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Field Testing of Biological Spectral Weighting Functions for Induction of UV-absorbing Compounds in Higher Plants^{†¶}

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ABSTRACT

Action spectra are typically used as biological spectral weighting functions (BSWF) in biological research on the stratospheric ozone depletion issue. Despite their critical role in determining the amount of UV supplied in experiments, there has been only limited testing of different functions under realistic field conditions. Here, we calculate effective radiation according to five published BSWF and evaluate the appropriateness of these BSWF in representing the induction of UV-absorbing compounds. Experiments were carried out in the field using both ultraviolet-B radiation (280–320 nm) supplementation and selective filtering of solar UV radiation. For the four species tested, BSWF that extend into the ultraviolet-A radiation (320–400 nm) (UV-A) with moderate effectiveness best represented the observed results. When compared with the commonly used generalized plant response, these BSWF suggest that simulations of ozone depletion will require more radiation than in the past experiments. However, they imply lower radiation supplements than a new plant growth BSWF that has a greater emphasis on UV-A wavelengths.

INTRODUCTION

In addressing the stratospheric ozone depletion issue, biological spectral weighting functions (BSWF) have been widely used and have received considerable attention (1–5). Yet, it is only recently that there have been a few attempts to test how well these functions predict the performance of plants under field conditions. In Tierra del Fuego, Rousseaux *et al.* (6) took advantage of changes in solar UV spectral irradiance as the Antarctic vortex containing the

“ozone hole” passed overhead. They found that DNA damage (formation of cyclobutane pyrimidine dimers [CPD]) was predicted better by BSWF that declined steeply with increasing wavelength than by BSWF with significant weighting in the ultraviolet-A radiation (320–400 nm) (UV-A) waveband (6). In a test of growth inhibition in an *Arabidopsis* flavonoid mutant, Fiscus and Booker (7) used a series of three types of UV-exclusion filters. They found that the seedling DNA damage (CPD induction) function (8) predicted growth inhibition better than did the generalized plant response. Another experiment examining plant growth responses used combinations of ultraviolet-B radiation (280–320 nm) (UV-B) lamps together with filters removing different amounts of solar UV-A. This experiment tested a new BSWF for plant growth that has a significant tail into the UV-A (9). This new BSWF was found to be the best predictor for growth changes seen in 2 years of field treatments when compared with a variety of other BSWF (10). To date, however, we know of no specific tests of how well different BSWF predict the induction of UV-absorbing compounds.

The UV-absorbing pigments in leaves have long been considered important in selectively absorbing much of the solar UV-B radiation before it reaches the chloroplasts (11–14). Besides their importance in screening UV-B, these compounds also have a role in ecosystem dynamics. They have been shown to mediate levels of herbivory (*e.g.* Warren *et al.* [15]; see also review by Caldwell *et al.* [16]), and there is the potential for changes in phenolic metabolism to influence decomposition (17) and plant disease (18). Plants grown under reasonably high levels of photosynthetically active radiation (PAR) already have considerable quantities of these pigments, even if nearly all UV radiation (both UV-B and UV-A) is removed (*e.g.* Phoenix *et al.* [19]). Experiments providing UV-B supplementation induce a small, but often statistically significant, increase in these compounds. Under field conditions, a meta-analysis showed a statistically significant 10% increase in these compounds with supplementation of UV-B above ambient levels (20).

Under field conditions, these incremental changes in UV-absorbing pigments are primarily induced by UV-B, and other wavebands, such as the UV-A, are relatively less effective (21,22). An exception has been shown with specific kaempferol compounds, where solar UV-A appears more effective in inducing these compounds than UV-B (23). However, quantitative assessment of the ability of different BSWF to predict this pigment induction in the field has not been attempted.

Plant growth responses in the field are influenced by both UV-B and UV-A, but it is not certain which is the most appropriate

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Abbreviations: BSWF, biological spectral weighting function; CPD, cyclobutane pyrimidine dimer; PAR, photosynthetically active radiation, 400–700 nm; RAF, radiation amplification factor; UV-A, ultraviolet-A radiation, 320–400 nm; UV-B, ultraviolet-B radiation, 280–320 nm; UV-B_{BE}, biologically effective UV-B.

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BWSF for induction of UV-absorbing compounds. This presents a dilemma for experimental design. It is necessary to use BWSF to set irradiance in experiments designed to test ozone depletion effects using UV supplementation from lamps (24). But if two very different BSWF both represent realistic plant responses, there is no clear guide on which BSWF to select. Using different BSWF can have a large influence on the amount of supplemental UV-B radiation that is necessary to supply with lamps (1,24).

To help resolve this issue, we tested different BSWF in the field for their ability to predict the accumulation of UV-absorbing compounds in leaf tissue. We tested the spectral response of plants using an experiment that combined UV-B supplementation with filtering of solar UV-A and another experiment where treatments were effected by selectively filtering solar UV. The irradiances of the different treatments, when weighted with different BSWF, are compared with the observed relative responses from these different combinations of spectral irradiance.

METHODS

Plant culture. Experiments were conducted outdoors near Logan, UT (41.5°N, 1.5 km elevation), during late spring through early fall in 1996 with cultivated oat (*Avena sativa* L. cv Otana), the weed kochia (also known as summer cypress) (*Kochia scoparia* [L.] Schrad.), cultivated green bell pepper (*Capsicum annuum* L. var. California Wonder), and sorghum (*Sorghum bicolor* [L.] Moench var. DK 18). As in previous experiments (10,25), plants in all experiments were grown in Ray Leach containers (0.15 L conically shaped containers; Stuewe and Sons, Corvallis, OR). More containers were planted than were needed for the experiments so that uniform seedlings could be selected as experimental plants. Additional plants provided an unsampled edge row. Containers were arranged in racks with an interplant spacing of 4.5 cm, producing a density equivalent to 494 plants m⁻². This density simulates more natural plant growth conditions (and canopy light climate) than would the use of isolated plants.

Supplemental UV-B and filtered sunlight experiment. This experiment was conducted with cultivated oat from 13 June to 11 July. This was the second of two field seasons used to test plant responses during evaluation of a new BSWF for plant growth (10), but the pigment data were not published in that report. A full description of the lamp adjustments and irradiation conditions is given in Flint and Caldwell (10). Briefly, supplemental UV-B radiation was supplied by UV-B fluorescent lamps encased in different types of plastic film filters, providing a UV-B treatment and a control with minimal UV-B. The two lamp filter conditions were combined with two types of plot covers (to influence primarily the UV-A radiation) and two lamp emittance levels controlled by an electronic modulation system (26). All lamps were continually adjusted by the electronic system so that emittance tracked ambient UV-B radiation during the day (26). A broadband UV-B sensor (27) provided the ambient UV-B signal to this system as well as a record of ambient UV-B flux. Although the lamp control system has considerable ability to adjust lamp output to accommodate and compensate for changes in filter transmittance due to aging (26), all lamp filters were changed weekly.

Because lamp emittance in the UV-A is minimal compared with solar UV-A, plastic filters were used over all plots to create spectrally different solar UV-A conditions. Llumar (0.13 mm thick) (Norton Performance Plastics, Wayne, NJ) was used to remove all UV-A (wavelength <400 nm). To avoid creating different microclimates, it was necessary to have a filter over the near-ambient UV-A plots also. Clear polyester (0.10 mm thick, transmits wavelengths above 315 nm) was used for this. The filters were domed to shed rainfall. The spectral irradiances for the treatments in this lamp-filter system are shown in Fig. 2a. Both filters transmitted high levels of PAR.

Lamps were electronically adjusted to provide a simulation of 15% and 30% ozone depletion over Logan, UT. These treatments were based on the generalized plant weighting function (in Caldwell [28] as formulated by Green *et al.* [29]). Because the covers removed most short-wavelength UV-B, the generalized plant weighted UV in the control plots was negligible, and nearly all UV-B in the UV-B treatment plots was provided by the lamps.

Eight lamp arrays were used in this experiment, and each array was split into two different UV-B treatments (10). Racks of plants were rotated within that treatment daily, and on alternating days half the racks were exchanged with the same treatment under the other lamp array with the same cover type. This permitted us to use the racks as the experimental replicates.

Filtered sunlight experiments. Three species were exposed to three different spectral irradiances of filtered sunlight. Plastic films were used to provide the different UV radiation environments. The near-ambient UV treatment used premium cellulose triacetate, which transmitted all solar wavelengths equally (similar to the Aclar used in other studies [30]). Because of the high transmittance of this material in the visible waveband (as indicated by testing with an array of 32 photodiodes [31]), it was necessary to use three layers of this 0.12 mm thick material so that the visible waveband was identical in all treatments. An intermediate UV level was provided by two layers of clear polyester (each 0.025 mm thick), and a very low UV level was provided by a combination of vinyl (press-polish firm, 1 mm thick) and one 0.025 mm thick layer of polyester. Spectral irradiances are shown in Fig. 2d. As in the lamp-filter experiment, the filter material was domed to shed rainfall. Plots were surrounded with fiberglass screen to protect plants from insects.

The first experimental plant, kochia, was exposed under the filters from 5 to 29 August, after emergence in the field. Pepper was exposed from 12 August to 25 September and sorghum from 19 August–13 September, after seedlings emerged in the greenhouse (to avoid insect damage). The UV-exclusion filters were not replicated in this experiment; rather, the filter tents and their corresponding treatment plants were rotated to different locations in the field twice weekly. Individual racks were considered the experimental replicates and were rotated daily to different portions of the experimental plot.

Radiation fluxes. Because the two types of experiments were conducted during different parts of the summer, ambient UV-B differed substantially during the two periods. For the lamp-filter experiment with oat, even though the filters removed much of the solar UV-B, treatment UV-B levels were controlled by the modulation system that tracked ambient UV-B radiation. Under the treatment simulating 30% ozone depletion, plants received an average of 10.1 kJ m⁻² day⁻¹ biologically effective UV-B (UV-B_{BE}, weighted with the generalized plant spectrum [28] normalized at 300 nm). In the moderate UV-B supplement, simulating 15% ozone depletion, they received 7.2 kJ m⁻² day⁻¹ UV-B_{BE}. In the second experiment the UV-B-exclusion treatments were initiated when ambient UV-B was still relatively high (>5 kJ m⁻² day⁻¹ UV-B_{BE} on clear days), but solar UV declines rapidly at this time of year at our latitude. During the course of the experiment, under the triacetate filter, UV-B_{BE} (in kJ m⁻² day⁻¹) averaged 2.9 for kochia, 2.3 for sorghum, and 2.0 for pepper.

Phenolic assay. For oat, leaf disks were placed in 5 ml 99:1 (vol/vol) ethanol-glacial acetic acid immediately after harvest and were refrigerated until extracted. The UV-absorbing compounds were extracted by refluxing (32). A total of 140 oat plants were sampled individually.

For the three species used in the exclusion study, leaf disks were placed in 5 ml of 99:1 methanol-HCL, stored at -20°C to rupture the leaf cells, and analyzed after warming to 20°C (33,34). For each species, 96 plants were sampled individually. For all plants, absorbance in the UV region was measured in a Beckman Model 35 double-beam spectrophotometer and recorded on a chart recorder between 260 and 360 nm. Species-specific absorbance peaks within this range were used to quantify the amount of UV-absorbing compounds present.

Calculations and statistics. In all experiments, means for each plant rack were used in the analysis. Thus, except where some treatments were combined in the lamp and filtered sunlight experiment (see below), n = 4 replicates per treatment. Data were analyzed by analysis of variance followed by Duncan's new multiple-range test. Analyses were conducted on the absorbance data, but here we present the relative changes between treatments (Fig. 2) to show more clearly the results in relation to the predictions of the different BSWF.

In the lamp and filter experiment with oat, the two treatments with control lamps and Llumar sunlight filters were pooled and used as the lowest UV level. The two treatments with control lamps and polyester sunlight filters were also pooled. (This was done because the irradiation conditions in these treatments were equal and thus induced similar responses.) The high-UV lamp supplement with polyester sunlight filters was the most severe UV treatment. The difference in plant response between the two most extreme treatments (end members) was calculated. This difference was then partitioned among the intermediate treatments as

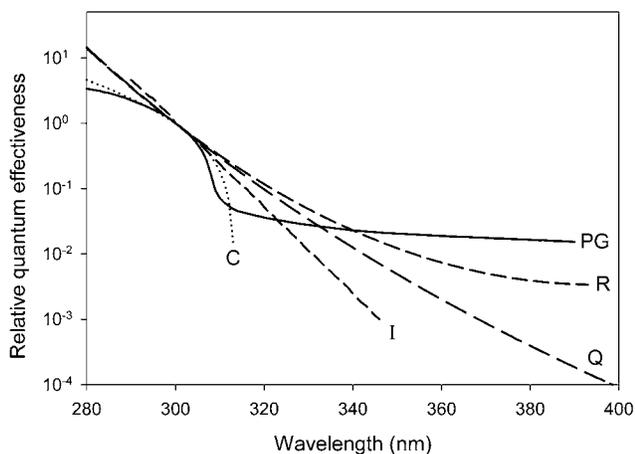


Figure 1. BSWF discussed in this article. All are normalized at 300 nm. C, generalized plant response of Caldwell (28); I, flavonol accumulation spectrum for *Mesembryanthemum* of Ibdah *et al.* (37); Q, DNA damage (pyrimidine dimer induction) spectrum for alfalfa seedlings of Quaita *et al.* (8, as formulated by Musil [35] and extrapolated to 400 nm); R, spectrum for inhibition of leaf-level photosynthesis in *Rumex patens* (1,36); PG, plant growth response spectrum (10).

a proportion of the total response that the individual treatments represented. Average plant response for each treatment was then compared with the relative response predicted by weighting midday spectral irradiance using several different BSWF (as in Flint and Caldwell [10]). In limited situations where the change in pigments did not follow the expected sequential response to increasingly severe irradiance, no predictions were attempted. For the UV-exclusion study, similar computations were made with the vinyl and triacetate filters as the end member treatments. In this case the only prediction that could be made was for the effect of the polyester (UV-B removal) filter treatment.

Five different published BSWF are presented (Fig. 1) as an array of different spectral responses that could potentially be appropriate for representing the induction of the UV-absorbing compounds. For each of the four species the predicted response from the BSWF most closely fitting the measured response is also indicated (Fig. 2).

RESULTS AND DISCUSSION

For all four species, statistically significant results were seen among some of the UV treatments (Fig. 2b,c,e,f). Expressing the data on a foliage area basis (Fig. 2b,e) and sometimes on a foliage mass basis (Fig. 2f) generated patterns of increasing pigment levels with increasingly severe UV irradiance. Expressing the oat data on a mass basis (Fig. 2c) produced more erratic results that did not precisely follow the expected pattern, although the higher UV levels still tended to show greater pigment induction than the lower UV levels.

Overall, a BSWF with a modest decline from the UV-B into the UV-A, such as the Ibdah or Quaita functions (from Fig. 1), best fit the observed results. The relative change in pigment levels is presented in Fig. 2b,c,e,f with the one (or in one case, Fig. 2f, two) BSWF that most closely predicts the measured response. Of the 11 predictions in Fig. 2, the generalized plant response predicts one observation (kochia, mass basis, Fig. 2f), the *Rumex* spectra predict one observation (pepper, area basis, Fig. 2e), and the new plant growth spectrum predicts one (oat, area basis, moderate UV-B with solar UV-A removed, Fig. 2b). The Ibdah or Quaita functions, which are intermediate between the other spectra (Fig. 1), best predict most (eight) of the results.

For oat pigments expressed on an area basis the Quaita function, closely followed by the Ibdah function, came nearest to predicting

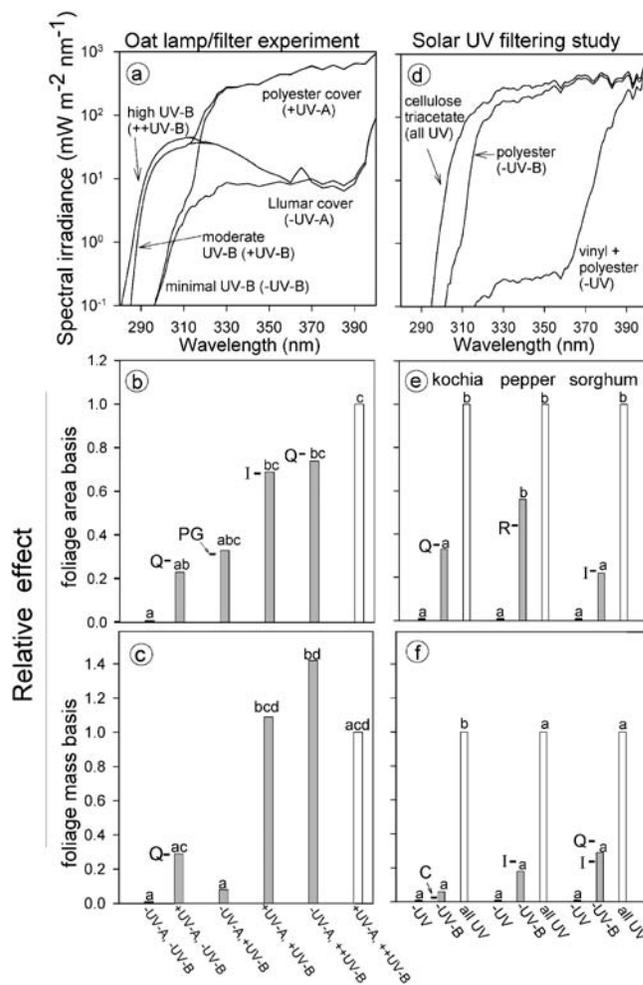


Figure 2. Spectral irradiance for the two different experiments (a,d) and relative UV-absorbing compound changes in response to the different irradiance treatments expressed on a foliage area (b,e) or foliage mass (c,f) basis. For each species different lowercase letters atop bars indicate statistical significance at $P < 0.05$ for the original absorbance data. End members are shown as a black line for the least severe UV treatment (0 relative effect) or an open bar for the most severe UV treatment (relative effect = 1). Short horizontal lines indicate the relative predicted value of the BSWF (designated by uppercase letters from Fig. 1) that most closely predicts the observed values. Note that for sorghum in (f), the measured value fell halfway between two predicted values.

the observed trend (Fig. 2b). When the oat data were expressed on a mass basis (Fig. 2c), considerably more variation was evident, and the expected response pattern was not observed. Although there were no statistically significant differences in leaf mass per area (data not shown), it appears that this was one source of variation causing some intermediate UV treatments to have pigment levels surpassing those of the maximum UV level (Fig. 2c). The Ibdah function, closely followed by the Quaita function, best predicted the observations for the three species in the UV-exclusion experiment (Fig. 2e,f). Of the six predictions, only two were predicted better by other BSWF.

Of the two BSWF providing the best fit, the Ibdah function was specifically developed for flavonol induction in *Mesembryanthemum*, using a solar simulator with polychromatic UV radiation and high levels of PAR (37). This type of solar simulator can provide radiation conditions as close to natural sunlight as has been

accomplished (38). Thus, it is not surprising that this BSWF fit the pigment response better than the ones developed for photosynthetic inhibition or plant growth (shown in Fig. 1). The Quate DNA damage function (8) was also a good predictor of pigment response. Whether there is any mechanistic connection between pigment induction and DNA damage is uncertain, although Beggs *et al.* (39) have suggested that DNA damage might lead to the induction of one isoflavonoid compound.

The importance of conducting the evaluation of BSWF under realistic field conditions cannot be overemphasized. Under either laboratory conditions or other unnatural situations, plants may respond very differently. For example, in the laboratory, UV-A may decrease the level of extractable flavonoids (40), in contrast to our results. In another example, *Carex* plants grown in the greenhouse showed decreased levels of flavonoids when treated with UV-B, whereas under UV-B supplementation in the field, flavonoids in this species increased or remained constant (41). Thus, despite the increased variation inherent in field experiments, they are a critical step in testing BSWF for the stratospheric ozone depletion issue.

Considering that it is not always possible to elicit changes in UV-absorbing compounds by lamp or UV-exclusion treatments, it is not surprising that we have not shown statistically significant separation among many of the individual UV treatments. However, all four species showed similar patterns with increasingly severe UV irradiance. To test BSWF under field conditions with statistical rigor, increased replication and repetition of experiments in time may be necessary. The possibility of different spectral responses between species also needs examination. Our limited data set suggests a likely range of BSWF for this phenomenon, but it is not possible to discern whether outlying results (*e.g.* kochia on a mass basis or pepper on an area basis) represent differences in species-specific spectral responses or are simply due to random variation.

Despite testing the accumulation of UV-absorbing compounds with two very different types of experiments, the conclusions as to the most appropriate BSWF are similar. The oat experiment was conducted during the time of maximum UV-B and used fluorescent lamps to provide a severe UV-B treatment. In contrast, the UV-exclusion experiment was conducted later in the season, and plants in the highest UV treatment in this experiment received less than 30% of the UV-B_{BE} received by those in the most severe UV treatment of the oat experiment. Thus, this spectral relationship appears to hold over a wide range of radiation conditions.

If the Ibdah or Quate functions are used to assess the ozone depletion issue, there are implications for both estimating radiation amplification factors (RAF—the increment of biologically effective UV resulting from a specific degree of ozone depletion) and simulating different scenarios of ozone depletion. These two functions provide a lower RAF than the generalized plant response, suggesting that ozone depletion will provide a smaller increment of effective radiation and existing latitudinal gradients of UV (in the absence of ozone depletion) will be smaller. However, this also means that in past experiments a smaller quantity of effective UV was applied than was anticipated at the time. These changes, however, are not as large as suggested by the comparison of the generalized plant response with the new BSWF for plant growth (10).

CONCLUSIONS

A BSWF extending into the UV-A, such as the Ibdah or Quate functions, appears most appropriate for the induction of UV-

absorbing compounds. However, these functions are substantially different from both the commonly used generalized plant response and the new plant growth function that we recently found to reflect best the spectral response of plant growth changes. If the Ibdah or Quate functions are used in experimental ozone depletion studies, more radiation will need to be applied than when the generalized plant response was used to set UV spectral irradiances. Although continued testing of BSWF on diverse taxa is warranted, for the present it is advisable to follow the suggestion of Cullen and Neale (42) to provide effective UV doses according to several published BSWF. This would allow retrospective reinterpretation of results as new data on spectral responses of organisms become available.

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