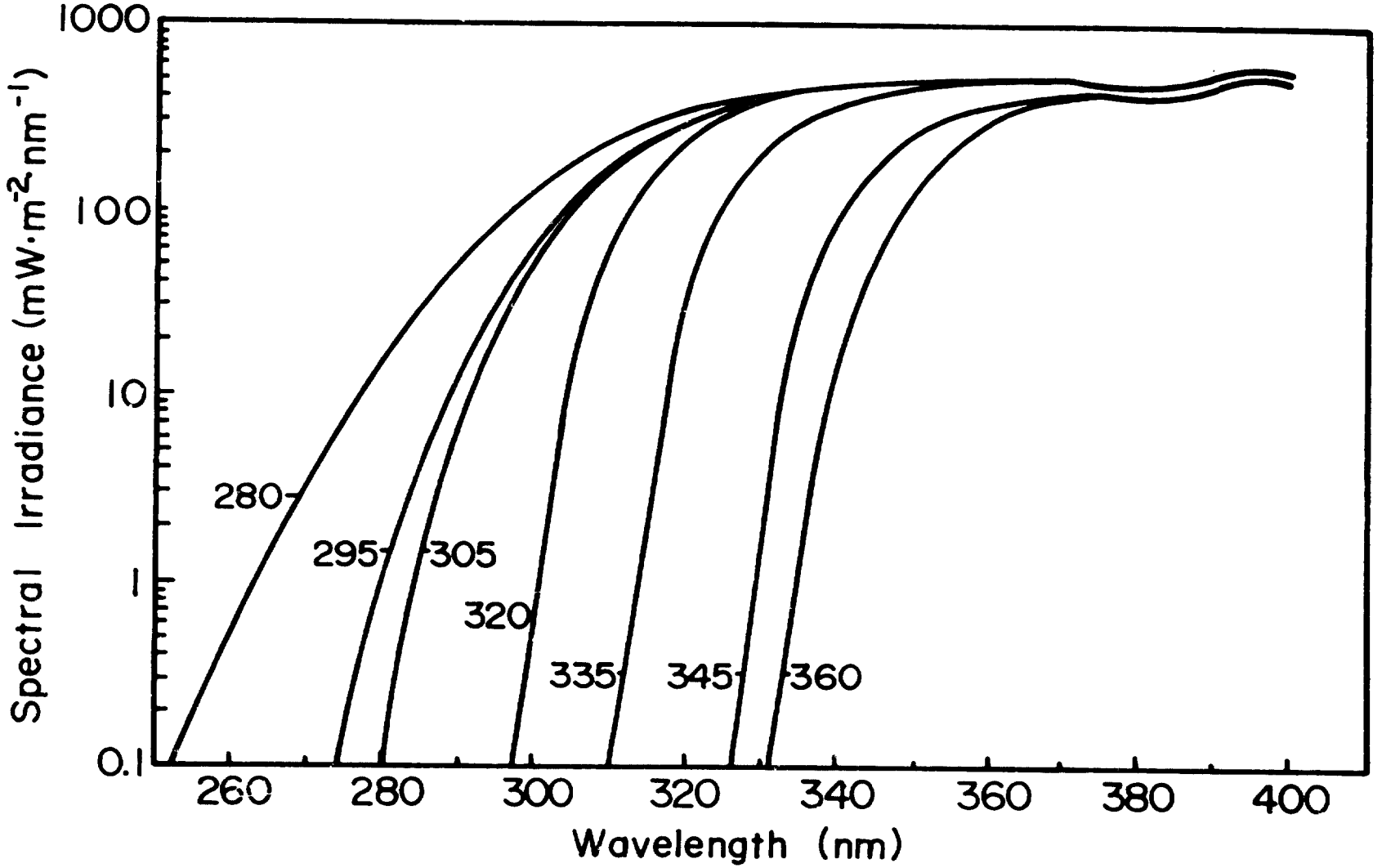

1000
100
10
1
0.1
Spectral Irradiance (mW·m⁻²nm⁻¹)
280
295
305
320
335
345
360
260
280
300
320
340
360
380
400
Wavelength (nm)

Fig. 1.

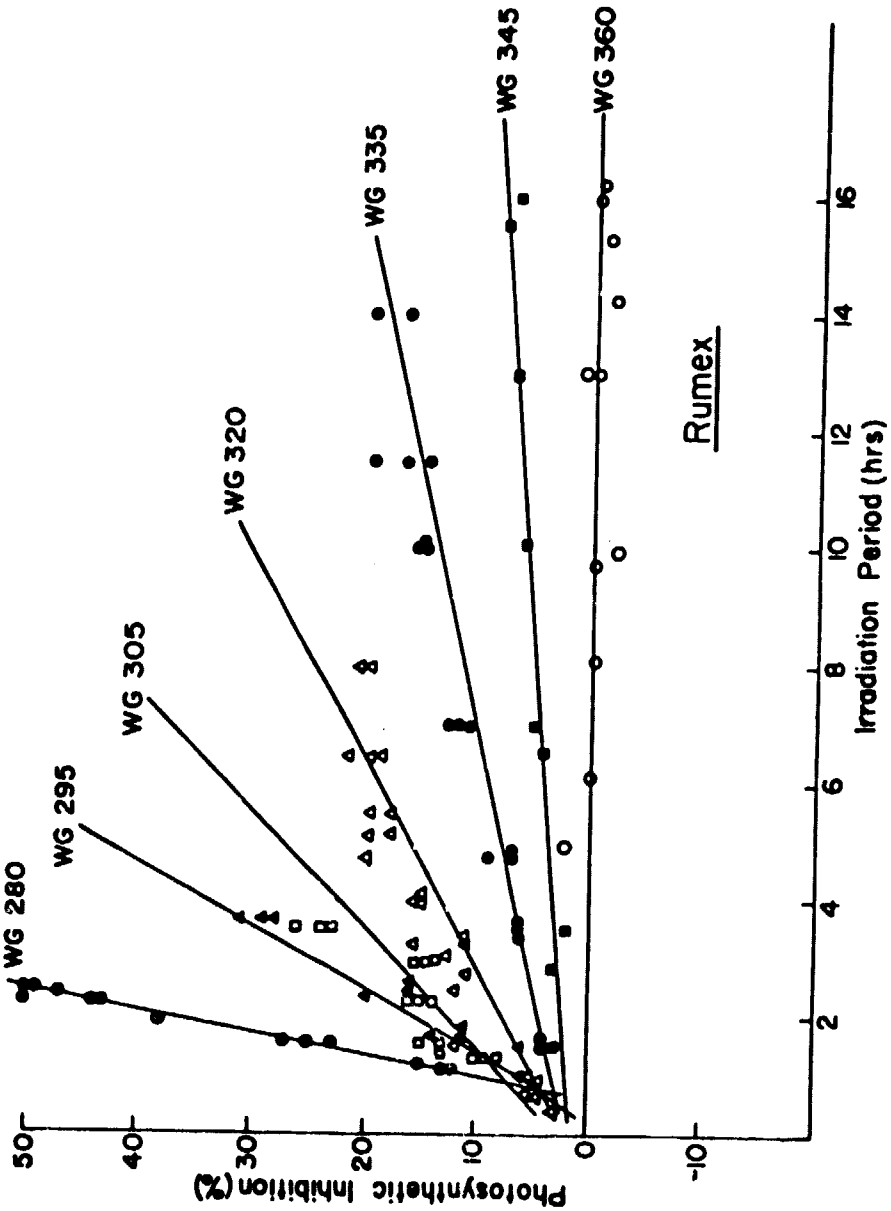

WG 280
WG 295
WG 305
WG 320
WG 335
WG 345
WG 360
Rumex
Photosynthetic Inhibition (%)
Irradiation Period (hrs)
50
40
30
20
10
0
-10
2
4
6
8
10
12
14
16

Fig. 2.

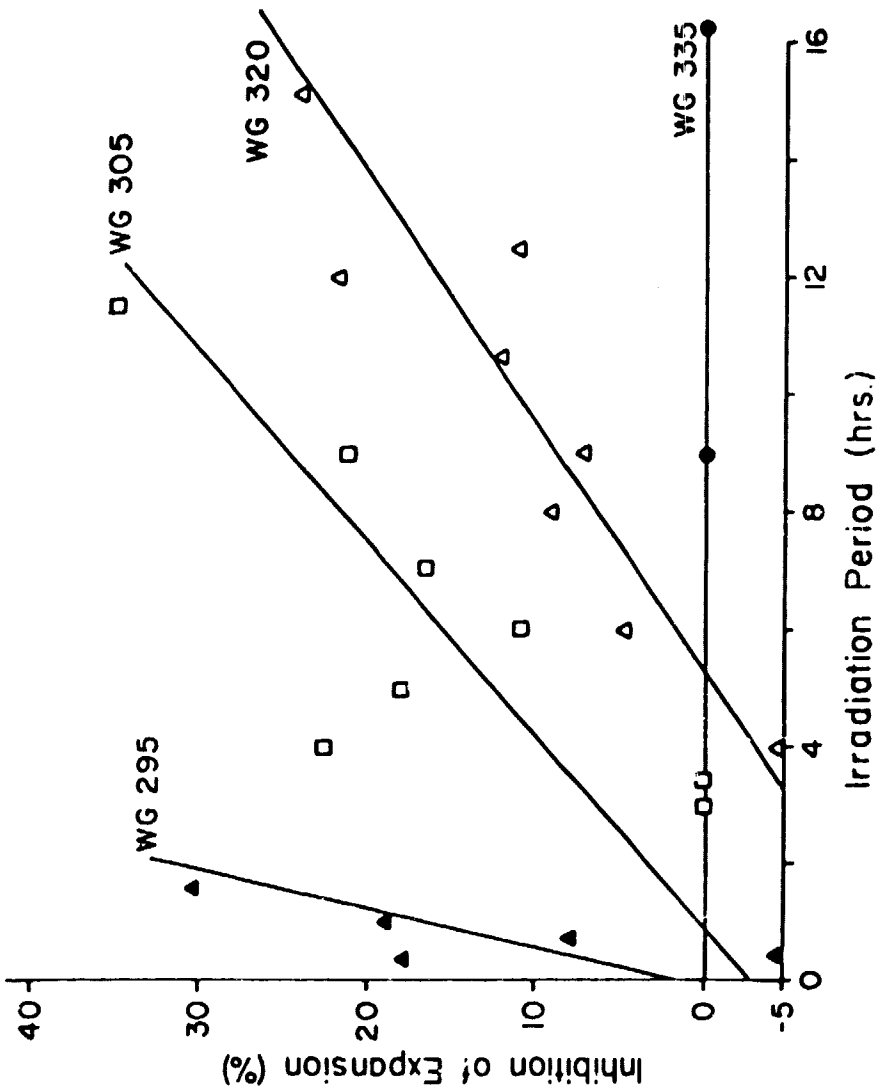

WG 295
WG 305
WG 320
WG 335
Inhibition of Expansion (%)
Irradiation Period (hrs.)
40
30
20
10
0
-5
0
4
8
12
16

Fig. 3.