Comparisons of spore dosimetry and spectral photometry for measurement of biologically effective doses of solar UV radiation

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ABSTRACT

Since our major concern on the stratospheric ozone depletion is possible adverse effects on the biosphere, it is important to establish the way to determine biologically effective doses of solar UV radiation. The "spore dosimetry" system measuring the lethality of dry bacterial spores on membrane filters has been developed to meet this purpose. The methodology to evaluate experimental correlation with spectral measurements based on the effectiveness calculation has been applied in several field comparisons carried out at Nea Michaniona (Greece), Brussels (Belgium), and São Martinho (Brazil). When plotted against UVB irradiance (total energy below 320 nm), the calculated values of MED (minimal erythema dose), SID (spore inactivation dose) and DND (DNA damage dose) exhibited increasing exponents in power regressions, while the exponents from spore dosimetry exceeded those of the calculated values. The results of calculated versus observed values of SID indicate a general convergence at low to modest dose rates, but at high dose rates the calculated ones tended to yield lower values than those obtained from direct biological measurements.

Keywords: solar UV radiation, spore dosimetry, field comparison, erythema dose, DNA damage dose

1. INTRODUCTION

Solar radiation is polychromatic and the irradiance at the shorter wavelength region (generally referred to as UVB between 280 and 320 nm) varies greatly dependent on various meteorological and environmental factors. Therefore, physical measurements of fine wavelength resolution are required for comprehensive description. The spectrophotometers to meet such demands have been developed, and operated at various places. Although the global standardization has yet to be attained, regional comparisons (such as carried out in Europe, North America and Japan) seemed to confirm the majority of these instruments function in a reasonable congruence.

There are several problems with spectrophotometers that need be addressed from the point of the assessment of biological risks. The first one is that the spectral irradiance needs be converted to some values relevant to biological effectiveness. For this purpose, the methodology proposed by Setlow[1] has been widely employed. This is based on the premise that biological effects are represented as the sum of the effect of each component wavelength. To obtain spectral effectiveness ("effective spectrum"), several action spectra such as for minimal erythema induction, DNA damage induction or plant productivity have been introduced and used. However, since these spectra are not entirely derived from actual experiments based on absolute fluence but produced by some consensual concoctions, the real-life meaning of the "doses" derived from such calculation is often dubious. The second problem is that the spectral measurements are made in very short intervals (practically instantaneous at each wavelength), whereas many biological effects are dependent more on cumulative doses. Under conditions of changing weather, the discrepancy becomes evident. This necessitates that the spectral measurements are supplemented with continuous or cumulative measurement. Thirdly, the instruments are bulky and expensive, and require much resources and dedicated scientists for routine maintenance and operation; the conditions are difficult to be met for example in tropical regions where high UV doses are expected.

A small and robust dosimeter using *Bacillus subtilis* mutant spores has been introduced to complement and extend the usefulness of physical dosimetry[2]. The mutant spores exhibited high sensitivity to killing with UV radiation, and the inactivation (determined as the loss of colony-forming ability) follows strict exponential kinetics at any wavelength. This has been exploited to obtain a detailed action spectrum for inactivation in the entire UV wavelength range between 254 nm and 400 nm using the Okazaki large spectrograph[3,4]. Using the experimentally determined action spectrum, the quantitative comparisons with Brewer-type spectrophotometers have been successfully performed at three sites in Japan and two sites in Europe[3,4].

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In this work, further field experiments were conducted at European and South American sites and the dosimetric comparisons are made between UVB (total irradiance between 280 nm and 320 nm), MED (minimal erythema dose) and DND (DNA damage dose) in addition to SID (spore inactivation dose).

2. MATERIALS AND EXPOSURE PROTOCOLS

Preparation of spore dosimeter samples has been described before[2-4]. Briefly, four spots of about 10^6 spores of *B. subtilis* strain TKJ6312 (*spl uvr*) are made on a membrane filter, of which two spots were covered with cardboard to serve as unexposed controls. After exposure to sunlight, spores from each spot were suspended in water, and the colony-forming survivals were determined by standard microbiological methods. Spore inactivation dose (SID) was determined as the natural logarithm of surviving fraction; SID = -ln (Ne/Nc), where Ne and Nc represent the average number of colony-formers recovered from exposed and control spots, respectively.

Experiment at Nea Michaniona (40.47°N, 22.85°E, alt. 30 m) near Thessaloniki in Greece was conducted in BIODOS campaigns on July 20 and 21 in 1997 as described[4]. Exposure of spore dosimeters was performed from 8:45 A.M. to 6:15 P.M. (local time) for 60, 30 or 15 min dependent on the presumed intensity. In this report, only mid-day data of 15-min exposure were used to keep the consistency of data. A Brewer spectrophotometer (MK-III) was operated at a 30-min interval scanning between 287.5 and 366.0 nm in step of 0.5 nm during the exposure period, and each spectrum was corrected using the values of erythema irradiance determined at every minute with a UVB broadband detector (UVB-1, Yankee Environmental Systems)[5].

Experiment at Belgian Institute for Space Aeronomy (50.80°N, 4.35°E, alt. 120 m) in Brussels, Belgium was conducted on July 13 and 14, 2000. Exposure of spore dosimeters was performed for 30 min between 10:00 A.M. to 5:00 P.M. (local time). A spectroradiometer (modified Jobin-Yvon HD-10 double monochromator) was operated at a 15 min interval scanning between 280 and 550 nm in step of 0.5 nm. Also, integrated irradiance over UVB was determined with a broadband UVB meter (Yankee Environmental Systems) at every second[6].

Experiment at the INPE's Southern Space Observatory (29.43°S, 53.82°W, alt. 500 m) in São Martinho da Serra, near Santa Maria, Brazil (hereafter referred to as São Martinho) was performed on December 20, 22 and 25, 2000. Spore dosimeters were exposed for 10 min during the noontime from 12:50 P.M. to 2:10 P.M. (local time). A Brewer spectrophotometer (MK-II) was operated at a 30-min interval scanning between 280.0 and 326.0 nm in a step of 0.5 nm. Unfortunately, due to the troubles in operation, the data are available only for December 22. Concomitantly, a UVB radiometer (MS-210W, Eiko Seiki) was in operation yielding integrated UVB irradiance at every min.

Action spectra used for the effectiveness calculations are shown in Fig. 1. The action spectrum of spore dosimeters is from interpolation of the values of inactivation rate constants experimentally determined using Okazaki large spectrograph[4]. The action spectrum for erythema induction is a reference established by the Commission International de l'Eclairage (CIE)[7] and the value is normalized as 1 MED is equivalent to 210 J/m^2. The DNA damage spectrum is originally compiled by Setlow[1] and adapted to the formula[8]: spectral action = exp {13.82[(1.0/D)-1.0]} , where D = 1.0 + exp [(L-310)/9] (L, wavelength in nm). The value is 1.0 at 254 nm.

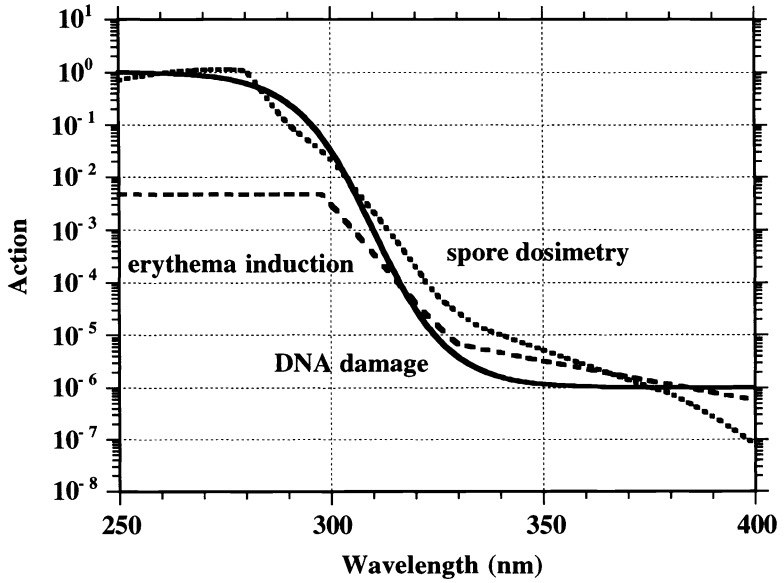

Fig. 1. Three action spectra used for effectiveness calculation.

3. RESULTS

3.1 Experiment at Nea Michaniona

From spectral irradiance obtained with 30-min interval scans and MED measurements at every minute, spectra for each 15-min interval were constructed, and used to calculate the effectiveness spectra by multiplication with action spectra. The spectral effectiveness was summed up for the wavelength range of 290 and 400 nm (a uniform value of irradiance was assumed between 367 and 400 nm) and the values of spore inactivation dose (cal SID), minimal erythema dose (MED), and DNA damage dose (DND) were derived. The calculated values of UVB (J/m^2) were obtained as the sum of spectral irradiance below 320 nm. For a comparison purpose, all the derived doses were divided by 15 min and expressed as the dose rate. The maximum value of observed dose rates was 0.48 (SID/min) when the calculated one was 0.33. In Fig. 2, these doses together with the values of spore inactivation dose (obs SID) directly measured by spore dosimeters are plotted against UVB irradiance and regressed by power functions.

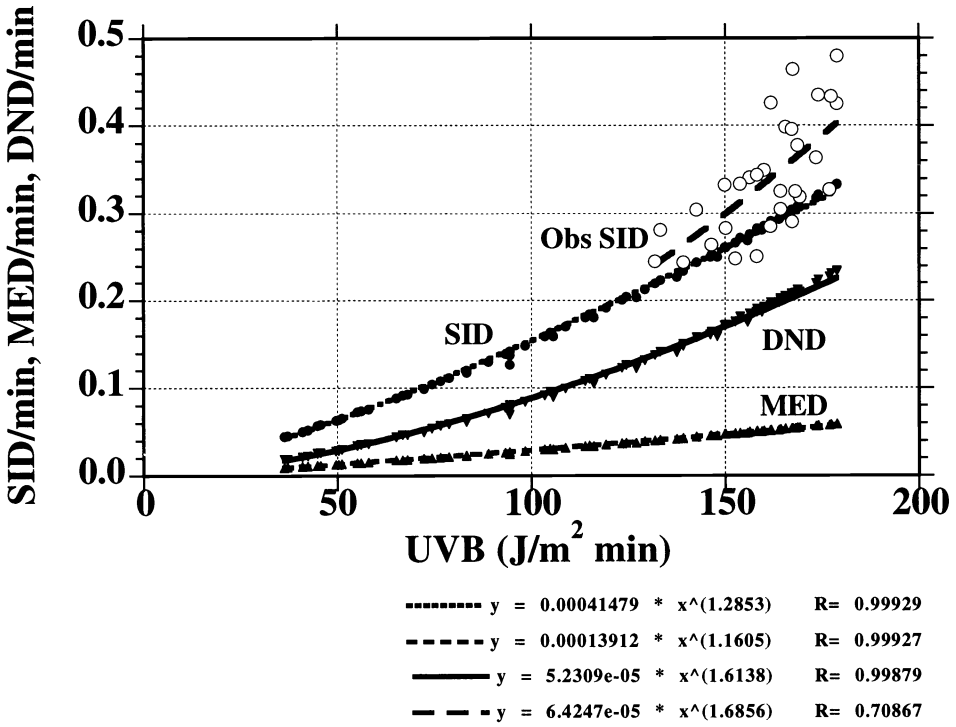

Fig. 2. Calculated UVB irradiance *versus* calculated SID (filled circle), DND (down-triangle), MED (up-triangle), and observed SID (open circle) from experiment at Nea Michaniona.

All the calculated doses can be fitted to power functions of UVB irradiance with maximal regression coefficients (>0.99). As expected from the action spectra, the exponent of the regressions is smallest for MED (1.16), middle for SID (1.29) and largest for DND (1.61). On the other hand, the observed doses of SID exhibit large scatters and the exponent is larger (1.69) than that for DND. As previously argued, it is likely one cause for the scatters of the observed values is weather changes: the correction method of spectral construction does not work perfectly under rapid weather changes. On the other hand, higher ratios (1.18 ± 0.16 SD for 30 data points) of the observed doses to the calculated ones are not easily explained.

3.2 Experiment at Brussels

Exposure of spore dosimeters for continuous 30-min intervals was carried out from 10:00 A.M. to 5:00 P.M. on two consecutive days in July 2000. On the first day (13^{th}), it was generally cloudy, and on the second day (14^{th}), it was extremely variable from moments of clearness to heavy clouds and hard rain. The variable weather is seen as alternate ups and downs in the Fig. 3 (the right panel). The maximum value of observed dose rate was 0.20 (SID/min) when the calculated one was 0.19. Close examination reveals that under such variable weather, two observed values of SID and

UVB and two calculated values of SID and UVB exhibit parallel changes. This illustrates the difficulties of a spectrophotometer to follow rapid weather changes even for the instrument that makes measurements at every 15-min interval. Under such conditions, continuous measurements of either UVB or MED are needed to estimate biological doses.

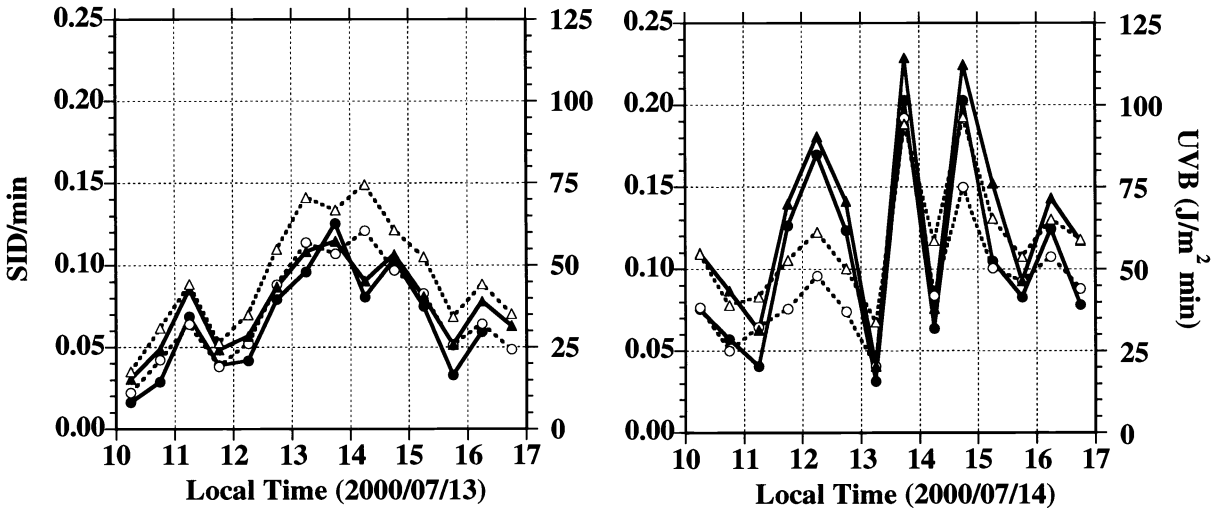

Fig. 3. Diurnal measurements of observed (filled circle) and calculated (open circle) SID and observed (filled triangle) and calculated (open triangle) UVB at Brussels.

The result of the effectiveness calculations is shown in Fig. 4. In the regressions to calculated UVB irradiance, similar but less extensive tendencies than seen at Nea Michaniona are observed. The exponents of the regressions are in the increasing order, 1.09 for MED 1.13 for calculated SID, and 1.28 for DND, and 1.48 for observed SID. The values of observed SID exhibited large scatters against calculated UVB, but the mean ratio of observed to calculated SID was close to one (1.01 ± 0.31 SD for 27 data points). This indicates that when the spectral measurements were corrected using the data of continuous measurements of MED or UVB, the biological and physical dosimeters were very congruent.

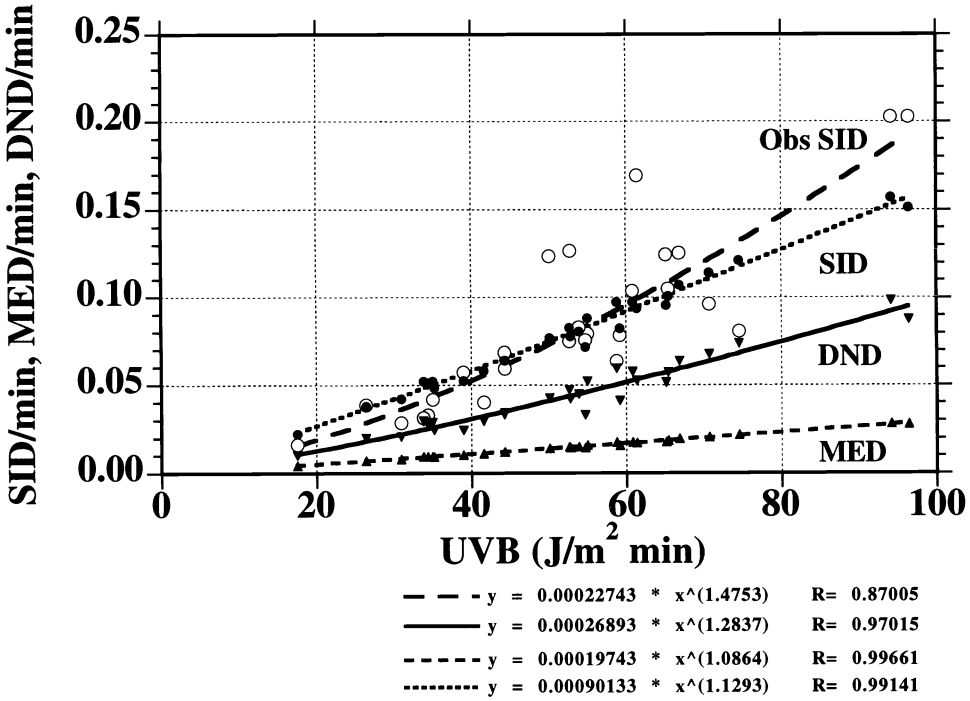

Fig. 4. Calculated UVB irradiance *versus* calculated SID (filled circle), DND (down-triangle), MED (up-triangle), and observed SID (open circle) from experiment at Brussels.

3-3 Experiment at São Martinho

Previous measurements at two European sites suggested that the physical dosimetry based on spectral measurements and the spore dosimetry were concordant when the solar intensity was relatively low. It is thought necessary to extend such comparison under higher solar altitudes. We have chosen three relatively clear days around the solar solstice (minimal solar zenith angle = 6.3° at 1:30 P.M. on 22nd) in December 2000 at São Martinho in Southern Brazil. On each day (20th, 22nd and 25th), exposure of spore dosimeters was performed continually for 10 min from 12:50 P.M. to 2:10 P.M. The maximal value of the observed dose rates was 0.77 (SID/min). The UVB values were obtained from continuous measurements with a UVB meter and generally paralleled the observed values of SID as shown in Fig. 5.

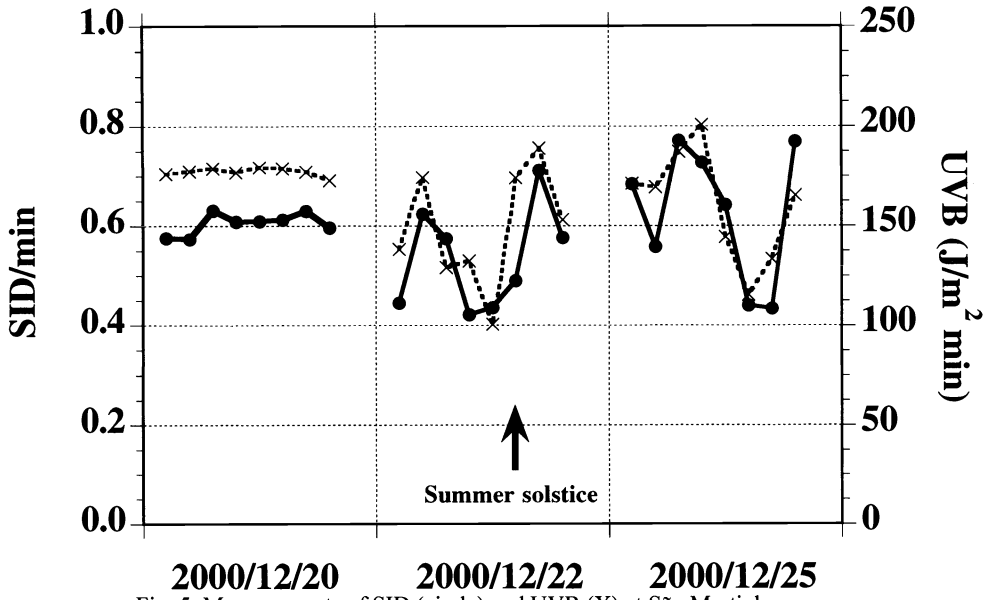

Fig. 5. Measurements of SID (circle) and UVB (X) at São Martinho.

The Brewer data were available only for 22nd, and the results of dose calculations are shown in Fig. 6. The exponents of the power regressions 1.15 for MED 1,22 for calculated SID, 1.44 for DND; the values were in the midst of those observed at Brussels and Nea Michaniona. However, three observed values of SID/min exceeded much the calculated ones with the ratios of 1.66, 1.37 and 1.73. Unfortunately, the scarcity of available data seems to preclude detailed analysis at this point and further experiment is in progress.

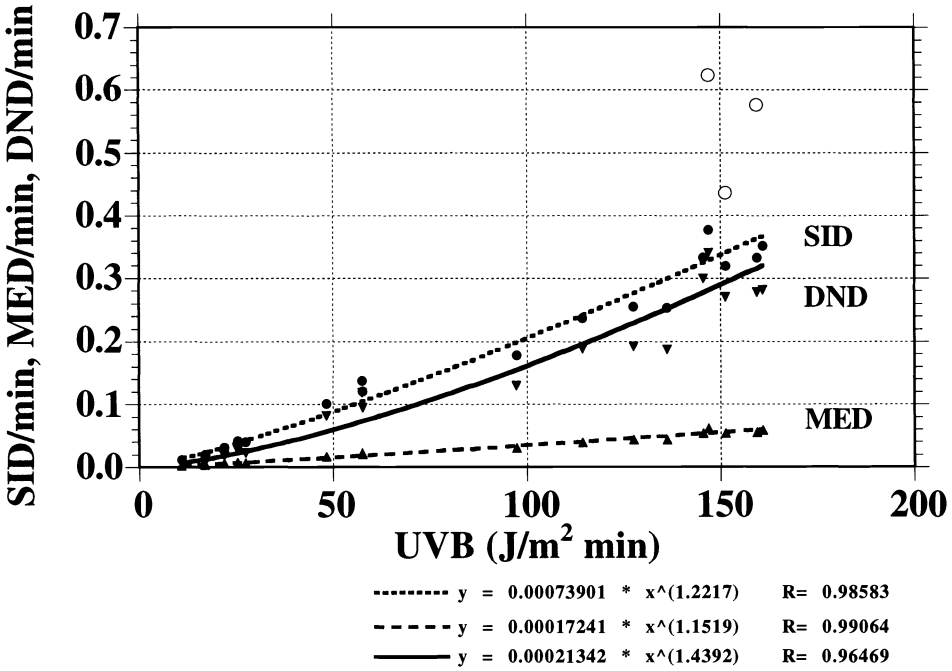

Fig. 6. Calculated UVB *versus* calculated SID (filled circle), DND (down-triangle), MED (up-triangle), and observed SID (open circle) from experiment at São Martinho.

4. DISCUSSION AND CONCLUSIONS

Among the physical measurements discussed in this paper, the simplest one is integrated UVB irradiance. The UVB irradiance in energy fluence can be calculated from spectral irradiance and also obtainable with well-calibrated meters dedicated for continuous monitoring. On the other hand, none of the doses relevant to biological effectiveness display proportionality with the UVB values: instead, it is possible to regress biological doses to power functions of UVB, and the exponents of such function were generally higher than one. This is reasonable, since the higher UVB value means higher irradiance of the shorter wavelength components, which amplifies biological effectiveness as seen from the action spectra. The profiles of the three effectiveness spectra and solar spectral irradiance are shown in Fig. 7.

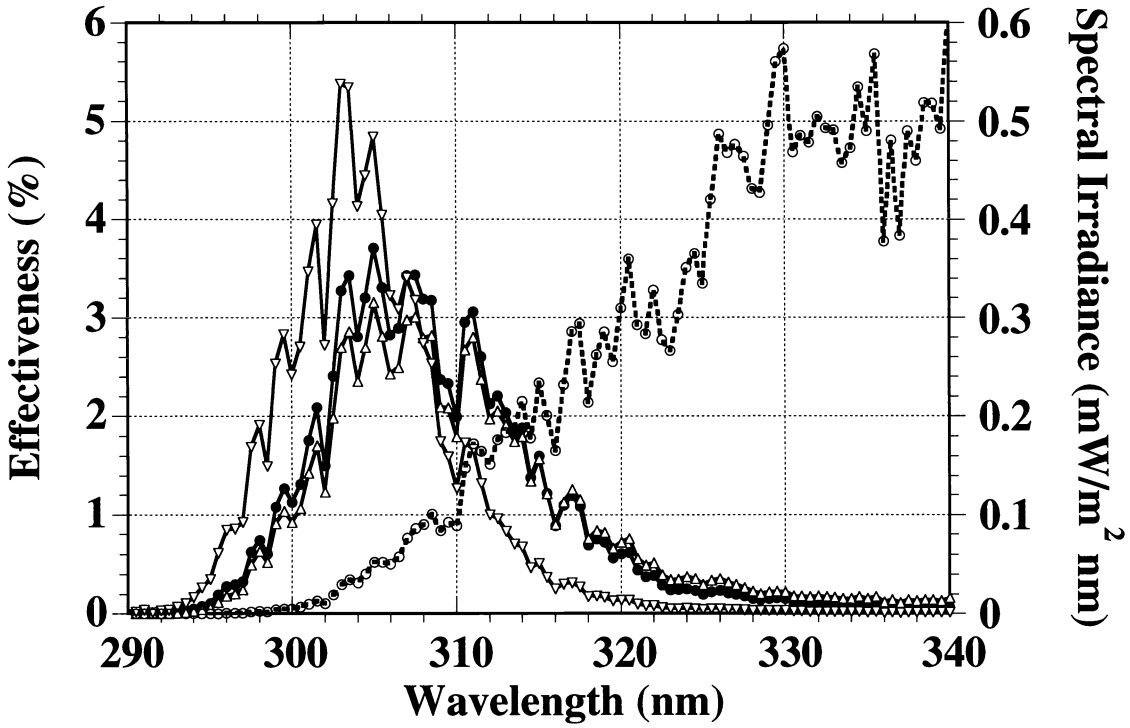

Fig. 7. Effectiveness spectra for SID (filled circle), DND (down-triangle), and MED (up-triangle), and an irradiance spectrum (open circle) observed at 1:15 P.M. on July 20, 1997 at Nea Michaniona.

As has been noted for SID effectiveness spectra, the spectra exhibit fine structures of peaks and troughs originating from Fraunhofer lines of the solar spectrum. The peaks in shorter wavelengths are higher for the DND spectrum in comparison to SID and MED spectra; the maximum peak for the former one is at 303 nm, while those are at 305 nm for the latter ones. These are explained from the differences in the action spectra. The DND spectrum exhibits the steepest slope in UVB wavelengths, while the SID and MED spectra exhibit less steep slopes. On the other hand, the DND and SID spectra continue to increase the action in the shorter wavelengths than 300 nm, while the MED spectrum stays constant. It is notable that none of the major peaks of the effectiveness can easily be discernible in the irradiance spectrum.

Fig. 8 summarizes the comparisons of spore dosimetry and UVB measurement. In the figure, the UVB values at Nea Michaniona and Brussels are obtained from calculated ones, whereas those at São Martinho are from the meter readings because of the lack of Brewer data in two days. The data points at three sites cover different dose ranges and seem to be divergent in complex ways. Some of the scatters are explainable by weather changes, but the large differences between the data obtained at Nea Michaniona and São Martinho can not easily be explained. One important factor seems the differences in ozone thickness: daily representative values of ozone column in Dobson Unit were 314 and 320 at Nea Michaniona, 355 and 377 at Brussels, and 267, 271, and 268 at São Martinho. At this point, we do not have a satisfactory model to incorporate ozone thickness in the relationship between UVB and SID. When all the data are forced to regress to a power function, we obtain, y = 0.00021*x^(1.50) (R=0.86), where x is UVB (J/m^2 min) and y is the observed value of SID/min.

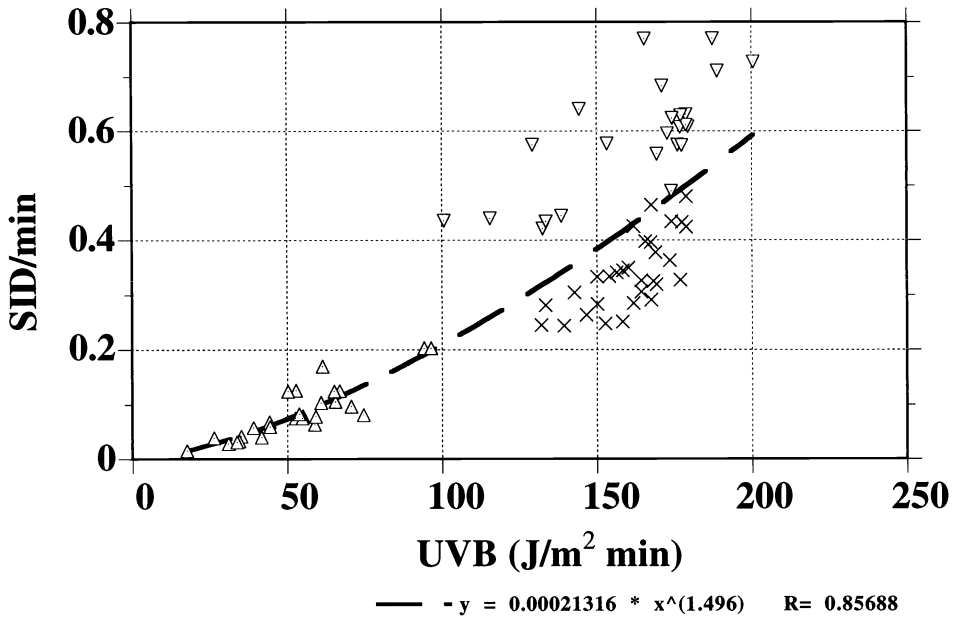

Fig. 8. UVB irradiance *versus* observed SID at Nea Michaniona (X), Brussels (up-triangle), and São Martinho (down-triangle).

In conclusion, comparisons of physical and biological dosimetry have been extended at two European sites and one South American sites covering a large dose range. The doses calculated from biological effectiveness spectra were nonlinear to the integrated UVB irradiance. Moreover, the direct biological measurements with spore dosimeters exhibited the largest amplification to the increase of UVB irradiance. The reasons for the lower calculated values of SID than the observed ones shown clearly under high intensity conditions are not known. It may suggest uncharacterized deficits in spectral determination in the short wavelength region.

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