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The Use of Biodosimetry to Measure the UV-C Dose Delivered to a Sphere, and Implications
for the Commercial Treatment of Fruit.

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Abstract

Commercialization of UV-C treatment of horticultural produce in order to induce beneficial responses in the produce following treatment requires both accurate dose delivery and a method of treating large quantities of produce efficiently. Furthermore, it has long been assumed that such effects require the entire surface of the horticultural commodities - typically fruit - to be exposed to UV-C. This has invariably been achieved by manually rotating the fruit in a UV-C field whilst reducing the dose delivered at each rotation in direct proportion to the number of rotations. However, the resulting UV-C dose distributions achieved under these circumstances are generally not reported in the literature. In the work described here a polystyrene sphere (Dia., 70 mm) was used to simulate fruits such as tomatoes, apples, peaches etc., that have an approximately spherical form in order to provide a means of measuring the total doses of UV-C accumulated during treatment and comparing such estimates to theoretically-derived ones. This was achieved using dosimetry based on spores of *B. subtilis* in which spore-impregnated membranes were attached to the surface of the sphere. The fraction of spores surviving exposure was used to estimate dose from a dose-response curve for the spores. Under irradiation conditions leading to a theoretically calculated dose of 10.6 J, spore dosimetry yielded estimates of 9.1, 10.7 and 6.1 J for UV-C delivered in respectively, one, two or four exposures. In the case of exposure of the sphere during continuous mechanical rotation for the same length of time (80

s) a value of only 3.5 J was obtained. Irradiation conditions resulting in the spores being subject to intermittent exposure to UV-C led to dose estimates below the theoretically derived ones. The circumstances under which spore dosimetry can be used to obtain surface dose distributions are discussed.

Keywords: UV-C Hormesis, UV-C Dose Measurement, *Bacillus subtilis* spores, Biodosimetry, Commercial UV Processing.

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1. Introduction

The proportion of post harvest losses of fruits and vegetables has been conservatively estimated as being of the order of 20 % in developed countries (Wilson and Wisniewski, 1989; Dal Bello et al., 2008) and as high as 50% in developing countries (Wilson and Wisniewski, 1989). Whilst these estimates conceal geographic, crop-specific and other variations, such levels of losses can no longer be accepted as inevitable, or indeed sustainable, when viewed in terms of the scarce resources needlessly consumed at a time when the world's demand for food is failing to be met.

One proposal that is attracting attention for increasing the shelf life of horticultural commodities, and hence reducing food wastage, is the application of low doses of shortwave ultraviolet light (UV-C). This form of treatment has been referred to as 'hormetic' i.e. providing beneficial outcome from an agent (UV-C in this case) that at high doses can prove detrimental (Calabrese and Blain, 2009). This type of application needs to be differentiated from the more conventional application of UV-C conducted to directly inactivate micro-organisms present at, or near, the surface of the horticultural commodities. Hormetic treatment is intended to result in the induction of anti-microbial plant metabolites that occurs over a period of time *following* the application of UV-C treatment (Shama, 1999). The potential that hormetic UV-C treatment holds for the horticultural sector has recently been reviewed (Shama and Alderson, 2005), and one benefit in particular is that decreased reliance would be placed on exogenously applied chemical agents such as fungicides (Escalona et al., 2010).

Optimal UV-C doses are typically obtained as a result of experimental studies conducted at a small scale and often, by the treatment of individual commodities –typically fruit. Because, as inferred above, UV-C has the potential to damage plant tissue at sufficiently high doses, it is important to be able to accurately deliver doses that have been experimentally found to elicit hormetic effects. Such considerations would be crucial in commercializing UV-C treatment (Shama, 2007). However, it is first necessary to investigate whether the modes by which fruit

have been treated in previous experimental studies are all equivalent. In the majority of previous studies workers have attempted to ensure that the entire surface of the fruit receives exposure to UV-C by manually rotating the fruit 2 or 4 times. In such cases the dose delivered to each 'side' of the fruit is reduced in direct proportion to the number of times it is rotated (Stevens et al., 2005; Charles et al., 2008; Yang et al., 2009). However, in none of these studies were surface dose distributions experimentally determined.

One method of achieving this is through the application of spore dosimetry (Tyrrell, 1978). In this method the dose-response behaviour to UV-C of microbial spores is first obtained and then the fractional survival of spores is determined under conditions where it is desired to estimate the UV-C dose. Doses may then be computed from the dose-response curve. Spores of *Bacillus subtilis* have frequently been used for this purpose owing to the fact that they are not pathogenic (Gardner and Shama, 1999). Spore dosimetry itself comes under the general category of 'biodosimetry', i.e. measuring the response of a biological agent to the effects of electromagnetic radiation. In the work described here we examine whether spore dosimetry can be used to estimate the doses of UV-C delivered to the surface of a polystyrene sphere under conditions of exposure designed to emulate those mentioned above that have been used in laboratory studies with fruit. Manual rotation of fruit would obviously not constitute a viable commercial method of treatment, and therefore we extended our investigation to include one method (mechanical rotation) that could potentially enable different types of produce to be irradiated with UV-C ensuring both that consistent doses are achieved and that the dose distribution is relatively even over the surface of the produce. In all cases the integrated UV-C dose was estimated by attaching membranes onto which spores of *B. subtilis* had been deposited at various points on the surface of the sphere, and these are compared with computed estimates of doses.

2. Materials and methods

2.1 UV Apparatus

The apparatus used for irradiating polystyrene spheres with UV is shown in Figure 1. The UV source used was a low pressure mercury burner (GX018TSL, Voltarc Tubes Inc., Fairfield, CT., USA) having principal emission at 253.7 nm and rated at 42 W. This source was located within a parabolic reflector fabricated from anodised aluminium. Immediately below the source was a roller assembly driven by a variable speed electric motor (not shown). The entire source-reflector assembly could be raised or lowered above the rollers to change the UV-C intensity. For static treatment of polystyrene spheres, the spheres were placed on the cylindrical rollers but with the motor turned off. For irradiation of membranes impregnated with spores (see below), the roller assembly was removed and the membranes were treated on a stainless steel plate placed centrally below the source. The intensity at the membrane surface was measured using a radiometer (Model UVX, UV Products Ltd., Cambridge, Cambs.).

2.2 Preparation of Dose-Response Curve

Spores of *B. subtilis* (ATCC 6633) were produced according to the method described by Gardner and Shama (1999) and stored at 4°C until needed. Spore suspension (1 mL) was filtered through a 13 mm dia. Durapore® membrane with a retention of 0.22 µm (Millipore (UK) Ltd., Watford, Herts) and then dried for 5 minutes in a laminar flow hood. This procedure was highly consistent and resulted in the deposition of from 3.0 to 3.2 x 10⁶ spores per membrane. After treatment, spores were recovered by placing the membrane in tubes containing 1 mL Ringer's Solution and 5 glass ballotini beads (4 mm) and agitated using a vibratory mixer for 5 minutes and the spore suspensions thus obtained were serially diluted as necessary. Aliquots (100 µL) were then plated onto the surface of Tryptone Soya Agar (Oxoid Ltd., Basingstoke, Hants). The plates were then incubated at 30°C overnight and then counted. All experiments were conducted

in duplicate. Plots were then made of the log of reduction in spore viability ($\log N/N_0$) against delivered dose to give the Dose-Response Calibration Curve for *B. subtilis*.

2.3 Preparation and Irradiation of Polystyrene Spheres

Shallow indentations (0.5 mm deep) were made in the surface of polystyrene spheres (dia. 70 mm; Fred Aldous Ltd., Manchester, Lancs.) using a stainless steel rod of 15 mm dia. This enabled the membranes prepared as described above to be securely attached to the surface of the spheres. The membranes were further secured in place by 50 μ m thick discs of UV-C transparent perfluoroalkoxy (PFA) film (Polyflon Technology Ltd., Stone, Staffs) held in place by narrow strips of double-sided adhesive tape. Imagining the 'north pole' of a sphere to represent 0°, membranes were placed at 0, 45, 90 135 and 180° (Figure 2a). For static treatment the spheres were irradiated as follows; a) irradiation for 80 seconds b) irradiation for 40 sec. after which the sphere was rotated through 180° before receiving a further irradiation of 40 sec. c) irradiation for 20 seconds followed by three rotations of 90° at which irradiation was for 20 seconds at each rotation.

For treatment under rotation, spheres were treated singly for either 80 or 160 seconds at the same intensity at a rotational speed of 10 rpm. In a further series of experiments spheres were treated as above but with identical 'blank' spheres either side of the test sphere. These spheres did not contain spore-laden membranes at their surfaces but were introduced to establish whether their presence would reduce the amount of UV-C energy incident on the test sphere.

2.4 Estimating the Total UV-C Dose Delivered to Spheres by Measuring Spore Survival

Figure 2b depicts a sphere within a UV-C field; if a spherical segment has an area dA then the energy falling on the surface of the segment is given by:

$$dE = D(y)dA \quad (1)$$

where $D(y)$ is a function denoting the variation of UV-C dose at the surface. Substituting the area of a segment of thickness dy into equation (1) gives:

$$dE = D(y) * 2\pi y(x) \sqrt{dx^2 + dy^2} \quad (2)$$

Because the object in the UV-C field is a sphere, the function $x(y)$ may readily be computed. The total UV-C energy falling on the sphere is obtained by integrating (1):

$$E = 2\pi r \int_0^{2r} D(y) dx \quad (3)$$

In the work conducted here the dose was determined using spore dosimetry at points 1-5. $D(y)$ was obtained by fitting a polynomial function to the experimental points.

2.5 Theoretical Estimation of the Total UV-C Dose Delivered to Spheres

Knowledge of the UV-C intensity at any point on the sphere enables the intensity at any other point to be calculated using the inverse square law:

$$I_2 = I_1 \left(\frac{y_1^2}{y_2^2} \right) \cos \theta$$

Where I_1 is the intensity at distance y_1 from the UV-C source and I_2 is the intensity at distance y_2 from the source and θ is the orientation of a tangent drawn at the surface of the segment with the x-axis.

In the work reported here the sphere was divided into 5 segments and I_1 (3.1 mW/cm^2) was measured using a UV-C radiometer.

2.6 Statistical Analysis

Analysis of variance (ANOVA) was carried out using commercially available software (SIGMAPLOT 11; Systat Software Inc., San Jose, USA) on all experimental determinations of delivered UV-C doses.

3. Results and discussion

The dose response curve for spores of *B. subtilis* is shown in Figure 3. Using this figure the measured log reductions in spore viability were ‘translated’ into UV-C doses expressed as mJ/cm².

Table 1 depicts the reductions in spore viability at each position of the sphere at which membranes were attached along with the corresponding UV-C dose estimates. The values shown represent the means from two separate experiments. For the case of a single exposure for 80 s, the highest dose recorded (178 mJ/cm²) was at position 1. The dose at position 3 is only 10 % of that at position 1, whilst at positions 4 and 5 no reduction in spore viability was detected implying a zero dose.

Delivering the UV-C dose in 2 exposures each of 40 s resulted in doses at positions 1 and 5 of 92.0 mJ/cm², that is, 52 % of that for a single exposure. Where the dose was delivered in 4 consecutive exposures each of 20 s duration with rotation through 90 ° after each exposure, the doses at positions 1, 3 and 5 ranged from 49.2 to 58.2 mJ/cm², which represented 30 % of the value for a single exposure. Using the methods described above in Materials and Methods the total, or integrated, UV-C dose delivered to spheres were calculated from experimental measurements and also from theoretical considerations and are displayed in Table 2. Although based on five experimental point readings of dose, the geometric symmetry of the test object (a perfect sphere) enabled these predictions to be made with confidence. The theoretically-derived doses are all equal to 10.6 J, however, the dose distribution is markedly different for each case and is depicted in Figure 4. As expected, rotation of the sphere in the UV-C field four times results in the most even dose distribution.

Good agreement with the theoretically-derived total dose is obtained from the spore dosimetry experiments when the sphere was irradiated either once or twice (Table 2). However, for the case

of four rotations the method employed here gave a total dose of only 6.1 J - considerably below the calculated value. The errors shown alongside the doses were computed using the polynomial used to fit the data in the dose response curve (Figure 3) and from estimates of the errors in determining the reductions in spore viability. For the former cases (no rotation of the sphere, or only one rotation) each of the spore-laden membranes received only a single exposure to the UV-C source, however, for four rotations each of the membranes would have received two exposures of correspondingly reduced doses of UV-C with a short time interval between each exposure.

In experiments conducted using the mechanical rollers it was observed that although the weight of the polystyrene spheres (c. 5.6 g) was considerably lower than that of typical fruit of the same diameter – an orange, for example, would weigh approximately 200 g – at the speed of rotation employed here the spheres did not display a tendency to roll or spiral in a lateral direction. Under these conditions of irradiation the total apparent dose for 80 s exposure was only 3.5 J. This was the same time of exposure used for the spheres that were manually rotated and is only 33 % of the theoretical dose. Doubling the exposure to 160 s gave an increased dose of 10.2 J – close to the values obtained above. In order to establish whether this form of irradiation employing rollers could form the basis of a practical, commercially-based process for treating produce, the effect of interference from adjacent spheres was evaluated. To do this a sphere with spore-laden membranes attached to it was placed on the rollers and on either side of it were placed blank spheres – i.e. without membranes. A reduction in spore inactivation was observed at positions 1 and 5 (Table 3), that is along the axis of rotation, but the total dose delivered was 8.9 J which represents only a relatively small reduction compared to the case above for a single sphere.

The case of the sphere given 4 exposures to UV-C and the spheres rotated on the rollers are similar in that the spores located on the membranes were subject to, in the first case, as pointed out above, 2 exposures to the UV-C source separated by a short time interval, and in the latter

case multiple exposures separated by somewhat shorter time intervals. The effects of intermittent exposure to UV-C on microbial inactivation have previously been studied. Harm (1980) found that survival in such instances was greater than if the dose were delivered in a single exposure. This was attributed to the operation of DNA repair mechanisms during those intervals when the microbial cells were not actually exposed to UV-C. Significantly, spores of *B. subtilis* are known to possess the facility for repairing UV-C induced damage (Slieman and Nicholson, 2000).

This phenomenon constitutes in effect a limitation to the application of spore dosimetry for UV-C dose determination. For cases where spores would receive only a single exposure to UV-C the results presented here show that the method should prove useful and readily applicable. Spore biodosimetry could be used to obtain estimates of dose distribution on the surface of objects of irregular geometry or in cases where an object receives irradiation by more than one UV source where mathematical predictions would become complex. However, limitations could arise if the conditions of dose delivery result in an interval between UV-C dose accumulation at the surface of an object. Apart from the roller device described here, this could arise if the object were being conveyed in a UV tunnel with a discrete number of sources resulting in intervals of time when the surface of the object were not being irradiated (Shama, 1999).

It has become the convention in experimental studies to cite UV-C doses in terms of energy delivered per unit area – e.g. J/m² (Shama and Alderson, 2005) rather than in terms of *total* UV-C dose delivered. The former are obtained by multiplying the UV-C intensity by the time of exposure. The reluctance to give total doses stems from the fact that whilst it is possible to calculate the total dose delivered for objects of regular geometry, horticultural produce rarely conforms to this mathematical convenience. Notwithstanding, certain fruits such as apples, tomatoes, citrus fruit and peaches could be considered to a first approximation as perfect spheres. Calculating the total UV-C dose delivered to a head of broccoli would prove more

challenging, whilst calculating the dose delivered to a bunch of grapes would require a considerably greater mathematical effort. Irrespective of this, the methods described here should permit delivered doses to be measured when objects of irregular geometry – i.e. fruits and vegetables – are exposed to sources of UV-C.

The issue of whether it is even necessary to irradiate the entire surface of horticultural products is one that requires consideration. Mercier et al. (2000), attempting to prevent *Botrytis cinerea* infection of carrots, found that UV-C did not have a systemic effect, and that it was necessary to ensure full surface exposure. Moreover, these workers showed that resistance to infection was closely associated with the accumulation in the carrot tissue of 6-methoxymellein which only accumulated where the tissue had received direct irradiation. In such cases it would be useful to have surface dose distribution plots such as are shown in Figure 4 in order to ensure that the threshold UV-C dose for eliciting the plant response was being achieved over the entire surface. On the other hand, Stevens et al., (2005) showed that for apples, peaches and tangerines the greatest resistance to a variety of mould-induced rots were obtained by delivery of the UV-C dose at the stem end of the fruit without rotation. It may turn out that whether or not full surface exposure to UV-C is necessary may be dependent on the type of produce and it is evident that further studies are required to determine this.

257 **Acknowledgement**

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302 **Figure Captions**

303 Figure 1 Schematic of UV Equipment

304 Figure 2 Polystyrene Sphere used in Experimental Studies

305 Figure 2a Location of Spore-laden Membranes

306 Figure 2b Estimation of the Total UV Dose Delivered to a Sphere

307 Figure 3 UV-C Dose Response Curve for Spores of *Bacillus subtilis*

308 Figure 4 Theoretically-Derived UV-C Dose Distributions for Spheres under Different

309 Conditions of Exposure

310 a Single Exposure (The UV-C Dose was delivered in one exposure of 80 s)

311 b Two Exposures (The sphere was irradiated for 40 s, rotated through 180 ° and irradiated for a further 40 s)

312 c Four Exposures (The sphere was irradiated for 20 s then rotated through 90 °; this was repeated a further 3
313 times).

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315

Position		Mode of Exposure to UV-C (number of exposures x time at each exposure)					
		1 X 80 s		2 X 40 s		4 X 20 s	
		log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)
Number ¹	Angle (Degrees)						
1	0	-1.5	178.1 _a ±3.2	-1.22	92.0 _a ±7.5	-0.96	54.5 _a ±0.1
2	45	-1.4	129.1 _b ±5.2	-1.10	73.8 _b ±0.1	-0.64	27.2 _b ±3.2
3	90	-0.4	18.7 _c ±0.5	-0.60	24.9 _c ±2.7	-0.92	49.2 _a ±5.4
4	135	0.0	0.0 _d	-1.13	83.2 _b ±9.8	-0.66	27.2 _b ±2.7
5	180	0.0	0.0 _d	-1.22	92.0 _a ±7.5	-1.00	58.2 _a ±3.6

317

318 Table 1: UV Doses² Delivered to a Sphere (Dia., 70 mm) following Different Modes of
319 Exposure as Estimated from *B. subtilis* spore dosimetry

320 1 Refer to Figure 2

321 2 Average of two readings with standard deviation

322 Within the same column dose values bearing different subscripted letters are significantly different (P ≤ 0.05)

323

Number of Rotations in the UV Field	UV Dose (J)	
	Experimental ¹	Theoretical
Single	9.1 _a ±0.9	10.6
Two	10.7 _a ±1.0	10.6
Four	6.1 _b ±0.6	10.6

Table 2: Comparison of Total UV-C Doses Delivered to Spheres under Different Conditions of Exposure as Determined by *B. subtilis* Spore Dosimetry and by Calculation.

‘Single exposure’ denotes that the sphere was irradiated by the UV-C source for 80s; ‘Two Exposures’ that the sphere was irradiated for 40 s, rotated through 180 ° and irradiated for a further 40 s; ‘Four exposures’ that the sphere was irradiated for 20 s and rotated through 90 ° and that this was repeated a further 3 times.

¹ Experimentally determined UV dose values with percentage errors from dose response plot (Figure 3)

324 Within the same column dose values bearing different subscripted letters are significantly different ($P \leq 0.05$)

325

Conditions and Time of Exposure		Single Sphere (80 Seconds)		Single Sphere (160 Seconds)		Sphere with Neighbour (160 Seconds)	
Position	Angle (Degrees)	log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)	log (N/N ₀)	UV Dose (mJ/cm ²)
1	90	-0.47	18.6 _a ±1.5	-0.79	35.6 _a ±1.0	-0.39	15.5 _a ±0.6
2	45	-0.65	27.7 _b ±0.6	-0.85	45.9 _b ±5.7	-0.82	39.5 _b ±4.5
3	0	-0.91	49.2 _c ±0.2	-1.39	136.9 _c ± 2.6	-1.34	135.9 _c ±1.6
4	45	-0.66	27.7 _b ±0.6	-0.85	45.9 _b ±5.7	-0.82	39.5 _b ±4.5
5	90	-0.49	18.6 _a ±1.5	-0.79	35.6 _a ±1.0	-0.42	17.2 _d ±1.0

Table 3: UV-C Doses¹ Delivered to a Sphere Rotated at 10 rpm under Different Conditions.

The notation “Single Sphere” indicates that only one sphere was present on the roller assembly during treatment, whereas “Sphere with Neighbours” denotes that blank spheres were placed either side of the test sphere.

1. Average of two readings with standard deviations

Within the same column dose values bearing different subscripted letters are significantly different ($P \leq 0.05$)

326

327

328

Figure 1

Figure 2

Figure 3

Figure 4