Utah State University

# DigitalCommons@USU

5-1981

# Effect of Mild Water Stress and Enhanced Ultraviolet-B Irradiation on Leaf Growth of *Rumex obtusifolius* L. and *Rumex patientia* L. (Polygonaceae).

Steve R. Holman
*Utah State University*

Follow this and additional works at: https://digitalcommons.usu.edu/etd

Part of the Ecology and Evolutionary Biology Commons, Environmental Sciences Commons, and the Plant Sciences Commons

EFFECT OF MILD WATER STRESS

AND ENHANCED ULTRAVIOLET - B

IRRADIATION ON LEAF GROWTH

OF RUMEX OBTUSIFOLIUS L. AND

RUMEX PATIENTIA L. (POLYGONACEAE)


by

Steve R. Holman


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


of

MASTER OF SCIENCE

in

Range Ecology.


Approved:




Utah State University
Logan, Utah

1981

## ACKNOWLEDGMENTS

This research was funded by the National Aeronautics and Space Administration.

I wish to sincerely thank my major advisor, Martyn Caldwell, for his invaluable assistance and continual support at every stage of the research. Working with him was a stimulating and enjoyable experience. Herman Wiebe and Doug Johnson provided valuable advice and constant support and enthusiasm during the project.

I am also extremely grateful for the comments, criticisms and suggestions provided by Ron Dzurec, Sue Lindoo, Jim Leithliter, Bob Nowak, Ron Robberecht and Harvey Neuber. Lee Camp gave me many willing hours of technical assistance and his contribution is gratefully acknowledged.

I would finally like to thank my wife, Jeannette Holman for typing the manuscript and for putting up without me all those months.

<div align="right">Steven R. Holman</div>

TABLE CF CONTENTS

# LIST OF FIGURES

# ABSTRACT

Effect of Mild Water Stress and

Enhanced Ultraviolet-B Irradiation

on Leaf Growth

of <u>Rumex obtusifolius</u> L. and <u>Rumex</u>

<u>patientia</u> L. (Polygonaceae).

by

Steven R. Holman, Master of Science

Utah State University, 1981

Major Professor: Dr. Martyn M. Caldwell
Department: Range Science

Leaves of <u>Rumex obtusifolius</u> L. and <u>R</u>. <u>patientia</u> L.
were exposed to combinations of mild water stress and en-
hanced ultraviolet-B irradiation during their ontogeny.
Two UV-B treatments (enhanced UV-B and control) and three
water stress treatments ($-0.0$ MPa, $-0.2$ MPa and $-0.4$ MPa
rooting medium matric potentials) were employed. The
impact of the stress interaction was assessed on the basis
of changes in leaf area, average adaxial epidermal cell
size, and total number of adaxial epidermal cells per leaf.
Although the level of UV-B irradiation applied was in-
sufficient to significantly alter leaf growth at any given
water stress, UV-B did interact with water stress to alter
the pattern of plant response to water stress. The inter-
action was only apparent when the water stress was greater

than -0.2 MPa root matric potential. For both species UV-B irradiation exacerbated the depression of leaf growth due to -0.4 MPa water stress. For R. obtusifolius the basis of the reduction in leaf growth was likely a reduction in the rate of cell division during the early phase of leaf growth. For R. patientia the effect of the interaction on cell division was less clear. Cell expansion was not directly affected by UV-B irradiation in either species, although the reduction in cell size with increasing water stress was apparent. In terrestrial ecosystems, mild water stress is a common occurrence and with predicted anthro-pogenic modifications of the atmospheric ozone layer, UV-B radiation reaching the earth's surface can be expected to increase. The effect on higher plants of the stress inter-action may thus be of considerable significance under natural conditions.

(45 pages)

# INTRODUCTION

The manner in which a plant responds to its physical environment may be strongly influenced by the interaction of many different environmental factors. These factor interactions can produce plant responses quite different from those due to the effect of any of the individual factors alone. Plant responses to two or more factors may either be additive, when the response to the combination of factors is equal to the sum of the responses to the individual factors, or synergistic, when the plant shows a response either more or less than additive (Salisbury, 1975).

Recent research into anthropogenic modifications of the stratospheric ozone layer (Molina and Rowland, 1974) has led to concern that a reduction in the ozone layer would lead to an increase in ultraviolet radiation reaching the Earth's surface (Green et al., 1974). This increase would occur mainly in the ultraviolet-B (280-320 nanometer) region of the spectrum. Because radiation in this waveband is quite actinic, even a slight increase in terrestrial UV-B could significantly affect higher plants.

In order to fully understand the impact of enhanced terrestrial UV-B on plants, factor interactions must be considered. Most investigations to date have examined UV-B as an isolated stress factor, although Fox and Caldwell (1978) have examined the interaction of competitive stress

and enhanced UV-B.

Among the effects of enhanced UV-B radiation on higher plants so far observed has been a significant reduction in leaf growth (Sisson and Caldwell, 1976; Dickson and Caldwell, 1978). Although UV-B radiation can cause a decrease in the photosynthetic rate of exposed leaves which could indirectly limit leaf growth, Sisson and Caldwell (1976) demonstrated that the depression of early leaf expansion in Rumex patientia was greater than the level solely attributable to photosynthate limitation. Dickson and Caldwell (1978) determined that UV-B irradiation depresses leaf growth in Rumex patientia by reducing the rate of epidermal and mesophyll cell division in young leaves. Brown and Klein (1973) found that "near-UV" (300-400 nm) repressed cell division in pea root meristems by increasing the length of interphase period between divisions. Similar responses to near-UV have been observed in many procaryotic and eucaryotic cells (Klein, 1979).

Water stress is a widespread and common natural stress factor. There are few terrestrial ecosystems where water is universally abundant and most plants are subjected to occasional water stress. Even plants growing in well watered soil may suffer mild water stress when evaporative demands are high.

The most frequently observed effect of water stress on plants is a reduction in leaf growth (Slatyer, 1967;

Hsiao, 1973). At the cellular level this reduction has been correlated primarily with a reduction in cell size due to depressed cell expansion (Hsiao, 1973; Slatyer, 1967) although water stress has been reported to reduce cell division in some cases (Hsiao, 1973; McCree and Davis, 1974).

The rate and duration of leaf growth is a function of the rate and duration of both cell expansion and cell division (Milthorpe and Newton, 1963). Cell division is most important during the early phases of leaf growth, but division ceases when the leaf is from 1/6 to 1/2 final size, depending on the species (Avery, 1933; Maksymowych, 1963; Milthorpe and Newton, 1963; Saurer and Possingham, 1970). Sunderland (1960) however, reported that in sunflower leaves, cell division may continue until the leaf is from 1/2 to 3/4 final size. Cell expansion continues throughout the growth of the leaf.

These two growth processes are closely linked. During the interphase period between cell divisions, cells must expand to reach a 'threshold' size before the next division can take place (Hsiao, 1973). After division ceases, further leaf growth depends solely on the rate and duration of cell expansion.

Because UV-B irradiation and water stress have a significant impact on the processes of cell expansion and division and inasmuch as these processes are closely tied to the

early phase of leaf growth, it seems likely that the com-
bined effects of the two stresses would have a significant
effect on leaf growth.  The purpose of this study was to
investigate the nature of this potential interaction by
testing the hypothesis that the two stresses would interact
to produce a synergistic reduction in leaf growth.

This hypothesis seems reasonable if, during the cell
division phase of leaf growth, water stress increased the
amount of time necessary for a cell to expand to the
'threshold' size and divide.  If UV-B irradiation exerted
an independent but concurrent effect on the cell division
process, the combination of increased time for cell en-
largement and decreased cell divisions per unit time would
likely result in a synergistic reduction of leaf growth.

Two closely related species were chosen for the study;
Rumex patientia, a plant sensitive to UV-B irradiation and
Rumex obtusifolius, which is relatively less sensitive to
elevated UV-B according to data from Sisson (unpublished).
Both leaf size and the changes in size and number of the
upper epidermal cells were monitored for stress-induced
effects.  It was expected that the synergistic reduction
of leaf growth due to the interaction would be more
apparent in the UV-B sensitive species.  The results of the
investigation made a comparison of the species' responses
difficult, although the data generally support the

hypothesis that UV-B and water stress interact synergisti-
cally.  Cell division appeared to be the primary growth
parameter affected by the interaction.

METHODS

## Plant Growth

Seeds of Rumex patientia and R. obtusifolius from
field collections made near Logan, Utah were germinated on
moist filter paper. The seedlings were planted in 22 cm x
4 cm conical plastic containers (Ray Leach Conetainer Co.)
in vermiculite and placed in a controlled environment
chamber under conditions identical to those under which the
experiments would be conducted, with the exception of UV-B
irradiation. Plants were watered every other day with 1/2-
strength modified Hoagland's nutrient solution until the
initiation of the 7th leaf. At that time the plants were
placed in the controlled water stress system devised by
Tingey and Stockwell (1977). The plants were transferred
into conetainers with approximately 75% of the surface area
removed leaving an open plastic framework around the vermi-
culite root mass. The frame and root-vermiculite mass were
then enclosed in two layers of cellulose acetate semi-
permeable membrane with an upper molecular weight cut-off
of 8000-9000. (Spectrapor t.m. dialysis membrane #1,
Spectrum Medical Industries, Inc.) Transplanting was ac-
complished with minimal disturbance to the plants since, by
the time of the transfer, the vermiculite rooting medium
was thoroughly permeated by roots and the entire mass was
easily manipulated.

## Water Stress System

The plant-membrane systems were equilibrated for 24 hours in distilled water, then placed in 1/2-strength Hoagland's solutions to which had been added varying amounts of polyethylene glycol 20,000 (PEG) (J. T. Baker Chemical Co.). The PEG was used to control the osmotic potential of the solutions at different levels to provide different levels of water stress. The PEG solutions controlled water movement through the membrane so that the matric potential of the vermiculite rooting medium was in equilibrium with the osmotic potential of the solutions. The plants were subjected to three different levels of water stress; no water stress, or zero megapascals (MPa) solution osmotic potential, achieved by immersing the plant-membrane system into 1/2-strength Hoagland's solution to which no PEG was added, low water stress, -0.2 MPa solution osmotic potential, and high water stress, -0.4 MPa solution osmotic potential. The PEG concentration needed to achieve the low and high water stress treatments was determined by a calibration curve provided by Tingey (personal communication).

During the investigation, these osmotic solutions maintained midday leaf water potentials of the test plants at -1.3 MPa ($\pm$0.1 MPa), -0.96 MPa ($\pm$0.05 MPa), and -0.65 MPa ($\pm$0.2 MPa) for the -0.4 MPa, -0.2 MPa and zero MPa treatments respectively. Pre-dawn leaf water potentials ranged from -0.4 MPa to -0.6 MPa for all treatments. All

water potential measurements were made with a P. M. S. pressure chamber. For each experiment, twelve plants, all at approximately the same stage of leaf development, were subjected to each stress.

The plants were allowed to equilibrate for 48 hours in the solutions. Then for the following three days the length of the 7th leaf on each plant was measured daily. The rate of leaf growth and the absolute leaf length were used as a basis of similarity for choosing five pairs of plants from each water stress treatment. Although this method of pairing plants was considered the most practical one, some difficulty was encountered in insuring synchronous leaf ontogeny within each pair and with each treatment. This difficulty was reflected in relatively high variability in some of the data.

## Growth Chamber Conditions

The effect of UV-B irradiation on the experimental plants was assessed under growth chamber conditions. One member of each plant pair was randomly assigned to a growth chamber with an enhanced UV-B irradiation regime and the other was placed in an identical chamber, but under control (low UV-B irradiation) conditions.

Apart from UV-B radiation, both growth chambers were maintained at identical environmental conditions. A 6000-W Osram Co. Xenon arc provided 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (400-700 nm) as measured with

a Lambda Co. Model LI-190-SR quantum sensor.  Photoperiod
was nine hours.  Growth chamber temperatures were maintained
at constant 25$^o$C in order to maintain the temperature-
dependent osmotic potentials of the PEG solutions at con-
stant levels.  Humidity remained constant at approximately
20% relative humidity.  Daytime leaf temperatures in both
chambers, as measured with a copper-constantan thermocouple,
remained between 22 and 24$^o$C.

The enhanced UV-B treatment was achieved by placing
the plants 40 cm below three Westinghouse FS-40 sunlamps
fitted with 5 mil (0.13 mm thickness) cellulose acetate
plastic filters.  These filters transmit ultraviolet
radiation down to approximately 290 nm.  The control treat-
ment consisted of sunlamps filtered with Mylar Type D (5
mil, 0.13 mm, DuPont Co.) plastic film which transmits no
radiation below 315 nm.  Sample leaves were held hori-
zontally with thread to ensure maximum irradiation.  Ultra-
violet lamps were engaged for the middle seven hours of the
day.  Dose rates were determined with a Gamma Scientific Co.
spectroradiometer and weighted for biological effectiveness
based on a relationship reported by Caldwell (1971).
Biological effective UV-B (UV-B$_{BE}$) dose rates were
$2.4 \times 10^3$ effective $J \cdot m^{-2} \cdot day^{-1}$ and $1.0 \times 10^2$ effective
$J \cdot m^{-2} \cdot day^{-1}$ for the enhanced UV-B and control treatments,
respectively.

## Data Collection and Analysis

The eighth leaf, which was the youngest leaf at the start of the experiment, was examined periodically during the experiment for changes in leaf size and in size and number of the adaxial epidermal cells. Data were collected for up to 14 days after the start of UV-B irradiation, the duration of the experiment being dependent on the longevity and growth rates of the test leaves. Cellulose acetate dialysis tubing is subject to bacterial degradation over time. Many workers have estimated the effective life of dialysis membrane in PEG solutions. The estimates include: 2 1/2 to 3 weeks (Painter, 1966), 12 days to 2 weeks (Zur, 1966), 13 days (Kaufmann, 1969), 5 to 10 days (Wisbey, et. al., 1977), and 7 days (Tingey and Stockwell, 1977). In order to insure the continual integrity of the water stress system used in this investigation, the dialysis membrane was changed for all treatments on the 7th day of each experiment. In preliminary investigations it was noted that visible signs of membrane decay did not occur until at least the 14th day after immersion in the PEG solution. Experiments were repeated twice for each species.

Leaf area was measured with light sensitive blueprint paper held closely appressed to the leaf and briefly exposed to the light. When developed in ammonia vapor, the silhouette of the leaf was precisely reproduced. This was then cut out and used to measure leaf area with a Lambda Co.

model LI-3000 photoelectric area meter.

Epidermal cell density was determined by taking a rubber impression of the upper leaf surface each day by a modification of the technique described by Groot (1969). Dow Corning brand RTV silicon rubber encapsulant was used to make the impression. The liquid rubber was mixed with a catalyst, spread on the leaf, and could be peeled off as a solid impression within two minutes without harming the leaf. Clear fingernail polish was used to make a secondary impression from the silicon rubber and this was mounted and inspected at 100 power under a microscope. A reticule grid was used to count the number of cells per square millimeter. For each leaf 10 counts of cell density (cells$\cdot$mm$^{-2}$) were made at the tip, middle and base of the leaf.

Continuous transects through the long axes of repre-sentative leaves of both Rumex patientia and R. obtusifolius were counted and from this it was determined that a linear relationship existed between cell density and relative position along the leaf. The slope of the relationship changed with leaf size, but the relationship remained linear. It was also noted that for both species, the relationship between leaf length and leaf width for a given leaf size class could be described by a quadratic equation. The product of the linear equation of cell density at each position along the leaf and the quadratic equation of leaf

width at each position along the leaf would give, for a
leaf of given size class, an estimate of the total number
of adaxial epidermal cells across the leaf at each position
along the leaf length. Integrating over the entire leaf
length would give the total number of cells per leaf or,

$$Y = \int_1^L W_x \cdot Z_x dx$$

where Y = total number of adaxial epidermal cells per leaf,
$W_x$ is the cell density per relative length and $Z_x$ is the
leaf width per relative length.

To solve this expression the leaf length and the slope
of the cell density-leaf length relationship were required.
Because the resultant cell number was only an estimate of
the actual cell number, the equation was also used to cal-
culate the estimated leaf area based on the measured leaf
length. The ratio between the actual leaf area and esti-
mated leaf area was applied as a correction factor to the
estimated number of cells per leaf. The corrected cell
total was then divided into the actual leaf area to give
the average cell size for each leaf.

To test for significant differences in mean values of
leaf area, cell size, and cell number among the three
water stress treatments under either UV-B or control condi-
tions, a two factor analysis of variance was applied (Zar,
1974). For all treatment combinations measured each sample
day, the three sample parameters were tested for significant

differences ($P \leq 0.05$) due to enhanced UV-B irradiation
alone, water stress alone, and to the treatment interaction.

RESULTS

## Rumex obtusifolius

The results of this investigation indicate that there was an interaction between enhanced UV-B irradiation and water stress as measured by leaf growth and epidermal cell dynamics. Ultraviolet radiation apparently acted to alter the response of R. obtusifolius to the different levels of water stress used in this study.

Figure 1 (A, B) illustrates the pattern of leaf growth for plants exposed to the three levels of water stress (-0.0 MPa, -0.2 MPa, and -0.4 MPa) under both enhanced UV-B and control conditions. Under control conditions (Fig. 1-B) by day eight of the experiment the leaves under both -0.2 MPa and -0.4 MPa water stress were significantly smaller than the unstressed (-0.0 MPa) leaves, but were not different from each other. Under enhanced UV-B irradiation (Fig. 1-A) a different pattern emerged. By day six and for the remainder of the experiment, the leaves under the greatest water stress were significantly smaller than those under the two lesser stresses. Leaves under -0.0 MPa and -0.2 MPa stress were not significantly different from each other until day 13. At no time did leaf area respond significantly to UV-B alone, or to the stress interaction. There was, however, an apparent change in plant response to increasing water stress between the UV-B and control groups. The impact of the -0.4 MPa water stress treatment compared

Figure 1.  Patterns of leaf growth, cell division and cell
expansion for Rumex obtusifolius exposed to en-
hanced UV-B (graphs A, C, and E) and control
(graphs B, D, and F) treatments while under three
levels of water stress (-0.0 MPa, -0.2 MPa, and
-0.4 MPa rooting medium matric potential).
Average values which are not significantly
different from one another at $P \leq 0.05$ are
connected with vertical bars. Asterisks denote
days on which a significant ($P \leq 0.05$) inter-
action was observed between water stress and
UV-B irradiation.

16
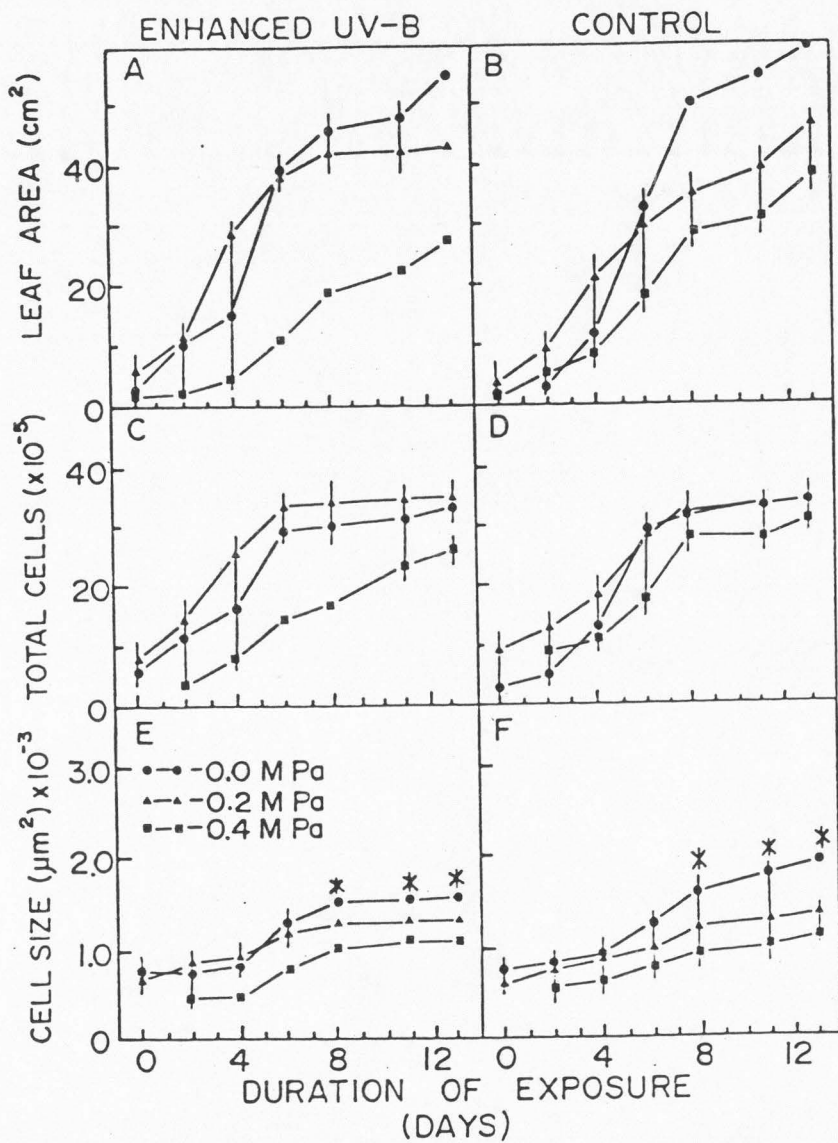
to the -0.2 MPa treatment was exacerbated by UV-B irradiation. In addition, the depression of leaf growth due to -0.2 MPa water stress as compared to the unstressed leaves was manifest five days later under UV irradiation than under control conditions. In a replicate experiment, however, the difference between the leaf area of plants under -0.0 MPa and -0.2 MPa stress was apparent approximately six days into the experiment under both UV-B and control conditions (Appendix 1-A, B), suggesting that the differences in timing observed here were probably not due to supplemental UV irradiation.

In an effort to quantify the relative influence of both epidermal cell division and cell expansion on the pattern of leaf growth, the total number of adaxial epidermal cells and the average epidermal cell size was determined at intervals throughout the experiment.

Figure 1 (C, D) illustrates the pattern of cell division as influenced by water stress and UV-B irradiation. When the three water stress treatments were compared under control conditions, there were no significant differences, and only small apparent differences between the average numbers of cells per leaf (Fig. 1-D). Under enhanced UV-B the apparent differences were much larger (Fig. 1-C). The total number of cells in the leaf epidermis of plants subjected to -0.4 MPa was significantly less than for the two lower water stress levels on days six and eight. This transient difference, and the small increase in cell

numbers over time for the -0.4 MPa treatment as compared
to the other treatments indicates that the rate of cell
division was proceeding more slowly at -0.4 MPa water stress
under supplemental UV-B than under any of the other stress
combinations tested.  As for leaf area, no statistical inter-
action between the stress effects was observed.  One factor,
however, suggests that a subtle form of interaction may have
been manifest.  First, since cell numbers for the -0.4 MPa
plants under enhanced UV-B increased steadily throughout
the investigation, while division ceased earlier under the
other treatments, UV-B irradiation seemed to act to prolong
the cell division phase of leaf growth in the most severely
water stressed plants.  The overall rate of cell division
was also slower under -0.4 MPa and UV-B irradiation than for
any other treatment.  The replicate experiment, though of
a shorter duration, repeated this trend (Appendix 1-C, D).
It thus seems possible that, although the interaction is
subtle, the effect of UV-B and water stress is to depress
the rate and to prolong the duration of cell division in
R. obtusifolius.

Changes in average epidermal cell size during the
experiment are illustrated in Figure 1 (E, F).  Under en-
hanced UV-B irradiation, the reduction in cell size with
increasing water stress was quite apparent (Fig. 1-E).
Plants subjected to the three water stress treatments ex-
hibited significantly different cell size from day eight.
The control plants did not show the same relationship in

cell size as a function of water stress (Fig. 1-F). At no time were the leaves under -0.2 MPa and -0.4 MPa water stress significantly different in cell size. Beyond day eight the unstressed leaves were different from those at -0.4 MPa and by day 13 they were different from both -0.2 MPa and -0.4 MPa treatments. On days 8, 11, and 13 of the experiment, a statistical interaction between water stress and UV-B irradiation was observed. Under UV-B irradiation, cell size decreased with decreasing rooting medium matric potential. This did not occur until day 13 under control conditions and then only between the unstressed (-0.0 MPa) and stressed (-0.2 MPa and -0.4 MPa) groups. The trends observed here were confirmed by the replicate experiment. This relationship indicates that the response of cell expansion processes to increasing water stress is, at least within the range tested here, influenced by UV-B irradiation.

## Rumex patientia

The response of R. patientia to the combination of stresses was somewhat similar to that of R. obtusifolius. The leaf growth patterns are presented in Figure 2 (A, B). Under control conditions, the only significant differences in leaf area were observed between the unstressed (-0.0 MPa) and stressed (-0.2 MPa and -0.4 MPa) plants (Fig. 2-B). Leaves under -0.2 MPa and -0.4 MPa stresses were never different from each other. Under enhanced UV-B irradiation, however, leaves under -0.4 MPa stress were different from

either of the other two water stress treatments from day six
through day ten (Fig. 2-A). On day 10 under UV irradiation,
leaf area began to decline in the two stressed treatments.
This was due to an early onset of senescence in these leaves.
After day 10, leaves from both treatments began to develop
small chlorotic areas along the leaf blade and attrition
of the leaf margin was noted in many cases. This decline
in living leaf area undoubtedly influenced the relationship
between the leaves under the three water stresses during
the latter part of the experiment (days 10 through 14) and
may have been responsible for the lack of difference be-
tween -0.2 and -0.4 MPa stressed plants on days 13 and 14.
This early leaf senescence did not appear in any plant
under control conditions. No statistically significant
effect of UV-B irradiation was observed within any water
stress level and no interaction between the two stresses
was demonstrated.

There were no significant differences in total cells
per leaf among plants from any water stress level under ei-
ther UV or control treatments and no interaction was apparent
for R. patientia (Fig. 2-C, D).

For R. patientia enhanced ultraviolet radiation did
not appear to have an important effect on the rate or
magnitude of cell expansion (Fig. 2-E, F). The relation-
ship between cell size under the three water stresses
appeared to be very similar for both UV and control groups.
The only clear differences occurred on days 10 and 14,

Figure 2.   Patterns of leaf growth, cell division and cell
expansion for *Rumex patientia* exposed to en-
hanced UV-B (graphs A, C, and E) and control
(graphs B, D, and F) treatments while under three
levels of water stress (-0.0 MPa, -0.2 MPa, and
-0.4 MPa rooting medium  matric potential).
Average values which are not significantly
different from one another at $P \leq 0.05$ are
connected by vertical bars.
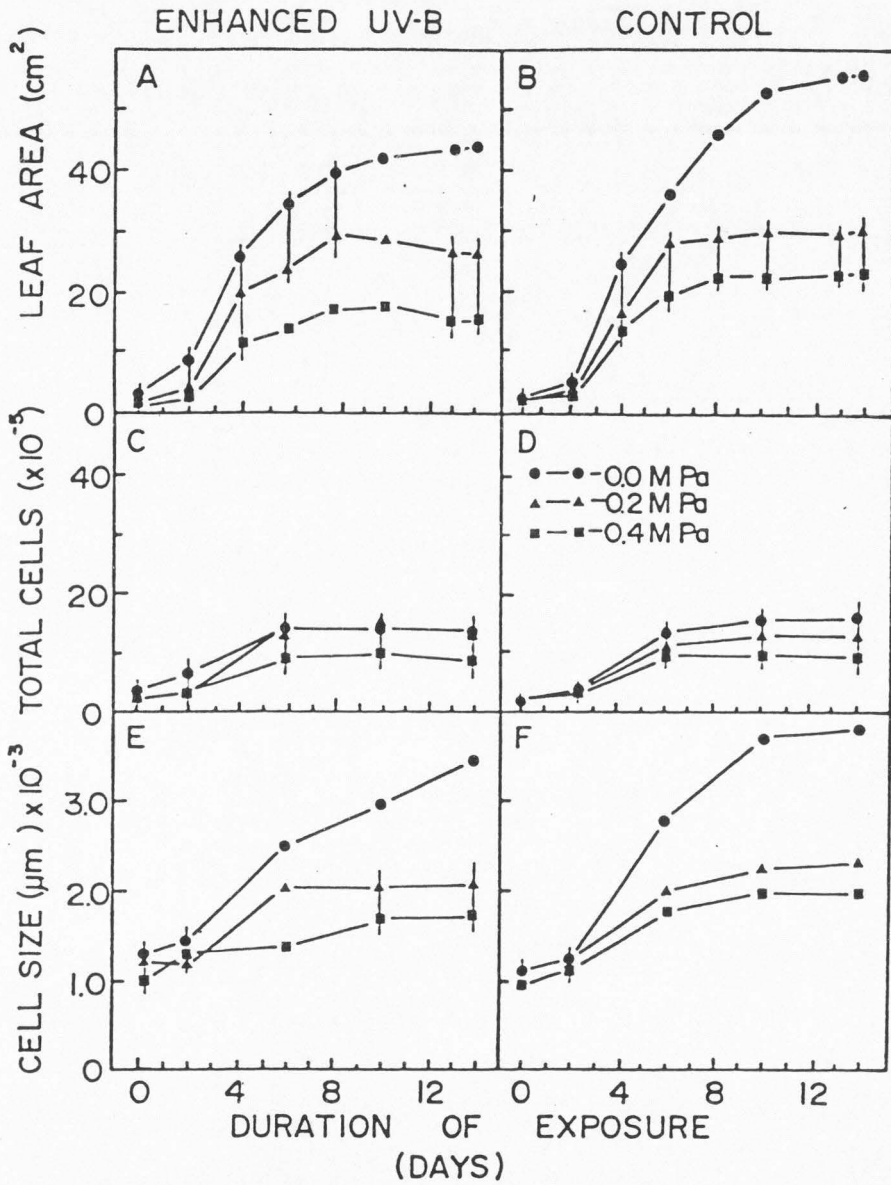
when under UV irradiation the -0.2 MPa and -0.4 MPa treat-
ment groups were not statistically different (Fig. 2-E)
while under control conditions they were (Fig. 2-F).
Because senescence was occurring in both treatments under
UV it is possible that this was partially responsible for
the difference.  In addition, on day 10, the average cell
size for the unstressed plants under enhanced UV-B irradia-
tion was significantly smaller than for the corresponding
plants under control irradiation.

## DISCUSSION

The combined effect of enhanced UV-B irradiation and water stress on leaf growth of Rumex obtusifolius resulted in an unexpected interaction. The UV-B dosage applied to R. obtusifolius in this investigation was insufficient to significantly suppress leaf growth or alter most leaf cell characteristics of irradiated plants when these were compared to control plants under any given water stress. The nature of the stress interaction was evident in the differential plant response to water stress under UV irradiation and control conditions (Fig. 1). Under UV-B irradiation, leaf growth and total epidermal cells per leaf were depressed by -0.4 MPa water stress relative to the -0.0 MPa and -0.2 MPa treatments (Fig. 1-A, C). Under control conditions leaves did not respond differently to -0.2 MPa or -0.4 MPa water stresses in any growth parameter (Fig. 1-B, D, F). In most cases the relationship between leaf growth parameters at -0.0 MPa and -0.2 MPa water stresses was the same under both UV and control conditions (Fig. 1), indicating that in this water stress range, UV does not alter the response of R. obtusifolius to water stress.

Without the additional stress of UV-B radiation, the change in the severity of water stress imposed by a drop in the matric potential of the rooting medium from -0.2 MPa to -0.4 MPa was not sufficient to affect leaf growth, cell division, or cell expansion (Fig. 1-B, D, E). Superimposi-

tion of UV-B irradiation subtly altered this relationship.
At some point between -0.2 MPa and -0.4 MPa, UV-B began
to interact with water stress to amplify the leaf growth
depression due to water stress.

After the initiation of the leaf primordium, the rate
and duration of cell division and cell expansion are solely
responsible for leaf growth (Milthorpe and Newton, 1963).
An analysis of both these processes in this experiment re-
vealed that under water stress and UV-B irradiation cell
division and expansion were both to some extent affected
by the interaction.

Ultraviolet radiation did not directly affect epidermal
cell size except on the final day of the experiment at
-0.0 MPa.  However, UV-B did alter the cell size response
to water stress during the latter part of the experiment
(Fig. 1-E, F).  It is notable that by the final day of the
experiment the pattern of differences in cell size was simi-
lar to the differences in leaf area (Fig. 1-A, B) indicating
that the relationship between the treatments due to the
interaction observed in the whole leaves may be explained
in part by the patterns in cell expansion.  It is clear,
however, that the patterns of cell division must also be
important in determining leaf growth response to the stress
interaction.  It must be remembered that cell expansion
plays two roles in leaf growth. Expansion takes place dur-
ing interphase (Hsiao, 1973) and after the cell division
phase of leaf growth has ceased (Maksymowych, 1963).  If the

rate of cell division was reduced by the stress interaction then a long-term reduction in cell size might be expected since the cell expansion phase of leaf growth would be delayed. At any given time, therefore, plants so affected would have had less time for the cells to expand to their final size and would have, on average, smaller cells, than unstressed plants. If, alternately, the stress interaction primarily acted to reduce cell expansion, then with longer interphase periods required to allow the cells to grow large enough to divide, cell division rates would be reduced. It is difficult to seperate these two possibilities based on the data presented here. There is, however, strong evidence to suggest that cell division may be most directly sensitive to the stress interaction.

The effect of the stress combination on total number of cells in the leaf seems clear. The smaller rate of increase in total cells per leaf and the extended duration of the cell division phase of leaf growth resulted in significantly fewer cells produced by the middle portion of the experiment in plants under the greatest water stress and UV-B irradiation (Fig. 1-C). The manner in which UV interacted with -0.4 MPa water stress to produce the observed effect on cell division is not clear. Either UV-B irradiation directly affected cell division and this level of water stress indirectly reduced the ability of the leaf cells to overcome the UV-B injury or both stresses combined to directly affect the division process.

A previous attempt to analyze UV-B-mediated reduction
in leaf growth on a cellular basis (Dickson and Caldwell,
1978) revealed that the rate of palisade and adaxial epi-
dermal cell division in Rumex patientia was reduced under
supplemental UV-B. For R. obtusifolius a similar response
was observed, but only under a stress combination, not due
to UV-B alone. In addition, the duration of the cell divi-
sion phase of leaf growth was increased under the stress
interaction in this investigation, while the earlier work
suggested no change in the duration of cell division under
UV-B. Although a direct comparison of the two studies is
difficult, it is clear that in both cases, UV-B irradiation
influenced the process of cell division.

Brown and Klein (1973) working with "near UV," a wave-
band from 300 to 400 nm overlapping both UV-B (290-320 nm)
and UV-A (320-400nm) spectral regions, found that irradi-
ation increased the length of the mitotic cycle in excised
pea root meristems by lengthening the $G_1$ or pre-DNA synthesis
period of mitotic interphase. It thus seems reasonable to
postulate that UV-B may have acted directly to slow the
mitotic cycle of the leaf cells under -0.4 MPa water stress
in this study.

The role of water stress in the interaction is unclear.
Although a direct effect of water stress on cell division has
occasionally been reported (Terry et al.; 1971, McCree and
Davis; 1974; review by Hsiao, 1973), the true nature of the
relationship is far from certain. Hsiao (1970), however,

has reported that the number of polyribosomes and hence the rate of protein synthesis is reduced in the cytoplasm of cells of <u>Zea</u> <u>mays</u> coleoptiles exposed to mild water stress (-0.65 MPa to -1.1 MPa plant water potential). Because enzyme and structural protein synthesis are closely linked to cell division, the effect of water stress on protein synthesis has important implications concerning the effect of water stress on cell division.

There is evidence, therefore, to suggest that water stress and UV-B irradiation may have an affect on cell division. Data presented here also suggest a role of UV-B in affecting the cell expansion process. Because these two processes are closely tied, the relative effects of the two stresses are difficult to separate in a study of this nature. As suggested above, the observed effect of UV-B irradiation on cell expansion under water stress may be simply a consequence of a direct effect on cell division. Conversely; by a mechanism as yet unknown, a direct affect of UV-B on cell expansion of plants under water stress may be responsible for the observed response of cell division. Based on the data presented here a definitive answer is not possible. Experimental evidence gathered by other workers, however, lends strong support to the possibility that the process of cell division is primarily and directly affected by the stress combination. To extend and refine this conclusion, detailed cytological studies of cell growth behavior under these stress conditions would be invaluable.

The pattern of the growth response of R. patientia to UV-B and water stress was generally similar to that of R. obtusifolius. As found for R. obtusifolius, leaves of R. patientia did not grow differently under -0.2 MPa or -0.4 MPa water stress under control conditions. However, under supplemental UV-B irradiation, leaf growth was significantly reduced by -0.4 MPa stress relative to the other two water stress levels (Fig. 2-A, B). The senescence that was observed in the water stressed leaves under enhanced UV-B affected the relationship between the -0.2 MPa and -0.4 MPa stressed leaves by day 13 and, thus, interpretations of the last two days of the experiment were difficult to make. It is interesting, however, that only under water stress and enhanced UV-B was this early onset of senescence noted. Sisson and Caldwell (1977) reported that leaf longevity of R. patientia was reduced substantially in a supplemental UV-B radiation regime that was slightly greater than that employed in this investigation. It is thus possible that under mild water stress, one effect of enhanced UV-B radiation is to reduce leaf longevity in this species. Unfortunately, a replication of this experiment was curtailed by technical difficulties after five days, well before leaf senescence could have occurred and, thus, this possibility awaits verification.

When the two growth parameters of cell size and cell number were examined for R. patientia, the same distinct relationship between water stress, UV-B irradiation and

cell division found for R. obtusifolius did not emerge.
At no time was the total number of cells different for
different water stress treatments under either enhanced
UV-B or control conditions (Fig. 2-C, D).  There was a
slightly greater difference in number of cells between
water stress treatments under control conditions, and a
slightly greater reduction in number of cells under -0.4
MPa stress in relation to -0.0 MPa and -0.2 MPa values with
supplemental UV-B, but the differences were not statisti-
cally significant.  Likewise, there was no clear indication
of an effect of the stress interaction on cell expansion
(Fig. 2-E, F).  Cell size seemed to respond similarly to
water stress under either UV-B regime.

Numerous studies have established the relatively high
sensitivity of R. patientia to UV-B irradiation (Sisson and
Caldwell, 1976, 1977; Dickson and Caldwell, 1978; Robberecht
and Caldwell, 1978).  Dickson and Caldwell (1978) have
linked this sensitivity partially to an effect of UV-B on
cell division rates.  In this investigation, however, high
UV-B sensitivity was not apparent even though approximately
the same effective UV-B doses were employed as in previous
investigations.  The difference in UV-B sensitivity may have
been due to differences in other environmental conditions
during the experiments.  One possible explanation may be
the soil moisture conditions of these experiments.  In this
study plants were grown under conditions much different
than those encountered by the soil-grown Rumex patientia

used in all other studies. Because of soil matric forces,
soil water potentials are rarely zero. It is undoubtedly
more difficult for plant roots to extract water and nutrients
from soil (especially slightly dry soil) than from the
solution-membrane system employed here, at least for the
-0.0 MPa solution and perhaps for the -0.2 MPa solution as
well. It may be that under slight or nonexistent water
stress, UV-B is for some reason unimportant and that only
under greater stress is the effect of the irradiation
manifest. If so, then water stress may have been an un-
recognized contributing factor to the effects previously
reported. To test this possibility for one study, soil
grown R. patientia were exposed to growth chamber conditions
closely approximating those employed by Sisson and Caldwell
(1977) and leaf water potentials were measured with a
pressure bomb periodically during the day. In this in-
stance under the relatively high midday chamber tempera-
tures used by Sisson and Caldwell ($37^{\circ}$C) leaf water poten-
tials dropped to approximately -1.1 MPa from a pre-dawn
(chamber temperature $16^{\circ}$C) level of -0.3 MPa. The plants
were watered to field capacity daily at the beginning of the
light period. The lowest leaf water potentials were recorded
seven hours after watering. The lowest water potentials for
the soil-grown Rumex were between the midday values recorded
for plants grown hydroponically under -0.2 MPa and -0.4 MPa
stress levels. Because this seems to be the zone in which
UV-B irradiation begins to interact with water stress, it is

possible that a hitherto unsuspected interaction between water stress and UV-B irradiation was at least partially responsible for the effects noted by Sisson and Caldwell (1977). Although this is only speculation, the evidence would seem sufficient to suggest that further research on UV-B radiation and leaf growth should include some consideration of the water status of the test plants.

The synergistic reduction in leaf growth, cell division and cell expansion due to water stress and enhanced UV-B irradiation suggested by this research has important ecological implications. It is possible, given sufficient leaf longevity, that the reduction in cell division rate observed here would only delay and not curtail the process of leaf growth. However, this early period of leaf growth is critical to the ultimate success of the plant. Photosynthetic carbon fixation is most rapid during this period for these species and a small, even transient decrease in leaf area could seriously reduce the carbon pool available for later growth and reproduction. The implications of this are clear. The water stress under which the interaction was manifest was quite mild, and may often be exceeded during the growing season in both natural and agricultural systems. Hence, if the relationship between water stress and UV-B irradiation observed here holds for other species, under an enhanced UV-B regime significant reductions in plant growth, carbon gain and reproduction might be expected. As a consequence of the effects on individual plants, altered competitive

interactions and community dynamics might be an even more important result of UV-B irradiation, as has been suggested by Fox and Caldwell (1978).

Further work is required to extend the results presented here, particularly in order to examine the mechanism of the interaction in finer detail and to examine the interaction over a broader range of stress combinations. Nevertheless, it does seem clear that an interaction does exist, an interaction with important ecological implications in view of the currently predicted anthropogenic reductions of the ozone layer.

LITERATURE CITED

Avery, G. S.  1933.  Structure and development of the
    tobacco leaf.  Amer. J. Bot. 20:  565-592.

Brown, S. J. and R. M. Klein.  1973.  Effects of near
    ultraviolet and visible radiations on cell cycle
    kinetics in excised root meristems of Pisum sativum.
    Amer. J. Bot. 60:  554-560.

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.

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(8):
    857-863.

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

Green, A. E. S., T. Sawada, and E. P. Shettle.  1974.  The
    middle ultraviolet reaching the ground.  Photochem.
    Photobiol. 19:  251-259.

Groot, D.  1969.  The use of silicone rubber plastic for
    replicating leaf surfaces.  Acta. Bot. Neerl. 18:
    703-707.

Hsiao, T. C.  1970.  Rapid changes in levels of poly-
    ribosomes in Zea mays in response to water stress.
    Plant Physiol. 46:  281-285.

Hsiao, T. C.  1973.  Plant responses to water stress.  Ann.
    Rev. Plant Physiol. 24:  519-570.

Kaufmann, M. R.  1969.  Effects of water potential on
    germination of lettuce, sunflower, and citrus seeds.
    Can. J. Bot. 47:  1761-1764.

Klein, P. M.  1979.  Plants and near-ultraviolet radiation.
    Bot. Rev. 44:  1-127.

Maksymowych, R.  1963.  Cell division and cell elongation
    in leaf development of Xanthium pensylvanicum.  Amer.
    J. Bot. 50:  891-901.

McCree, K. J. and S. D. Davis. 1974. Effect of water
    stress and temperature on leaf size and on size and
    number of epidermal cells in grain sorghum. Crop. Sci.
    14: 751-755.

Milthorpe, F. L. and P. Newton. 1963. Studies on the
    expansion of the leaf surface III. The influence of
    radiation on cell division and leaf expansion. J.
    Exp. Bot. 14: 483-495.

Molina, M. J. and F. S. Rowland. 1974. Stratospheric sink
    for chlorofluoromethanes: chlorine atom-catalyzed
    destruction of ozone. Nature 249: 810-812.

Painter, L. I. 1966. Method of subjecting growing plants
    to continuous soil moisture stress. Agron. J. 58:
    459-460.

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.

Salisbury, F. B. 1975. Multiple factor effects on plants.
    pages 501-520. in F. J. Vernberg, ed. Physiological
    adaptations to the environment. Intex Educational
    Publishers.

Saurer, W. and J. V. Possingham. 1970. Studies on the
    growth of spinach leaves (Spinacea oleracea). J.
    Exp. Bot. 21: 151-158.

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

Sisson, W. B. and M. M. Caldwell. 1977. Atmospheric ozone
    depletion: Reduction of photosynthesis and growth of a
    sensitive higher plant exposed to enhanced UV-B
    radiation. J. Exp. Bot. 28: 691-705.

Slatyer, R. O. 1967. Plant-water relationships. Academic
    Press, London. 366 pp.

Sunderland, N. 1960. Cell division and expansion in the
    growth of the leaf. J. Exp. Bot. 11: 68-80.

Terry, N., L. J. Waldron, and A. Ulrich. 1971. Effects of
    moisture stress on the multiplication and expansion of
    cells in leaves of sugar beet. Planta 97: 281-289.

Tingey, D. T., and C. Stockwell. 1977. Semipermeable
    membrane system for subjecting plants to water stress.
    Plant Physiol. 60: 58-60.

Wisbey, B. D., T. A. Black, and R. J. Copeman. 1977.
    Controlling soil water matric potential in root
    disease studies. Can. J. Bot. 55: 825-830.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall,
    Inc., New Jersey. 620 pp.

Zur, B. 1966. Osmotic control of the matric soil-water
    potential: I. Soil-water system. Soil Sci. 102:
    394-398.

APPENDIX

Figure 3    Patterns of leaf growth, cell division and cell
            expansion for the replicate experiment with
            Rumex obtusifolius, exposed to enhanced UV-B
            (graphs A, C, and E) and control (graphs B, D,
            and F) treatments while under three levels of
            water stress (-0.0 MPa, -0.2 MPa, and -0.4 MPa
            rooting medium matric potential). Average
            values which are not significantly different
            from one another at $P \leq 0.05$ are connected by
            vertical bars. Asterisks denote days on which
            a significant ($P \leq 0.05$) interaction was ob-
            served between water stress and UV-B
            irradiation.