# A STUDY OF THE TOTAL ULTRAVIOLET EXPOSURE TO ALL THE LEAVES FOR SMALL PLANT GROWTH

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**Abstract** - A dosimetric spectrum evaluator was employed to evaluate the total biologically effective UV (UVBE<sub>plant</sub>) on the surface of all the leaves of small plants. It was applied to evaluate the UVBE<sub>plant</sub> to soybean leaves (*Glycine max* [L.] Merr.), cultivar Essex. The plants received three levels of UVBE<sub>plant</sub> averaged over all the leaves (0, 8.1 and 15.8 J cm<sup>-2</sup>) from emergence to the V7 growth stage. As canopy closure occurred with plant growth, the ratio of the average of the UVBE<sub>plant</sub> to all leaves compared to that on the uppermost set of leaves changed from 0.85 to 0.50 to 0.29 for the V2, V4 and V7 stages respectively. Measurement of the UVBE<sub>plant</sub> at the top of the canopy would introduce errors of 18%, 100% and 245% at the V2, V4 and V7 stages respectively compared to the actual exposure to the plant leaves. The evaluation of leaf UVBE<sub>plant</sub> allows formulation of models defining plant response such as suppression of plant height and leaf area in terms of the UV exposure to all the leaves along with the effects on the fresh and dry weights of the leaves, stems and roots.

#### **1. INTRODUCTION**

Studies have confirmed that a portion of the earth's protective layer of stratospheric ozone is being depleted [1]. Upward trends in the levels of ultraviolet radiation at the earth's surface have been measured [2,3]. UV radiation, in particular, the UVB waveband (280 to 320 nm) can affect plant growth and physiology [4], for example, plant height, leaf area and photosynthesis are reduced to various degrees in UV sensitive plants. Of primary concern are the implications for global food production resulting from the effects of increased UVB radiation on plants. There have been differences reported in the effects of increased UVB on soybean (Glycine max [L.] Merr.). Teramura et al. [5] found an overall yield reduction of 19 to 25% in field grown soybean (cv. Essex) during 4 of the 6 years of the study with no reductions in the other two years which were generally hot and dry with prolonged periods of drought. Miller et al. [6] reported no UVB effects on the same cultivar. Teramura and Sullivan [4] attribute these differences in response to other microclimatic conditions such as precipitation patterns and solar irradiance. To allow formulation of a more reliable quantitative prediction of the anticipated influences on agricultural crop production as a result of decreased stratospheric ozone, experiments under field conditions, and in controlled greenhouse conditions are required [7].

Although the increase in total solar irradiance resulting from ozone depletion is minimal, any increase in UVB is important biologically due to the effectiveness of these wavelengths in producing plant damage [5]. This effectiveness of UV irradiation is expressed with the biological weighting function called the action spectrum. For UV plant damage, the action spectrum of Caldwell [8] normalised at 300 nm was developed as the generalised response for

several processes of higher and lower plants and is widely employed. For any biological process with an action spectrum,  $A(\lambda)$ , and a solar spectral irradiance of  $S(\lambda)$ , the biologically effective UV exposure (UVBE<sub>plant</sub>) for a period T, is provided by:

$$UVBE_{plant} = T \int_{UV} A(\lambda)S(\lambda)d\lambda$$
(1)

As a result, knowledge of the UV spectrum incident on the plant is required to calculate UVBE<sub>plant</sub> in any research on the effects of enhanced UVB on plants.

Previous studies have predominantly employed the measurement of the ambient spectrum on a horizontal plane with spectroradiometers to provide an indication of the UVBE<sub>plant</sub> incident on the plants. This approach could provide misleading information as the exposure spectra to the plant canopy may be substantially different from the ambient spectrum. Models have been developed for calculating the light penetration within a plant canopy, however, they cannot account for all the complex temporal and spatial variations of the visible and UV radiation environment within a plant canopy. No previous research has measured the total UVBE<sub>plant</sub> per area on the plant leaf surface and investigated the relationship to leaf expansion and other indicators of plant growth. A new device has been reported [9,10,11] for spectrum evaluation to assess the actual UVBE<sub>plant</sub> exposure to a model of the plant canopy in a study of soybean. However, these papers have not provided the total UV exposure to each leaf. As it has been proven [11] that the exposure varies from point to point over the canopy, the mean leaf exposure would be a better indicator for assessing the effect of solar radiation on the plant growth. This project aims to employ the spectrum evaluator to measure the total UV radiation incident on the surface of each leaf of a small plant to evaluate the mean plant leaf exposure

to  $UVBE_{plant}$  and to determine the relation of this mean leaf exposure to modifications of any plant growth parameters.

## 2. EXPERIMENTAL DETAILS

#### 2.1 Soybean growth and irradiation

Two seeds per pot (1 litre pots) of soybean cv. Essex were planted in late spring on 7 November 1996 in the University of Southern Queensland greenhouse in Toowoomba (27.5°S latitude), Australia. This soybean variety is a particularly UVB sensitive one [5]. An artificial irradiation environment was employed in this research in order to test the method of evaluating the UVBE<sub>plant</sub> on the surface of the leaves. The seeds were planted in a 1:1:1 mixture of peat moss, vermiculite and sand with the seedlings thinned upon emergence to leave the single strongest plant in each pot. Throughout the experiment, the plants were watered 3 days/week and fertilised weekly with Nitrosol (13% N, 2.3 % P, 10% K) mixed 10 mL to 2.5 L water. Plant protection was provided by regular inspection and physical control to remove lepidoptera larvae. Dyston 50 (active constituent 50 g kg<sup>-1</sup> Disulfoton) granules were placed in the pot to the base of each plant on 7 December to control white fly (dose rate of 30 g m<sup>-2</sup>). The natural photoperiod over the growing period averaged at 13.7 h.

Supplemental UV was provided to the treated group of plants by fluorescent Philips TL40/12 lamps (Lawrence and Hanson, Toowoomba, Australia) wrapped in 0.13 mm cellulose acetate (CA) film (Artery, Hobart, Australia) following the procedure of Parisi *et al.* [11]. For the control group of plants, the lamps were wrapped in 0.13 mm Mylar film (Cadillac Plastics, Australia) which allowed no UVB (280-320 nm) and UVC transmission (Figure 1). The CA

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filters were replaced every 4 days and the Mylar weekly due to photodegradation of the filters causing reduced UV transmission. UVA (320-400 nm) and visible radiation were available to both the treated and control group of plants from the solar radiation transmitted through the greenhouse glass. As a result of the absorption properties of the greenhouse glass there was no solar UVB in the greenhouse. The lamps were pre-burnt and the filter material pre-solarised for eight hours. The lamps were switched on daily with an electronic timer between 09:00 and 15:00 Eastern Standard Time (EST). Each treatment plot was separated with Mylar and the entire irradiation frame surrounded with Llumar (no UV transmission) (Scotchline, Australia). The Llumar and Mylar were maintained at about 20 cm above the bench top to allow air circulation and to maintain each of the irradiation plots at approximately the same temperature. Air temperature within each compartment was electronically recorded every 30 minutes using a data logger (La Trobe University, Australia). Temperature differences between compartments were not significant. Daily temperature ranged between 17 and 35 °C over the length of the trial.

A low UV (T56) and a high UV (T35) treatment was provided with the UV lamps at approximately 0.56 m and 0.35 m respectively above the plant tops with a control group for each of the low (C56) and high (C35) UV treatment plots. A cross sectional view of the irradiation facility is provided in Figure 2 with the two lamps in each plot spaced 0.3 m apart. Irradiation of the plants commenced on 15 November at seedling emergence and continued for 36 days till harvest. In each of the treated and control plots there were 18 to 19 plants and the plants were randomised weekly within each plot to minimise any positional effects. As required, the height of the lamps was adjusted weekly with the raiseable frame.

#### 2.2 UVBE evaluation

The emission UV spectrum was evaluated with a previously described spectrum evaluator [12] employing the four thin film UV dosimeter materials polysulfone, nalidixic acid (NDA), 8-methoxypsoralen (8MOP) and phenothiazine [13] in close proximity to one another in a holder of size 30 mm x 30 mm. For each material the  $\Delta A$  as a result of exposure to a UV emission spectrum for a period, T is:

$$\Delta A = T \int_{uv} \overline{S(\lambda)} R(\lambda) d\lambda$$
<sup>(2)</sup>

where  $R(\lambda)$  is the spectral response of each material and  $\overline{S(\lambda)}$  is the time averaged UV spectrum. For a UV exposure, measurement of  $\Delta A$  and knowledge of  $R(\lambda)$  for each material allows the time averaged emission spectrum to be evaluated with a numerical technique.

The emission UV spectrum was evaluated simultaneously at each set of leaves on a sample plant in each treated plot with the spectrum evaluator described above. The days selected for measurement were at the full expansion of the first, third and sixth trifoliate leaves above the unifoliate node (V2, V4 and V7 growth stages [14]). These stages corresponded to 13, 21 and 36 days from seedling emergence of UV irradiation. The emission spectrum was evaluated on each set of leaves in the morning, noon and afternoon at 09:00 to 10:00, 11:30 to 12:30 and 14:00 to 15:00 EST. For the V2 and V4 stage measurements, a spectrum evaluator was attached at the approximate plane of each set of leaves on a frame constructed with 2 mm steel. At the V7 stage, the plants were large enough to support a spectrum evaluator on each set of leaves as shown in Figure 3. The spectrum evaluators were on the plants for three hours only at each of the three growth stages and any interference with plant growth was considered

as insignificant. In this research, UV radiation incident on the leaves only was considered as the leaves form the largest surface area of the plant and the radiation falling on other parts of the plant such as stems was neglected. Following evaluation of the emission spectrum, the biologically effective UV for generalised plant damage, UVBE<sub>plant</sub> was calculated employing Equation (1) and the plant damage action spectrum.

#### 2.3 Plant measurements

At the V2 , V4 and V7 growth stages, the height of each plant in each treatment was measured. At the V2 stage, the length and width of every leaf was measured. At the V4 stage, the length and width of each leaf that had developed since the V2 stage was measured. The plants were harvested at the V7 growth stage (pre flowering) following 36 days supplemental UV exposure. Measurement of total leaf area was made using a calibrated planimeter (Licor, USA). The root systems were removed from the growth media by washing and blotted dry with paper towelling. Fresh weight of leaves, stems and root systems were made before oven drying at 80°C and measurement of dry weights. The specific leaf weight (SLW) defined as the ratio of leaf dry weight to leaf area was calculated. The length, width and area of a sample of sixty leaves of different sizes were measured to establish a regression equation relating the product of the length and width to the actual leaf area [15]. This regression equation was used to estimate leaf area of plants at the V2 and V4 growth stages. The Student's two sample t-test with P < 0.05 was used for attributing statistical significance to treatment differences in plant growth.

#### 3. RESULTS

#### 3.1 UVBE evaluation

The UV spectrum was evaluated on each set of leaves at the V2, V4 and V7 growth stages and Equation (1) employed to provide the total UVBE<sub>plant</sub> per area to each set of leaves. From previous research, comparison of the evaluated UVBE<sub>plant</sub> on a horizontal plane to those obtained by measuring the spectrum with a calibrated spectroradiometer provided an agreement to better than 20% [11]. The evaluation of the spectrum on each set of leaves with this method provides a more accurate representation of the total UV exposure incident on a plant than any obtained by measurement with radiometers or spectroradiometers of the ambient exposure on a horizontal surface [11]. The total UVBE<sub>plant</sub> per area on each set of leaves was interpolated between the measurement times of 09:00-10:00, 11:30-12:30 and 14:00-15:00 EST to provide the day exposure at that growth stage on each set of leaves. This day exposure is shown in Figure 4 for the low and high UV treatments. The spectrum evaluators were also employed in the control plots with no significant change in absorbance for the polysulfone dosimeter which responds only to UVB wavelengths. Consequently, no measurable UVBE<sub>plant</sub> was incident on the control plants. A point to note for the V7 growth stage is that as a result of canopy closure, shading by other leaves on the same plant and other neighbouring plants, there is not a significant difference between the UVBE<sub>plant</sub> incident on the L1 to L6 set of leaves for the T35 and T56 plants. Consequently, as the plant develops, the difference between the incident high and low UV irradiances is expressed predominantly on the uppermost part of the canopy. The differences in UVBE<sub>plant</sub> between the high and low exposure treatments was greatest for leaves with un-impeded access to the radiation source (lamps). Thus as the plants grew, competitive shading by leaves resulted in reduced UVBE<sub>plant</sub> levels (and treatment differences) for leaves other than those which were uppermost in the canopy (Figure 4).

This is further illustrated by averaging the total  $UVBE_{plant}$  per area for all leaves at each growth stage. The all day  $UVBE_{plant}$  for the high UV treatments when averaged over all the leaves for the V2 stage is approximately double that of the low UV treatments. This is expected due to the height difference of the UV lamps. In comparison, with canopy closure, at the V7 stage, the  $UVBE_{plant}$  averaged over all the leaves is practically the same for the high and low UV treatments as a result of the shading of the L1 to L6 leaves. Specifically, there is a dilution effect as a result of the increasing leaf area per unit of available radiation. The all day  $UVBE_{plant}$  have been interpolated between the measurement days to provide the cumulative average leaf exposure per area for each stage in the final two columns of Table 1.

#### 3.2 UVBE<sub>plant</sub> and plant height

At the highest UV dose level (lamps at 35 cm above the canopy), plant height was significantly reduced (P < 0.05, Student's t-test) at the V2, V4 and V7 stages when compared to the control treatment (Figure 5). The height difference between the treated and control groups increased steadily over the length of the experiment. At the lower UV dose (lamps at 56 cm above the canopy), plant height was significantly reduced (P < 0.05, Student's t-test) at the V7 stage only with no significant difference at the V2 and V4 stages.

#### 3.3 Cumulative average leaf UVBE<sub>plant</sub> per area and plant height and leaf area

The percentage changes in plant height and leaf area for the low and high UV treatment plants compared to the respective control plants as a function of the cumulative average leaf

UVBE<sub>plant</sub> per area (combined across UV treatments) are shown in Figure 6. The asterisks represent statistically significant differences in leaf area at all three stages for the high UV treatment. Although, there is no significant difference (P < 0.05) in leaf area for the low UV treatment, there is a general trend to increased reduction in leaf area with the number of irradiation days. This confirms previous research under greenhouse conditions that has also found soybean (cv. Essex) to be UV sensitive. Murali *et al.* [14] measured a reduction in leaf area of 19% at the V6 growth stage for a daily UVBE<sub>plant</sub> irradiance on a horizontal surface of 11.5 kJ m<sup>-2</sup> with Teramura and Sullivan [16] measuring reductions in plant height and leaf area for the V7 growth stage for daily UVBE<sub>plant</sub> irradiances on a horizontal surface of 11.5 and 13.6 kJ m<sup>-2</sup>.

The magnitude of the percentage reduction in plant height and leaf area increases with increasing average leaf UVBE<sub>plant</sub> per area up to a total exposure of approximately 15 J cm<sup>-2</sup> where the effect begins to plateau for these vegetative stages of soybean (cv. Essex). For the high UV treatment, the exposure of 15 J cm<sup>-2</sup> occurs after approximately 20 days of irradiation. The amount of exposure required to saturate the response was not obtained prior to harvest for the low UV treatment. Regression curves fitted to the data in Figure 6 allow estimation of the response in plant height and leaf area for a given leaf exposure of UVBE<sub>plant</sub>. For the percentage change in plant height, (%  $\Delta$ H):

$$\%\Delta H = 0.087x^2 - 4.006x + 12.9 \tag{2}$$

and for the percentage change in leaf area, ( $\%\Delta LA$ ):

$$\%\Delta LA = 0.218x^2 - 6.28x + 23.5 \tag{3}$$

where x is the UVBE<sub>plant</sub> in units of J cm<sup>-2</sup>. Both dose response relationships have an R-squared value of 0.95.

#### 3.4 Growth parameters at V7 stage (harvest)

The plant growth parameters at the V7 growth stage when the plants were harvested are provided in Table 2. The means followed by asterisks are significantly different at P < 0.05according to Student's t-test. Plant height was significantly reduced in both the high and low UV treatments with the greater reduction for the high UV treatment. The number of leaves, stem fresh weight and SLW were significantly different in both UV treatments. In soybean, SLW is correlated with the leaf thickness [14]. Both the high and low UV treatment plants demonstrated a response of the thickening of the leaves compared to the control plants. Total leaf area, leaf fresh weight, stem dry weight, root fresh and dry weights were significantly reduced in the high UV treatment with no significant difference for the low UV treatment. The largest differences occurred for root weight with differences of 36% and 43% for fresh and dry weight respectively. Although not statistically significant, the total leaf area and leaf fresh weight of the low UV treatment were reduced compared to the control.

#### 4. DISCUSSION

The total biologically effective ultraviolet radiation on all the leaves of a small plant has been evaluated and correlated to plant growth parameters. This has not been done previously and evaluation of the total  $UVBE_{plant}$  to the plant leaves with the method in this paper provides a more accurate representation of the UV exposures incident on a small plant in studies of the

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effects of enhanced UV levels. This method is applicable to small plants and is not feasible for large plants with many leaves. The method allows simultaneous measurement at multiple sites on a plant and as a result takes into account shading by neighbouring plants and shading by other leaves in the plant. The spectrum evaluators employed require an exposure of approximately one hour for greenhouse conditions and consequently, any variations in lamp output and filter transmission during this time are taken into account. The measurement of the  $UVBE_{plant}$  with the spectrum evaluator at the same position as the actual plant leaves takes into account the dynamics of the plant canopy as the plant grows. For example, the ratio of the whole plant mean of the UVBE<sub>plant</sub> compared to that on the uppermost set of leaves changes from 0.85 to 0.50 to 0.29 for the V2, V4 and V7 stages respectively. As canopy closure occurred with plant growth, the differences between a high and low UV treatment were incident predominantly on the top leaves of the plants. On the other hand, a spectroradiometer or radiometer can only take a single reading on a predominantly horizontal surface. Measurement of the UVBE<sub>plant</sub> at the top of the canopy would introduce errors of 18%, 100% and 245% at the V2, V4 and V7 stages respectively compared to the actual exposure to the plant leaves. This error cannot be corrected by a scaling method. Consequently, as plants develop, the difference between the incident high and low UV irradiances is expressed predominantly on the uppermost part of the canopy. Thus as plants grow, competitive shading by leaves results in reduced UVBE<sub>plant</sub> levels (and treatment differences) for leaves other than those which are uppermost in the canopy. Photobiologically, this may possibly mitigate against some of the detrimental effects on plants of increased UVB.

The response of soybean, cv. Essex, was shown to be dependent on the level of UV exposure with the effects increasing with higher UV exposures. The plant growth parameters of plant height, number of leaves and stem fresh weight were significantly reduced by 11%, 6% and 13% respectively for the low UV treatment. This corresponded to a cumulative UVBE<sub>plant</sub> to each set of leaves of 8.1 J cm<sup>-2</sup>. Total leaf area, leaf fresh weight, stem dry weight and root fresh weight exhibited a reduction trend of 12%, 10%, 4% and 6% respectively for the low UV treatment. These became statistically significant for the high UV treatment  $(15.8 \text{ J cm}^{-2})$ with reductions of 21%, 13%, 20% and 36% for the same parameters respectively. In addition for the high UV treatment, plant height, number of leaves, stem fresh weight and root dry weight were reduced by 27%, 4%, 20% and 43% with an increase in SLW of 13% and 17% for the low and high UV treatments. In general, these effects on plant growth parameters are consistent with the results of Teramura and Sullivan [16] under greenhouse conditions who measured reductions in plant height at the V7 stage of 10% and reductions in leaf area of 11% and 22% and an increase in SLW of 10% and 12% for low and high UV treatments respectively. The advantage of the method presented in this paper is that it evaluated the total UVBE<sub>plant</sub> exposure to the leaves and allowed models to be formulated defining plant response such as suppression of plant height and leaf area in terms of the UV leaf exposure.

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Growth Stage	All day	<b>UVBE</b> <sub>plant</sub>	Cumulative UVBE <sub>plant</sub>			
	(J	cm <sup>-2</sup> )	(J cm <sup>-2</sup> )			
	T35	T56	T35	T56		
V2 (13 days)	0.61	0.25	7.9	3.2		
V4 (21 days)	0.37	0.24	11.8	5.2		
V7 (36 days)	0.16	0.15	15.8	8.1		

Table 1 - Average leaf  $UVBE_{plant}$  per area calculated as all day and cumulative values for each treatment as a function of plant growth stage.

Table 2 - Plant growth parameters at the V7 growth stage (harvest). Each mean is the average of 18 to 19 plants with the error represented as one standard error. Means followed by an asterisk are significantly different at P < 0.05 according to Student's t-test.

Treatr	nent	Growth Parameter									
		Plant	Number	Total	Leaf fresh	Leaf dry	Stem	Stem dry	Root	Root dry	SLW
		height	of leaves	leaf area	wgt	wgt	fresh	wgt	fresh	wgt	(g m <sup>-2</sup> )
		(cm)		$(cm^2)$	(g)	(g)	wgt (g)	(g)	wgt (g)	(g)	
Low UV	Control	101±3	9.0±0.1	949±28	12.1±0.4	2.06±0.08	7.7±0.3	1.26±0.06	8.0±0.7	1.6±0.2	21.7±0.4
	Treated	90±2*	8.5±0.2*	835±49	10.9±0.7	2.08±0.14	6.7±0.4*	1.21±0.08	7.5±0.7	1.8±0.2	24.6±0.5*
High UV	Control	113±3	9.0±0.1	1053±43	12.9±0.6	2.15±0.12	8.7±0.4	1.36±0.08	10.0±0.7	2.3±0.2	20.3±0.3
	Treated	82±2*	8.6±0.1*	834±34*	11.2±0.5*	2.00±0.11	7.0±0.3*	1.09±0.06*	6.4±0.4*	1.32±0.08*	23.8±0.5*

## **Figure Captions**

- Figure 1 Emission spectra of the Philips TL40/12 lamps (1) unfiltered, (2) filtered by cellulose acetate and (3) filtered by Mylar [11].
- Figure 2 Cross section view of the irradiation facility.
- Figure 3 Soybean plant with a spectrum evaluator attached to each set of leaves.
- Figure 4 The all day UVBE<sub>plant</sub> at the V2, V4 and V7 growth stage incident on each set of leaves for the high (T35) and low UV (T56) treatment plants. The leaf position (L1, L2 etc.) denotes the leaf age from the first true leaf (unifoliate).
- Figure 5 The plant height for the low UV treatment plants (T56) compared to the control (C56) and for the high UV treatment plants (T35) compared to the control (C35).
- Figure 6 The percentage change in plant height and leaf area for the low (◆) and high
  (■) UV treatment plants compared to the respective control plants. The asterisks represent statistically significant differences at P < 0.05 according to the t-test. The solid lines are the regression curves fitted to the data points.</li>



Figure 1

(A.V. Parisi, J.C.F. Wong, V. Galea)



Figure 2

(A.V. Parisi, J.C.F. Wong, V. Galea)



# Figure **3**

(A.V. Parisi, J.C.F. Wong, V. Galea)



Figure 4

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Figure 5

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Figure 6

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